BIOPROSPECTING THE POTENTIAL OF

Taverniera cuneifolia (Roth) Ali.

THESIS SUBMITTED TO

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BY

POONAM S. MANGALORKAR

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Dr. PADAMNABHI S. NAGAR

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DEPARTMENT OF BOTANY
FACULTY OF SCIENCE
THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA
VADODARA 390 002, (GUJARAT), INDIA.
PHONE : (+91-265) 2791891

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CERTIFICATE

I Hereby Declare that the thesis entitled "Bioprospecting the potential of *Taverniera cuneifolia* (Roth) Ali" submitted for the award of the degree of Doctor of Philosophy (Botany) of the M.S. University of Baroda, Baroda, Vadodara, embodies the results of bonafide research work carried out by Ms. Poonam S. Mangalorkar under my Guidance and supervision.

(Dr. P. S. Nagar)

Guiding Teacher

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Chapter 1: Introduction

Chapter 2: Review of Literature

Chapter 3: Materials and Methods

Chapter 4: Results and Discussion

Introduction

1.1 INTRODUCTION

Bioprospecting is the "Exploration of biodiversity for commercially valuable biological and genetic resources" (Laird and Kate, 2002). This activity "involves the application of advanced technologies to develop new pharmaceuticals, agrochemicals, cosmetics, flavorings, fragrances, industrial enzymes, and other products from biodiversity" (Artuso, 2002).

Bioprospecting means the "search for useful biological materials in micro-organisms, plants, fungi, animals and humans" (Polski, 2005). Ever since mankind has started living in community be it small or large, the bioprospecting of nature for combating diseases has been a never ending process. The trial and error efforts directed towards the advancement of the quality of life was compiled orally or textually in due course of time, so that it can be communicated well down the generations. Today contemporary natural medicines in use are pure and well-defined chemotherapeutic chemical entities. The evolution from herbal remedies to novel chemical entities in clinical use today was a slow and gradual process that started with investigative minds at the beginning of the 19th century (Wetzel *et al*., 2010).

Friedrich Wilhelm Adam Serturner, a German pharmacist, reported the isolation of a white crystalline powder from Opium (*Papaver somniferum*), which he named morphine in earlier 18th century. This was the first isolation of a pure natural product that was commercialized two decades later by Heinrich Emanuel Merck. The same molecule and several classes of analogues are being used as analgesics even after 200 years. Later this led to isolation of several natural products as strychnine, colchicine and codeine (Sneader, 2005). The fact that 214,000 chemical entities are known to us indicates that mankind has traversed a long way in drug discovery and bioprospecting in a major way (DNP, 2010).

International Cooperative Biodiversity Groups (ICBG) program of the National Institutes of Health, has recognized bioprospecting as: (i) protects rather than degrades the biodiversity resource, (ii) promotes host-country capacity building and intellectual property rights, as well as (iii) seeking novel biological resources for profit. This formula has been adopted by many research groups that emphasize securing economic benefits for host countries or cultures and the promotion of biodiversity conservation (Kursar *et al*. 2006; INBio 2009).

With the growing demand of herbals, one of the main targets of bioprospecting is traditional medicine. Studies conducted by the World Health Organization (WHO) states that about 80% of the world's population relies on traditional medicine (Anonymous, 2002). GIA (Global Industry

Analyst) comprehensive global report on Herbal Supplements and Remedies markets shows that the global herbal supplements and remedies market is forecast to reach US$107 billion by the year 2017, spurred by growing aging population and increasing consumer awareness about general health and wellbeing. Additionally, the fact that herbal supplements and remedies cause little or no side effects and provide greater efficacy is also proving to be a major factor aiding market growth. (http://www.prweb.com) (Jose, 2012)

1.2 Overview of Industries Involved in Bioprospecting

Bioprospecting involves the use of a wide variety of species and variety of industries (Kate and Laird, 1999; Beattie and Ehrlich, 2004). The natural products are likely to provide the best lead-molecules in the future (Chapman 2004; Ortholand and Ganesan 2004).

1.2.1 Pharmaceutical Bioprospecting

Interest in novel products from biodiversity has varied greatly in the last decade, with general decline in pharmaceutical bioprospecting by major companies, although resurgence is expected (Chapman, 2004). Based on the knowledge that many important drugs, such as aspirin, were derived from natural products (Jack, 1997) — that is, generated in the tissues of native species— the industry has at various times invested heavily in the exploration of species-rich communities such as rain forests and coral reefs in search of commercially profitable pharmaceuticals (Ismail *et al.*,1995; Bailey 2001). Alarming levels of antibiotic resistance in many human pathogens is likely to provoke an increase in pharmaceutical bioprospecting, which remains a vital source of lead drug discovery (Wessjohann, 2000; McGeer and Low 2003; Newman *et al.*, 2003). Malaria, one of the world's most deadly diseases, has been treated historically with drugs derived from natural products—quinine, chloroquine, mefloquine, and doxycycline—and today the artemisinins derived from the Chinese herb Qinghao (*Artemisia annua*) are at the forefront of the battle against this parasite. The current assessment of bioprospecting by the large pharmaceutical companies is reflected in the focus of their research and development, where the major investment is in rational drug design and combinatorial chemistry (Olsen *et al.*, 2002; Hijfte *et al.*, 1999) rather than natural products.

1.2.2 Ethnobotanical Bioprospecting

Historically, most of the corporate drug discovery is dependent on indigenous knowledge delivered to modern science through ethnobotany. Over 50% of modern prescription medicines were originally discovered in plants, and plants continue to be the source of significant therapeutic compounds to this day (Pearce and Puroshothaman 1993; Cragg and Newman 2007). Farnsworth and Soejjarto, 1985 showed that at least 89 plant-derived medicines used in the industrial world were originally discovered by studying indigenous medicine. Among the best known is quinine, used in South America to treat fever. This has been the single most effective cure for malaria. Quinine comes from the bark of trees of the genus *Cinchona* that grows in the Andean region. More recently, the drugs vincristine and vinblastine were discovered in the rosy periwinkle (*Catharanthus roseus*) from Madagascar. Indigenous people generally have large pharmacopoeias, since plants are often the only source of medicine available to them. Ethnobotanical studies list a large number of plant species used medicinally (Cox and Balick, 1994; Balick 1994; Peters *et al.,* 1989; McCutcheon *et al*., 1992). The MA Sub-Global Mekong River Wetlands Assessment has identified 280 medically important plant species, 150 of which are in regular use. The ethnobotanical approach to drug discovery is more likely to succeed where people have lived in the same area over many generations and so have had more time to discover suitable medicines.

1.2.3 The Botanical Medicine Industry

Botanical medicines in commerce are generally whole plant materials as opposed to pharmaceuticals, which are often derived from specific biochemical compounds extracted from plants. Best-selling examples include *Ginkgo*, St. John's wort, Echinacea, Garlic, *Ginseng* and various yeasts. The structure of this industry varies according to the particular medicines being produced, but typically there are several stages: collection from the wild or cultivation, followed by the purchase of materials by exporters, importers, wholesalers, brokers, or traders. Materials may then be tested for contamination, powdered, or extracted by processing companies or by manufacturers of the finished products. These may then be handled by specialized distributors before retailing to consumers. Revenues from these products can be very large. For example, annual sales of medicinal *Ginkgo*, Garlic, Evening Primrose, and *Echinacea* in Europe average $350 million (Kate and Laird, 1999). The global sales of raw botanical materials by leading U.S.

suppliers amount to approximately $1.4 billion (Kate and Laird, 1999). The ‘‘nutraceuticals’’ industry sells food ingredients or products believed to confer health or medical benefits. These include dietary supplements; individual nutrients, foods enhanced in various biotechnological ways, and fortified foods. Major products of this industry include dietary additives. Products include teas with added ginseng, probiotic yogurts, fruit juices fortified with calcium, and flour fortified with folic acid. Various companies in the sweet-tasting proteins produced by plants such as *Dioscoreophyllum cumminisii, Thaumatococcus daniellii,* and *Richardella dulcifera,* all from West Africa, and *Capparis masaikai* from South China. The nutraceuticals market for 1996 was estimated at $16.7 billion (Kate and Laird 1999), and interest is rapidly growing worldwide.

1.3 Herbal medicine

The World Health Organization (WHO) has defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today (Anonymous, 1991). Traditional medicine is the synthesis of therapeutic experience of generations of practicing physicians of indigenous systems of medicine. The traditional preparations comprise medicinal plants, minerals, organic matter, etc. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparations for therapy. The earliest recorded evidence of their use in Indian, Chinese, Egyptian, Greek, Roman and Syrian texts dates back to about 5000 years. The classical Indian texts include *Rigveda*, *Atherveda*, *Charak Samhita* and *Sushruta Samhita*. The herbal medicines/traditional medicaments have, therefore, been derived from rich traditions of ancient civilizations and scientific heritage (Kamboj, 2000)

CAM is generally considered “holistic”, “natural”, and, it is inferred, safe. Its popularity is undeniable; each year an estimated 10% of the UK population received a CAM therapy (Thomas and Coleman, 2004). In 2003-04, $5bn was spent on herbal treatments alone in Western Europe (Anonymous, 2008). A UK study, found 20% of cancer patients had used herbal medicines (Damery *et al*., 2011). Globally, between 40,000 and 50,000 plant species are used for medicinal purposes in both traditional and modern medical systems (Heywood, 2011); only a tiny minority have been assessed scientifically, although there is some evidence for the effectiveness of many. A principal tenet of modern herbal medicine is that several compounds act on one another to

moderate, oppose or enhance an effect. An enhancement may be an additive or a "synergistic" action, whereby the combination of constituents is greater than would have been expected from the sum of individual contributions (Heinrich *et al*., 2012).

1.3.1 India's strength in Medicinal Plant Wealth

If we dwell for a moment on our glorious past, *Rigveda*, one of the oldest repositories of human knowledge written between 4500-1500 BC mentions the use of 67 plants for therapeutic use and *Yajurveda* enlists 81 plants whereas *Atherveda* written during 1200 BC describes 290 plants of medicinal values, *Charak Samhita* (900 BC) describes 341 medicinal plants and the next land mark in Ayurveda *Sushruta Samhita* (600 BC) mentions 395 medicinal plants. India unquestionably occupies the top position in the use of herbal drugs. It is one of the, foremost countries exporting plant drugs and their derivatives, and excel in home-consumption too and this is owing to following factors:

a. Great biodiversity and abundance of flora.
b. Variety of geographical climatic conditions most exotic medicinal plants can be grown here.
c. Indian systems of medicine dwell heavily on medicinal plants.
d. Long tradition of phytochemical research and scientific cultivation of medicinal plants.
e. Well-developed pharmaceutical industry.
f. Rapidly growing phytochemical and herbal drug industry.

India is thus in a vantage position to exploit this source both for meeting the domestic demand for drugs as also for export (Rajsekharan, 2001). Estimates for the number of species used medicinally vary from 35,000 to 70,000 worldwide (Schippmann *et al*., 2002; Hamilton *et al*., 2003) and 8,000 in India (AICRPE, MOEF, GOI 1995).

Table 1.1 : Profile of India's Medicinal Plants (Cross tabulation of the number of Medicinal Plant Species recorded across Medical Systems (AICRPE)

	AYURVEDA	FOLK	FOLK (V)	HOMEO.	SIDDHA	TCM	TIBETAN	UNANI	WESTERN
AYURVEDA	1539	776	310	176	758	359	248	429	74
FOLK	776	4765	283	161	773	673	187	332	80
FOLK (V)	310	283	547	47	300	138	82	111	14
HOMOEO.	176	161	47	490	145	129	69	137	102
SIDDHA	758	773	300	145	1152	289	211	337	59
TCM	359	673	138	129	289	881	109	206	80
TIBETAN	248	187	82	69	211	109	252	179	23
UNANI	429	332	111	137	337	206	179	496	63
WESTERN	74	80	14	102	59	80	23	63	190

Total : 6,198 species

1.3.2 Herbal medicine standardization

As per WHO definition, there are three kinds of herbal medicines: raw plant material, processed plant material and medicinal herbal products. Herbal drugs are finished labeled products that contain active ingredients such as aerial or underground parts of plant or other plant material or Combination thereof, whether in the crude state or as plant preparations. The use of herbal medicines has increased remarkably in line with the global trend of people returning to natural therapies. (Vaidya and Devasagayam, 2007).

Herbals are traditionally considered harmless and increasingly being consumed by people without prescription. However, some can cause health problems, some are not effective and some may interact with other drugs.

Standardization of herbal formulations is essential in order to assess the quality of drugs, based on the concentration of their active principles (Yadav and Dixit, 2008). The medicinal plant parts should be authentic and free from harmful materials like pesticides, heavy metals, microbial or radioactive contamination, etc. The extract should then be checked for indicated biological activity in an experimental animal model(s). The bioactive extract should be standardized on the basis of active principle or major compound(s) along with fingerprints. The next important step is stabilization of the bioactive extract with a minimum shelf-life of over a year. The stabilized bioactive extract should undergo regulatory or limited safety studies (Kamboj, 2000).

Herbal drug technology involves conversion of botanical materials into medicines where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge is employed, and various drug delivery technologies used for herbal drugs were reported (Agarwal and Paridhavi, 2007; Gulati and Ray, 2010).

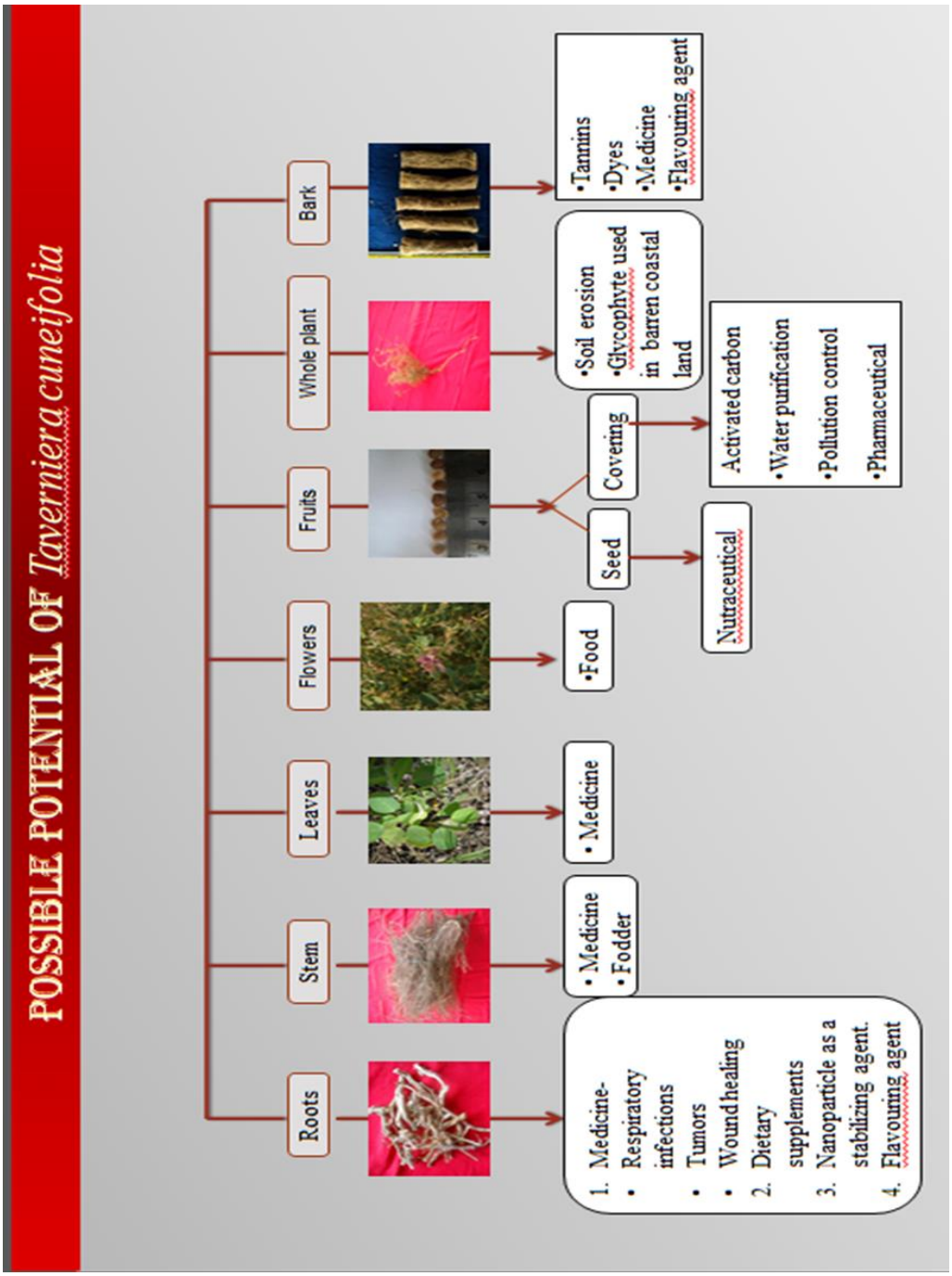


Figure 1.1 Possible potential of *Taverniera cuneifolia*

1.4 International Status

The roots of *G. glabra* are very widely used in traditional system of medicines all over the world (Sasaki *et al*., 2003). Licorice is not produced locally in Japan hence the licorice used in Japan is imported from countries such as China, Afghanistan, Turkmenistan, Uzbekistan, and Pakistan. Table 1.1 shows the trade statistics of licorice imported in Japan in 1987 and 2007 (http://www.customs.go.jp/toukei/info/index_e.htm). Although the total amount of licorice imported in Japan was 10,723,342kg in 1987, it decreased to 1,377,213kg in 2007. Medicinal licorice is more expensive than licorice used for the production of glycyrrhizin and other licorice products; the latter is imported from other licorice-producing countries such as Afghanistan and Australia. The fact that 144,710kg of licorice was imported from Australia in 2007 is remarkable (Hayashi and Hiroshi, 2009).

Table 1.2: Import of licorice to Japan in 1987 and 2007:

Year	Country of origin	Quantity (kg)	Value (JPY1000)	Price (JPY/kg)
1987	China	5,298,304	1,098,344	207
	USSR	2,730,619	325,310	119
	Afghanistan	2,304,547	215,739	94
	Pakistan	267,700	21,730	81
	Turkey	116,382	15,000	129
	Hong Kong	4,023	1,986	494
	Taiwan	1,767	734	415
	Total	10,723,342	1,678,843	157
2007	China	932,503	372,387	399
	Afghanistan	300,000	21,887	73
	Australia	144,710	12,463	86
	Total	1,377,213	406,737	295

These data are based on the trade statistics of Japan published by the Ministry of Finance and the Customs of Japan (http://www.customs.go.jp/toukei/info/index_e.htm).

Driven by shortages of licorice roots in all producing countries, particularly Iran and China, in the past 3 years prices have soared by more than a third for this natural sweetener and flavour used in a wide variety of sweets and beverages. While prices vary according to the block, paste or spray dried form for this popular extract derived from licorice roots, around 10 - 20 tonnes are demanding €4.75 (₹283.48) to €4.80 (₹286.46) a kilo (http://www.confectionerynews.com, 2008).

In many cases, the plant is cultivated, e.g. in Italy, Southern France and Spain, and also in Central Asia, Australia, and Brazil (Mansfeld, 1986), but licorice plants growing in the wild also continues to be exploited to a large extent. The USA was the leading importer of licorice roots with annual average import volume of about 17,887 metric tonnes, valuing to about US$ 10.5 million during 1994 to 1997. The main source countries were China, Afghanistan, Pakistan, Azerbaijan and Turkmenistan. The EU annual average import volume during 1994 to 1997 amounted to about 4,562 metric tonnes, valuing to US$ 3.6 million (Table 1.2). In 1996, the European countries imported 6,000 metric tonnes of roots, almost all from Asia. Main source countries were Azerbaijan, Turkmenistan, Afghanistan, Iran and Pakistan. Afghanistan exports licorice roots mainly to the USA, Japan, France and India with an annual export value of US$ 4.2 million. The EU exports of licorice roots amounted to 2,700 metric tonnes only, more or less evenly directed to North America, Africa and Asia. France dominates the import trade in Europe. Turkey is the biggest exporter of licorice, shipping 3,040 metric tonnes in 1991; 1,684 in 1992; 1,350 in 1993; 1,140 in 1994; 1,560 in 1995 and 1,730 metric tonnes in 1996, according to the UNCTAD Comtrade database (Lange, 1998). In addition, Turkey also exports licorice extracts to the USA, Egypt, Italy, France, and Israel. Spain also exports licorice, however the quantities involved are much less. It is obtained either from wild stock or from cultivation. In Bulgaria, licorice is regarded as being rare (WCMC, 1988).

Table 1.3: The USA and the EU import of licorice roots (commodity code HS 1211.10) during 1994-97

Source country	Annual average import			
	US		EU	
	Volume (MT)	Value (million US $)	Volume (MT)	Value (Million US $)
China	5,942	3.3	227	0.4
Afghanistan	4,712	2.1	454	0.3
Pakistan	4,323	2.1	205	0.1
Azerbaijan	966	0.5	753	0.3
Turkemenistan	502	0.3	1,050	0.6
Turkey	453	0.3	474	0.4
Syria	290	0.2	286	0.6
Others	699	1.7	1,111	0.8
Total	17,887	10.5	4,560	3.5

1.5 National status

Majority requirement of the *Glycyrrhiza glabra* in India is met through import from Afghanistan and Pakistan (Caraka, 1888). Cost of total Licorice roots imported in India is ₹218.52 lakhs during 2004-2005. We are importing 1603.20 metric tonnes of Licorice every year to suffice our requirements Cost of export of Licorice Roots Fresh/Dried W/N Crushed/ Powdered in 2003-2004 was ₹1,120,667 for 9789 kg but in 2004-2005 it increased to ₹3,851,182 for 6,420 kg. Cost of import of Licorice Roots Fresh/Dried W/N Crushed/ Powdered in 2003-2004 was ₹14,725,558 for 1,178,714 kg but in 2004-2005 it increased to ₹21,852,455 for 1,603,196 kg. (Directorate General of Commercial Intelligence and Statistics, 2006 nmpb.nic.in/FRLHT/ Chapter 6.Doc).

1.6 Significance of the study

With the above references there are sufficient clues that *T. cuneifolia* could be a potential substitute of *G. glabra*. There are also some clues as per the study of Zore (Zore, 2008) and preliminary studies (Solanki, 2009) that justify the statement. Some of the interesting parameters that were of significance are:

Table 1.4: Comparison of Parameters of *G. glabra* and *T. cuneifolia*

Parameters	***G. glabra***	***T. cuneifolia***
Glycyrrhizin concentration	15.88%	13.20%
Harvesting time	3 yrs	1yr
Soil type	Acidic	Saline
Root production	3 tonnes/acre	4-5 tonnes/acre

Licorice resources and future prospects at present, since commercial production of glycyrrhizin by plant cell culture is difficult (Hayashi *et al*., 1988, Henry *et al*., 1991). Glycyrrhizin is obtained from the licorice of wild or cultivated *Glycyrrhiza* plants. The recent over-utilization of wild *Glycyrrhiza* plants has resulted in a reduction in the natural reserves and desertification of the habitats of these plants, especially in China. Thus, in 2000, the Chinese government enforced restrictions on the collection of wild licorice, leading to a shortage of licorice in the market (Yamamoto and Tani, 2005). *Glycyrrhiza* cultivation has been undertaken to compensate for the reduction in the natural reserves of *Glycyrrhiza* plants; however, the glycyrrhizin content of the licorice obtained from these plants is often low. Researchers were successful in producing 4-year-old adventitious roots with glycyrrhizin levels that conformed to the Japanese Pharmacopeia standard (not less than 2.5%) (Yamamoto *et al*., 2003, Yamamoto and Tani 2005); moreover, licorice obtained from cultivated plants was also imported from Australia. Further studies are required to devise a method for increasing the glycyrrhizin content in the roots of cultivated *Glycyrrhiza* plants and to obtain a *Glycyrrhiza* strain that produces high amounts of glycyrrhizin (Yamamoto and Tani, 2005). The cDNA sequences of the enzymes involved in glycyrrhizin biosynthesis have been reported; therefore, the possibility of using metabolic engineering to generate glycyrrhizin-producing plants or microorganisms seems promising and should be considered in future studies (Hayashi *et al*., 2001, Seki *et al*., 2008).

However, the standardization is lacking and the only reference of Zore (Zore, 2008) is restricted to quantification of glycyrrhizin that to from the wild resource. Preliminary studies of *T. cuneifolia* have shown that other than Glycyrrhizin there are other glycosides in the roots that

have to be further elucidated. Above all the total potential of plant i.e.: root, stem, leaves, flowers, fruits are yet to be studied.

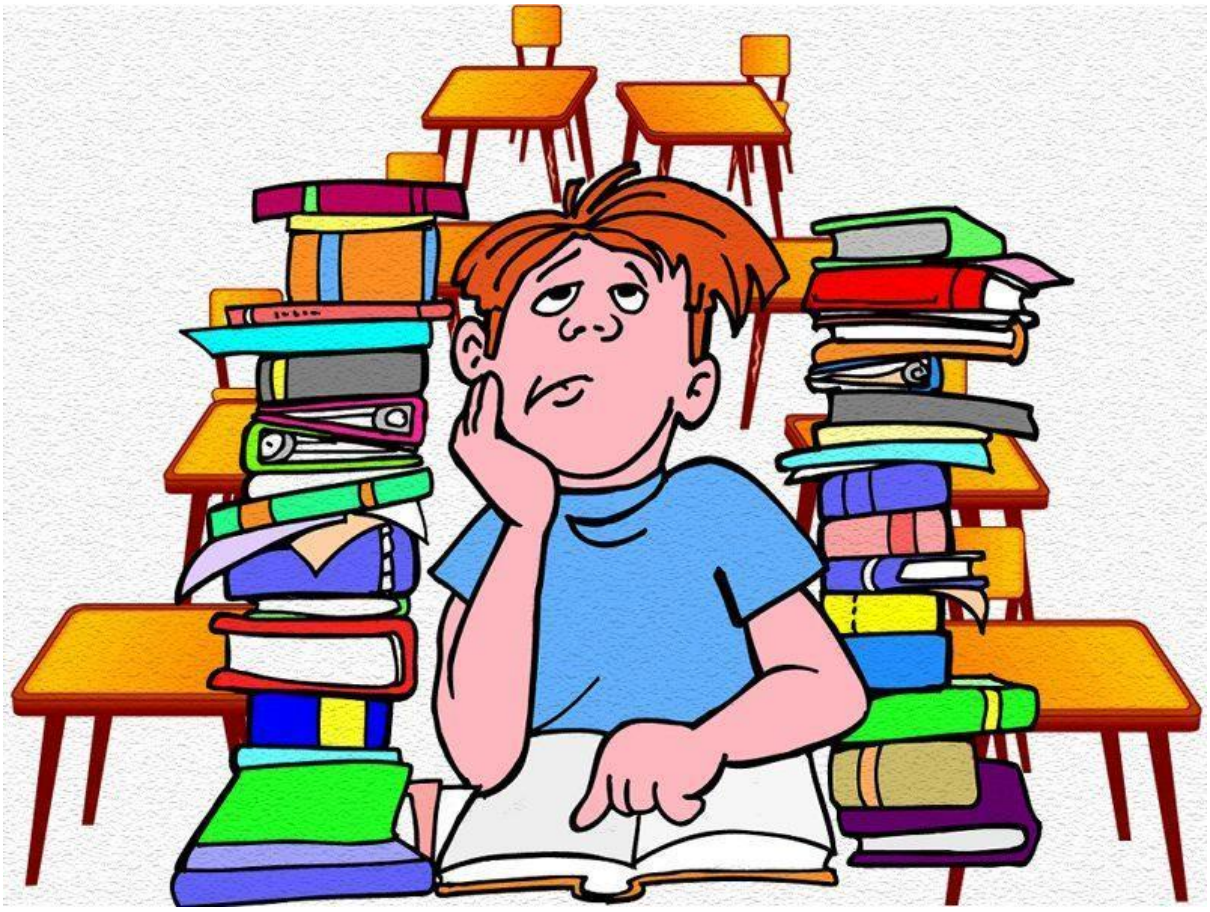
The potential of the plant can be studied only by developing Standards for good agriculture practices, good harvesting technology, SOPs for phytochemical and pharmacological analysis and there of understanding the possible total potential of the plant for therapeutic, nutraceutical, dietary supplement and other value added products.

On the basis of the above understanding the following objectives were undertaken:

1) To study the distribution pattern of the species within the state and across the country.
2) To study the ecological parameters of the indigenous site.
3) Macromorphological and micromorphological studies.
4) Determination of physicochemical characters
   Ash analysis, heavy metals, microbial contamination, etc.
5) Based on the morphological and ecological parameters understanding the seed germination techniques for developing cultivation practices of *T. cuneifolia*.
6) To study the phytochemical constituents
   Flavonoids, Phenolic acids, Steroids, Saponins, Glycosides, Anthocyanins, Alkaloids etc.
7) To study the potential of seeds and roots as a as Dietary and Nutraceutical supplement.
   (Protein, Fat (Omega-3 fatty acids), Carbohydrate, Vitamins, Bio Active Components - Phytosterols , etc.
8) Preclinical and toxicity studies would be done for wound healing, antitussive, role in PCOS models .


Quality Evaluation of Taverniera cuneifolia
Organo-leptic
Biological
Botanical
Chemical
Physical
Colour
Oour
Taste
Texture
Fracture
Macroscopic
• Shape
• External Marking
Microscopic
• Quantitative
• Qualitative
• SEM studies
• Powder studies
Microbial contamination
Preclinical studies
• Toxicological
• Pharmacological
• Other specific activities
Antagonistic
• Bacterial
• Fungal
Qualitative
Quantitative
• HPTLC fingerprinting
• Secondary metabolites
• DNA fingerprinting
Chromatography
• HPTLC
• GLC
• HPLC
Pesticide residue
Heavy metal
Mycotoxin
Moisture content
Ash values
Extractive values
Fluorescence analysis

Chapter 2: Review of Literature



2.1 Distribution and Ecology

Taverniera cuneifolia was initially recorded and reported from India by Roxburgh (1832), Graham (1839), Dalzell (1850), Palin (1880), Hooker (1872-1897), Gray (1886), Boissier (1888), Narine (1894), Libosa (1896), and Woodrow (1897-1901). Further, the detailed description and area of occurrence was specified in the Flora of Presidency of Madras (Gamble, 1901) and the Flora of Presidency of Bombay (1908). Borgeson (1929) mentioned about *T. cuneifolia* in "Notes on vegetation at Dwarka".

Post-Independence the description of the plant and its occurrence has been recorded in the Flora of Saurashtra (Santapau 1953, 1962). The detailed description of *T. cuneifolia* was given by Ali (Ali, 1977), based on which *T. cuneifolia* (Roth) Ali has been accepted by International Plant Name Index (IPNI). With the development of karyological studies the chromosomal number was described by Jahan *et al*. (1994). Reassertion of *T. cuneifolia* has been done by Sarwar (2002) in the Flora of Pakistan.

The genus *Taverniera* belongs to the family Fabaceae in the major group Angiosperms. There are 16 species belonging to this genus (ref: www.theplantlist.org, www.ildis.org, Table 2.1). With respect to *T. cuneifolia* most the earlier flora's (Hooker, 1879; Gamble, 1901; Cooke, 1997) have stated *T. cuneifolia* (Roth.) Arn. and *T. nummularia* DC. However, as per the International Plant Name Index (IPNI), details of *T. cuneifolia* as follows:

Genus: *Taverniera* DC.

Botanical name: *Taverniera cuneifolia* (Roth) Ali

Higher Taxon: *Hedysareae*

Synonyms:

Hedysarum cuneifolium Roth, *Taverniera ephedroidea* Jaub. & Spach, *Taverniera glabra* Boiss, *Taverniera nummularia* sensu Baker :auct. non DC., *Hedysarum gibsonii* Grah., *Onobrychis cuneifolia* DC., *Onobrychis diffusa* Camb., *Taverniera stocksii* Boiss.

Vernacular names:

English: East Indian moneywort (ILDIS), **Gujarati:** Desi jethimadh (Thaker, 1908), Jethimal, jethimadh,

Phlyogenetic status:

Cladistically *T. cuneifolia* belonging to family Fabaceae was initially kept in the order Rosales (Bentham & Hooker); however, according to some recent classification of Cronquist and APG III based on phytoconstituents and matK gene has kept it under the order Fabales. Status of *T. cuneifolia* as per various classifications is as follows:

Table: 2.1 Different Classifications of *Taverniera cuneifolia*

Bentham & Hooker (1862-1883)	**Cronquist (1988)**	**APG III (2012)**
Division: Spermatophyta	**Kingdom:** Plantae	**Clade:** Eudicots
Sub Division: Angiospermae	**Phylum:** Magnoliophyta	**Clade**: Core eudicots
Class: Dicotyledonae	**Class:** Magnoliopsida	**Clade:** Fabids (eurosids)
Sub Class: Polypetalae	**Sub class:** Rosidae	**Order**: Fabales
Series: Calyciflorae	**Superorder:** Fabanae	**Family:** Fabaceae
Order: Rosales	**Order:** Fabales	**Sub family:** Papilionoideae/Faboideae
Family: Fabaceae	**Family:** Fabaceae	**Tribe:** Hedysareae
Genus: *Taverniera*	**Genus:** *Taverniera*	**Genus:** *Taverniera*
Species: *cuneifolia*	**Species:** *cuneifolia*	**Species:** *cuneifolia*

Geographical Distribution:

Geographically various species of *Taverniera* are distributed in Asia, Africa and Middle East (Table 2.1). Maximum numbers of species of *Taverniera* are found in African continent Ethopia. While in Asian continent maximum 3 species has been reported from Pakistan while from Middle East four species has been recoded from Oman.

With respect to India there is only one species that has been recorded i.e., *T. cuneifolia*, which is distributed in African, Asian and Eurasian continent.

Internationally *T. cuneifolia* has been reported from Africa, Asia and Eurasia along coastal tracts. From Africa - Somalia (Thulin, 1985) while from Asia - Iran (Thulin, 1985); Pakistan (Thulin, 1985; Ali, 1977) and from Eurasia (Middle East): Oman (Thulin, 1985), United Arab Emirates (Thulin, 1985) and Farasan islands (Alfarhan, 2005).

Nationally, *T. cuneifolia* has been reported from **Gujarat** (Shah, 1978) - Shetrunjaya, Rozimata temple, Narara beyt, Rampara sanctuary, Hingolgadh sanctuary (Nagar, 2008), Kutch (Bhuj) Tapkeshwari Hill Range (Joshi *et al*., 2013), Gir forest, Ghumli, Dwarka (Santapau, 1962) **Maharashtra** (Karthikeyan & Kumar, 1993) - Majalgaon (Khan *et al*., 2012), Osmanabad (Jamdhade *et al*., 2013), **Karnataka -** (Bijapur, Madhbhavi, Raichur and Bellary (Singh, 1988), **Madhya Pradesh** (Sanjappa, 1992); **Orissa** (Bairiganjan *et al*., 1985); **Punjab** (Bhandari, 1978) - Plains of Punjab (Khare, 2007); **Rajasthan** (Bhandari, 1978, Shetty & Singh, 1987); **West Bengal** (Sanjappa, 1992), **Andhra Pradesh** (Gamble, 1918; Rao et al., 2006) - Betam cherala, Kurnool district, **Jammu and Kashmir:** North West Himalaya (Chauhan *et al*., 2003) (Map: 2.1, 2.2).

Ecologically the plant has been recorded Semi-arid tract and coastal tract of Gujarat (Rao *et al*. 1966, 1967) which is rocky sandy. From Jafarabad to Bhavnagar with special reference Victor Albert Port, Gopnath coast sub moist forest and evergreen deciduous forests of Maharashtra and Madhya Pradesh. The plant has been also recorded from the Mangrove forests of river estuaries of Orissa to tropical dry deciduous forests of Punjab. It is also found in semiarid regions of Rajasthan to moist temperate climate in North West Himalaya. The plant is also recorded in Karnataka's semiarid climate to tropical moist deciduous forests of Andhra Pradesh.

Table 2.2 : List of different species of *Taverniera* and their Geographical distribution

Species Name	**Synonyms**	**Geographical Distribution**
Taverniera abyssinica A.Rich. (Thulin, 1985)	*Taverniera schimperi* Jaub. and Spach var. *oligantha* sensu Cufod	Africa: Ethiopia (Thulin, 1985)
Taverniera aegyptiaca Boiss. (Thulin, 1985)	NA	Africa: Djibouti (Audru *et al*., 1987) Egypt, Ethiopia Sudan Middle East, Saudi Arabia (Thulin, 1985)
Taverniera albida Thulin. (Thulin, 1985)	NA	Asia; Middle East: South Yemen (Thulin, 1985)
Taverniera brevialata Thulin (Thulin, 1985)	NA	Asia; Middle East- Oman (Thulin, 1985)
Taverniera diffusa (Cambess.) Thulin (Thulin, 1985)	*Onobrychis diffusa* Cambess (Thulin, 1985)	Asia- India: Punjab, Rajasthan; Pakistan (Thulin, 1985)
Taverniera echinata Mozaff. (Mozaffarian, 1988)	NA	Asia; Iran (Mozaffarian, 1988)

Species Name	Synonyms	Geographical Distribution
Taverniera glauca Edgew. (Thulin, 1985)	NA	Asia; Middle East: South Yemen (Thulin, 1985); Yemen (Boulos, 1988)
Taverniera lappacea (Forssk.) DC. (Thulin, 1985; Ali, 1977)	*Hedysarum lappaceum* Forssk. (Ali,1977) Taverniera *stefaninii* Chiov. (Thulin, 1985; Ali, 1977)	Africa: Ethiopia, Somalia, Sudan; Asia (Thulin, 1985) Pakistan (Thulin, 1985; Ali, 1977) Middle East: Oman, Saudi Arabia, South Yemen (Thulin, 1985)
Taverniera longisetosa Thulin	NA	Africa: Somalia (Thulin, 1985)
Taverniera multinoda Thulin	NA	Africa: Somalia; Middle East: Oman (Thulin, 1985)
Taverniera nummularia DC. (Townsend, 1974)	*Taverniera persica* Boiss. and Hausskn.	Asia: Iran (Thulin, 1985; Townsend, 1974; Parsa, 1948; Rechinger, 1984) Iraq (Thulin, 1985; Townsend, 1974; Rechinger, 1984)
Taverniera oligantha (Franch.) Thulin (Thulin, 1985)	*Taverniera schimperi* Jaub. and Spach var. *oligantha* Franch.	Africa: Djibouti (Thulin, 1985)
Taverniera schimperi Jaub. & Spach	NA	Africa: Ethiopia (Thulin, 1985)
Taverniera sericophylla Balf.f.	NA	Africa: Socotra (Thulin, 1985)
Taverniera spartea (Burm.f.) DC. (Thulin, 1985; Ali, 1977; Rechinger, 1984)	*Hedysarum spartium* Burm. F., *Taverniera gonoclada* Jaub. & Spach (Ali, 1977) *Taverniera incana* Boiss. (Thulin, 1985)	Asia; Iran (Thulin, 1985; Parsa, 1948; Rechinger, 1984) Pakistan (Ali, 1977) Middle East: Bahrain, Oman, Qatar, Saudi Arabia, United Arab Emirates (Thulin, 1985)

NA- Not Applicable

Figure 2.1: Distribution pattern of *Taverniera cuneifolia* across the world

Africa: Somalia; **Asia**: India, Iran, Pakistan; **Middle East** Oman, United Arab Emirates, Farasan islands

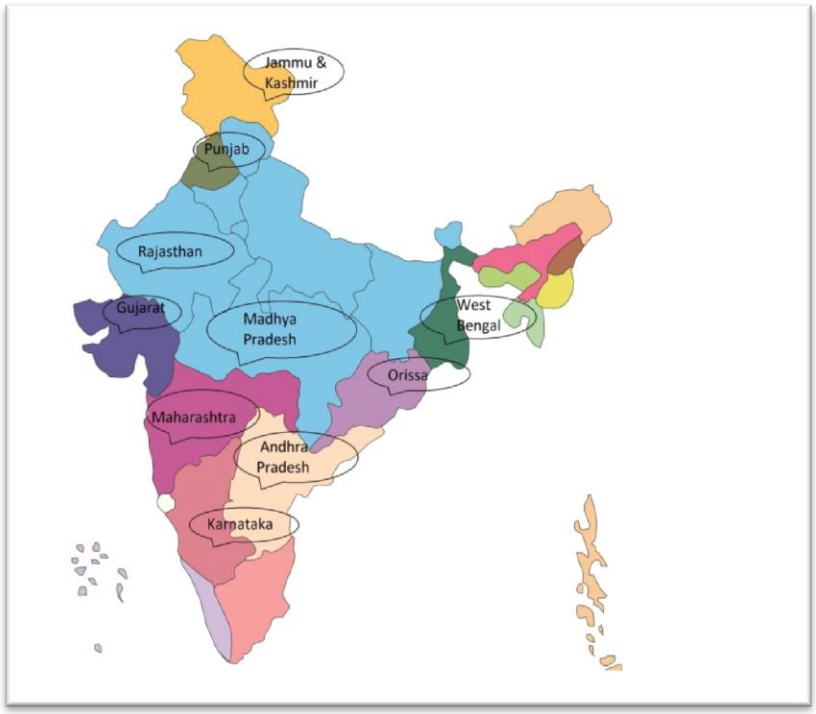


Figure 2.2: *T. cuneifolia* in different states of India

Jammu & Kashmir, Punjab, Rajasthan, Gujarat, Madhya Pradesh, Orissa, West Bengal, Andhra Pradesh, Maharashtra, Karnataka.

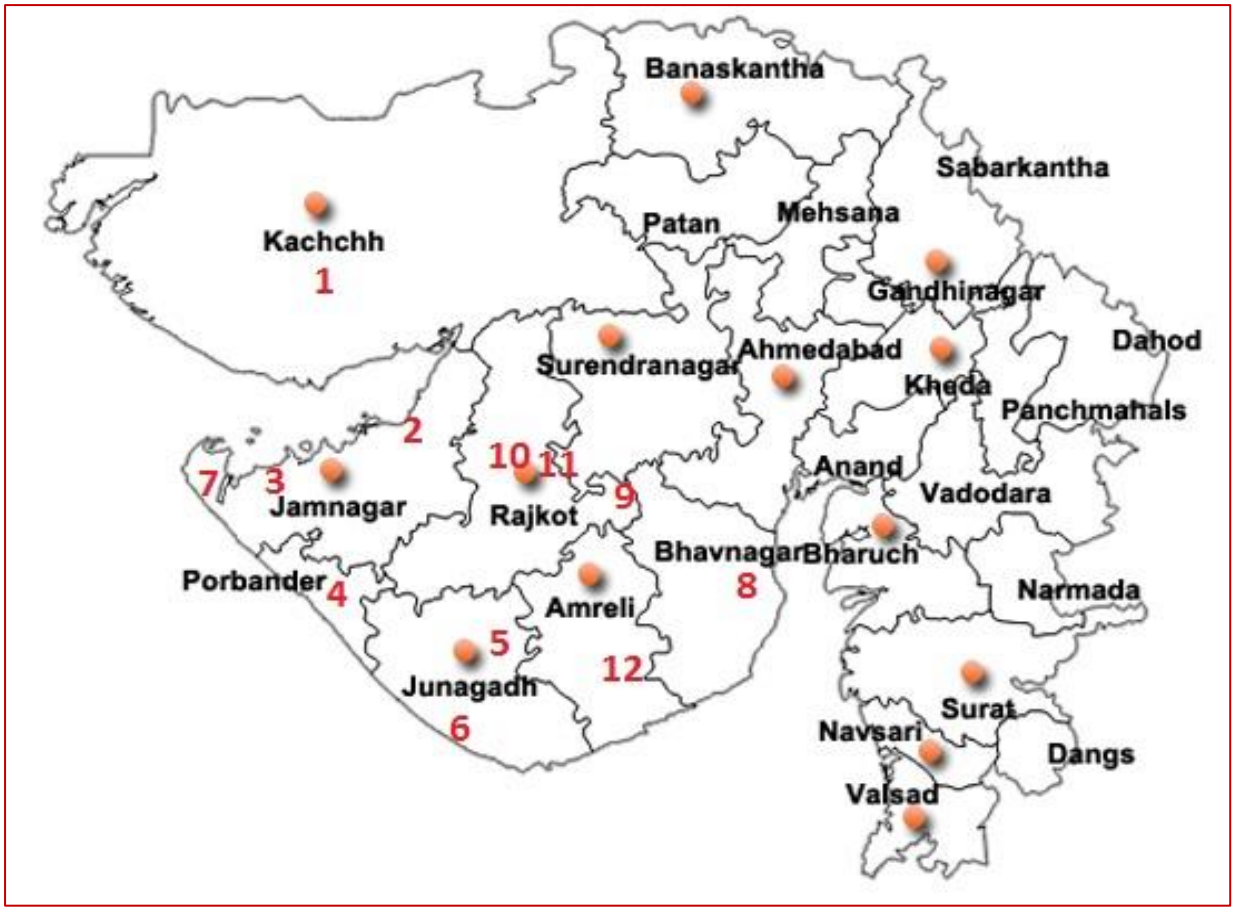


Figure 2.3: *T. cuneifolia* in different places of Gujarat

1. Kachchh (Kutch) - Bhuj
2. Jamnagar - Rozi Mata Temple,
3. Jamnagar - Narara beyt
4. Porbandar - Narsang tekri (Black soil)
5. Junagadh - Veraval to Gir forest along coastal track
6. Junagadh - Gumli
7. Dwarka- Sea coast
8. Bhavnagar- Shetrunjay Hill Range
9. Rajkot - Hingolgadh sanctuary
10. Rajkot - Munjka village
11. Rajkot - IMPLANT garden, Saurashtra University, Rajkot
12. Amreli- Way to sea coast

2.2 Anatomy:

Perusal of literature revealed no much information related to the pharmacognostic studies of *T. cuneifolia*, except Saluja (1979) and Notani (2012). The former author has compared roots of *G. glabra* with *T. cuneifolia* and reported that in *G. glabra* cork tissue is not lignified while in *T. cuneifolia*, cork cells showed suberine and lignin. Another striking difference is former species showed phloem fibres are arranged in form group and form tangential bands. However, this character is not apparent in *T. cuneifolia*. The phloem rays in *T. cuneifolia* are dilated; thus, show very broad rays as compared to *G. glabra*. In contrast, the ray cells in *G. glabra* are more or less radially arranged. Xylem elements are much more strongly lignified in *T. cuneifolia.* On the other hand, no significant variation was reported in the size of the starch grains of *T. cuneifolia* of both the species which is measured from 4-10µ only in that of *G. glabra* where it varies from 2-10µ whereas the size of the calcium oxalate prisms of both *G. glabra* and *T. cuneifolia* fall in the same range (Saluja, 1979). Study by Notani (2012) has not revealed much detail about anatomical features that may be used as markers to differentiate both the species. According to Notani (2012) roots show presence of cork, cortex, vascular bundles, medullary rays and xylem. Powder characters of the root showed group of simple and compound starch grains, bordered pitted and vessels, tannin in cork and cortex zone, oil globules, aleurone grains and parenchymatous cells.

The major difference in both the studies lies in the fact that Saluja (1979) has mentioned Ca-oxalate prisms and Notani (2012) did not mention about prisms. The former has mentioned about suberized cells in cork but the latter did not mention. Elongated sap in xylem ray is mentioned by Saluja but not mentioned by Notani. Notani has mentioned about oil globules and aleurone grains from cortex and stellar portion but no mention by Saluja (l.c.). Notani (l.c.) found that the pith has presence of parenchyma cells which had few starch grains in it, Saluja has not mentioned about this. Comparative study of the two major works is as follows:

	Saluja (1979)	**Notani (2012)**
Ca-oxalate crystal	Observed	Not recorded
Suberized cells	Observed	Not recorded
Oil globules	Not recorded	Observed in Cortex
Aleurone grains	Not recorded	Observed in stellar portion

Starch grains	Not recorded	Presence of Starch grains in parenchymatous pith

There are no references related to the anatomical or powder studies on any of the *Taverniera* species.

2.3 Phytochemistry

Ronbiquet (1809) for the first time analysed the root of *G. glabra* phytochemically and isolated glycyrrhizin. The name of glycyrrhizic acid was given later by Roussin (1876) *Glycyrrhiza glabra* popularly known as Liquorice was phytochemically isolated for the first time for 18β-glycyrrhizic acid (3-O-(2-O-β-d-glucopyranuronosyl–α-d-glucopyranurosyl)-3-β-hydroxy-11-oxo-18β, 20β-olean-12-en-29-oic acid) from the roots of *Glycyrrhiza glabra*. (Benigni *et al*., 1964).

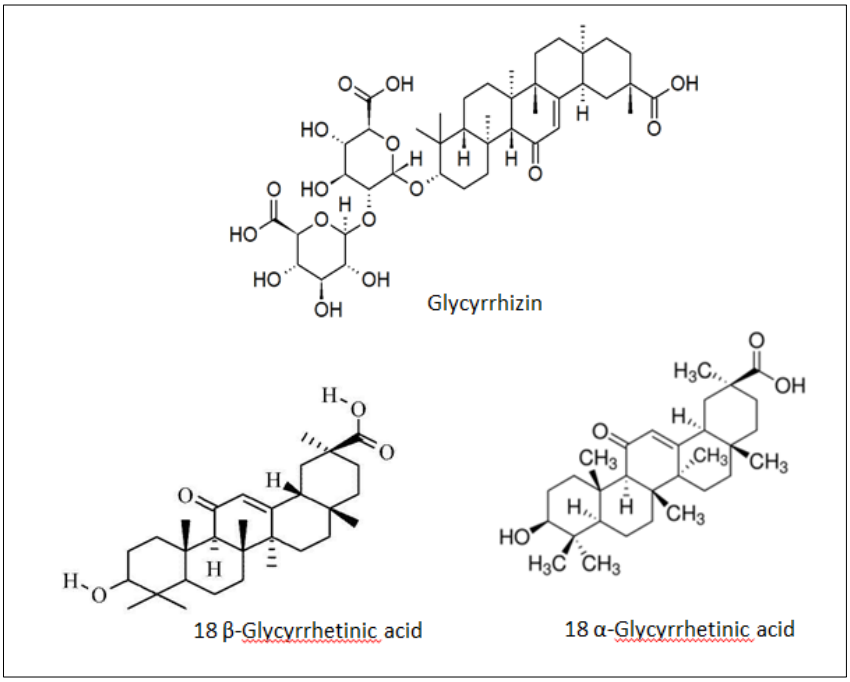


Figure : Glycyrrhizin and its isomeric forms

Glycyrrhizin and its aglycone, 18β-glycyrrhetinic acid (also known as 18β-glycyrrhetic acid or glycyrretic acid or glycyrrhetinic acid), have interesting therapeutic properties. Therapeutic potential of glycyrrhizin is mainly ascribed to the action of the steroid-like structure aglycone (18β-glycyrrhetinic acid) having immunomodulatory properties. Traces of the α-form of glycyrrhetinic acid are also present in liquorice roots but have no pharmacological activity (Claude *et al*., 2008).

Licorice is scientifically used for various metabolic disorders. Hence, due to extensive usage the plant was always in shortage in India. This has led to the possible substitutes of G. glabra. One of the main adulterant which was often used was *Abrus precatorious* however, it could not substitute the real licorice. However, there was some unnoticed substitute which did not get phytochemical preference however were locally used licorice or in other words Yashtimadhu. If we look into the phytochemical literature of licorice we see that it was later on various phytochemical analysis on *G. glabra* was done and that lead to various phytoconstituents.

T. cuneifolia due to sweet component in the roots for long has been used as substitute but the phytochemical analysis was missing. However, some efforts were made by Saluja, 1979 where it is indicated that the presence of glycyrrhizin by TLC. However the phytochemical characterization and validation was not done. Later after three decades the work was taken up by Zore, 2008 wherein HPTLC studies reported the presence of Glycyrrhizin. Notani, 2012 reconfirmed the same. All the preliminary studies states that the presence of Glycyrrhizin.

More than 300 flavonoids have been isolated from *Glycyrrhiza* species. These flavonoids belong to various types, including flavanones or flavanonols, chalcones, isoflavans, isoflavenes, flavones or flavonols, isoflavones and isoflavanones. Amongst them, flavanones and chalcones are the main types (Zhang & Ye, 2009). Various phytocomponents identified by various author in *G. glabra* are are as follows:

Table 2.3: Phytoconstituents present in *Glycyrrhiza glabra*

Sr. No.	**Phytoconstituent**	**Phyto Group**	**Reference**
1.	liquiritic acid	Triterpenes	Isbrucker and Burdock, 2006
2	Glycyrretol		
3	Glabrolide		
4	isoglaborlide		
5	liquorice acid		
6	Liquiritin (Flavanone)	Flavonoid	Williamson, 2003
7	Liquiritigenin (Flavanone)		
8	rhamnoliquiritin		
9	neoliquiritin		
10	chalcones		
11	Isoliquiritin (Chalcone)		
12	Isoliquiritigenin (Chalcone)		
13	neoisoliquiritin,		

Sr. No.	Phytoconstituent	Phyto Group	Reference
14	licuraside,		
16	Licoflavonol		
17	Glucoliquiritin apioside	Flavonoid	Rastogi and Mehrotra, 1990; Anonymous, 2005
18	Prenyl licoflavone A		
19	Shinflavanone		
20	1-methoxyphaseolin		
21	5,8-dihydroxy-flavone-7-O-beta-Dglucuronide		Li *et al*., 2005
22	glychionide A		
23	5-hydroxy-8-methoxyl flavone-7-O-beta-D-glucuronide		
24	glychionide B		
25	Glabridin (isoflavan)	Isoflavones	Kinoshita *et al*., 2005
26	galbrene		
27	glabrone		
28	shinpterocarpin		
29	LicoisoflavonesA and B		
30	formononetin		
31	glyzarin		
32	kumatakenin		
33	hispaglabridin A		
34	, hispaglabridin B,		
35	4′-O-methylglabridin		
36	3′-hydroxy-4′-O-methylglabridin		
37	glabroisoflavanone A		
38	glabroisoflavanone B		
39	glabroiso-flavanone B		
40	2-methylisoflavone	Isoflavone	Rastogi and Mehrotra, 1990
41	Semilicoisoflavone B	Isoprenoid substituted Phenolic compounds	
42	1 methoxyficifolinol		
43	Isoangustone A		
44	licoriphenone		
45	Kanzonol R	prenylated isoflavan	Anonymous, 2005
48	Genistein	Isoflavones	Ammosov and Litvinenko, 2003
49	Glicoricone		
50	Glisoflavone		
51	Isoangustone A		
52	Glycyrrhizoflavanone	Isoflavanones	Ammosov and Litvinenko, 2003
53	Glyasperin F		
54	Licoisoflavanone		
55	Glyasperin C	Isoflavans	Ammosov and Litvinenko, 2003
56	Glyasperin D		
57	Licoricidin		
58	liqcoumarin	Coumarins	Kinoshita *et al*., 2005
59	glabrocoumarone A and B		
60	herniarin		
61	umbelliferone		

Sr. No.	Phytoconstituent	Phyto Group	Reference
62	Glycyrin		
63	Glycocoumarin		
64	Licofuranocoumarin		
65	Licopyranocoumarin		
66	Glabrocoumarin		
67	lipocoumarin	Coumarins	Ammosov and Litvinenko, 2003
68	licocoumarone		
69	licoriphenone		
70	isoglycryol		
71	Licoarylcoumarin	Coumarins	Li *et al*., 2000
72	Coumarin GU-12		
73	C licocoumarin		
74	6- acetyl, 5-hydroxy, 4-methyl coumarin		
75	Asparagine		
76	dihydro-3,5-dihydroxy-4′-acetoxy-5′-isopentenylstilbene	Dihydrostilbenes (isolated from leaves of *Glycyrrhiza glabra*	Sultana *et al*., 2010.
77	dihydro-3,3′,4′-trihydroxy-5-O-isopentenyl-6-isopentenylstilbene,		
78	dihydro-3,5,3′-trihydroxy-4′-methoxystilbene		
79	dihydro-3,3′-dihydroxy-5beta-d-O-glucopyranosyloxy-4′-methoxystilbene		
80	Phenol	Phenols	Näf and Jaquier, 2006
81	Guaiacol		
82	4- methyl-γ-lactones,4-ethyl-γ-lactones	γ-lactones	
83	glucose,	Sugars	Näf and Jaquier, 2006
84	sucrose		
85	Xylose		
86	starch	polysaccharides	
87	arabinogalactants		
88	β-sitosterol	Sterols	
89	dihydrostigmasterol		
90	Tartaric acid	Organic acids	Sherif *et al*., 2013
91	Citric acid		
92	Malic acid		
93	Propanoic acid		
94	Butyric acid		
95	Formic acid		
96	Fumaric acid		
97	Acetic acid		
98	Pentanol	Volatile components	Anonymous, 2005
99	Hexanol		
100	Linalool oxide A and B		
101	Tetramethyl pyrazine		
102	Terpinen 4-ol		

Sr. No.	Phytoconstituent	Phyto Group	Reference
103	α-terpeniol		
104	Geraniol		
105	Propionic acid		
106	Benzoic acid		
107	Ethyl linoleate		
108	Methyl ethyl ketine		
109	2,3- butanediol		
110	Furfuraldehyde		
111	Furfurylformate		
112	1-methyl-2-formylpyrrole		
113	Trimethyl pyrazie		
114	Maltol		
115	α–pinene	Essential oils	Ali, 2013
116	β-pinene		
117	octanol		
118	γ- terpinene		
119	stragole		
120	isofenchon		
121	β-caryophyllene		
122	citronellyl acetate		
123	caryophyllene oxide		
124	geranyl hexanolate		

Following phytochemical components have been reported from *G. glabra* and a close associate of *T. aegyptiaca*:

Of the 12 species of *Taverniera* known *T. aegyptiaca, T. lappacea, T. abyssinica* are phytochemically studied. The comparative details of various phytocomponents occurring in these species have been depicted in Table. Of the various phytocomponents saponins, coumarins, glycosides, and volatile oil have been studied however other components such as alkaloids, phenolic acids etc. are yet to be studied. The available references necessitated the detailed phytochemical analysis of *T. cuneifolia* roots. Based on these references various methodologies relevant to study of glycyrrhizin (Kokate, 2007) analysis was carried. Further for analysing the phytoconstituents vigorous TLC, HPTLC, HPLC, GCMS, LCMS studies were done.

Table 2.4: Comparison of phytoconstituents of *T. cuneifolia, T. aegyptiaca, T. abyssinica and T. lappacea*

Phytochemical constituent	*T. aegyptiaca*	*T. cuneifolia*	*T. abyssinica*	*T. lappacea*
Part used – Root				
Glycyrrhizin	?	+	_	_

Saponins				
22β-hyroxyolean- 11, 13 (18)-dien-3β-ɤl-β-D-glucopyranoside	+(Hassanean, 1998)	-	_	--
1β,22β-dihydroxyolean- 11, 13 (18)-dien-3β-ɤl-β-D-glucopyranoside	+ (Hassanean, 1998)	-	_	_
Phytochemical constituent	***T. aegyptiaca***	***T. cuneifolia***	***T. abyssinica***	***T. lappacea***
1β,22β-dihydroxyolean- 11, 13 (18)-dien-3β-ɤl-β-D-xylopyranosyl (1→2) -β-D glucopyranoside	+ (Hassanean, 1998)	-	_	_
28-methyl serratagenate-3-β-O-β-xylopyranosyl (1--)2)-β-Glucopyranoside	+ (Ibrahim *et al*., 2002)	_	_	_
28-methyl serratagenate-3-β-O alpha-rhamnopyranosyl (1--)2)-β-Glucopyranoside	+ (Ibrahim *et al*., 2002)	-	_	_
3-β-O alpha-rhamnopyranosyl (1--)2)-β-glucopyranosyl - olean - 11, 13 (18)-dien - 1β,3β, 22β triol.	+ (Ibrahim *et al*., 2002)	-	_	_
3-β-O-β--glucopyranosyl(1--)2)--β-glucopyranosylolean-11,13(18)-dien-1β,3β, 22β triol	+ (Ibrahim *et al*., 2002)	-	_	_
3-β-O alpha-rhamnopyranosyl (1--)2)-β-glucuronopyranosyl - olean - 11, 13 (18)-dien - 1β,3β, 22β triol.	+ (Ibrahim *et al*., 2002)	-	_	_
3-β-O-β--xylopyranosyl(1--)2 β-glucopyranosylolean 11,13(18)-dien-1β,3β, 22β triol	+ (Ibrahim *et al.*, 2002)	-	_	_
Volatile oil				
β-sitosterol	_	+ (Khan Subur W. *et al.*, 2012)	_	_
Lupeol	_	+ (Khan Subur W. *et al*., 2012)	_	_

Pterocarpan				
Medicarpin	_	*?*	+ *(Duddeck et al., 1987)*	_
4-hydroxymedicarpin	_	*?*		_
Phytochemical constituent	***T. aegyptiaca***	***T. cuneifolia***	***T. abyssinica***	***T. lappacea***
Phenolic acids (Flavonoids)				
Isoflavonoids				
formononetin	_	*?*	+ (Duddeck *et al.*, 1987)	_
Afrormosin	_	*?*	+ (Duddeck *et al.*, 1987)	_
Quercetin-3,7- diglucoside	_	_	_	(Ibrahim and Mohamed , 2009)
Kaempferol 7 rhamnoside	_	_	_	(Ibrahim and Mohamed , 2009)
Rutin	_	_	_	(Ibrahim and Mohamed , 2009)
Apigenin 7 glucoside	_	_	-	(Ibrahim and Mohamed , 2009)
Daidzin	_	_	_	(Ibrahim and Mohamed , 2009)
Kaempferol - 3-O glucoside	_	_	_	(Ibrahim and Mohamed , 2009)
Quercetin	_	_	_	(Ibrahim and Mohamed , 2009)
Kaempferol	_	_	_	(Ibrahim and Mohamed , 2009)
Naringenin	_	_	_	(Ibrahim and Mohamed , 2009)

Apigenin	–	–	–	(Ibrahim and Mohamed , 2009)
Caffeic acid	–	–	–	(Ibrahim and Mohamed , 2009)
Phytochemical constituent	***T. aegyptiaca***	***T. cuneifolia***	***T. abyssinica***	***T. lappacea***
Ferulic acid	-	–	–	(Ibrahim and Mohamed , 2009)
Scopoletin	–	–	–	(Ibrahim and Mohamed , 2009)

Sugars

Based on various studies on Genus *Taverniera*, Glucose is present in the roots *T. lappacea* and *T. aegyptiaca* while Xylose has been reported *T. lappacea* and *T. aegyptiaca*. With regard to *Glycyrrhiza glabra* 12 different types are sugars have been recorded. There is no literature on *T.cuneifolia* regarding carbohydrates in roots.

Table 2.5: Comparison of sugars present in *T. lappacea, T. aegyptiaca, G. glabra*

Plant name	**Sugars**	**Reference**
Taverniera lappacea	Glucose and Rhamnose	Ibrahim *et al*., 2009
Taverniera aegyptiaca	Glucose and Xylose	Hassanean *et al.*, 1998
Taverniera aegyptiaca	Glucose, Xylose and Rhamnose	Ibraheim *et al*., 2003
Glycyrrhiza glabra	Ribitol, Glucofuranose, α-D-Fructose, β-D-Fructose, Sorbose, 2-O-Hydroxyethylglucose, β-D-Galactofuranose, D-Mannopyranose, D-Mannitol, β-D-Glucopyranose, Myoinositol, saccharose, β-D-Mannopyranosyl–D-glucitol	Denisova *et al*., 2003

2.4 Origin of the research problem

Taverniera cuneifolia (Roth) Ali is an ethnobotanically important traditional medicinal plant of semi-arid region of India belonging to the family of Fabaceae. The roots of *T. cuneifolia*

are a potential substitute of *Glycyrrhiza glabra* L. (commercial Licorice, Sankrit - Yashtimadhu). It is known for its sweet component from the roots which is similar to that of *G. glabra* (Zore, 2008). *G. glabra* (Sweet Root, Spanish or Italian Licorice), is the commonest source of licorice. *G. lepidota* is American wild licorice, while *G. violacea* and *G. glandulifera* are Persian/Turkish and Russian varieties, respectively. *G. uralensis* (Manchurian licorice) is the species favored for traditional Chinese Eastern Medicines (Maisch, 1893). *T. cuneifolia* could be a potential substitute of *G. glabra* owing to the presence of glycyrrhizin (13%) (Zore *et al*., 2008).

In addition, the plant species could have prime importance owing to the following factors:

1) *G. glabra* roots are widely used in traditional systems of medicines all over the world (Grieve, 1992). *G. glabra* is rich in bioactivities like antiviral, anticancer, anti-ulcer, anti-diabetic, anti-inflammatory, anti-oxidant, anti-thrombic, anti-malarial, anti-fungal, anti-bacterial, estrogenic, immuno-stimulant, anti-allergenic and expectorant, promoting expectoration, an agent that promotes expectoration activities (Olukoga and Donaldson, 2000; Baltina, 2003; Sasaki *et al*., 2003; Cinatl *et al.*, 2003; Rastogi and Mehrotra, 1989). These properties are even stated for *T. cuneifolia* by Thaker which states that it can be used as an expectorant, blood purifier, anti- inflammatory, wound healing, antiulcer and for treating spleen tumors (Thaker, 1910), the data given by Thaker was reconfirmed by (Nagar, 2005), leaves are used in sloughing wounds (Khare, 2007).
2) The commercial licorice has a huge demand in the Indian system of medicine, and is a major requirement of the Ayurvedic drug industry in India. This requirement was met through import from Afghanistan and Pakistan (Rastogi and Mehrotra, 1989). Owing to restricted cultivation in Haryana and Punjab there is a shortage of licorice roots and even the roots harvested are of inferior quality. Thus there is an urgent need to cultivate and harvest the high quality licorice that is the need of the hour or to search for suitable substitute.
3) Number of plants is often referred as Indian licorice and as a substitute of true licorice such as *Abrus precatorius, Alysicarpus longifolius, Maerua arenaria, Taverniera cuneifolia*, however, a potential indigenous alternative to *G. glabra* is not yet available (Maisch 1893, Brown, 1995, Baltina, 2003).

With the rising demand and rates there is an urgent need of an alternative source of Licorice. Though there are various alternatives of Licorice, from India's perspective *Taverniera*

cuneifolia could be one of the most potential plants of the future. The plant being used as alternative has been described in Vanaspati shastra *Kathiyawadna Barda dungarni jadibuti teni pariksha ane upyog* (Thaker, 1908). Few references found in Nighantu period has shown that there is a wide description of aquatic variety of licorice which is named as Klitanak. They have mentioned two types of licorice i.e. *Sthalaj* (terrestrial) and *Jalaj* (aquatic) varieties. Some texts have strongly mentioned Klitanak to be the name of *Jalaj* licorice (Yashtimadhu) variety. The availability of *T. cuneifolia* near the riverside and other aquatic/coastal zones indicates that it could be an aquatic variety of licorice. There are references available in Charak Samhita, Nighantu Adarsh, Bhavprakash Nighantu and other ayurvedic text (Sharma and Das, 2008; Misra and Vaisya, 2007; Sharma and Sharma, 2005; Sharma, 2006; Sharma, 2004; Prasad, 2004; Vaishya, 2002; Suri, 1968; Vaidya, 2007; Gauda, 2002; Jayantilak, 1918; Kamat, 2006; Sharma, 2003; Shastry, 2005; Thaker, 1910) written during the Nighantu period wherein there is reference of Klitanak as aquatic licorice.

The details of the same are as follows:

Historically ayurvedic treatments have been recorded during Vedic Period, Purana Period, Samhita Period, Nighantu Period and Modern Period. Of which the details of *Jalaj Yashtimadhu* has been not mentioned in Vedas and Puranas however it has been reported during Samhita, Nighantu and Modern period.

Samhita Period

The description of *Jalaj Yashtimadhu* was primarily found in Charak samhita as Acharaya Charak has clearly mentioned about the two different varieties of Yashtimadhu i.e. *Sthalaj Yashtimadhu* (Terrestrial Licorice) and *Jalaj Yashtimadhu* (Aquatic Licorice), wherein he has emphasized *Jalaj Yashtimadhu* as Klitanak. The description found in Charak samhita for this plant is as follows:

शङ्खिन्यथ विडङ्गानि त्रपुषं मदनानि च
धामार्गवमथेत्त्वाकु जीमूतं कृतवेधनम्
त्र्यानूपं स्थलजं चैव क्लीतकं द्विविदं स्मृतम् ८१

The verse means that there are two types of Klitanak (*Glycyrrhiza glabra*) the one which grows in marshy land and the other which grows in dry land.

Nighantu Period

Raj Nighantu

क्लीतनं मधुरं रुचयं बल्यं वृष्यं ब्रणापहम्।
शीतलं गुरु चक्षुष्यमस्त्र पित्तमहं परम् ।।१४८।।

(R.Ni. Pipalyadi Varga, 148)

In this verse the author emphasizes that "In the name of Klitanak there are various synonyms found i.e. Madhuvalli, Madhurlata, Madhuparni, Madhurasa, Atirasa and Shoshapaha. It was found that Klitanak was sweeter. The words emphasize that Yashtimadhu is madhur and partially tikta whereas Klitanak (Aquatic Licorice) is madhur and ruchya".

Bhavaprakash Nighantu

In this text a very confine description was found regarding the Jalaj variety of Yashtimadhu.

यष्टीमधु तथा यष्टीमधुकं क्लीतकं तथा।
अन्यत्कलीतनकं ततु भवेतोय मधूलिका ।।

(Bh.Pr.145)

The verse states that there are different names of Yashtimadhu like Yashtimadhu, Yashtimadhuk and Klitanak apart from that there is also one aquatic variety found in aquatic region which is Yashtimadhu and it is called as Madhulika.

Dhanwantry Nighantu

तल्लक्षणं क्लीतनकं कलीतनं क्लीतिका च सा।
यष्टीकायुगलं स्वादु तुष्णापित्ता स्त्रउजिद्धिमम् ।।

(Dh.Ni.140)

This is the only classical text which emphasizes the specific description with the name of Klitanak and it gives with the heading as 'Madhuyashti vishesha'. The phrase describes that "The characterisitcs of Klitanak are similar to madhuyashti, Klitanaka, klitinika, etc all the above mentioned names are synonyms of Klitanak. It is differentiated into two types Sthalaj and Jalaj whereas Klitanak is Sthalaj and the Jalaj variety is known as madhuparni or Madhulika. Both type of mulethi are madhur, shital and are useful in raktapitta."

Kaideva Nighantu

यष्टीमधु क्लीतनकं यष्टयाह कलीतकं मधु।
मधुयष्टचपराम्भोजा मधुपर्णी मधूलिका ।।२६।।

(Ka.Ni.29)

This text emphasizes the description with the different synonym of Yashtimadhu in which it has mentioned about the two different varieties of it. The phrase describes that "Yashtimadhuka, madhukam, madhuyasti, madhustrava, Klitanak, yastyavaha, klitak, madhu all of them are synonyms of Yashtimadhu. The second vareity of madhuyasti is found in aquatic region which is known as madhuparni and Madhulika."

Ashtang Nighantu

यष्टी मधुकयष्टयाहवा मधुकं क्लीतकाह्यम्।
परुषको मृदुफलो रोषजो धन्वनच्छदः ।। २६ ।।

(As.Ni.Sari.gana.2/29)

This text emphasizes the description with the different synonym of Yashtimadhu. The phrase describes that "Yasti, madhukam, madhukaiyastaha, madhukam, klitaka, all of them are synonyms of Yashtimadhu."

Shaligram Nighantu

मधुवल्ली द्विप्रकारजलजा च स्थलो द्रवा ।
सावृष्या मधुरा रुच्या बल्यागुर्व्यी च शीतला ।।

(Sh.Ni.)

This text states about the two different varieties of Yashtimadhu. It describes that "madhuvalli is of two type one is Sthalaj i.e. terrestrial variety and the other one is Jalaj i.e. aquatic variety among which both of them are madhur, ruchya, balya and shital in properties."

Madanpal Nighantu

मधुयष्टी क्लीतनकं यष्टीमधु मधूलिका ।
यष्टयाहं मधुकं यष्टिमधूकं जलजा मधुः । ८७

(Ma.Ni.87)

This text emphasizes about the synonyms and two different varieties of Yashtimadhu. The phrase describes that "madhuvalli is of two type one is Sthalaj i.e terrestrial variety and the other one is Jalaj i.e. aquatic variety among which both of them are madhur, ruchya, balya and shital in properties."

Nighantushesha

मधुयष्टी च यष्टी च यष्टीमधु मधुस्त्रवा
यष्टीमधुकं मधुकं यष्टयाहं मधुयाष्टिका ।।
तल्लक्षणं क्लीतनं च क्लीतकं क्लीतका च सा।
स्थलजा जलजाडन्यो च मधुपर्णी मधूलिका ।।

(Nighantushesha)

This text emphasizes about the different synonyms and two different varieties of Yashtimadhu. The phrase describes that Madhuyasti, yasti, Yashtimadhu, madhustrava, madhuka, yastayavaha etc are the synonym of the Yashtimadhu also the quote elaborates that the one with Sthalaj variety is known as Sthalaj Klitanak and the one with Jalaj variety is known as madhuparni or Madhulika."

Modern Period also states the two types of Yashtimadhu which are as follows

Adarsh Nighantu

The text Adarsh nighantu has emphasized about the aquatic variety of Yashtimadhu. In the text he elaborates that in the state of Gujarat there is a shrub that grows with the name of Yashtimadhu which is different from actual Yashtimadhu. The Yashtimadhu sold in the market of Gujarat is *Taverniera nummularia* (syn. *T. cuneifolia*).

Kaviraj Virjacharan has emphasized 'madhuyasti to be Sthalaj; but the name Sthalaj klitak will be more specific nomenclature and the representation with this name will be more appropriate as sthal refers to dry region. Similarly Anup should be confined with aquatic and

marshy areas. The Yashtimadhu found near aquatic areas should be denoted as Anup klitak". The author has described the reference of Raj Nighantu where he refers that Klitanak has various synonyms like Madhuvali, Madhurlata, Madhuparni, Madhurasa, Atirasa and Shoshapaha. He has even mentioned that Klitanak is sweeter'.

क्लीतनं मधुरं रुचयं बल्यं वृष्यं ब्रणापहम ।
शीतलं गुरु चक्षुष्यमस्त्र पित्तपहं परम् ।।१४८।।

(R.Ni.Piplyadi Varga, 148)

The words emphasizes that Yashtimadhu is madhur and partially tikta whereas Klitanak is madhur and ruchya.

Shankar Nighantu

This text emphasizes about the two different varieties of Yashtimadhu. The one which is found in aquatic region and the other found in terrestrial region.

Saraswati Nighantu

मधुकं क्लीतकं यष्टिर्मधुका मधुयष्टिका ।
वल्लीमधुकरं काष्टमधुक च समं मतम् ।।१४।।

(Sw. Ni. 14)

This text emphasizes about different synonyms of Yashtimadhu. The phrase describes "Madhukam, Klitakam, Yastih, Madhuka, Yastivmadhuka, Madhuyastika, Vallimadhukaram, Kasthamadhukam being the synonyms of Yashtimadhu".

Dravyaguna Vijnan

The author has mentioned the references found in different ayurvedic texts regarding the two different varieties of Yashtimadhu as Jalaj and Sthalaj. In which Jalaj is also called as Madhulika.

Dravyaguna Vijnan

In this text the author has described two different varieties of Yashtimadhu as Jalaj and Sthalaj. The author has given many references related to the synonym Klitanak and the

description of the two varieties of Yashtimadhu found in the different classical texts of Ayurveda.

Vanaspati Shastra

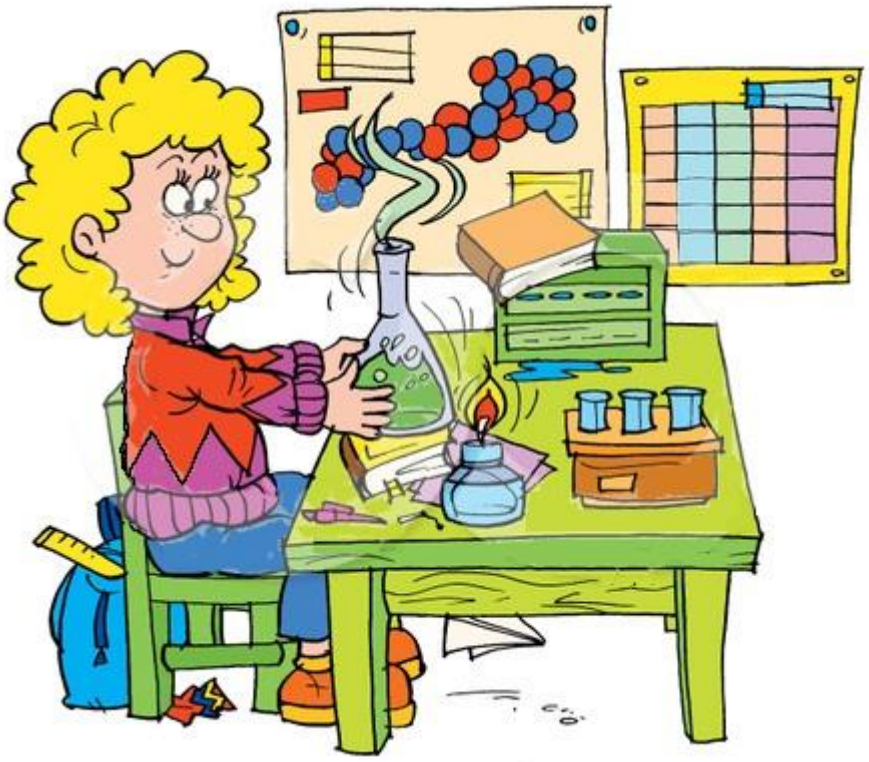
It is the only complete reference found with brief description about all the different aspects of the plant. The author has described the plant with detailed study of its habit, habitat, root, leaves, stem, inflorosence, fruiting, therapeutic dose, therapeutic uses etc.

Taverniera cuneifolia locally known as *Jethimadh* is used by the tribals of Barda hills and surroundings, Jamnagar as a substitute of Licorice or in other words the plant itself is considered to be *Glycyrrhiza glabra* (Nagar, 2005).

Dymock describes the Latin name of the plant *T. cuneifolia* to be taken as the variety for the Licorice root in Pharmacographia Indica (Dymock *et al*., 2005). However, there are few scattered references available for *T. cuneifolia* on pharmacognosy and phytochemistry but a detailed study is lacking. The phytochemical studies are restricted to the glycyrrhizin quantification in the roots and preliminary studies. Preliminary studies exhibited promising anti-inflammatory, anti-tumor, antigerm tube formation (in *Candida albicans*), protection from mutagen toxicity and cytotoxic activities comparable to *G. glabra*. (Zore *et al*., 2008) Above all the total potential of plant (root, stem, leaves, flowers, fruits) is yet to be explored and analyzed.

Standard Operating Procedures (SOPs) for phytochemical and pharmacological analysis should be developed for therapeutic, nutraceutical, dietary supplement and other value added products in order to understand the total potential of the plant. The present study gives an insight into the various aspects from the morphological characterization to that of phytochemical and pharmacological utility.

Chapter 3: Materials and Methods



Materials and Methods:

Section A:

3.1 Distribution and Ecology:

Based on the references available on occurrence *of T. cuneifoli*a in state and outside the state, various sites were visited and the plant material was identified in the field. The plants have been collected in the flowering and the fruiting stages and the plants were reconfirmed in the laboratory, during the field studies habit, habitat, height of plant, colour of flower, associations and other prominent features were also recorded. Further for comparing the phytoconstituents of *Taverniera abyssinica* with *T. cuneifolia,* the roots of *T. abyssinica* were collected from the market of Ethiopia (Fig. A4.6).

While visiting the sites various ecological parameters were studied. For the climatic data the meteorological data was collected. The soil was collected from the site. For this a pit of 1 x 1 x 1 ft. was made and thus the soil collected from various strata (upper, middle and lower) were collected and mixed. The soil collected was studied for the physical and chemical properties. The physical property of studies includes color, texture and water holding capacity. The following are the chemical property study in the soil.

Carbon (Chromic acid Method) (Perur, 1973)

Principle:

The oxidisable matter in finely ground sample is oxidized by Cr_2O_7 ions of $K_2Cr_2O_7$. The eaction is facilitated by spontaneous heat evolved by adding conc. H_2SO_4 in proportion to 2:1. The Cr_2O_7 ions will reduce from Cr^{+6} to Cr^{+3} and give green colour due to formation of chromus trivalent ions. Green colour of Cr^{+3} is measured with photoelectric colorimeter using 660mμ red filter. The green colour of reduced Cr^{+3} is used as a direct measure of quantity of carbon oxidised and the carbon present is red from the graph prepared from the known quantity of organic carbon.

Apparatus: centrifuge machine**,** analytical balance**,** Photoelectric colorimeter

Reagents:

- Standard 1N $K_2Cr_2O_7$ solution: Dissolve 49.04g $K_2Cr_2O_7$ in distilled water and dilute to one litre.

- Conc. Sulphuric acid A.R. containing 1.25% Ag_2SO_4 which is used to precipitate out soluble chlorides.
- Sucrose (A.R. anhydrous)

Preparation of Standard Curve:

A standard solution of sucrose is prepared by weighing accurately 1.25g of A.R. grade sucrose and dissolving in 1N $K_2Cr_2O_7$ solution and making up the volume to 250ml. Mix it well. Pipette out 1, 2, 3, 4, 5 and 6ml aliquots from the stock solution of sucrose to 50ml test tubes. Dilute the aliquots with 1N $K_2Cr_2O_7$ to 10ml and add 20 ml of concentrated H_2SO_4. Keep for 30 minutes and measure the green developed at 660mμ in a photoelectric colorimeter. Draw standard curve by plotting the colorimetric readings against carbon value calculated from the quantities of sucrose in the aliquots by multiplying with 0.42**.**

Procedure:

To each test tube or flask containing 1.00g 0.5mm sieved soil sample, add 10ml of 1N $K_2Cr_2O_7$ solution and 20ml of conc. H_2SO_4 with automatic pipette or with multiple dispenser. Stir the reaction mixture and allow to stand overnight. Alternatively, allow to stand for half an hour and then centrifuge ten minutes at 200rpm. Read the green chromous colour of the clear supernatant liquid on the photoelectric colorimeter, using 660mμ red filter, after setting the blank prepared in the same manner to zero.

Read the carbon from the standard curve. Express it as percent of the soil by the multiple factor.

Observations and calculation:

- Weight of the soil = Xg
- Colorimeter reading= Y
- % Organic carbon= 0.19 x 100/Wt. of soil = 0.19 x 100/1000mg soil

- %O.M. = O.C. x 1.724 (O.M. contains 58% O.C.)
- % N = O.M. x 0.05
  = O.C. x 0.0862

Rating of organic carbon:

Low < 0.50%, Medium 0.50-0.75%, High > 0.75%

Available Phosphorous in soil: (Ghosh *et al.*, 1983)

Available P. is determined by Olsen's extractant.

Principle:

The heteropoly complexes are thought to be formed by co-ordination of molybdate ions, with phosphorous as the central co-ordinated atom, the oxygen of the molybdate radicals being substituted for that of PO_4.

$$H_3PO_4 + 12\ H_2MoO_4 \longrightarrow H_3P\ (Mo_3O_{10})_4 + 12\ H_2O$$

These heteropoly complexes give a faint yellow hue to their water solution, which on reduction with stannous chloride gives blue colour. The intensity of the colour is read from a colorimeter at a wavelength of 660mμ using a red filter.

Olsen's extractant:

Mode of extraction:

The 0.5 M $NaHCO_3$, with pH 8.5 is designed to control the ionic activity of Ca, through the solubility product of $CaCO_3$ during the extraction of calcareous soils. S the carbonate activity in soil I sraised by this solution, the calcium activity is decreased. Thus, some phosphate from the surface of calcium phosphate is extracted through the solubility product of $Ca_3\ (PO_4)_2$. As Ca^{++} activity decreases, phosphate activity increases. The reagent also reacts with some phosphate from the surface of Al^{+++} and Fe^{+++} phosphate, which are more abundant in acid or neutral soils.

Apparatus

Klett summerson colorimeter or spectronic 20,

Reagents

- 0.5N Sodium bicarbonate: Take accurately 42g of NaHCO3, dissolved and diluted to one litre with distilled water. Adjust the pH of this solution to 8.5 with 1 M Naoh.
- Darce G-60 or carbon black or activated charcoal (phosphorous free).

- 1.5% ammonium molybdate: Dissolve 15g of ammonium molybdate in 300ml of wrm distilled water. Filter the water if necessary and allow it to cool. Then add 342ml of concentrate HCl gradually with mixing. Dilute to 1 liter with distilled water.
- Stannous chloride: (Stock solution) Dissolve10g of $SnCl_2$ in 25ml concentrated HCl. Store this solution in refrigerator. Working solution: Take 0.5ml stock solution of concentrated $SnCl_2$ and make to 66ml with distilled water.

Standard curve of Phosphorous

Reagents:

- 50 ppm standard solution of KH_2PO_4: take accurately 0.2195g of KH_2PO_4, dissolve and add 25ml of 0.7N H_2SO_4 and dilute with distilled water and make volume upto 1 litre. This solution contains 0.05mg of P/ml.
- 2ppm P working solution: Take 40ml of the standard 50ppm P solution dilute to one litre with distilled water. This solution contains 2 µg of P/ml.

Procedure for standard curve:

Take 0.0, 0.5, 1.0, 2.5, 5.0, 7.5, 10.0, 12.5, 20.0 and 25.0ml from the working solution of 2 ppm P and make the final volume of 50ml after addition of 5ml of ammonium molybdate, 1ml of working $SnCl_2$ and distilled water. Blue colour will develop. Take the intensity of blue colour on Klett summerson. The final 50ml solution contains 0, 0.2, 0.4, 1.0, 0.2, 0.3, 0.4,

0.5, 0.6, 0.8 and 1.0 ppm P, respectively.

Procedure:

Take 5g of 2mm sieved soil into a 250ml conical flask. Add one teaspoon of activated charcoal and 100ml of 0.5M $NaHCO_3$ solution. Shake the conical flask for 30 minutes on a mechanical shaker. Filter the suspension through a Whatman No.42 filter paper. Take 5ml aliquot of the extract in a 25ml volumetric flask. Ad 5 ml of ammonium molybdate solution and a little quantity of distilled water and shake well. Add 1ml of working $SnCl_2$ solution in each 25ml volumetric flask, make volume upto 25ml with distilled water and shake well. Measure the transmittance of the solution in a colorimeter during the tie of 5 minutes after and 20 minutes before the addition of SnCl2 solution. Use 660mµ incident light in colorimeter or red filter in Klett summesion.

Observation and calculation:

- Weight of the soil taken: 5g
- Volume of added 0.5M $NaHCO_3$: 100ml
- Aliquot taken=5ml
- Klett reading=X
- Blank reading=Y
- Actual reading=(X-Y)=Z
- Ppm P=G.F.*Z*100/5*25/5
- P Kg/ha=ppm P*2.24
- P2O5kg/ha=Pkg/ha*2.29.

Observations:

Rating of available P (Kg/ha)

Low < 11, Medium 11-25, High > 25

Available Potassium in soil (Perur, 1973)

Principle

Soil is shaken with neutral normal ammonium acetate. During the extraction ammonium ions release potassium ions adsorbed on the soil colloids.

(Colloid) K^+ + CH3COONH4 ↔ (Colloid) NH_4^+ + CH3COOK

Being the almost similar ionic radii, K^+ is more effectively replaced by NH_4^+. The extract is then filtered and potassium is determined by Flamephotometer.

Instrument

Electric shaker, flame photometer.

Reagents:

Normal neutral ammonium acetate solution: Dissolve 74.5g of ammonium acetate in 1 litre of distilled water. Test with bromthymol blue or with a ph meter. If not neutral, add either ammonium hydroxide or acetic acid to neutralize it to pH 7. Alternatively dilute 108ml concentrated ammonium hydroxide to 1 litre and dilute 115ml glacial acetic acid to 1 litre and dilute 115ml glacial acetic acid to 1liter and mix the two. Adjust the pH to 7.

Standard Potassium solution:

Dissolve 1.907g pure KCl in 1 litre of distilled water. This solution contains 1mg K perml i.e. 1000ppm K alternatively for 1000ppm K_2O 1.5851g pure dry KCl can be dissolved in one litre of distilled water. Preserve this solution as a standard stock solution of potassium. Working solutions: Take 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 ml of the stock solution, containing 1000ppm K_2O separately in 100ml volumetric flasks and make the volume with the N ammonium acetate solution. Add few drops of butyl alcohol to -ncl to improve spraying properties of the solutions. These solutions contain 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 ppm K_2O respectively.

Preparation of standard Curve:

Set up the flame photometer. Plot the meter readings against the respective potassium content and draw the curve.

Procedure:

Take 5g soil in 150ml conical flask. Add 25ml of ammonium acetate solution and shake for 5 minutes on electric shaker. Filter the contents through a Whatman No. 1 filter paper. Add 2 drops of butyl alcohol to each filterate. Feed the filterate to the flamephotometer and note the reading.

Observation and calculation:

- Wt. of the soil taken = 5g
- Volume of ammonium acetate added= 25ml
- Dilution=5 times
- Reading shown by flame photometer = X
- Ppm of available K2O against X
- As read from standard curve=Y
- Ppm of available K2O in soil= Y* dilution

  = Y*5=Z

- Kg/ha of available K2O= Z ppm * 2.24

Rating of available K_2O

Low < 120 kg/ha, Medium 120-280 kg/ha, High > 280 kg/ha.

1. **Soil pH:** (Perur, 1973)

Principle:

pH meter works on the principle of Nernst equation. It consists of two electrodes. Glass electrode and Calomel electrode. When the both electrodes are dipped in aqueous solution under test, the potential is developed in the solution. The potential between both the electrodes is measured by pH meter.

Instrument: pH meter, multiple dispensers, multiple stirrer, 50 ml beaker

Preparation of buffer solution:

- Dissolve 3.391g of citric acid and 23.3844g of Na2HPO4 in one litre of distilled water.
- Add 296.3ml of 0.1N NaOH and 500ml of 0.1M potassium hydrogen phthalate and dilute to 1 litre with distilled water.

Buffer solution (pH 9.18)

0.01M sodium tetraborate: 3.81g of Na2B4O7.10H2O is dissolved in 1 litre of water.

Procedure:

Place the rack having eleven 50ml beakers, each containing 10g soil sample under the multiple dispensers. Add 20 ml of distilled water and stir with multiple stirrer for 30 minutes. Determine the pH of the soil suspension with pH meter.

Preparation of saturation extract:

Saturated soil paste is prepared by distilled water to the sample of the soil.the soil mixture is consolidated from time to time by tapping the container on the bench. After mixing the sample should be allowed to stand for an hour. Transfer the paste into the filter funnel and apply vacuum. Collect the extract in a test tube.

2. **Electrical conductivity of soil: (Perur, 1973)**

Instrument:

Conductivity meter and a conductivity cell with known cell constant, suction machine.

Reagents:

- Saturated solution of calcium sulphate

- 0.01N KCl solution: dissolve 0.7456g of potassium chloride in distilled water and dilute to 1 litre**.**

Procedure:

The saturation extract of the soil was prepared as per method given in the procedure for ph determination. The same soil suspension used for pH is used fr salinity determination. After determination of soil pH allow the suspension to settle for 30minutes. Pour the supernatant in the conductivity tube. Dip the conductivity cell on conductivity meter.

3. Available Sulphur in soil: (Chesnin and Yein, 1950)

Principle:

The fact that much of the soil sulphur is present in organc forms and fraction of this sulphur is also soluble in water, hence sulphur may be gently released by hydrolysis.Water was added to the soils and evaporated to dryness. Then extraction was done with sodium chloride hence further fraction of sol sulphur was released.. heat is the main factor in releasing the additional sulphur.

Apparatus:

Kett Summerson, water bath, etc.

Reagents:

- Morgan's reagent: 100gm of sodium acetate dissolved in 600ml of distilled water and 30ml of glacial acetic acid. The final volume is made up to 1 litre and pH adjusted to 4.8.
- 1% NaCl
- Gum acacia solution: 0.5g pure gum is dissolved in 200ml distilled water and the solution is filtered.
- Barium chloride crystals
- Standard sulphur solution: 0.5434g potassium sulphate is dissolved in distilled water and dilute to 1 litre. This stock solution contains 100ppm sulphur.

Preparation of standard curve:

Different quantities of aliquot from stock solution i.e. 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0ml were taken in 50ml volumetric flasks to give 0,1,2,3,4,5,6 ppm sulphur respectively. 20ml of the extract and 2ml gum acacia are added and diluted to about 45ml distilled water. The flasks

are shaken for a minute and 1g barium chloride is added. The final volume is made and flaks shaken for 3 minutes. The flask allowed standing for 20 minutes. The developed turbidity is measured using Klett Summerson colorimeter using blue filter (430mμ) wavelength.

Prepare a standard curve for sulphur.

Procedure:

Weigh 5 g of soil and put in a silica basin and add 20ml distilled water. Allow it to boil till dryness. Then heated for 60 minutes in a hot air oven at 102˚C. After cooling the soil is transferred to 150ml conical flask and add 33ml 1%NaCl. Shake it for an hour. Filter it. Take suitable aliquot (10ml) in 25ml volumetric flask, add 10ml Morgan's reagent and 1ml gum. It is shaken for a minute and 0.5g barium chloride are added. The final volume is made. The final volume is made and flaks shaken for 3 minutes. The flask allowed standing for 20 minutes. Take reading on Klett summerson using Blue filter.

Observation and calculation:

- Wt of soil= 5g
- Aliquot taken for turbidity development=10ml
- Klett reading= R
- ppmS in soil= graph factor*33/wt. of soil*25/aliquot taken*Klett reading
- =0.0911*33/5*25/10*R
- ppmS in soil= 1.503*R

4. Available micronutrients in soil: (Ghosh *et al.*, 1983)

Micronutrients play many complex roles in plant nutrition, but most of them are used in the functioning of a number of enzyme systems. However, there is considerable variation in the specific functions of the various micronutrients in plants and in microbial growth processes.

Apparatus: Atomic absorption spectrophotometer (AAS), Electrical shaker.

Reagents:

Diethylene-triamine penta acetic acid (DTPA) 0.005M solution, Triethanolamine (TEA) 0.1M, CaCl2.2H2O 0.01M solution, Zinc metal, Dilute HCL (1:5) with doble distilled water. The extracting reagent is prepared by taking 1.967g of DTPA and 1.470g CaCl2.2H2O in a beaker. To this 20-25ml distilled water is added and 13.3ml of TEA followed by 100ml

distilled water. This is transferred to 1 litre volumetric flask with 3-4 washingsand volume made upto mark with water. The pH of the solution is adjusted to 7.3 with dilute HCl.

Preparation of standard curves:

Zinc: 1g of pure zinc metal is dissolved completely in minimum amount of dilute HCl and made upto 1 litrewith distilled water nd then treansferred to a plastic bottle. This stock solution contains 1000µg Zn/ml. In 6 100ml volumetric flask required aliquots are taken and standards of 0, 0.5, 1.0, 1.5, 2.0, 2.5ppm zinc solution are made and standard curve prepared against the readings of atomic absorption spectrophotometer.

Copper: A stock solution of 1000ppm Cu is obtained by dissolving 1g of Cu metal in 50ml of dilute (1:1) nitric acidand finally diluted to 100ml distilled water. From this working solutions (0.25, 0.5, 1.5, 2.0, 2.5 and 3ppm) are prepared in volumetric flask.

Iron: A stock solution of 1000ppm Fe is obtained by dissolving 1g of Fe metal in 50ml of dilute (1:1) nitric acidand finally diluted to 1 litre distilled water. Working solutions (1, 2, 4, 6, 8, 10 ppm) are made in 100ml volumetric flasks.

Manganese: 1g of pure Mn metal dissolved in 50ml dilute nitric acid and made upto 1litre gives a stcck solution of 1000ppm Mn. In 100ml volumetric flask working concentration of 1, 2, 4, 6, 8, 10 ppm Mn are taken.

Procedure:

To 10g of soil in 100ml conical flask, 20ml DTPA reagent is added and shaken for 2 hours. The extract is filtered and micronutrients are estimated with AAS.

Rating of available micronutrients:

Zinc: Low<0.5ppm, Medium0.5-1.0ppm, High>1.0ppm

Iron and Manganese: Low<5ppm, Medium5-10ppm, High>10ppm

Copper: Low<0.2ppm, Medium0.2-0.4ppm, High>

SECTION 2: PHARMACOGNOSY

Samples from the roots were of 2.2 to 2.8cm in diameter and the stem measured 0.8 to 1 cm. Plants were collected from the plants growing in Rajkot (Munjaka). The fresh plant samples were cut into suitable size of 4-5cm in length and were immediately fixed in Formalin Acetic Alcohol (FAA) solution (Berlyn & Miksche, 1986).Trimmed blocks were sectioned in transverse planes at a thickness of 12-15µm by using Leitz sliding microtome. Sections were stained with Saffranin: fast green combination (Johansen, 1940) and mounted in dibutyl phthalate xylene (DPX) after passing through the ethanol-xylene series.

Fully grown Fresh leaves were also collected simultaneously at the time of stem and root samples collection. After arriving to lab they were washed thoroughly with dist. water to obtain transverse section for anatomical study. Fresh leaves were cut into small pieces and boiled in 90% ethanol for about 3-5minutes to remove chlorophyll. After boiling, they were washed 2-3 times with distilled water to remove all the traces. An epidermal layer was peeled off with the help of pointed needles and forceps and washed in water. Subsequently peels were stained with 0.5% saffranine (Saas, 1940). Leaf constants, such as stomatal index / mm^2, vein islet number / mm^2, vein termination number / mm^2 and palisade ratio were calculated using standard procedures (Anon. 2006). For each parameter, 30 measurements were taken randomly to obtain mean.

Powder studies

Completely dried plant material was powdered. The fine powder obtained was stained with 0.5% Safranine (Sass, 1940) and observed under a microscope to locate and identify the important characters. Important results were micro-photographed with the help of Leica DME trinocular research microscope with digital camera.

SECTION 3: SEED GERMINTION

Seeds of *T. cuneifolia* were collected from Munjka region, Rajkot, Gujarat, India in April 2012. Seeds were separated from pod in the laboratory and surface sterilized by treating with sodium hypochlorite. Seeds were germinated on 3 sheets of 9 cm diameter filter paper in10 cm diameter. Petri dishes with 10 ml of distilled water. The Petri dishes were sealed in plastic bags. Twenty five seeds were used for each treatment with three replicates each. Effect of

GA_3: Seeds were soaked in 100, 250, 500 ppm GA3 for 48 hours and 1000, 2000 ppm GA3 for 72 hours. (Wiese and Binning, 1987; Auld *et al*., 1988)

The germination rate was calculated as follows (based on Wiese and Binning, 1987):

$$\text{Germination rate} = \sum_{n=1}^{5}(\text{number germinating since } n-1)/n$$

Where, *n* is the days of incubation.

The effect of different salts (KCl, $MgCl_2$, NaCl, Na_2CO_3, $MgSO_4$) were tested with different concentration each. The experiment was carried out at 31ºC. Germination was recorded at every alternate day and after 9 days un-germinated seeds were transferred to distilled water to determine the recovery of germination. The percent recovery was calculated using the following index:

Percent recovery = a-b / b-c x 100

where, a = total number of seed germinated after being transferred to distilled water, b = total number of seed germinated in saline solution and c = total number of seeds (Khan, 2002).

3.2 Cultivation of *Taverniera cuneifolia* (http://affordable-antimalarials-for-the-poor.net/resources/Growing_Artimisia.pdf)

FIRST TASK: Germinating the seeds in seed trays:

• Method for germinating seeds:

1. Prepare in a big cooking pot a mixture of one quarter of sand, one quarter of old earth , one half of hot water (for instance: 10 tins of sand, 10 tins of old earth and 20 tins of water) and boil this mixture for five minutes. Allow it to cool
2. **Build portable germination-trays**: -The tray can be of plastic (for instance the lid of a bucket) or wood, or a basket. - A germination tray of 50 cm length, 50 cm width (20 inches X 20 inches) would be a good size for 1/3 of a gram of seeds, (for a 1 g package you need three such seeds trays of 50 sq cm (20"), - Its rim should be at least 5 cm (2") high. - Make a number of holes into the bottom, large enough so that water can get in.
3. Fill the soil/sand/water mixture into the germination tray almost up to the rim (5 cm or 2 inches) and wait until the water has drained away

4. Sprinkle the right amount of tiny seeds (depending on the size of your germination tray) with a sieve on top of the mixture; do not bury them (for a box 20 square inches one third of a gram of seeds (about 300 seeds) is needed.)

5. Put the tray in a warm and sunny place, if temperature is higher than 30 ° Celsius, put it in the shade. Protect the seeds from chicken, ants or birds (if necessary) by covering the tray with a piece of mosquito netting or mosquito gauze and/or placing it on a table.

6. Keep the soil of your seed moist by inserting the tray in water two to three times a day for ten minutes (You may create a small pond with a plastic sheet next to the place where you keep the trays, and then fill this pond with the amount of water you need for the trays). If you have a spraying device you may also water the tray with a very fine spray (mist) from the top, but never use a watering can before the plants have taken root and keep the tray out of the rain since larger drops of water will dislocate the tiny seeds.

7. After 3-7 days shoots should begin to appear. They should have two leaves. If only one leaf appears, this is a grass seedling. If some seedlings are too close together, transplant one of them with a tweezer to another place in the seed tray.

If the plantlets grow very slowly use a small amount of liquid fertilizer ("kic-start", 1.5 cups to 3 litres of water) to make up for nutrient deficiency.

SECOND TASK: (about 4 weeks after sowing) transplant seedlings to polyethylene bags

When the seedlings will be 3 cm (1.2 ") high they are ready to be transplanted:

- Put them in a polyethylene bags filled with a bottom layer (1/2) of well-rotted compost and a top layer of sand.

- Use small sticks to make a hole into the bags and to pick out the plantlets.

- Put these pots into water once a day or water with a sprayer.

The seedlings grown from seeds are ready for transplanting approximately 7 weeks after sowing, when they have reached a height of about 10-12 cm.

1. **Hardening**:

- When seedlings are ready for transplanting, they need hardening for about one week: this is done by reducing watering to a minimum and exposing the plants to sunlight.

- Care must be taken not to overdo the hardening process as this can result in stress to the plants which leads to premature flowering and greatly reduces leaf. Before pulling them out, soak the plants in water and keep them moist until they have reached their final destination.

2. Land preparation:

- Deep ripping with tined implements to allow the tap root to penetrate any hard pan in the soil while at the same time conserving soil moisture is recommended.
- If furrow irrigation is proposed ridging of the soil before planting may be required.
- Pre-planting weed control is essential, using chemical herbicide where necessary. Adding natural manure to the planting holes is important and land preparation should be completed at least one week before transplanting to allow the land to settle.
- On the day before transplanting the field should be thoroughly watered.

THIRD TASK: 3. Transplanting into the field: (about 8 weeks after sowing): Planting the Seedlings out into the field.

This is best done at the start of the rains or before the rain stops totally (monsoon end) - depending upon the climatic condition, since the water content of the soil must be high at transplanting and for several weeks thereafter to avoid moisture stress and premature senescence.

METHOD OF PLANTING THE SEEDLINGS OUT IN THE FIELD

- Plant the seedlings in rows, one foot apart, and each plant 1foot apart within the row in zigzag manner.

- Planting holes should be 30 cm (12 inches) deep, filled with soil mixed with leaves and small branches and dung.

- The soil should be very loose and wet. If the rains at the period of transplanting are not reliable, irrigation is essential.

- When transplanting great care must be taken to avoid **bending the tap-root**.

- The soil must be pressed firmly around the plant to prevent wilting and to help the plant to recover from the shock of transplanting.

- Use some mulch on top to prevent soil from drying out.

Section B:

3.3 Section 1: Standardisation of the herbs

3.3.1 Collection and processing of plant samples:

The plant material was collected from Munjka village, Near Saurashtra University Campus, Rajkot and Rosy port area, Jamnagar, Gujarat, India. The plant material was authenticated at BSI (Botanical Survey of India) Jodhpur, Rajasthan, India. Ref. no. BSI/AZC I.12012/ Tech./2011-12 (PL.ID)-55. The shade dried roots were used for the investigation of qualitative phytochemical test, physicochemical tests and microbial contamination.

Organoleptic evaluation:

Organoleptic evaluation of drug shows that it has a sweet taste but slightly bitter sensation at the end. The roots are hard, odour of the powder when boiled are like groundnut seed

3.3.2 Determination of water soluble extractive for Powder:

Accurately weighed 5.0g each of roots and aerial parts were taken and transferred to a 100ml capacity ground glass stopper conical flask. To this 100ml of water was added. It was shaken well for 3h. Then filtered through Whatman filter paper. 25ml of filtrate was evaporated in preweighed evaporating dish. Then it was dried in a steam oven (800ºC) for 15 min. The evaporating dish was cooled in a dessiccator and then weight of the evaporating dish with the residue was taken.

The percentage of water soluble extractive was calculated using the formula

$$\% \text{ of water soluble extractive} = \frac{\text{Wt. of residue}}{\text{Wt. of sample (A)}} \times 100$$

3.3.3 Determination of Alcohol soluble extractive:

Accurately weigh 5.0g (note the exact weight) of sample and transfer it to a 100ml capacity ground glass stopper conical flask. To this add 100ml of alcohol or suggested ration of alcohol and water. Shake well for 3 hr. Filter it through Whatman filter paper. Evaporate 25ml of filtrate in preweighted evaporating dish and evaporate the filtrate on water bath.

Then dry in a steam oven (800ºC) for 15 min. Cool the evaporating dish in dessicator and then the evaporating dish with the residue was weighed.

Calculate the percentage of water soluble extractive using the formula

$$\% \text{ of alcohol soluble extractive} = \frac{\text{Wt. of residue}}{\text{Wt. of sample (A)}} \times 100$$

3.3.4 Determination of Moisture by Karl Fischer apparatus:

Sufficient quantity of methanol was added to dip the electrodes in the beaker and methanol was neutralized with Karl Fischer Reagent. The equipment was operated as per the equipment operating procedure.10µL water was added and addition of Karl Fischer Reagent was started till end point. From this the factor was calculated.

$$\text{Karl Fischer factor} = \frac{\text{Weight of water in mg}}{\text{Volume of Karl Fischer Reagent consumed}}$$

100mg of sample (each roots and aerial parts) was accurately weighed and added in the beaker with constant stirring. After the sample dissolved uniformly in the solution, the addition of Karl Fischer Reagent was started till the end point. The reading displayed on the screen was noted and % of moisture was calculated the by the given formula.

$$\% \text{ of moisture content} = \frac{\text{Factor x reading}}{\text{Weight of the sample in mg}} \times 100$$

3.3.5 Determination of Total ash:

Accurately weighed 1.0g of the air dried sample (each of roots and aerial parts) was transferred to a preweighed crucible. Then crucible with the sample was weighed and exact weight was noted. The sample was incinerated, gently at 4500˚C, until it gets free from carbon. The crucible was removed from the furnace and cooled in a dessicator and then crucible was weighed. Difference in the weight is the weight of the ash.

the total ash content was calculated in the sample using the formula

$$\% \text{ of Total Ash} = \frac{\text{Wt. of Ash}}{\text{Wt. of sample}} \times 100$$

3.3.6 Determination of acid insoluble ash:

The ash obtained from total ash was taken to determine the acid insoluble ash content. 25ml conc. HCl was added and boiled on water bath for 10 min. Filtered through the ashless filter paper. The insoluble matter was washed with hot water. The insoluble matter of ash was transferred with the filter paper in the earlier taken crucible and dried on hot plate. It was ignited for 1 hr at 8000ºC in furnace. The crucible was removed from the furnace and cooled in a dessicator and then the weight of the crucible was taken. The acid insoluble ash content was calculated in the sample using the formula

$$\% \text{ of Total Ash} = \frac{\text{Wt. of acid insoluble ash}}{\text{Wt. of ash}} \times 100$$

3.3.7 Determination of Water soluble ash:

The ash obtained from total ash was taken to determine the water soluble ash content. 25ml of water was added and boiled on a water bath for 10 min. It was filtered through the ashless filter paper. The insoluble matter of ash was transferred with the filter paper in the earlier taken crucible and dried on a hot plate. It was ignited for 20 min at 4500˚C in furnace. The crucible was removed from the furnace cooled in a dessicator and the crucible was weighed. The water soluble ash content was calculated in the sample using the formula,

$$\% \text{ of water soluble Ash} = \frac{\text{Wt. of water soluble matter}}{\text{Wt. of ash}} \times 100$$

3.3.8 Determination of sulphated ash:

The ash was taken from the total ash obtained to determine the sulphated ash content. The ash was moistened with 1 -2ml sulphuric acid. It was heated gently until white fumes no longer evolved. It was then ignited for 1 hr at 8000˚C in furnace. The crucible was removed from the furnace and cooled in a dessicator and then the crucible was weighed.

The sulphated ash content was calculated,

$$\% \text{ of Sulphated Ash} = \frac{\text{Wt. of residue}}{\text{Wt. of ash}} \times 100$$

3.3.9 Heavy metals

Preparation of Standard solutions:

Lead (Pb)

10ppm Pb (Working standard): 0.25ml standard solution of Pb (1000 ppm) was pipetted out accurately in 25ml volumetric flask and the volume was made up with distilled water. 0.1ppm Pb: 0.25ml solution form working standard (10 ppm) was pipetted out in a 25ml volumetric flask. The volume was made up with distilled water. 0.5ppm Pb: 1.25ml solution from working standard (10 ppm) was pipetted out in a 25ml volumetric flask. The volume was made up with distilled water.1.0ppm Pb: 2.5ml solution from working standard (10 ppm) was pipetted out in a 25ml volumetric flask. The volume was made up with distilled water. 2.0ppm Pb: 5.0ml solution from working standard (10 ppm) was pipetted out in a 25ml volumetric flask. The volume was made up with distilled water. 3.0 ppm Pb: 7.5ml solution from working standard (10 ppm) was pipetted out in a 25ml volumetric flask. The volume was made up with distilled water.

Cadmium (Cd):

10ppm Cd (Working standard): 0.25ml standard solution of Cd (1000 ppm) was pipetted out accurately in 25ml volumetric flask and the volume was made up with distilled water.

0.1 ppm Cd: 0.25ml solution form working standard (10 ppm) was pipetted out in a 25ml volumetric flask. The volume was made up with distilled water. 0.2 ppm Cd: 0.50 ml solution from working standard (10 ppm) was pipetted out in a 25ml volumetric flask. The volume was made up with distilled water. 0.3 ppm Cd: 0.75ml solution from working standard (10 ppm) was pipetted out in a 25ml volumetric flask. The volume was made up with distilled water. 0.4 ppm Cd: 1.0ml solution from working standard (10 ppm) was pipetted out in a 25ml volumetric flask. The volume was made up with distilled water. 0.5 ppm Cd: 1.25ml

solution from working standard (10 ppm) was pipetted out in a 25ml volumetric flask. The volume was made up with distilled water.

Arsenic (As):

1000ppb As: 0.1ml standard solution of As (1000 ppm) was accurately pipetted out in 100ml volumetric flask and the volume was made up with distilled water. 100ppb As (Working standard): 10ml solution of Arsenic (1000 ppb) was accurately pipetted out in 100ml volumetric flask and the volume was made up with distilled water. 5ppb As: 2.5ml working standard (100 ppb) was accurately pipetted out in 50ml volumetric flask and the volume was made up with distilled water. 10ppb As: 5.0ml working standard (100 ppb) was accurately pipetted out in 50ml volumetric flask and the volume was made up with distilled water. 15ppb As: 7.5ml working standard (100 ppb) was accurately pipetted out in 50ml volumetric flask and the volume was made up with distilled water.20ppb As: 10.0ml working standard (100 ppb) was accurately pipetted out in 50ml volumetric flask and the volume was made up with distilled water.

Mercury (Hg):

1000ppb Hg (Working standard): 0.1ml standard solution of Hg (1000 ppm) was accurately pipetted out in 100ml volumetric flask and the volume was made up with distilled water. 20ppb Hg: 1.0ml working standard (1000 ppb) was accurately pipetted out in 50ml volumetric flask and the volume was made up with distilled water. 40ppb Hg: 2.0ml working standard (1000 ppb) was accurately pipetted out in 50ml volumetric flask and the volume was made up with distilled water. 60ppb Hg: 3.0ml working standard (1000 ppb) was accurately pipetted out in 50ml volumetric flask and the volume was made up with distilled water. 80ppb Hg: 4.0ml working standard (1000 ppb) was accurately pipetted out in 50ml volumetric flask and the volume was made up with distilled water.

3.3.10 Microbiological tests:

Pre Treatment of the Sample: 10g/ml of the samplewas dissolved in 50ml buffered sodium chloride peptone solution having a pH of 7.0. Simultaneously10g/ml of the sample was dissolved in 50ml of SCDB for the determination of pathogens and it was incubated at 30-35°C for 18-24h.

Primary Treatment: 0.1g/ml of the sample was pipetted out from buffered sodium chloride peptone solution and spread onto SCDA in duplicates to determine the Total Bacterial Count and 0.1 ml onto SDA in duplicates for Total Fungal Count.

SCDA plates were then inverted and incubated at 30-35°C for 3 days after which the number of colonies were counted and multiplied by the dilution factor and were represented in the form of cfu/g/ml, whereas the SCDA plates were inverted and incubated at 25°C for 5 days after which the number of colonies were counted and multiplied by the dilution factor and were represented in the form of cfu/g/ml.

Negative Control: 0.1ml of buffered sodium chloride peptone solution was aseptically plated onto sterile SCDA plates and was incubated at 30-35°C for 3 days.

Positive Control: 0.1ml of a bacterial suspension containing approximately 105 - 108 (0.5 McFarland solution) was aseptically plated and incubated at 30-35°C for 3 days.

Test for Pathogens

Test for *E. coli*

1ml of the solution was pipetted out from (SCDB) and transferred to 100ml of (MCB) and incubated at 42-44°C for 24-48h. They were then sub cultured on MCAP and incubated at 30 -35°C for 18 - 72h. A Positive control with the Sample *E. coli* ATCC 8739 was carried out and a Negative control with only the sterile media was incubated at the required temperature.

Test for *Salmonella*

0.1ml of the solution was pipetted out from SCDB and transferred into 10ml of tubes containing *Salmonella* enrichment broth and incubated at 30-35°C for 24-48h. They were then subcultured on a plate of Wilson and BBS Agar and incubated at 30-35°C for 24-48h. A Positive control with the Sample *Salmonella* NCTC 6017 was carried out and a Negative control with only the sterile media was incubated at the required temperature.

Test for *Pseudomonas aeruginosa*

0.1ml of the solution was pipetted out from SCDB and spread onto Cetrimide agar plates and incubated at 30-35°C for 18-72h. A Positive control with the Sample *Pseudomonas aeruginosa* ATCC 9027 was carried out and a Negative control with only the sterile media was incubated at the required temperature.

Test for *Staphylococcus aureus*

0.1ml of the solution was pipetted out from SCDB and streaked onto Mannitol Salt Agar and incubated at 30-35°C for 18-72h. A Positive control with the Sample *Staphylococcus aureus* ATCC 6358 was carried out and a Negative control with only the sterile media was incubated at the required temperature.

Section B: Phytochemistry and Pharmacology

3.4 Section 1: Phytochemistry

Based on the available literature, the phytoconstituents of *T. cuneifolia* were analysed for their preliminary and sophisticated test. The plant material collected was dried and powdered (coarse). The coarse powder was further used for preliminary, chromatographic, spectroscopic and analytical studies. The prime focus of phytochemical studies was flavonoids, phenolic acids, saponins and fatty acids. The licorice is known for its sweetness and bioactive components i.e. glycyrrhizin and hence the sugar analysis and were studied separately for its presence. Nutraceutical properties were analysed based on sugars, vitamins, sterols and amino acid. Further, an effort was made for analysing the proteins and glycoproteins in the roots. The methodology used for various phytoconstituents are as follows:

3.4.1 Methodology for Preliminary Qualitative Chemical Tests:

The methods employs to isolate active substance are termed as extractive method. Crude extracts obtained from such extraction can be qualitatively tested to ascertain the presence of different types of components. Qualitative tests are used to detect the presence of functional groups, which play important role in the expression of biological activity. Using various solvents such as methanol, water and chloroform the solubility of the extracts were decided for the qualitative tests. These tests indicate the types of phyto-constituents present in the sample (Baxi *et al*., 2001). Plant material used for preliminary study was root.

Tests for Alkaloids:

Powdered plant was moistened with ammonium hydroxide and kept in a stopper flask for about one hour. This was then extracted with chloroform 2 to 3 times. About 5ml chloroform extract was taken in a dish and chloroform was evaporated. The dried substance was tested for the presence of alkaloid.

- With Mayer's reagent: The substance to be tested is treated with few drops of dilute 2N HCl and 0.5 ml Mayer's reagent, alkaloids give a white precipitate.
- With Wagner's reagent: The substance is treated with few drops of dilute 2N HCl and 0.5 ml Wagner's reagent, brown flocculent precipitate with alkaloids.
- With Dragendroff's reagent: The substance is treated with few drops of dilute 2N HCl and 0.5ml Dragendroff's reagent. Brown precipitate is obtained.

Test for Flavonoids:

Shinoda test: To dry powder or extract, 5 ml 95% ethanol was added to that few drops of conc. HCL and 0.5 gm Magnesium added, solution turns pink. To small quantity of residue, lead acetate solution was added. Yellow colored ppt was formed. Addition of increasing amount of NaOH to the residue shows yellow coloration decolorizes after addition of acid.

Test for Tannins:

Powdered 1gm root was boiled with 95% alcohol and alcoholic extract was filtered while hot. The extracts were dried on boiling water bath and the resulting residue was dissolved separately in about 5 ml of water and this solution was used for further qualitative tests.

- To a portion of the solution, few drops of lead acetate was added, presence of white precipitate indicates the presence of tannins.
- To an aliquot 5% Ferric chloride solution was added, greenish to black colour indicates the presence of tannins.

Test for Saponins:

Foam test: Shake the drug extract or dry powder vigorously with water. Persistent foam indicates the presence of saponins.

Test for Resin:

Dissolve 0.1 gm of powdered sample in 10 ml of acetic anhydride. Add one drop of H_2SO_4 using glass rod carefully, a purple colour rapidly changing to violet is produced.

Test for Phenol: Fresh plant sample was collected, the sliced tissue was immersed in 2N HCl in a test tube and heated for 30 min. on a boiling water bath, cooled, extract with ether, the ether extract was pipetted and evaporated to dryness. The residue was used for test.

Neutral $FeCl_3$ Test:

The residue was treated with neutral $FeCl_3$, formation of blue, green, red or violet color indicates the presence of phenolics.

Test for Terpenoids:

1g of powder was extracted with 10 ml of petroleum ether (40-60°C) and the extract was subjected for following test.

Leibermann burchard test:

To 1 ml of extract, 1 ml of chloroform was added first and then 1 ml of acetic anhydride followed by gentle addition of few drops of sulphuric acid from the side wall added. Purple colour ring at the junction of two layers indicated the presence of triterpenes.

3.4.2 Quantitative Estimation:

Estimation of Total Tannin Content: (Rajpal, 2005),

For the Tannin estimation, two methods were employed for analysis.

A. Indigocarmine Method (Titration Method) and

B. Folin- Denis Method(Spectrophotometric)

In the present study The Indigocarmine Method was carried out for Total Tannin estimation.

Extraction of Tannin:

5g of dry powdered plant material containing tannin was taken in 400ml distilled water and the mixture wasshaken thoroughly every 10 minutes for 1hour. It was allowed to stand for 1 hour at room temperature. The extract was filtered. The filtrate was taken in a 500ml of volumetric flask and the volume was made up. This was the stock solution containing tannin. The tannin thus extracted was estimated by Indigocarmine method.

Requisites:

0.5% Indigo carmine reagent, 0.1 N Standard KMnO4 (3.16g in 1000ml of distilled water), NaCl acid solution: 5% H2SO4 solution saturated with NaCl and Gelatin (2%): Dissolve 2g of Gelatin in 100ml of hot distilled water.

Procedure:

Part I: Standardization of $KMNO_4$:

A solution containing 2.5 ml concentrated H_2SO_4, 4.5 ml distilled water and 5 ml of 0.1N oxalic acid was prepared. The solution was warmed on a water bath (70°C) and this hot solution was titrated with 0.1N $KMNO_4$ till the appearance of a persistent light pink color. The $KMNO_4$ solution was diluted by the value of the burette reading and the titration was repeated till 5 ml of KMNO4 drop was required to neutralize 5 ml of 0.1N oxalic acid. This diluted $KMNO_4$ solution is the standard $KMNO_4$.

Part II: Titration with Tannin:

10 ml of the extracted Tannin was taken in a 1000 ml volumetric flask. Add 25 ml Indigocarmine reagent to the extract and make the volume up to 800 ml (with distilled water). This solution is titrated against 0.1N $KMNO_4$ solution. The end point is indicated by the presence of yellowish green color. Let the volume of $KMNO_4$ required to neutralize the acid content of Tannin= X ml.

Part III: Titration without Tannin:

50 ml of the extracted tannin was mixed with 50 ml of NaCl acid solution (5% H_2SO_4 solution saturated with NaCl) and 25 ml gelatin solution. The mixture was shaken vigorously for 5 min. The precipitate was filtered (brown solid particles) and to the 10 ml of the filtrate add 25 ml Indigocarmine reagent. Make up the volume to 800 ml and titrate with 0.1 N $KMNO_4$ as in Part II. Let the volume of $KMNO_4$ required in this part of the expt. = Y ml.

Calculation:

500 ml of the filtrate contains 5g of the sample.

Now, 10 ml of the filtrate (taken for titration with KMNO4) = 0.1 g of the sample

Actual amount of acid present in the sample

(X-Y) × $KMNO_4$ factor = gm Tannin present in 0.1 g of dry plant material.

($KMNO_4$ factor = 0.00415 gm for 0.1 N KMNO4). Multiply this value by 1000 to get the percentage of Tannin in the experimental sample.

b) Estimation of Saponin:

Procedure:

5gm sample extract was taken with 90% v/v methanol (25ml) and it was refluxed for half an hour. The residue was extracted two more times by taking 25ml methanol. The methanolic extract was combined and the solvent was distilled off. The soft extract was treated and left after distillation of alcohol. After removing alcohol 25 ml of petroleum ether 60-80^oC extract was refluxed for half an hour. It was cooled and the solvent was decanted. Further, the soft extract was treated successively with chloroform (25ml) and ethyl acetate (25ml). and these solvents were decanted **(**Rajpal, 2005).

The soft extract (after three extractions cited above) was dissolved in 25ml of 90% v/v methanol. It was filtered and concentrated to 5 ml. It was added drop by drop with constant stirring to 25ml acetone in order to precipitate the saponins. The precipitates were filtered, collected and dried to a constant weight at 105^oC.

Calculation: - Percentage of saponin= weight of residue/weight of sample×100%

Estimation of flavonoids:

3 g extract was refluxed with 50 ml alcohol on a water bath for half an hour. The above process was repeated twice. The alcohol was evaporated under vacuum. The filtrate and the residue were repeatedly shaken with 25, 20 and 15ml of hot water. The above aqueous extract was shaken repeatedly with 25, 20, 15 and 15ml of ethyl acetate, the ethyl acetate extract was collected and washed with water. It was then evaporated to dryness and weighed (Rajpal, 2002).

Calculation: - Percentage of Flavonoid= weight of residue/weight of sample×100%

3.4.3 Qualitative tests for Flavonoids and Phenolic acids:

All the procedures followed in the present work were separately performed for all the four parts of the plant i.e. root, stem, leaves and flowers. Their extraction, isolation and identification of flavonoids are described below.

Flavonoids:

Fifty grams of aerial and root powder were extracted in a Soxhlet's apparatus with methanol for 48hrs or till the plant material became colourless. The methanolic extract was concentrated to dryness in a water bath. 25-30ml of water was added to the dry residue and the water soluble phenolic glycosides were filtered out. The filterate was hydrolysed in a waterbath for one hour using 7% HCl.

This hydrolysate was extracted with diethyl ether/solvent ether, whereby the aglycones got separated into ether fraction (fraction A). The remaining aqueous fraction was further hydrolysed for another 10 hours to ensure the complete hydrolysis of all the O-glycosides. Aglycones were once again extracted into diethyl ether (fraction B) and the residual aqueous fraction was neutralized and evaporated for the analysis of glycoflavones.

Ether fractions A and B were combined and analysed for aglycones using standard procedures (Mabry *et al*., 1970; Harborne JB, 1984). The combined concentrated extract was banded on Whatman No.1 paper. The sample system used was 30% glacial acetic acid. The developed chromatograms were dried in air and the visibly colour compounds were marked out. These chromatograms were observed under ultra-violet light (360nm) and the bands were marked. Duplicate chromatograms were then sprayed with 10% Na_2CO_3 and 1% $FeCl_3$ and the colour changes were reported. The marked bands of the compounds were cut out from unsprayed chromatograms and were eluted with spectroscopic grade methanol. The UV absorption spectra of these compounds were recorded in methanol using 'Perkin Elmer Lambda 25 UV/Vis' spectrophotometer.

Phenolic acids:

Analysis of combined ether extract (A and B) was carried out by two-dimensional ascending paper chromatography. Toluene: acetic acid: water (6:7:3 v/v/v, upper organic layer) in the first direction were used as irrigating solvents. The sprays used to locate the compounds on the chromatograms were diazotized p-nitroniline or diazotised sulphanilic acid and a 10% Na_2CO_3 overspray (Ibrahim and Towers, 1960).

Diazotization:

0.7gms of p-nitraniline/sulphonic acid was dissolved in 9 ml of HCl and the volume made up to 100ml. Five ml of 1%$NaNO_2$ was taken in a volumetric flask and kept in ice till the temperature was below 4°C. The diazotized sprays were prepared by adding 4ml of p-

nitraniline/sulphanilic acid stock solution to the cooled $NaNO_2$ solution. The volume was made up to 100ml with ice cold water. The various phenolic acids present in the extract were identified based on the specific colour reactions they produce with the spray reagents.

3.4.4 Fatty acids

The fresh fruits of *T. cuneifolia* were collected from Munjka region, Rajkot, Gujarat, India during the favourable season between March and May. The fresh fruits (containing seed) were dried and powdered.

Extraction

The oil from the fruit powder was extracted completely with petroleum ether (60-80◦C) in a Soxhlet apparatus. The resulting extract was filtered and distilled off in a rotary evaporator leaving behind dark greenish oil with pungent odour. It was evaluated for its various chemical properties as per AOCS standard methods. Three replicates were taken for the analysis of chemical properties.

GLC Analysis

Three samples were taken for the analysis of the fatty acid composition. The methyl esters of extracted oil were prepared in accordance with the Bureau of Indian Standards (BIS). (548, part III). A NUCON-GLC chromatograph with a flame ionization detector (FID) was employed for the analysis using nitrogen as the carrier gas. The column used was 30M x 0.53 mm I.D. 5.0 µm DB-1 Type MXT-1 capillary column. The injection port temperature was 250◦C and the detector temperature was 280◦C. Sample injection was done at 60◦C and the temperature programming was set for 2◦C rise per minute to a maximum of 280◦C.The total run time was 40 min. Identification of each component was made by comparing its retention time with that of a Sigma-Aldrich standard fatty acids mixture.

3.4.5 Paper chromatographic analysis of amino acids

Take 5 g each of fruits, roots, leavs and fruit cover powder with 50 ml of methanol in each sample in round bottom flask. Keep the samples for refluxing for an hour. The methanol extract is filtered, concentrated and the aqueous solution is directly spotted on chromatographic papers. Standard solution of amino acids were taken. 100 mg dissolved in 1-2 ml of methanol) was spotted on the side of the plant sample. The papers were developed in

the solvent system mentioned below. When the run was over, the paper was dried, sprayed or dipped in the spraying reagent. The sprayed papers were then heated over a hot plate for 5 -10 minutes. The amino acids in samples were identified by comparing the color and Rf values of the spots with those of the standard

Solvent system:- BAW (n-butanol-acetic acid-water 4:1:5) While the upper organic layer is used as developing solvent, the lower aqueous layer is used to saturate the chromatographic chamber. Spray reagent used was ninhydrin **(**Dissolve 1gm ninhydrin in 100 ml acetone).

3.4.6 HPTLC analysis of Amino acids

Sample preparation: Accurately weighed powder (0.5 g) was extracted with methanol (5.0 mL). The mixture was vortexed for 1 min and kept standing overnight. The mixture was sonicated for 20 mins and then filtered through Whatmann filter paper no.1. The filtrate obtained was evaporated to dryness in water bath at 65° C and reconstituted in 1 ml methanol. The concentrated sample was subjected to HPTLC analysis.

Mobile phase: n- Butanol: 70% Ethylene glycol: Ethyl acetate 5: 2: 3 (v/v/v)

Derivatizing agent: Ninhydrin reagent (110 °C for 5 min)

HPTLC analysis of Sugars

Method of preparation: Take 2 g Diphenylamine, add 2 ml aniline and dissolve in 80 ml methanol. To this add 15 ml of 30 % orthophosphoric acid and make the volume to 100 ml with methanol. Analysis of various sugars was done with the various sugar standards procured from Sigma Aldrich.

Mobile phase: Isopropanol: water: Ethyl acetate 4: 0.5: 0.5 (v/v/v)

Derivatizing reagent: Diphenylamine aniline reagent (110 °C for 5 min)

3.4.7 Proteins:

Extraction of proteins from dry root and seed powder

The root powder of *T. cuneifolia* and *T. abyssinica* and seeds of *T. cuneifolia* was added in (2gms) extraction 0.1 M phosphate buffer containing 0.1 M NaCl having pH 7. The preparation kept on shaker for 1-1.5 h (100 rpm) at room temperature. The extraction medium was centrifuged (10,000 rpm, 20 min at 4°C), the supernatant was taken. Total protein in the supernatant was precipitated by ammonium sulphate (80% saturation) precipitation and kept at 4°C for 2 h. The protein precipitates were separated by centrifugation (10,000 rpm, 20 min

at 4°C) and dissolved in a minimum amount of 10 mM phosphate buffer (pH 7). Protein estimation as described by Lowry et al., 1951.

Protein estimation:

Protein was estimated according to Bradford method, and BSA (Bovine Serum Albumin) used as standard (Bradford, 1976).

SDS-PAGE

12% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was used as described by Laemmli (Laemmli, 1970). Electrophoresis was carried out in a Banglore Genei (India) SDS-PAGE unit and electrophoresed at 30 mA using Banglore Genei pack system (USA). 70 µl aliquot of protein sample was equal volume of sample buffer and heated for 10 min in boiling water bath. Molecular mass standards were from Biorad. One gel was stained with Coomassie Brilliant Blue R-250. The gel was kept in 1% CBB R- 250 for 1 h and the gel was destained using methanol: acetic acid: water (45:10:45) until the gel bands become visible against the clear background. The gel was then stored in 3% acetic acid.

Reagents (Laemmli, 1970)

(i) Acrylamide stock solution

Acrylamide 15.0g

Bisacrylamide 0.4g

Dissolved in water and the final volume was made 50ml. Filtered the solution through whatman no. 1 filter paper and stored in brown bottle at 4˚C.

(ii) Stacking gel buffer stock (Tris-HCl, pH 6.8)

Tris 1.5 g

Distilled water 20ml

Adjust the pH with 1M HCl and final volume was made upto 25ml.

(iii) Separating gel buffer stock (pH 8.8)

Tris 18.15 g

Distilled water 75ml

Adjusted pH to 8.8 and the final volume was made 100ml.

(iv) Tank buffer (Tris-glycine, pH 8.3)

Tris 0.328g

Glycine 1.441g

SDS 0.1g

Distilled water 100ml

Adjusted pH to 8.3 and the final volume was made 100 with distilled water.

(v) Polymerising Agents

Ammonium persulphate 1 per cent (w/v) 0.1g/10ml, prepared freshly before use TEMED

Fresh from refrigerator

(vi) Staining solution

Coomassie brilliant blue R-250 0.25g

Methanol 50ml

Periodic Acid-Schiff staining

PAS staining was performed as modified by Dubray and Bezard (Dubray and Bezard, 1986). Gels were run as described above. Briefly, gel was soaked in 7.5% (v/v) acetic acid for 30 min and then with 0.2% (w/v) periodic acid for 1 h at 4°C. The periodic acid solution was removed and the Schiff reagent added and incubated for 1 h at 4°C. Reddish-pink bands of stained glycoprotein would then be visible. The PAS reagent was removed and the gels were soaked in 7.5% acetic acid for 1 h and subsequently stored in water.

Gel preparation

Two plates were taken and spacers were kept between the edges of two plates which were joined with the help of clamps. For gel preparation, the contents of separating gel were mixed in a glass beaker and poured between two glass plates. The gel was allowed to polymerize under a layer of water. After polymerization of the gel, the layer of water was removed and comb was adjusted in such a way that it remained above the gel. All the ingredients of stacking gel were mixed in glass beaker and poured onto polymerized separating gel with the help of pipette and allowed to polymerize. Comb was removed from the polymerized gel such that fine wells were obtained and the prepared sample was loaded into the wells. One well was loaded with a standard (97.4 kDa to 14.3 kDa, Genei) medium range protein molecular marker. Electrophoresis was carried out with diluted running buffer until the tracking dye was almost at the end of the gel. Gel was removed and washed with distilled water. Gel was stained, using Coomassie Brilliant Blue. The gel was photographed with a camera (Laemmli, 1970).

3.4.8 HPTLC Analysis for seasonal, regional, species and elutropic fingerprinting

Samples of *T. cuneifolia* collected from Rajkot during summer, monsoon and winter were subjected to similar extraction conditions as well as application conditions on the TLC plates, in order to study the seasonal variation in HPTLC fingerprints. Exactly same procedures were also followed for the plant samples collected from Kutch, Jamnagar, Rajkot (Gujarat), Jodhpur (Rajasthan) and Kurnool (Andhra Pradesh). Similar procedure was even applied to species variation where *T. cuneifolia* was compared with *T. abyssinica* which was collected from the local market of Ethiopia. (Powder and roots of *T.abyssinica* in plant verification studied. Chapter 4). Sample collected from Rajkot was used for elutropic series. It was diluted in their respective solvents and HPTLC fingerprinting was done for petroleum ether extract, dichloromethane extract, ethylacetate extract, methanolic extract and water extract.

Experimental details:

Instrumentation:

a) HPTLC plates: Merck, aluminium sheet precoated with silica gel 60 F254 procured from Anchrom laboratories, Mumbai, India.
b) Applicator: CAMAG Linomat 5 sample spotter (Camag Muttenz, Switzerland) equipped with syringe (Hamilton).
c) Developing chamber: CAMAG twin trough chamber.
d) Densitometric scanner: Camag TLC Scanner 4 conjugated with winCATS software.
e) Photo documentation: Camag Reprostar 3

Solvents:

Organic solvents of analytical grade were procured from Merck specialties Private Limited, India.

Standard:

Reference compounds (standard) β-sitosterol (98% purity) and lupeol (95% purity) was procured from Sigma Aldrich, Steinheim, Germany.Glycyrrhizin was procured from Sigma Aldrich Chemicals Pvt. Limited, Bangalore, India. 18β – Glycyrrhetinic acid (≥97.0% purity) was procured from Sigma Aldrich (97% pure).

Regional variation, seasonal variation and Species variation (Sherbanoo, 2012)

Sample preparation: Accurately weighed powder (0.2 g) was extracted with methanol (5.0 mL). The mixture was vortexed for 1 min and kept standing overnight. The mixture was filtered through Whatmann filter paper no.41 and the filtrate obtained was diluted with

methanol in equal proportion (1:1) and subjected to HPTLC analysis. (regional, species, seasonal variation)

- Mobile phase: Toluene: Methanol: : 8: 1 (v/v)
- Saturation: 30 min
- Derivatizing Reagent: 10 % methanolic sulphuric acid

Elutropic series (Shailajan *et al*., 2011)

Sample preparation: The given samples (5) were diluted with respective solvents in equal proportion (1:1)

- Mobile phase: Cyclohexane: Ethylacetate: Formic acid: 6:4.5:0.2 (v/v/v)
- Saturation: 30 min
- Derivatizing Reagent: 10 % methanolic sulphuric acid

Seasonal variation with glycyrrhizin (Gantait *et al.*, 2010)

Sample preparation: Accurately weighed powder (0.2 g) was extracted with methanol (5.0 mL). The mixture was vortexed for 1 min and kept standing overnight. The mixture was filtered through Whatman filter paper no.41 and the filtrate obtained was subjected to HPTLC analysis.

- Mobile phase: Chloroform: Methanol: Water : : 65: 36:7.5 (v/v/v)
- Saturation: 20 min

3.4.9 HPLC analysis Glycyrrhizin.

Two methods were employed for the analysis of Glycyrrhizin in *G. glabra* and *T. cuneifolia*:

Method 1:

Shade dried roots were finely pulverized. To 100mg of the root powder10ml methanol was added and subjected to overnight extraction (Wang *et al*., 2004). The slurry was subsequently filtered and the extract was used for HPLC analysis.

Method 2:

To about 20g of powdered drug, 50 ml of acetone and 2 ml of dilute nitric acid was added. The solution was mixed thoroughly; the flask was corked and macerated for about 2 hours, with occasional shaking. The contents were filtered. To the marc, 20ml of acetone was added,

warmed on water bath for 10 minutes, and filtered. The filtrates were combined, and concentrated under vacuum. To the dry extract, dilute ammonia solution was added in sufficient quantity so as to completely precipitate glycyrrhizin in the form of ammonium glycyrrhizinate. The precipitate was separated by filtration, followed by two washings of acetone (Wagner and Bladt, 1996).

The chemicals and HPLC methodology for the comparative study is as follows:

Standards and chemicals:

Glycyrrhizin was procured from Sigma Aldrich Chemicals Pvt. Limited, Bangalore, India (CAS number -53956-04-0). HPLC grade methanol, Formic acid, Liquor ammonia was purchased from Qualigens, Mumbai, India. HPLC grade water, Acetone, Nitric acid was purchased from Loba Chemie, Mumbai, India.

Instrumentation:

HPLC analysis was carried out using a Shimadzu LC 20AT liquid chromatograph, equipped with a photodiode array detector. The analytical column used was Thermofischer BDS, Hypersil C18 (250x 4.6mm; 5µm). Mode of elution was isocratic and the mobile phase employed was methanol: water: formic acid (70:30:0.1 v/v/v). Flow rate was maintained at 1.0 ml per minute for a total run time of 10 minutes. Detection wavelength was set at 254nm, wherein glycyrrhizin shows maximum absorbance. All samples were filtered through a 0.45µm Acrodisc syringe filter (Pall Corporation, Mumbai, India) before filtration. Glycyrrhizin standard solution (100 µg per ml) was prepared in methanol. Presence of glycyrrhizin in samples was identified by congruent retention times and UV spectra when compared with those of the standard. All samples were prepared and analyzed in duplicates.

3.4.10 HPLC Method for anthocyanidins (Harborne, 1984)

Fresh petals were heated in 2M HCl in a test tube for 40 min at 100˚C. The coloured extract was cooled and decanted from the plant tissue and was further filtered. The filterate was washed twice with ethyl acetate to remove flavones, the ethyl acetate layers were discarded and the remaining aqueous layer was heated at 80˚C for 3 min to remove the last traces of ethyl acetate. The separation was done with a separating funnel. The pigment was then extracted into a small volume of amyl alcohol. Then spotted on whatman no.1 paper. The paper was allowed to run in 30% Acetic acid in a dark room. The anthocyanidin in the residue is dissolved in 2-4drops of methanolic HCl and then its HPLC analysis was done.

3.4.11 Column chromatography and HPLC analysis for analysing saponins and proteins

Procedure for the isolation of glycosides:

Powdered 500g roots were mixed with 5 liters of distilled water and kept overnight at room temperature. Next day only 3 litres were left. Then the solvent was heated on a hot plate (100°C) till it got reduced to 120ml. It was then allowed to cool. Remaining water was removed by Dean Stark apparatus by adding butanol to it. The ratio of the sample and butanol was 1:3. The coloured butanol was decanted which was approximately 20ml and kept aside and sticky mass (40-44g) was obtained. Then the solid material was then kept in the refrigerator at 5°C. The sticky solid was given a washing of acetone approximately 2-3 times to remove its stickiness. 20g of methanolic washing was used as a sample for column chromatography with sephadex LH 20 100 (Sigma Aldrich). The ratio taken up for column was 1:10 (sample: sephadex). The first run was with methanol for column chromatography followed by methanol: water and lastly with water. 14g sample of methanolic fraction gave 4.66g, methanol: water (1:1) 1.8g, water fraction was 0.7g. Then each sample was tested for its melting point by DSC (Differential Scanning Colorimeter) and elemental analysis was done by Elemental Analyser. Then, re-column chromatography in sephadex was done taking methanolic fraction 1.362g and water fraction 0.666g where 11 fractions were obtained. The following fractions below:

- Methanolic fraction from methanolic fraction
- Methanol: water (1:1) from methanolic fraction
- Water fraction From methanolic fraction
- Methanolic fraction From water fraction
- Methanolic fraction From water fraction
- Methanolic fraction From water fraction
- Methanolic fraction From water fraction
- Methanolic fraction From water fraction
- Methanol: water (1:1) from water fraction.
- Methanol: water (1:1) from water fraction.
- Methanol: water (1:1) from water fraction.

These fractions were further analysed by HPLC where it was found that all the fractions were similar and hence HPLC of the combined fraction was conducted.

3.4.12 Analysis for Saponins and Saikosaponins

For analysing Saponins and Saikosaponins the methodology developed by Hassanean, 1998; and Ibraheim *et al*., 2003 was followed. Wherein the air dried powdered roots (500gms) were powdered and extracted with methanol 3 times at room temperature. After removal of the solvent at reduced pressure the syrupy extract (37.79gms) was diluted with distilled water and extracted with CH_2Cl_2, the aqueous fraction was further chromatographed over Diaion HP 20 column and eluted with distilled water, methanol: water (1:1) and with methanol. The saponin fraction eluted with methanol: water was lyophilised (semi lyophilised sample 20gms) and used for doing column with silica gel using $CHCl_3$: MeOH gradient. The different gradients used for $CHCl_3$: MeOH were as follows:

1. Chloroform: methanol – 100:0
2. Chloroform: methanol – 95:5 (Fraction 1)
3. Chloroform: methanol – 90:10 (Fraction 2)
4. Chloroform: methanol – 85:15 (Fraction 3)
5. Chloroform: methanol – 72:28 (Fraction 4)
6. Chloroform: methanol – 65:35 (Fraction 5)
7. Chloroform: methanol – 60:40 (Fraction 6)

Then fractions were subjected to HPLC for further chromatographic analysis.

Chromatographic conditions for HPLC:

Solvent system: A: Acetonitrile: water: formic acid (96: 4: 100microlitre)

B: Water: Acetonitrile: formic acid (96: 4: 100microlitre)

- **Mode of Elution:** Gradient.
- **Detector wavelength:** 254nm.
- **Flow rate:** 1ml/min.
- **Detector:** PDA (Photo Diode Array).
- **Total run time**: 60 minutes.
- **Start wavelength:** 190nm
- **End wavelength:** 370nm.
- **Lamp type:**D2

Time Program:

Table 3.1: Concentration of B at different time intervals

Time	Value of B concentration
0.01	100
15.00	85
20.00	70
30.00	60
40.00	50
50.00	40
55.00	100
55.90	100
60.00	100

- HPLC chromatograms of S3, S4, S5 and S6, which was further elucidated for its Mass through LCMS

3.4.13 SPECIFICATIONS OF LC-MS:

- LC- Perkin Elmer PE Series 200pump, Autosampler, UV-detector
- MS- applied biosystem QTrap 2000.
- MS Parameters:
- Compound Parameters:
    1. Declustering Potential – 50v.
    2. Entrance Potential – 10v
- Gas Parameters:
- Curtain Gas: 20psi
- Ionspray Voltage: 5500ev
- Temperature of Curtain Plate: 400˚C
- Heater Gas (GS1): 50 psi
- Nebuliser Gas (GS2): 50 psi
- Column: Cosmosil C18 (150x4.6mm, 5µ)

3.4.14 GC-MS Analysis for Fatty acid analysis and other phytocomponents

GC-MS analysis was done for two extracts, one was for ethyl acetate extract for analysis of medicarpin and other fraction was the methanolic extract. The specification for GC-MS analysis for the two fractions is as follows:

GC-MS I:

The powdered roots of *T. cuneifolia* (190 g) were extracted with 100% ethyl acetate. After filtration, solvents were evaporated under vacuum (56˚C). Column chromatography of the crude extract was done by using Petroleum ether/Ethyl acetate as a solvent and ten fractions were collected. The crude ethyl acetate extract was adsorbed on silica gel and applied on the column which was packed with of silica. Column chromatography was done using the mixture of petroleum ether and ethyl acetate as solvent system. Ratios using 70:30 (Petroleum ether: ethyl acetate) and 60:40 (Petroleum ether: ethyl acetate) was taken.

GC-MS Analysis II:

500 gms of root powder was taken with methanol (3litres) which was kept overnight. It was then Filtered through a nylon cloth. The extract obtained was sticky after removal of the solvent by vacuum.

Specification for GCMS is as follows:

Shimadzu QP 2010 Ultra Gas Chromatography and Mass Spectrometry (With Quadruple detector). The Chromatographic separation was achieved using gradient programming on Rxi-5ms; 30m x 0.25mm ID x 0.25 µm dt., phase 5% diphenyl and 95% dimethyl polysiloxane is used as a stationary. The separated peaks were matched with the National Institute of standard and Technology (NIST) library.

3.4.15 HPLC analysis of Vitamins (Aslam *et al*., 2008)

Extraction solution was made by mixing 50 ml of acetonitrile with 10 ml of glacial acetic acid and the volume was finally made up to 1000 ml with double distilled water.

10 g of each sample was homogenized, weighed and transferred into conical flasks and 25 ml of extraction solution was added, kept on shaking water bath at 70˚C for 40 min. Thereafter, the sample was cooled down, filtered and finally the volume was made up to 50 ml with extraction solution.

Chromatographic conditions for HPLC:

Instrument name: Waters HPLC

Solvent system: A: 0.6% H_3PO_4 (pH-1.9)

B: Acetonitrile

Column: Sun fire, C-18, 250 x 4.6mm & 5µ particle size.

Mode of Elution: Gradient.

Detector wavelength: 254nm.

Flow rate: 1ml/min.

Detector: PDA (Photo Diode Array).

Total run time: 25 minutes.

Temperature: 30˚C

Time Program:

Sr. No	Time in min.	B conc.in %
1	0	2
2	7	2
3	15	15
4	18	15
5	19	2
6	24	2

Section 2: Preclinical and Toxicity studies

3.5.1 Methodology:

Adult female Charles foster rats weighing 200-225 g were chosen for the study. Rats were individually housed under standard environmental conditions (12 hr day: 12 day night). These animals were treated with *T. cuneifolia* root aqueous extract orally at doses of -125 mg/kg, 500 mg/kg, 1g/kg, 2.5g/kg, 5g /kg body weight for 30 days.

Parameters that were monitored are:

1. Serum Glutamate Pyruvate Transaminase (SGPT) [Marker for liver toxicity]
2. Serum Creatinine [Marker for renal toxicity]

Serum Glutamate Pyruvate Transaminase (SGPT)

Principle:-Enzyme Glutamate Pyruvate Transaminase (ALT) catalyses the exchange of the amino group of alanine for alpha-keto group of keto glutarate forming pyruvate & glutamate as end products. Dinitrophenylhydrazine was added to form hydrazones of pyruvate. These hydrazones react with sodium hydroxide to form a coloured compound that was read at 540 nm spectrophotometrically.

Reagents:

Buffered substrate (pH=7.4): Dissolve 15 g dipotassium hydrogen phosphate; 2 g of di hydrogen potassium phosphate; 300 mg of α ketoglutarate; 17.8 g of alanine in 800 ml of distil water. pH is adjusted and volume is adjusted to 1 l.

Color developer -0.02% w/v DinitroPhenyl hydrazine, 8.6% HCl, 1.6% w/v NaOH; Sodium Pyruvate (44 mg %) is used as standard.

Reagents	**Test**	**Blank**
Serum	50µl	50µl
Substrates	0.25ml	----
D/W	Incubate at 37° C For 1hr	0.25ml
DNPH	0.25 ml	0.25ml
	Incubate at RT For 20’	
0.4N NaOH	2.5 ml	2.5ml
O.D read at 540 nm spectrophotometrically		

Calculation: It was done according to slope obtained from standard graph.

Units: µmoles of puruvate formed /min/l.

Serum Creatinine (Jaffe’s method)

Principle: Creatinine present in serum or plasma directly reacts with alkaline picrate resulting in the formation of a red colour, the intensity of which is measured at 505nm/green filter.

Reagents:

1. Saturated picric acid (40 mM)
2. 0.75 M NaOH
3. Standard Creatinine is prepared in range of 15-50 µg.
4. Stock is 100 mg/dl

   Sample preparation: 1.5 ml of picric acid was added to 0.5 ml of serum and tubes were incubated and centrifuged at 3000 rpm/ 15 min.

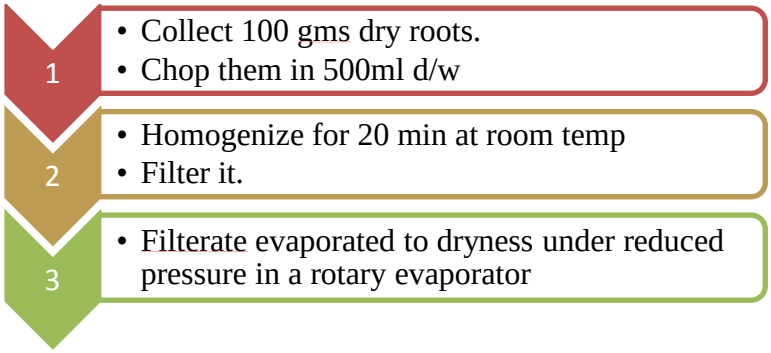
Reagents	**Test**	**Blank**
Supernantant of sample	1 ml	--
Distill water	1 ml	
Picric acid	1 ml	1 ml

0.75 M NaOH	1 ml	1 ml
Incubate for 20 minutes at RT and Absorbance recorded at 520 nm		

Calculation: It was done from slope obtained using standard.

Unit: mg/dl

Method For Preparing Plant Extract



Section 3: Wound healing

Test drug:

The well matured roots of *Taverniera cuneifolia* were collected. The fine powder (mesh120) of root of *Glycyrrhiza glabra* was purchased from the market and authenticated in the pharmacognosy Department, Jamnagar Ayurveda University. *T. cuneifolia* was authenticated by BSI, Jodhpur and Baro, Department of Botany, The Maharaja Sayajirao University of Baroda as per (Chapter 4).

Animals:

Wistar strain albino rats (200 ± 20g) and Swiss albino mice (30 ± 4g) of either sex were taken.

Source:

Animal house attached to Institute of Post Graduate Teaching & Research in Ayurveda, Gujarat Ayurveda University, Jamnagar.

Housing:

All the selected animals were kept under acclimatization for 7 days before dosing. Six animals were housed in each cage made of poly-propylene with stainless steel top grill. The dry wheat (post hulled) waste was used as bedding material and was changed every morning.

Environment:

The animals were exposed to 12 hour light and 12 hour dark cycle with the relative humidity of 50 to 70% and the ambient temperature during the period of experimentation was 22 ± 03°C.

Approval:

The experimental protocol was submitted to the animal ethics committee of the institute and approval was obtained for conducting the experimental study (Approval Number: IAEC/10/12/19).

Diet:

Amrut brand rat pellet feed supplied by Pranav Agro Ltd. was provided throughout the study period. The drinking water was given ad libitum in polypropylene bottles with stainless steel sipper tube.

Experimental Models

Effect on excision wound:

Prior to the operative procedure all the instruments (scissor, forceps, etc.) were autoclaved.

The area to be excised (on the back portion of the rat –suprascapular region) was shaved carefully by scissor prior to the procedure without causing any abrasions. The rats were anaesthetized with diethyl ether and they were inflicted with excision wounds as described by (Morton and Malone, 1972). The dorsal fur of the animals was shaved and the area of the wound to be created was outlined on the back of the animals with marker using a circular coin. A full thickness of the excision wound of circular area 220 -300mm^2 and 2mm depth was created along the markings with a surgical blade. The animals were randomly divided in to three groups of six each and were kept in separate cages. First group served as control. Second and third groups were treated with test drugs *G. glabra* and *T. cuneifolia* respectively.

The control group was treated with simple ointment base B.P. The standard group was treated with *G.glabra* (2% w/w). The test groups were treated with ointments with concentrations of *T. cuneifolia* viz. 2% (w/w) incorporated in simple ointment base. The vehicle and test drugs were applied to respective groups daily once covering the entire wound area after cleaning the wound with normal saline. The measurement of the wound areas was taken before applying the test drug on first day and at an interval of 3 days on a tracing paper. The area of the wound area was calculated in terms of square mm area with the help of graph paper. Duration required for complete healing was also noted down.

Section 4: Antitussive activity

3.6.1 Methodology

The selected animals were divided into three groups of six animals each. Group I received tap water and served as control. The test drugs *G. glabra* and *T. cuneifolia* were administered to the groups II and III. Dose of the test formulations for the animals was calculated by extrapolating the human dose (2g/day) to animals (400mg/kg) based on the body surface area ratio by referring to the standard table of Paget and Barnes (Paget and Barnes, 1964). The test formulation of both positive control *G. glabra* and test sample *T. cuneifolia* was suspended in distilled water to the recommended concentration and administered orally at a volume of 0.1 ml/10g body weight with the help of gastric catheter of suitable size sleevedon to a syringe nozzle. The test drugs were administered one hour before the SO_2 exposure. The anti-tussive effect of the test formulations was evaluated in mice against sulphur dioxide induced cough by following the procedure of Miyagoshi *et al. (*1986). In brief, the assembly comprises of a 500ml three-necked flask containing aqueous saturated sodium hydrogen sulphite (Na HSO_3; Nice Chemicals Pvt. Ltd.) solution was taken. To the bottle, concentrated Sulphuric acid (Merck, India) was introduced drop by drop; the reaction involved is as follows:

$2NaHSO_3 + H_2SO_4 = 2SO_2 + Na_2SO_4 + H_2O$

SO_2 was filled previously in the column of water manometer by opening the three-way cork such that the SO_2 can enter the water manometer but without any exit way until the pressure generated reads 75 mm of water as recorded by the water manometer. Then the three-way cork is rotated in such a way that the volume of SO_2 collected in the water manometer escapes into the desiccators and not into the flask containing sodium hydrogen sulphite solution. These procedures are operated in a drift. The mouse to be tested was placed in

1ldesiccators and covered with the lid. Certain amount of SO_2 is introduced to the desiccators by this procedure. The mice, after exposure to SO_2 for one minute in the desiccators, were taken out of the desiccators and confined in an up-turned filter funnel. The free end of the funnel is attached to a stethoscope, by the help of which the cough reflex of the mice was heard and the number of cough episodes in 5 minutes was enumerated. To avoid the observer bias, cough episodes were independently counted by two observers using digital counters and stopwatches.

Statistical Analysis

Results were presented as Mean ± SEM. Significance between different groups were statistically determined by paired and unpaired student's 't' test as well as One Way ANOVA with the level of significance set at $P<0.05$. The level of significance was recorded and interpreted accordingly.

Chapter 4: Results and Discussion

4.1.1 Distribution and Ecology

The plants have been collected in the flowering and the fruiting stages and the plants were reconfirmed in the laboratory, Department of Botany, Vadodara, with the Flora of West Pakistan (Ali, 1977), Flora of Presidency of Bombay (Cooke, 1908) and Flora of Gujarat State (Shah, 1978). During the field studies habit, habitat, height of plant, colour of flower, associations and other prominent features were also recorded. The herbarium sheets were preserved and later checked up with authenticated herbarium sheets of Botanical Survey of India, Jodhpur. The plant species was compared with following specimens: BSI, Arid Zone Circle, Flora of Bhilwara (Collector, A. N. Singh) Acc. no.-2320, Sardarsamand, Flora of Jodhpur (Collector, A. N. Singh) Acc. no.-5692, Sardar samand Farm, along canal, Flora of Pali, Rajasthan (Collector, B.V. Shetty) Acc. no. 4069, Blatter Herbaria 23241, BARO Herbarium, The Maharaja Sayajirao University of Baroda: Acc. no.-JVJ 1576 (Locality Udhana), Acc. No. JVJ 1456 (Locality Udhana - Bhestan along the rail line), Acc. No. - JVJ 1389 (Locality Udhana), Acc. No. 1658 (Locality Udhana).

Macroscopic characters

The macromorphology of *Taverniera cuneifolia:*

Bushy undershrub, 60-100 cm, length of tap root ranges from 30-60 cm while the girth varies between 2-4cm. Stem is greenish brown, smooth and shiny, with an average thickness of 0.5-0.8 cm. Leaf uni-trifoliolate, leaflets 0.6-2.5 cm long, obovate to oblanceolate, entire, mucronate, sparsely pubescent, becoming subglabrous, margin brownish; stipules connate, amplexicaul, 3 mm long. Flowers Purple-pink, Fruits Ovoid, fruit cover rugose and subreticulately echinate, size 0.4×0.6 mm, Pods with 1-2 seeded joints. Seed kidney shaped, size 0.25×0.4 mm, yellowish brown in colour.

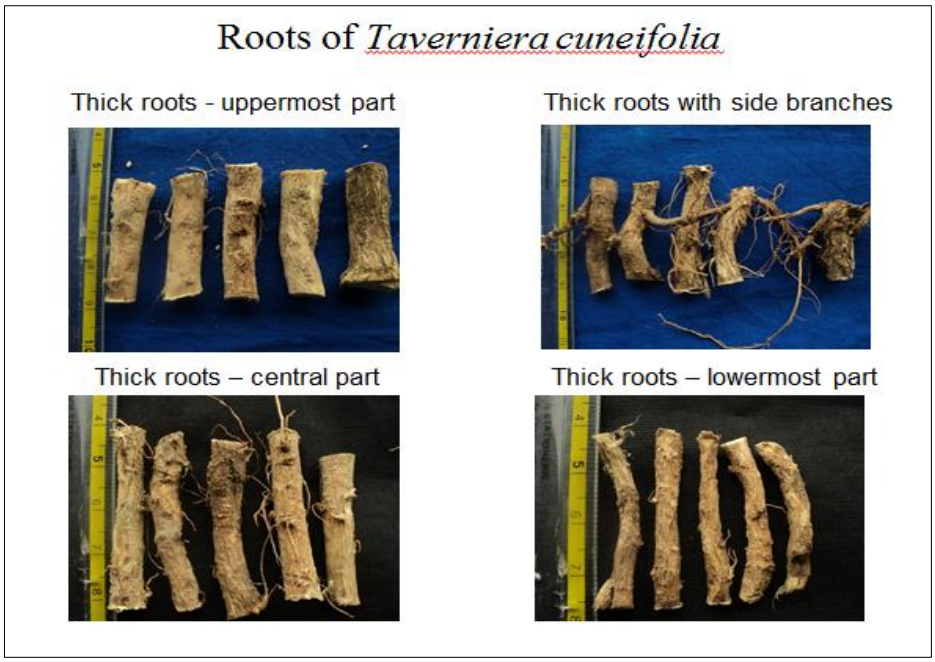


Figure A4.1: Thick roots

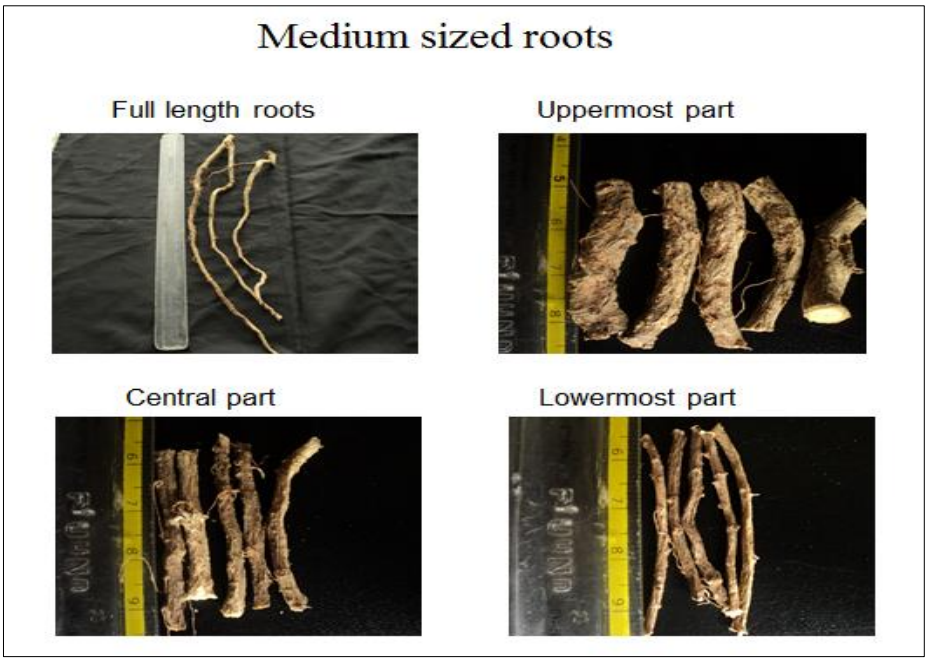


Figure A4.2: Medium Sized Roots

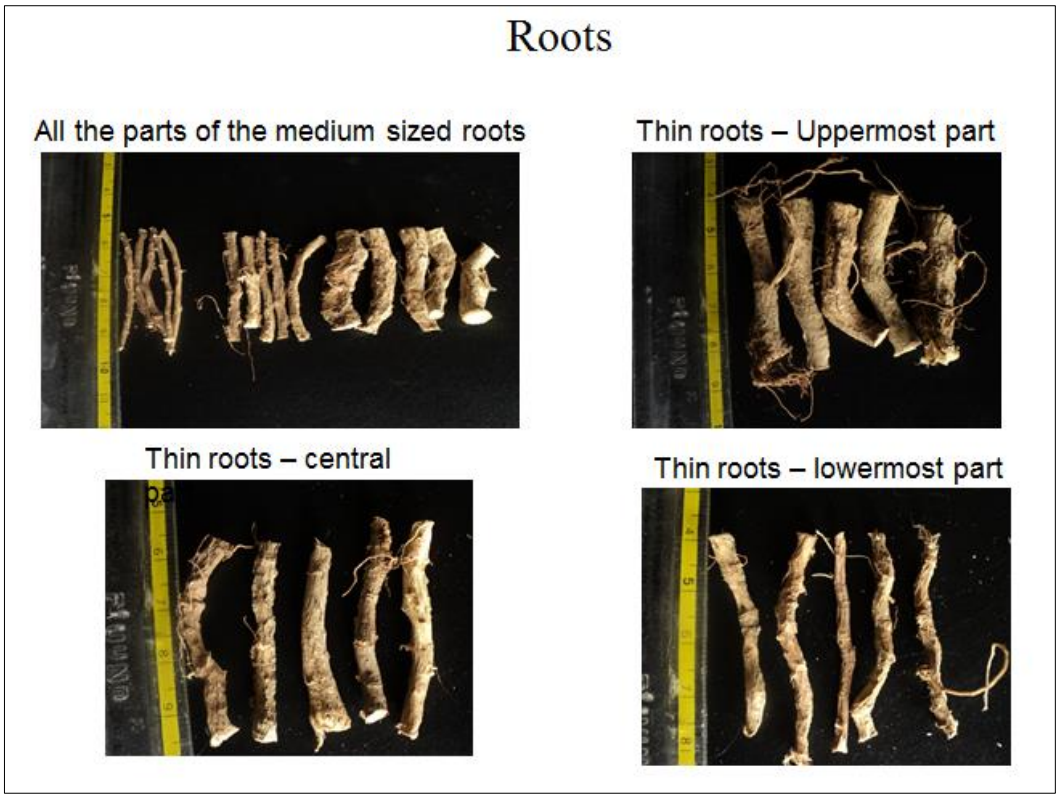


Figure A4.3: (C) Thin roots

Table 4.1: Measurement of the roots in cms.

Measurement of the roots in cms.		
Thick roots (A)	Medium roots (B)	Thin roots (C)
3.3	2	1.4
3.8	2	1.4
2.5	2.5	1.4
2.9	2.5	1.6
3.1	2.4	1.4
2.8	2	1.6
2.6	2	1.5
2.7	2.4	1.7
3.5	1.8	1.4
3	2	1.4
Range-2.5-3.8	Range-1.8-2.5	Range-1.4-1.7

Measurement of roots

Measurement of Thick roots in cms		
Uppermost	Central	lowermost
3.9	2.7	1.8
4.4	1.8	1.2
3.3	2.5	1.5
3.1	2.2	1.3
2.7	2.2	1.5

Measurement of medium roots in cms		
Uppermost	Central	lowermost
2.8	1.7	1.3
2.2	1.7	1.3
2.5	1.8	1.3
2.5	1.8	1.1
2.5	1.8	1.0

Measurement of Thin roots in cms		
Uppermost	Central	lowermost
1.8	1.1	0.8
1.4	0.9	0.8
1.6	0.9	0.7
1.6	1.0	0.7
1.4	1.2	0.6

Table 4.2: Measurement of all sized roots

4.1.2: Important characters for identification are as follows:

Description: Height: 60-100 cm

Plant Habit: shrub

Plant density: open

Growth rate: slow

Leaf arrangement: Alternate

Leaf type: compound

Trunk/bark/branches: smooth

Leaf margin: entire

Stem/twig color: greenish brown

Leaf shape: obovate

Stem/twig thickness: 0.8 cm

Leaf venation: reticulate

Leaf type and persistence: Deciduous

Leaf blade length: 0.6-2.5 cm long

Leaf color: glaucus

Fall color: green to yellow

Flower: Purple-pink

Fruit: Fruit shape: Ovoid

Fruit size: 0.4×0.6 mm

Fruit cover: Outer surface is rugose and subreticulately echinate

Fruit color: Purple pink

Fruit characteristic: Pods with 1-2 seeded joints.

Seed- Seed size: 0.25×0.4 mm

Seed shape: kidney shape

Seed weight: 0.16 µg

Roots: Tap root

Length: 17.2 cm

Width: 2.3 cm

Ecological details

Light requirement: plant grows in part shade/part sun; plant grows in the open areas

Soil tolerances: slightly alkaline; acidic; clay; sand; loam

Drought tolerance: high

Soil salt tolerances: moderate

Plant spacing: 12 to 18 inches

Winter interest: Plant has winter interest due to unusual form, nice persistent fruits,

Invasive potential: not known to be invasive

Pest resistance: no serious pests are normally seen on the plant (Usually rats are seen eating the roots of the plants)

The difference between the description of Ali (1977) and Cooke's (1908) flora is with regards to the leaves, wherein Ali has mentioned that the leaves are uni-trifoliolate while Cooke has stated it to be uni-foliolate. Usually uni-foliolate condition is common in most of the plants however, trifoliolate condition is occasionally seen (Figure A4.7) usually in lower nodes.

भारतीय वनस्पति सर्वेक्षण
शुष्क क्षेत्र परिमंड़ल
खेमे के कुआं के पास
पाल-बासनी केनाल लिंक रोड़
पी.ओ.नंदनवन
जोधपुर 342008 (राजस्थान)

भारत सरकार
Government of India
पर्यावरण एवम् वन मंत्रालय
Ministry of Enviornment & Forest

Botanical Survey Of India
Arid Zone Circle
Near Khema ka Kuan
Pal - Basni Canal Link Road
P.O.Nandan Van
JODHPUR-342008 (Rajasthan)

संख्या : भा.व.स./शु.क्षे. परि./
No. BSI/AZC/ 1.12012/Tech./2011-12(Pl.Id.)-551

सत्यमेव जयते

दिनांक/ ***Date*** 23.12.11

CERTIFICATE

This is to certify that Plant specimen no. 1 collected by **Ms. Poonam S. Mangalorkar** Research Scholar, Dept. of Botany, M. S. University of Baroda (Gujarat) identified as ***Taverniera cuneifolia*** (Roth) Arn. belonging to Family : **Fabaceae**

23/12/2011

(Dr. P. M. Padhye)

Scientist "E" & H.O.O

FAX No. 91-291-2741736 • Telephone : 2740415, 2747163 • E-mail ID : bsiazc@yahoo.com

Figure A4.4: Authenticated Herbarium sheet

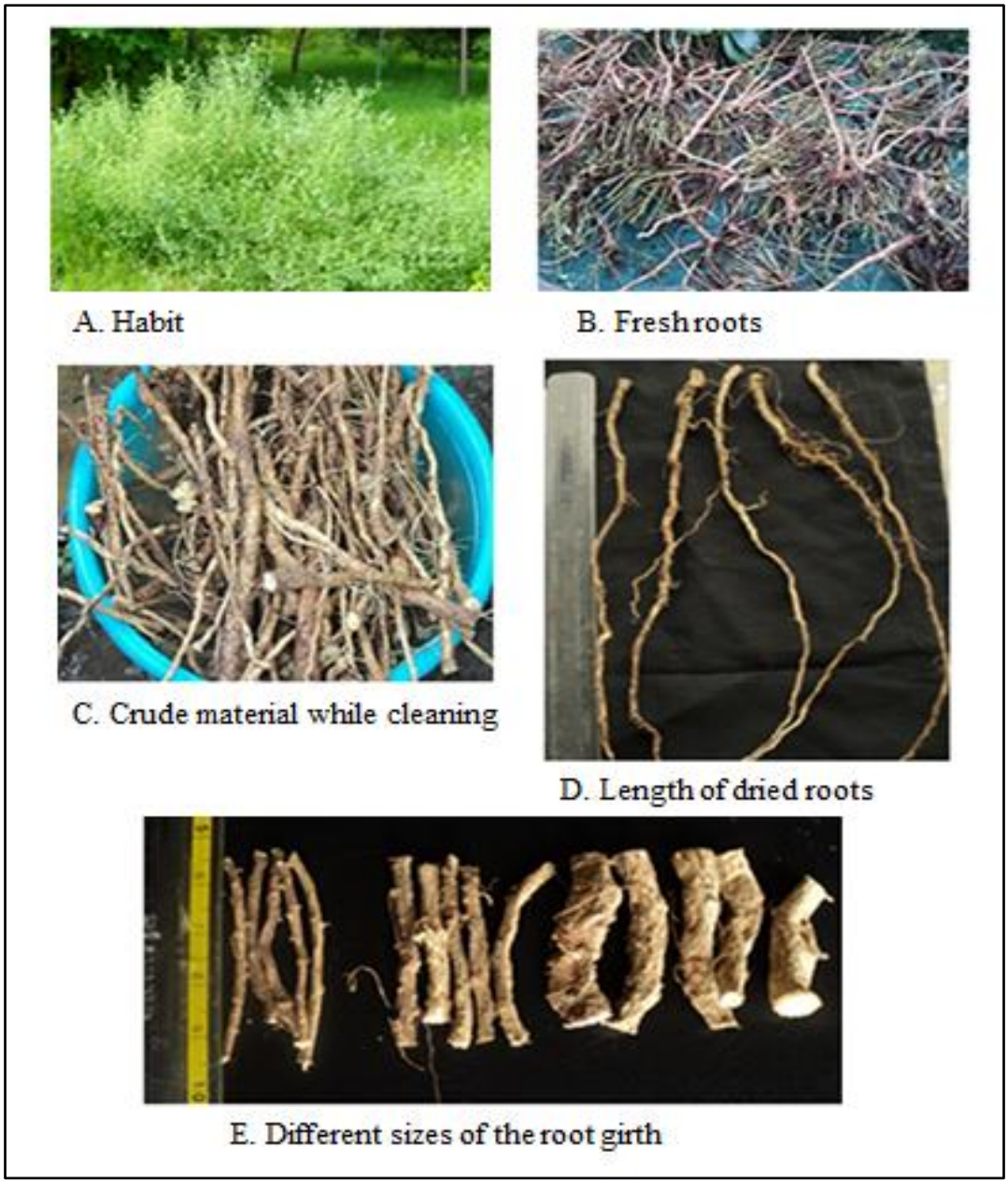


Figure A4.5: Habit and underground part of *T. cuneifolia*

Figure A4.6: *Taverniera abyssinica*; A) and B) roots; C) coarse root powder.

Figure A4.7: Different aerial parts of *T. cuneifolia*

Table 4.3: Ecological parameters of the indigenous site Arboretum (Vadodara), Kutch (Bhuj), Rajkot Munjka Region, Rajkot (IMPLANT Garden Campus).

Site	Arboretum (Vadodara)		Kutch (Bhuj)		Rajkot (Munjka Region)		Rajkot (IMPLANT Garden Campus)	
Parameter	Concentration	Quality	Conccntration	Quality	Concentrat ion	Quality	Concentr ation	Quality
Total nitrogen/Organi c carbon (%)	1.32	Higher	0.32	Less	1.16	High	0.76	High
Available Phosphorous/A cre	6.00	Less	6.00	Less	6.00	Less	5.00	Less
Total Potash/Acre	215	Higher	170	Higher	90.00	Medium	60.00	Less
pH	8.30	Alkaline	7.79	Normal	7.90	Normal	8.70	Alkaline
EC	0.31	Normal	2.21	Highest	0.59	Normal	0.91	Normal
Sulphur (ppm)	18.00	Medium	75.40	High	17.10	Medium	15.40	Medium
Micro nutrient								
Zn	3.52	High	0.24	Less	0.54	Medium	0.50	Medium
Fe	11.54	High	7.26	Medium	11.92	High	8.24	Medium
Mn	25.16	High	21.72	High	30.64	High	25.54	High
Cu	1.84	High	1.40	High	2.22	High	1.90	High

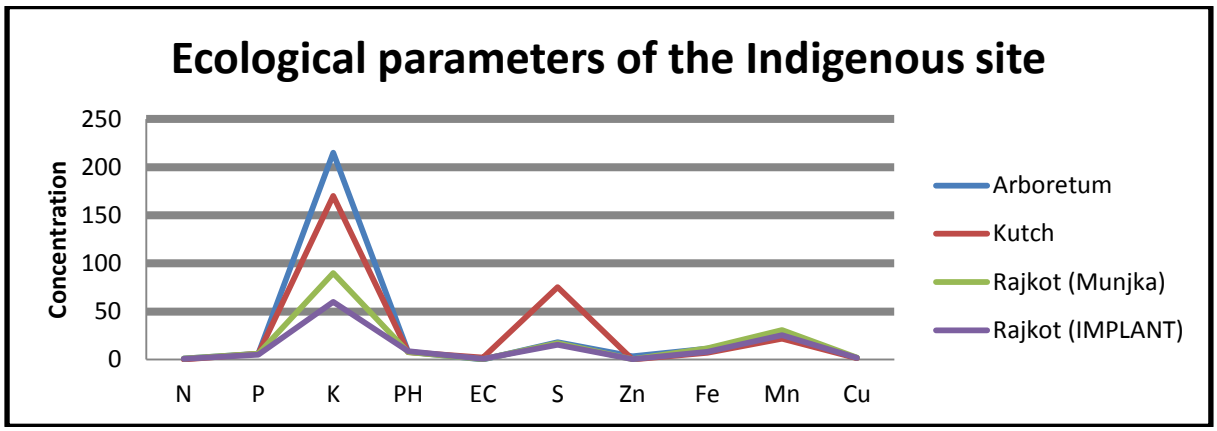


Figure A4.8: Ecological parameters of the Indigenous site

Ecology

Taverniera cuneifolia is pollinated primarily by insects (entomophilous) and the flowers are usually showy to attract pollinators. Particularly the honey bee, butterflies, which extracts the nectar produced by the flowers. Insects may also be responsible for pollination. Seeds are produced in abundance and this serves as a survival mechanism. They are small, brown to yellow to 2mm in diameter. The success of germination is relatively low giving rise to the low abundance of the species in wild. The leaves are acclimatising to saline and terrestrial conditions. The leaves are somewhat fleshy (swollen) on the coastal tract while membranous in terrestrial regions. Another interesting adaptation shading of leaves during the winter and hot summer months, however the photosynthesis continues with the green stem. Probably this is the cause during the offseason even the green twigs can be observed and the storage of sugars continues.

The associated species occurring with *T. cuneifolia* in grassland/fallow lands are *Aristida sp., Helandia latebrosa, Zizyphus nummularia, Alyscicarpus vaginalis, Bothriochloa pertusa, Indigofera cordifolia*, Pulicaria wightiana,

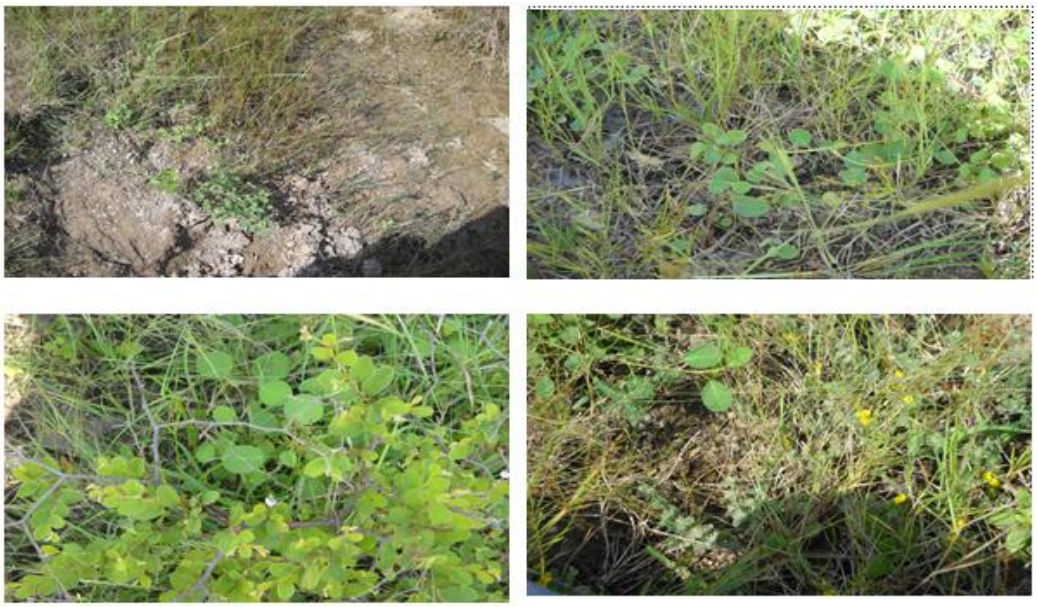


Figure A4.9: Associated plants growing with *T. cuneifolia* in Rajkot (IMPLANT garden)

Climatic parameters:
Based on the available meteorological data from 2002-12 the climatic data are as follows:

Rainfall:
The mean annual rainfall recorded is 697 mm and the mean number annual rainy days is 21 days in last 10 years (2002-2012), with monthly maximum average rainfall in month of July and August.

Temperature:
On an average temperature is 30-35°C in Saurashtra, with temperature going as high as 43-44°C. Temperature along coastal track is between 30-35°C.

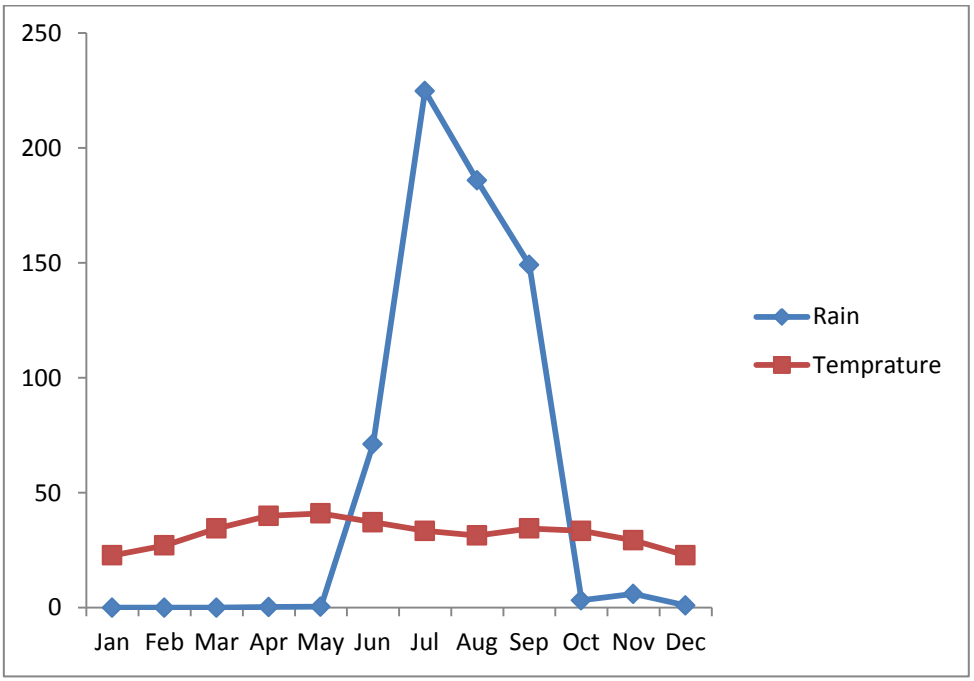


Figure A4.10: Average rainfall and temperature at Rajkot district

Humidity:
Humidity is maximum along coastal track of Jamnagar, Porbandar, Junagadh and Bhavnagar. Humidity goes to a maximum of 80-85% during monsoon with minimum humdity during the months May-July. However, along the coastal track which is most suitable track along which T.cuneifolia grows has an average of 40-45% humidity.

Wind speed:
Wind fall along coastal track is 20 km/hr. However, in the terrestrial zones of Rajkot, Girnar the wind fall is 14 km/hr.

Soil Physical properties:

The plant has been observed growing in following type of soils:

1) **Black Soil** ➡ 

2) **Moderate Black Soil** ➡ 

3) **Gravelly Soil** ➡ 

4) **Sandy Soil** ➡ 

5) **Salty Soil** ➡ 

Figure 4.11: Soil type of Rajkot

Soil type:

Soil in this region is usually sandy silty loam. Black and moderately black soil was recorded in Rajkot, Jamnagar, Porbandar and Surat. While gravelly soil was recorded in Rajkot, Bhavnagar, Jamnagar. Sandy soil was seen Dwarka, Porbandar and on Junagadh beach. Salty soil was seen in Roji (Jamnagar), Munjaka (Rajkot), Narsangtekri (Porbandar).

Soil Chemical characters:

Soil pH - The pH value in all the different ranges at different habitats ranges from 7.79-8.70. The pH value in Rajkot (IMPLANT garden) and Vadodara is slightly alkaline (8.9), both these regions are the zones wherein regular cultivation is a practice and the Total dissolved

solids (TDS) in water is very high. In grassland land coastal tracks the pH was comparatively normal. However, the plant has shown adaptation to various pH conditions.

Electrical Conductivity - The EC value is normal in Arboretum (0.31) and Highest in Bhuj (0.59). The high EC is indicative that the soil of Bhuj is rich in minearls while in other sites it was normal.

Total nitrogen/Organic carbon (%) -There is variations in the percentage of organic carbon content at different regions. The organic carbon is higher in Arboretum as the site is undisturbed by any external means. The range of variation is between 0.32-1.32 percent. The nitrogen content is high around the *T.cuneifolia* as it is a legume and the root nodules are prominent in mature roots.

Available Phosphorous - The pattern of variations of available phosphorous in all regions is poor and does not show any fluctuation. Probably as these plants are more common in grassland the available phosphorus is poor. Even these sites are prone to over-grazing and trampling.

Total potash - The total potash in comparatively less in Rajkot region as compare to higher content in Bhuj and Vadodara. As such the soil of Saurashtra is rich in Potash and additional Potash is not required in the soil.

Macro nutrients:

Sulphur (ppm) - The sulphur content is quite high in Kutch (75.40) as compare to the other zones. It is necessary to understand that the high content of sulphur in the soil affects the phytoconstituents of the root or not.

Micronutrients -

Zinc - Zinc was seen in a moderately high amount in Arboretum (3.52) and least in Bhuj area (0.24). Probably the high concentration of Zinc is affecting the growth of the saplings in initial stages as the plants were not able to establish under cultivation.

Iron - The content of iron was slightly high in Arboretum (11.54) and Munjka region, Rajkot (11.59) but was found medium in the other two regions.

Manganese - Manganese content had no much fluctuation and all the regions showed very high content. The highest amount was found in Rajkot Munjka (30.64).

Copper - The Copper content was found to be high in all regions but Rajkot Munjka region was highest (2.22) amongst all other regions.

Gopnath coast and victor Albert Port - The soil is sandy clayey to loamy. pH values exhibit mild to moderate alkalinity. Organic contents range between 2.14-3.57% which is considerably high. Values of total dissolved solids and sodium chloride indicate that at Gopnath coast, the soil is not under the direct influence of sea water. The calcium carbonate content of the soils of this habitat is high (23.72-36.95%) (Rao *et al*., 1967) .

Recommended Areas for Cultivation In Gujarat

- Kachchh (Abdasa, Bhachau, Bhuj, Lakhpat, Mandvi, Nakhatrana, Mundra, Rapar)
- Saurashtra (Jamnagar, Rajkot, Bhavnagar, Amreli, Surendranagar, Junagadh)
- South Gujarat (Surat)

Figure A4.12: A: Water body, B and C: Habitat of *Taverniera;* D: *T. cuneifolia* in Rajkot

Figure A4.13: **E. and F**. *T. cuneifolia* in its natural habitat (IMPLANT, Garden, Rajkot); **G. and H.** Collection site of *T. cuneifolia* from Rosy Beyt area, Jamnagar

Figure A4.14: I. *T. cuneifolia* in its natural habitat from Tapkeshwari, Bhuj; **J.** A single plant of *T. cuneifolia* from Tapkeshwari, Bhuj; **K**. Presence of water body where *T. cuneifolia* was found.

4.2 PHARMACOGNOSY

The observations pertaining to the roots, stems and leaves of *T. cuneifolia* were recorded and described as follows:

4.2.1 Root

In transverse view, the root was circular in outline, with the xylem forming the entire section. The outermost layers were quite disrupted and were composed of 5-7 layers of barrel shaped suberised cork cells. Underneath the suberised cork cells, a reduced cortex composed of thin walled parenchyma cells was observed. These cortical parenchyma cells were heavily loaded with simple starch grains. Endodermis and pericycle was indistinct. Phloem was composed of sieve tubes and companion cells. In the phloem region, bast-fibres were arranged in the form of alternate bands with the sieve elements intermixed with axial phloem parenchyma. The secondary phloem was composed of sieve elements, companion cells, axial and ray parenchyma cells. The vascular cambium was present between the secondary xylem and phloem. The cambium was storied and composed of vertically elongated fusiform cambial cells while ray cambial cells were arranged as cuboidal clusters of more or less iso-diametric cells. Next to the vascular cambium, secondary xylem was present. It was composed of vessels, tracheids, axial and ray parenchyma cells. The secondary xylem rays were uni-multiseriate but biseriate seriate rays were more frequent and showed heavy accumulation of starch grains (Figure A4.15C).

In transverse view, xylem vessels were mostly solitary and oval to oblong while in longitudinal view they showed simple perforation plate and presence alternate bordered pits on the radial walls. Vessels elements were measured from 284.4µm - 581.2µm in length and 42.18µm – 95.3µm in width. Xylem rays were mostly heterocellular and measured about 366.9 – 884.3µm in height and 25.09– 100.3µm in width with the ray cells were oval to polygonal with varying dimensions that was measured from 43.9µm - 76.56µm in diameter. Prismatic crystals (17.25µm - 61.15µm × 15.68µm – 54.87µm) of calcium oxalate were found to be abundant in fibres (Figure A4.15E).

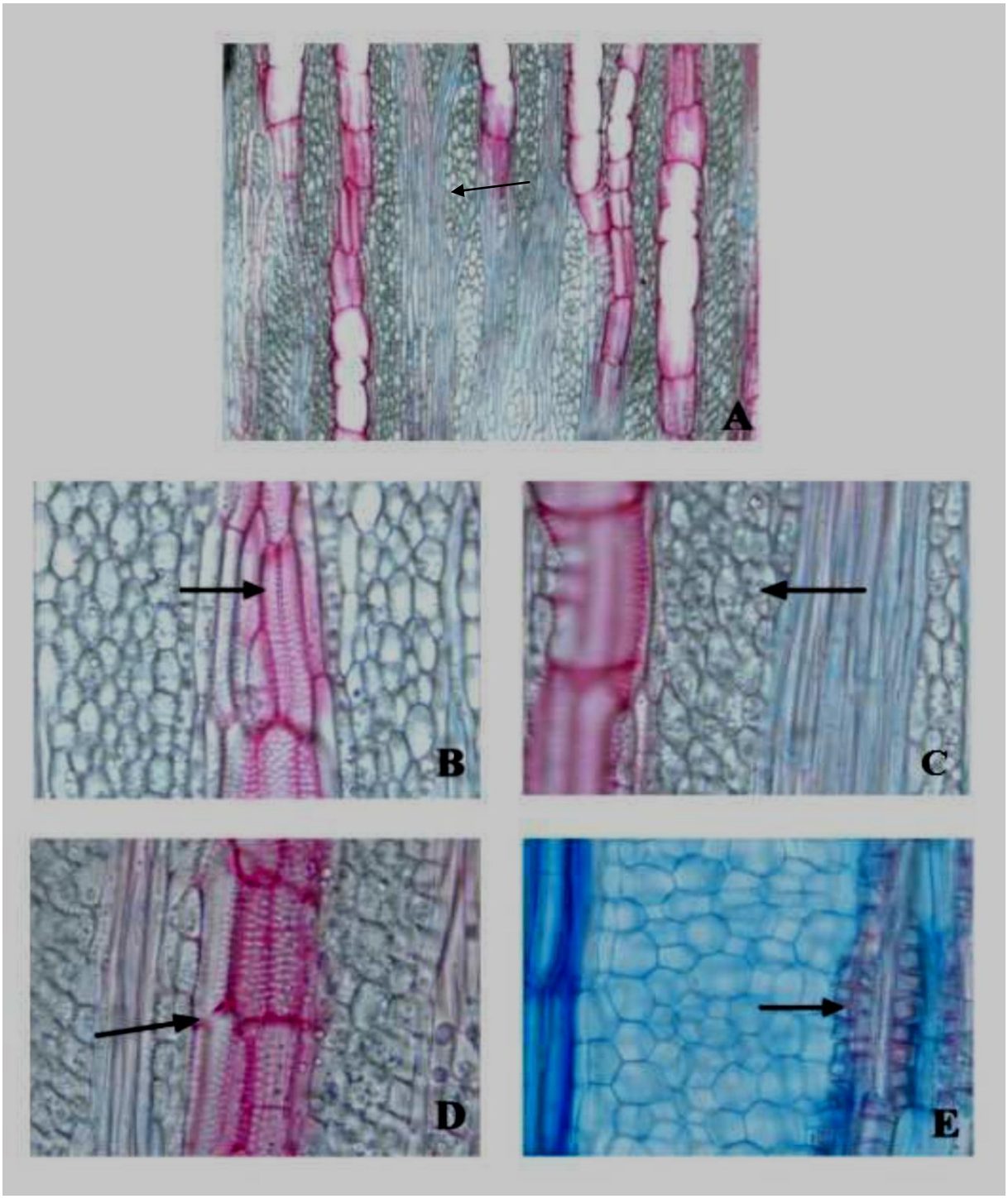


Figure A4.15: Longitudinal section of the root of *Taverniera cuneifolia*

A. Section of the root in 10x (200µm), Tracheids B. Narrow vessel elements. C. Starch grains in ray cells. D. Vessel elements and perforation plate in vessel elements E. Prismatic crystals. B-E 40x (200µm)

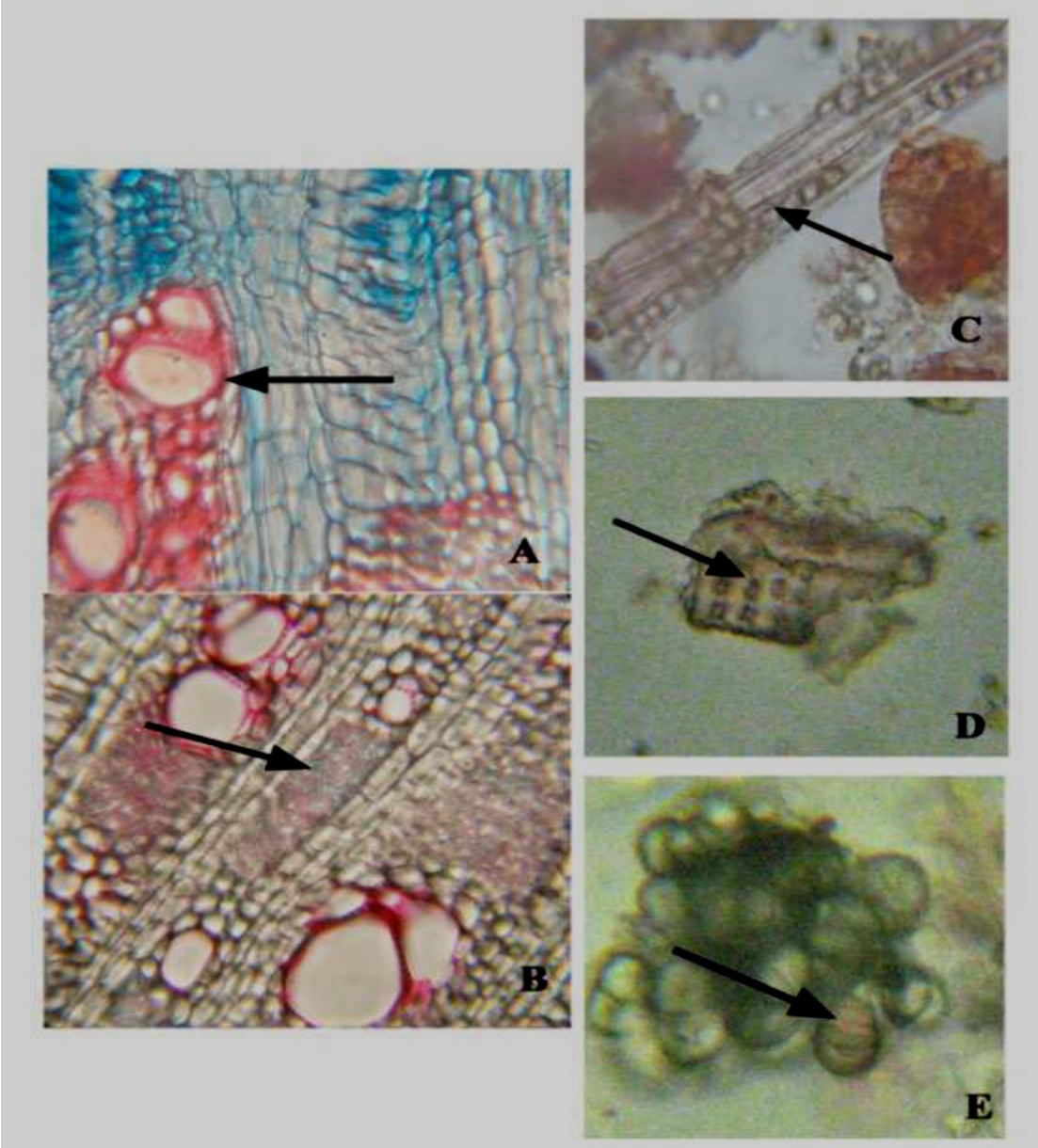


Figure A4.16: Transverse section and powder study of the root A. Xylem vessel. B. Fibres. C. Prismatic crystal. D. Pitted ray parenchyma. E. Starch grains (100µm)

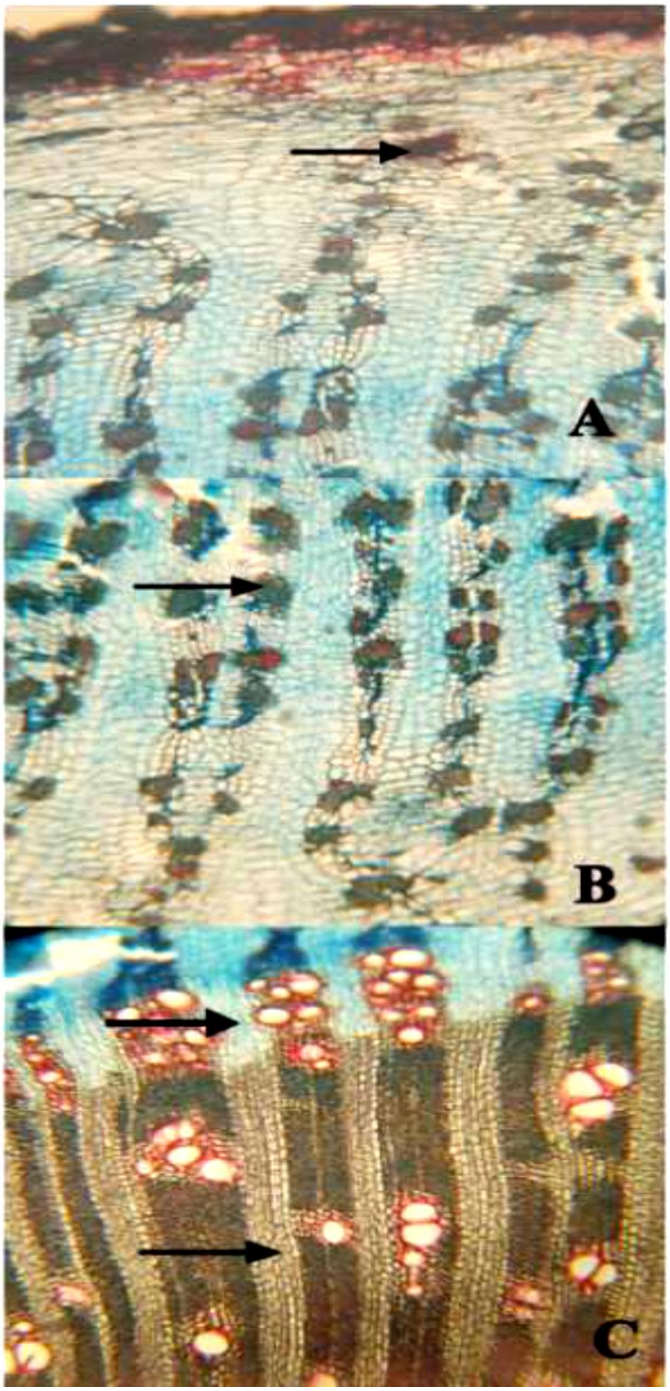


Figure A4.17: Transverse section of the root of *Taverniera cuneifolia*
A. Stone cells. B. Bastfibres. C. Xylem parenchyma and rays (100µm)

4.4.2 Stem

In transverse view, the stem was circular in outline and was covered with thick layer of cuticle. The layers below the epidermis consisted of colourless parenchyma cells, which were subsequently followed by 2-3 layers of oval chlorenchyma cells. Endodermis and pericycle was not distinct. However, pericycle fibres formed oval to spherical patches. Rays were uni to multiserriate. In the thick stems, epidermis was replaced by the by 10-15 cell layered radial files of cork cells. Subsequent to the cork was the secondary phloem and cambium. Secondary xylem was diffuse porous with indistinct growth rings and was composed of vessels, trachieds, xylem fibres and, axial and ray parenchyma cells. The medulla portion of the stem was occupied by large pith.

The longitudinal sections of the stem showed xylem vessel elements possessing simple pits. Xylem Tracheid elements were 148.9µm -649.1 µm long and 32.92 µm – 86.23 µm broad. Xylem rays were 84.66µm – 166.2µm long ×14.11 µm –78.39µm broad (40x 200µm) with ray cells having varying dimensions 210.1µm -788.6µm ×28.22 µm-65.85µm. (10x 200µm).

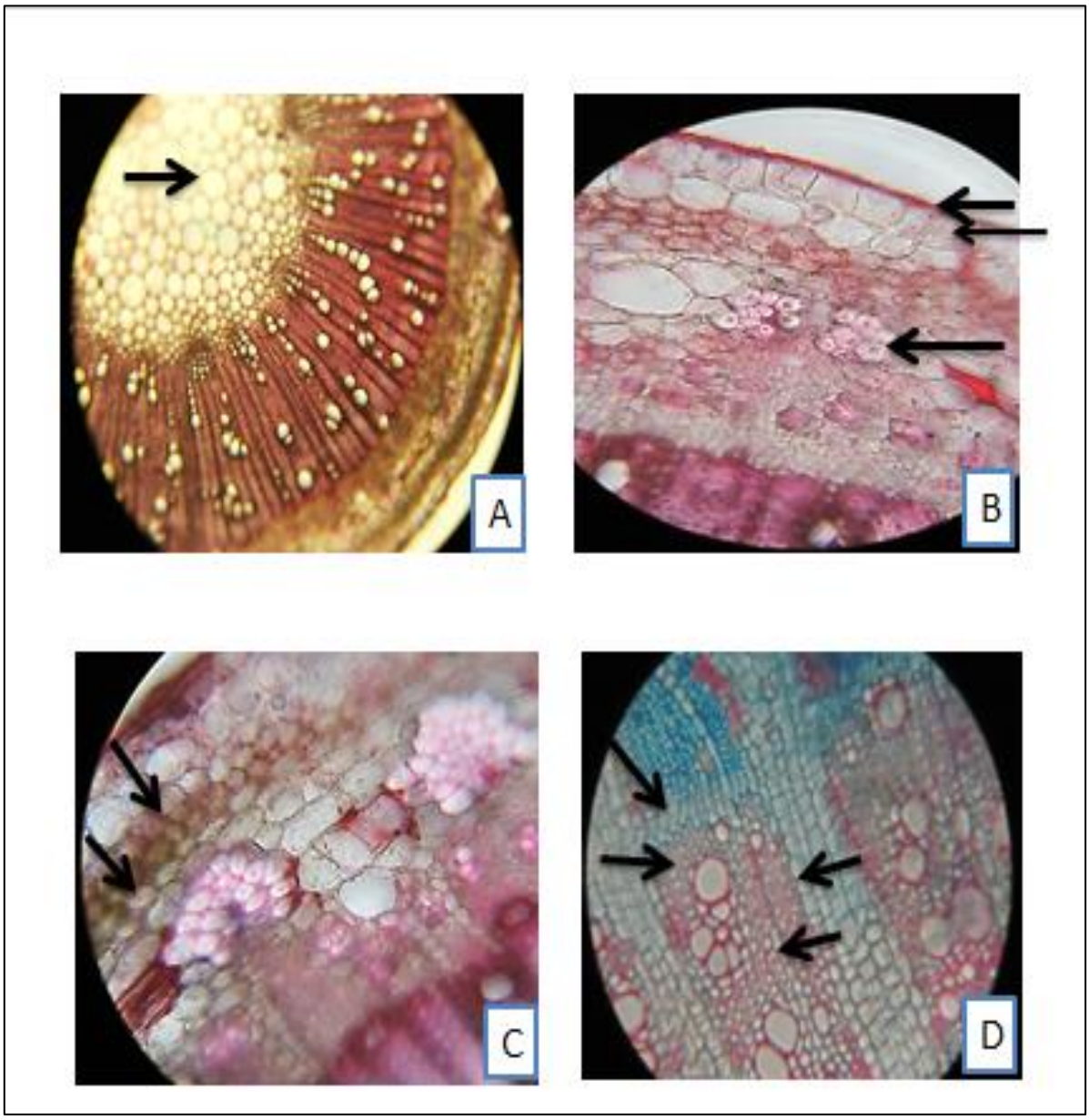


Figure A4.18: A. Circular nature of the transverse section of stem and large pith B. Cuticle, Epidermis and sclereids C. Simple parenchyma cells and Chlorenchyma D. Phloem, Cambium, Medullary rays and xylem portion.

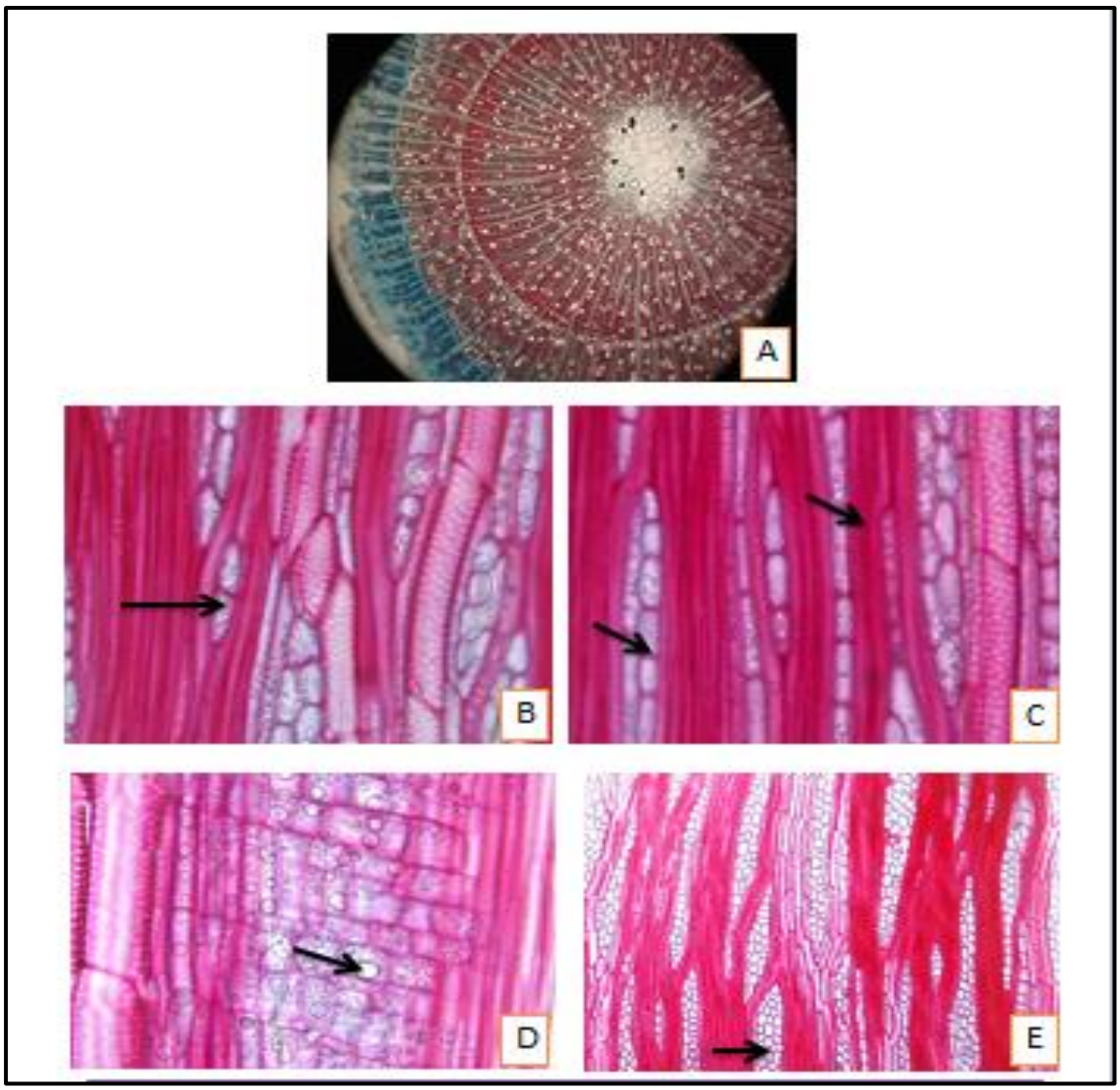


Figure A4.19: A. T.S. of stem (4x) (secondary growth) B. Uniseriate ray cells

C. Biserriate ray cells and Tracheids D. Starch grains in ray cells

E. Multierriately ray cells

4.4.3 Transverse section of leaf:

The transverse view, the leaf was dorsiventral and showed distinct palisade and spongy parenchyma. At the midrib region, lamina showed a slight concave cavity on the upper side while on lower surface, the mid vein bulge out as a hemispherical structure. There was a crescent shaped vascular bundle in the centre of the midrib, with the xylem towards the upper side and phloem on the lower side. The cells of the ground tissue on the upper side of the vascular bundle were smaller and spherical while those on the lower side were composed of little larger isodiametric parenchyma cells. The laminar portion had 2-3 layered palisade tissues which is covered almost half of the lamina and spongy tissue which were small and circular in shape. The palisade tissue had many long oil bodies in the form of a bag or sac like structure known as oleosomes. They were 570.7µm -1019.1µm in length and 29.79µm - 117.6 µm broad with few to many oil globules within it.

4.5 Micromorphology

Leaf constant such as stomatal index/mm^2 of the abaxial epidermis, stomatal index/mm^2 of the adaxial epidermis, trichome index/mm^2, vein islet number/mm^2, vein termination number/ mm^2, the palisade ratio were calculated and data is represented in Table 4.4. The data obtained are given in the Table 4.4.

Table 4.4: Calculated values of leaf constants*

Leaf constant	
Stomatal index (abaxial)	22.60±1.11
Stomatal index (adaxial)	24.57±2.18
Trichome index	4.91±0.48
Vein islet number	1
Vein termination number	3-4
Palisade ratio	2.55±0.68

*Each value is a mean of 20 readings except vein islet number and vein termination number

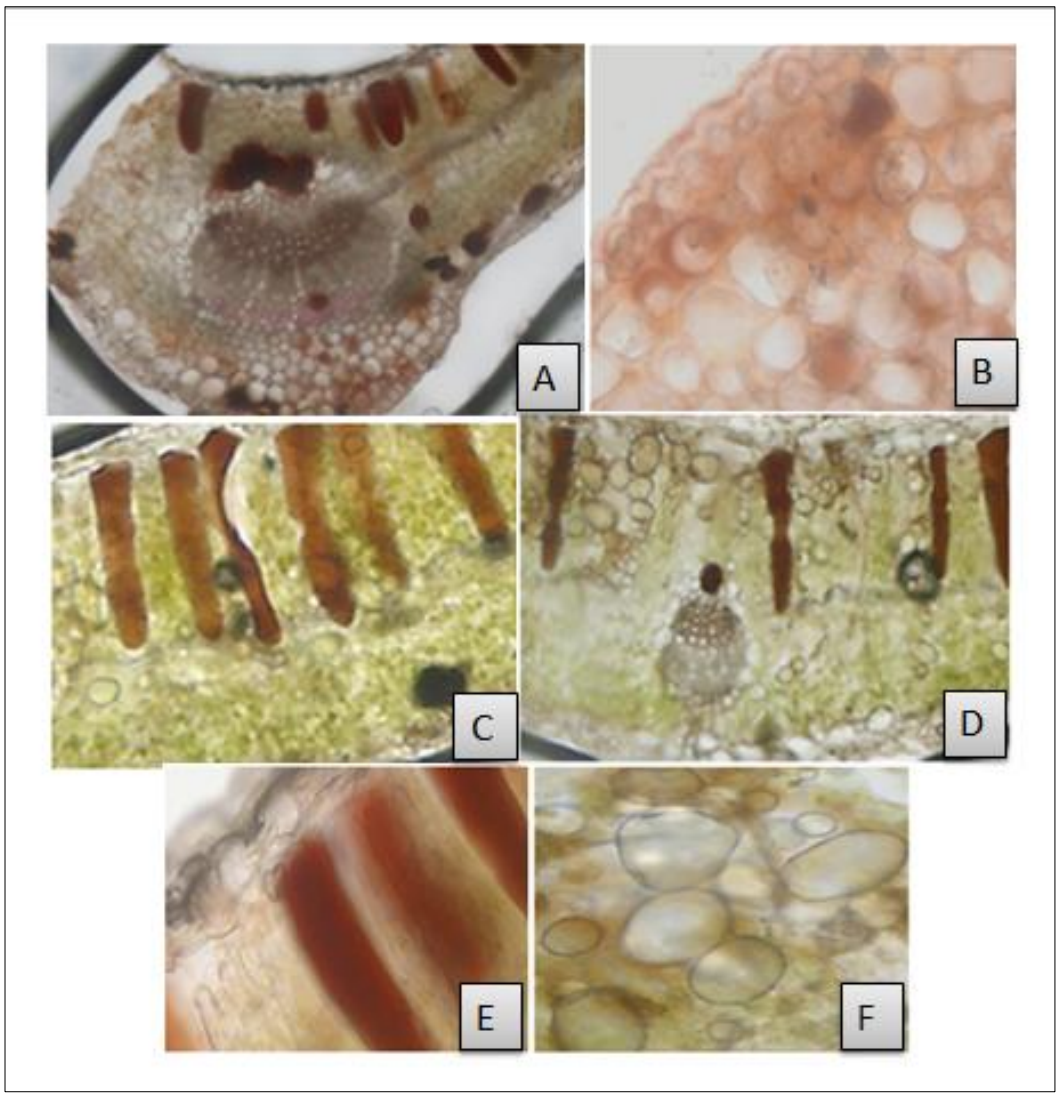


Figure A4.20: Transverse section of Leaf

A. Midrib showing Tracheid from the terminal end of xylem vessels (10x)
B. Cuticle in leaf (40x)
C. Palisade tissue showing oil bags (10x)
D. Midrib portion showing oil bags and oil globules (10x)
E. Stomata in epidermis (40x)
F. Storage material/Oil globules (40x200µ)

4.6 DISCUSSION

The present study brings out a number of distinguishing features of the roots of *Taverniera cuneifolia*. Pericyclic fibres, bast-fibre, prismatic crystals, starch grains and vasicentric tracheids (Figure A4.15, A4.16) which form the key characters for identification. The multiseriately arranged rays and the pitting in xylem elements are noteworthy. All these characters could be used as diagnostic biomarkers for *T. cuneifolia*, in both fresh materials as well as in powder and also for checking adulteration of the same.

The anatomical features of the root are first of its kind ever studied. A detailed study of longitudinal sections of the root has been carried out in the present study. Lignification of Xylem rays was observed in *G. glabra* and was not a feature of *T. cuneifolia* however, Saluja (l.c.) has stated lignification of xylem rays at a number of places and appearance of a side, radially elongated sap in a xylem medullary ray seen in *T. cuneifolia* and are totally absent in *G. glabra*. Further Sulaja (l.c.) stated that the starch grains of *T. cuneifolia*, measured from 4-10µ while in *G. glabra* varied from 2-10µ. However, the size of starch grains varies with the maturity of roots. Notani (2012) has shown presence of cork of roots, however, the cork is not a characteristic feature of roots. Though some time the roots exposed outside the soil may imitate like cork. Powder characters of the root showed aggregate of simple starch grains, bordered pitted and vessels, tannin in cork and cortex zone, oil globules, aleurone grains and parenchymatous cells.

The stem can be identified by its uni-multiserriate rays in longitudinal sections-presence of sclereids in transverse view. Xylem is endarch and pith is very large.

Transverse view of the leaf shows typical oil bodies/oleosomes in the palisade tissue region which is characteristic of the plant. The micromorphological features such as bundles of oil globules, amongst other features for the judgment of identity, purity and general quality of the plant. It can be reasonably speculated that oil bodies of mesophyll cells in most species probably act as intermediate storage products of photosynthesis (Chonan *et al.*, 1984). Another possible function for oil bodies is adaptation to cold temperatures. According to Pihakaski et al. (1987) in *T. cuneifolia* it could be the adaptation of the plant to the xeric condition. Oil globules are also recorded in the family of Fabaceae in *Baptisia Alba and B.tinctoria* (Lersten *et al.*, 2006). Leaf anatomy is quite responsive to climatic conditions, for example *Eucalyptus camaldulensis* plants from more arid locations have thick leaves and high oil gland density (James & Bell, 1995).

Glycyrrhiza glabra a well-known licorice could be differentiated from *Taverniera cuneifolia* on the basis of presence of prismatic crystals in stolon cortex while *T. cuneifolia* shows presence of starch granules. Prismatic crystals of calcium oxalate were found to be abundant in the fibres of *T. cuneifolia.* Pharmacognostical studies of *T. cuneifolia* and *G. glabra* reveals some similar characteristics like bordered pits, prismatic crystals, starch grains, and fibers. *G. glabra* has collapsed sieve tube elements called as ceratenchyma (Joshi and Aeri, 2009) which was found to be absent in *T. cuneifolia*.

4.7 SEED GERMINATION:

4.8 Good Agriculture practices:

- The most important issue is irrigation: Particularly during the **first months** ***Taverniera cuneifolia*** **needs moderate water** and however, the soil should never be entirely dry. In wild conditions also the plant is commonly found near aquatic pockets with high humidity, however, precaution should be taken that water logging should not take place.

4.9 Plant density:

- The current recommendation with F1 variety is to plant at a spacing of 1ft x 1ft (40,000 plants per acre).

4.10 BASIC PARAMETERS:

Variety: Seeds collected by smearing the fruits

Season: April 2012

Spacing: 1 X 1 ft

Area covered: one acre

No. of plants grown: 40,000

Manuring: NPK

Foliar application: None

Irrigation: Flood irrigation

Harvest:

Average root yield is 1-2 tons/acre. 1,12,48,000 seeds/acre can be produced.

Description	Data
Number of bunches/plant	25-30 bunches
Number of fruits/bunch	10 fruits
Number of fruits /plant	250 fruits
Number of seeds/ plant	480 seeds
Number of seeds/ gm	180 seeds
No of seeds/fruit	2 seeds (occasionally one)
No of seeds/kilogram	1,80,000 seeds/kg
Seed yield/plant in gms	2.5 gms
No of plants/acre	40,000 saplings
No of seeds/acre	1,12,48,000 seeds (74% viability)
10kg of roots	300 plants
Fresh weight of a root	50gm
No of roots in 100gm	2 plants
No of roots/kg	20 plants
Production per acre : Weight of 40,000 plants	2 tons
Dry weight of a root	25gm
No of roots in 100gm	4 plants
No of roots/kg	40 plants
No of roots/ton	40,000 plants

The parameters that need to be taken care while cultivation is as follows:

4.11 Fertilizer application:

- Soil testing is recommended in each location to assess the appropriate fertilizer requirement.
- Manure is always helpful: **20 tons of well-rotted manure per hectare** is strongly recommended.
- **A nitrogenous fertilizer** is often required but is best applied **as organic manure**. (Fertilization with N.P.K of 80-70-90 kg/ha.). However, in second year the Nitrogen and Potassium should be analysed before application. As the plant is a legume the nitrogen concentration may be more in the soil and Potash usually higher in Rajkot, Jamnagar and Porbandar.
- Top dressings of urea, Superphosphate and certain micronutrients such as Magnesium Sulphate ($MgSO_4$) as a source of Sulphur assist in better seed germination and establishment of the plant.

4.12 Weed control:

- Young *Taverniera* seedlings are very susceptible to competition from weeds, so good weed control at the early stages after transplanting has a big impact on yield**.**
- **Field application of herbicide is not recommended,** so transplanting into a clean field is the best way of avoiding the need for later hand weeding.
- By 12-16 weeks after sowing, provided crop establishment is good, the ground will be covered and the weed problem is at an end.

4.13 Pests and diseases:

- One of the attractions of *Taverniera* is that until now it has had few natural enemies in - one of major concern is at maturity of the roots when the sweet component attracts the rodents to borrow the soil and eat the roots. Application of Castor seed cake or *Jatropha* seed cake can resolve this problem as rotendicide.

4.14 Irrigation:

- *Taverniera* can withstand dry to saline conditions once it is established,
- But any **moisture stress in the early stages tends poor establishment and even death of the plant.**
- Soil testing is recommended in each location to assess the appropriate fertilizer requirement.
- Manure is always helpful: **20 tons of well-rotted manure per hectare** is strongly recommended.
- **A nitrogenous fertilizer** is often required but is best applied **as organic manure**. (Fertilization with N.P.K of 80-70-90 kg/ha.). However, in second year the Nitrogen and Potassium should be analysed before application. As the plant is a legume the nitrogen concentration may be more in the soil and Potash usually higher in Rajkot, Jamnagar and Porbandar.
- Top dressings of urea, Superphosphate and certain micronutrients such as Magnesium Sulphate ($MgSO_4$) as a source of Sulphur assist in better seed germination and establishment of the plant.

4.15 Harvesting:

- The time of harvesting *T. cuneifolia* is critical as most evidence suggests that the glycoside content of the roots falls off sharply when flowering begins. However, in

our study we have found the winter period (Jan-March) to most suitable time for harvesting, that is during post flowering and fruiting period.

4.16 Drying:

- The plants are stooked in an upright position (like rice or corn stooks) in the field for air drying to a **maximum moisture content of 12% or less**. This normally takes from 3 to 5 days depending on weather conditions**. Exposure to bright sunlight should be avoided** as this can affect Artemisinin content.

- The stems (tillers) should be chopped from the roots as soon as possible as after drying the stems becomes stiffer/hard to be chopped easily.

- Plants should not be harvested in rainy season as the **moulds grow rapidly and affect the whole harvest will be spoiled**.

- Roots should be dried in open sun for a period of 2-3 days; however, the blackening of the roots should be avoided with frequent turn over across the day.

Figure A4.21: Good Agricutural and cultivation Practice of *Taverniera cuneifolia*

Figure 4.4: Good Agricutural and cultivation Practice of *Taverniera cuneifolia*

Figure A4.22: Good Agricutural and cultivation Practice of *Taverniera cuneifolia*

Figure A4.24: A. Cultivation plot (Leveling and weeding); B. Mixing Sand and Organic

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Figure A4.25: E. Plots for the establishment of saplings; F. Fully grown plant in Arboretum, MSU, Baroda.

Plot of *Taverniera cuneifolia* cultivated in Saurashtra University

Plant in flowering condition in arboretum

Figure 4.26 *T. cuneifolia* cultivated at the local sites

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Roots Kept for washing

Figure 4.27: Harvesting of roots

SEED VIABILITY AND SEED STRESS STUDIES

Salinity is a major environmental stress factor that affects seed germination in coastal salt marshes (Khan, 2002) where salinity ranges from 0.8% to 2.4%. Seeds of *T. cuneifolia* showed 56% germination in non-saline control. Seed germination decreased with increase in

KCl, $MgCl_2$, NaCl, $MgSO_4$, Na_2CO_3 salts. Na_2CO_3 inhibited germination more than other salts and 0% seeds germinated, whereas 26.67% seeds germinated at 300mM NaCl solution. The study showed that the inhibition to seed germination of *T. cuneifolia* is in the order of NaCl > $MgCl_2$ > $MgSO_4$ > KCl. Recovery of germination of *T. cuneifolia* seeds after treating it with different salt solutions was observed. Here, Na_2CO_3 treated seeds also showed recovery with 11% at 300mM solution. Maximum recovery germination was seen in $MgSO_4$ at 100mM with 70.45%.

Germination rate was higher in those seeds which were treated with concentrated sulphuric acid with the germination percentage to be 80. The second highest germination percentage was 62.67% seen in boiling water and the third highest percentage germination was seen in lukewarm water with 57.33%.

As per Raunkiaer classification system, *T. cuneifolia* belongs to the life form - Chaemophyte and its adaptability class is Glycophyte and salinity limit is 14 dSm^{-1} (Dagar and Singh, 2007). Showing the adaptation of the plant to grow in saline and terrestrial zones.

The rooting of in vitro developed shoots (4~6 cm length) was best in MS medium containing 3% sucrose and 2 mgl-1NAA. Micropropagated plants were successfully acclimatized (60%) within 6 weeks after rooting. (Jhamdade *et al*., 2012) Even in this experiment low level of Gibberelic acid showed a good amount of growth. The shoot length had increased.

Propagation of *Glycyrrhiza glabra* is usually carried through stolons, cuttings of about 10-15 cm. Seed can be used, but seed-set is poor in India and seed germination is low (Anonymous 2012).

Table 4.5: Seed germination of *Taverniera cuneifolia* when treated with different salt solutions

	Germination (%)				
Salt concentration (M)	KCl	MgCl2	NaCl	$MgSO_4$	Na_2CO_3
0.10	78.67	50.67	24.00	73.33	0.0
0.15	65.30	64.00	50.67	46.67	0.0
0.20	69.33	62.67	17.33	60.00	0.0
0.25	58.67	25.33	26.67	30.67	0.0
0.30	09.33	21.33	26.67	21.33	0.0

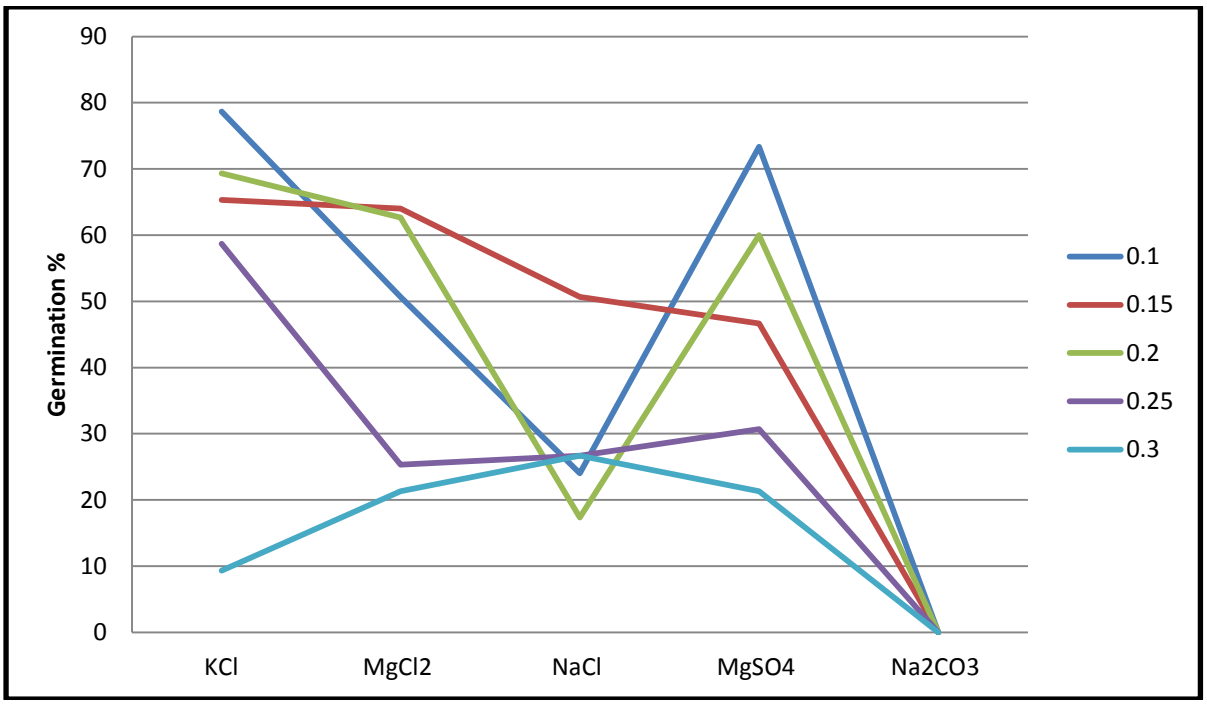


Figure A4.28: Seed germination when treated with different salt concentration

Table 4.6: Recovery of germination of *T. cuneifolia* when treated with different salt solutions

Salt concentration (mM)	Germination (%)				
	KCl	$MgCl_2$	NaCl	$MgSO_4$	Na_2CO_3
100	50	27.03	5.26	70.45	4.17
150	23.08	22.22	27.03	62.5	7.14
200	21.74	21.43	9.68	80	5.63
250	45.16	21.43	25.45	32.69	7.14
300	35.29	32.2	0	10.17	11.94

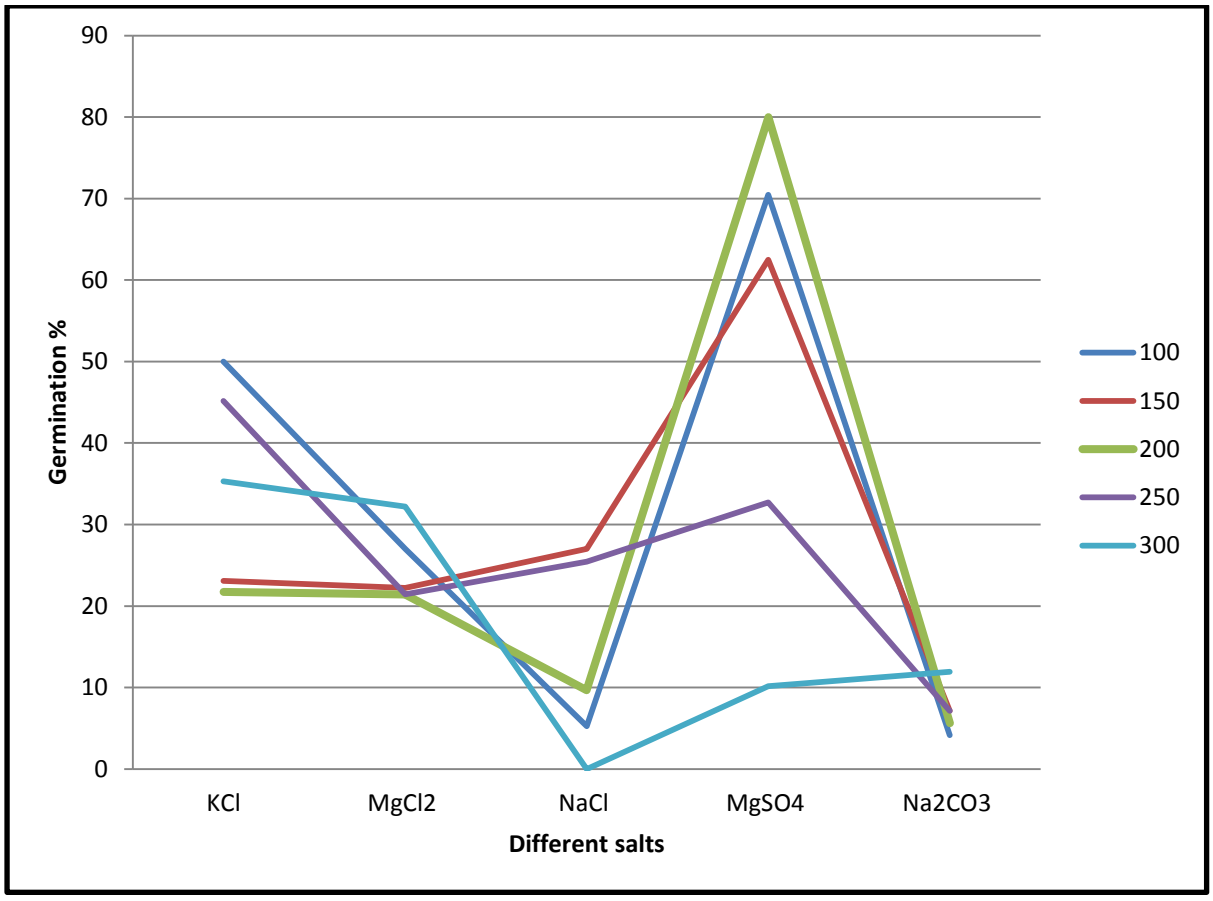


Figure A 4.29: Recovery of germination after the removal from different salt concentrations

Sr.no		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day10	Germination rate (seeds per day)	Germination (%)
1	GA 2000	0	2	4	7	8	10	11	11	12	14	0.19	18.67
2	GA 1000	1	6	10	14	16	17	19	19	21	25	0.33	33.33
3	GA 500	5	8	15	20	27	28	30	31	33	35	0.47	46.67
4	GA 250	4	10	18	23	25	29	35	36	36	38	0.51	50.67
5	GA 125	5	13	22	24	26	26	27	27	29	33	0.44	44.00
6	Luke warm H_2O	9	19	26	30	33	35	38	39	40	43	0.57	57.33
7	Cool H_2O	3	4	11	16	21	21	22	26	30	35	0.47	46.67
8	HNO_3 10min.	4	7	15	17	18	19	19	21	24	24	0.32	32.00
9	HNO_3 30min.	2	7	11	13	16	25	27	32	38	38	0.51	50.67
10	H_2SO_4	4	8	30	47	47	49	49	51	59	60	0.80	80.00
11	Boiling H_2O	0	36	46	47	47	47	47	47	47	47	0.63	62.67
12	Control	0	6	17	24	24	25	26	28	29	29	0.39	38.67

Table 4.7: Different solvents used in seed germination

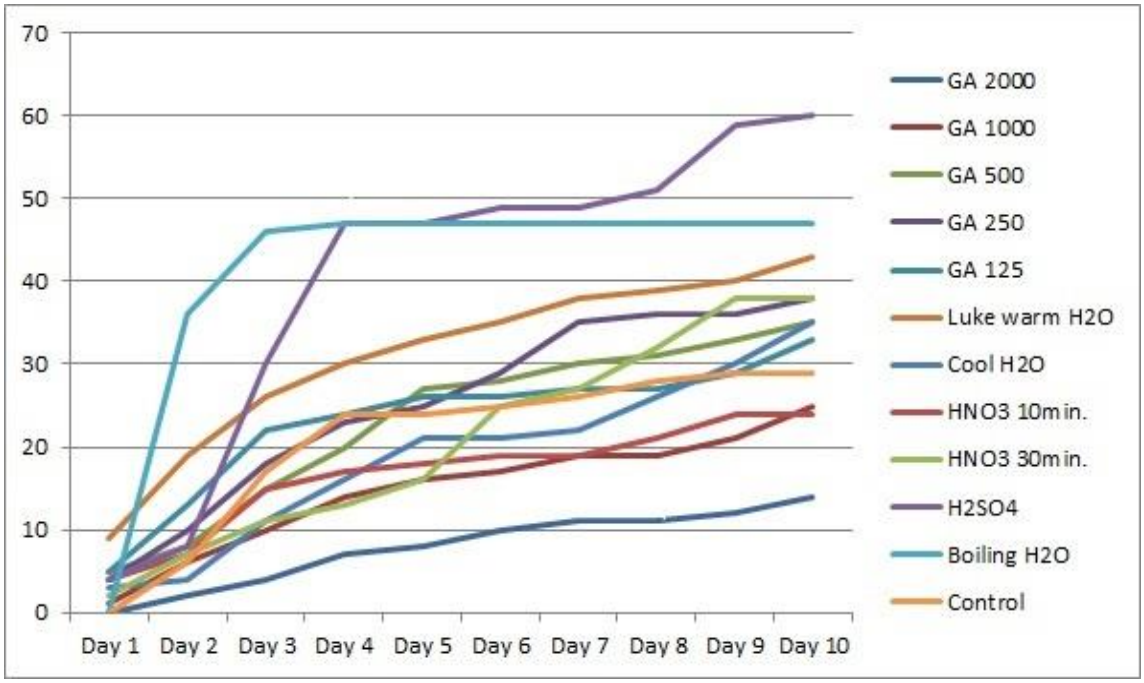


Figure A4.30: Germination rate in different chemical and solvent treatments.

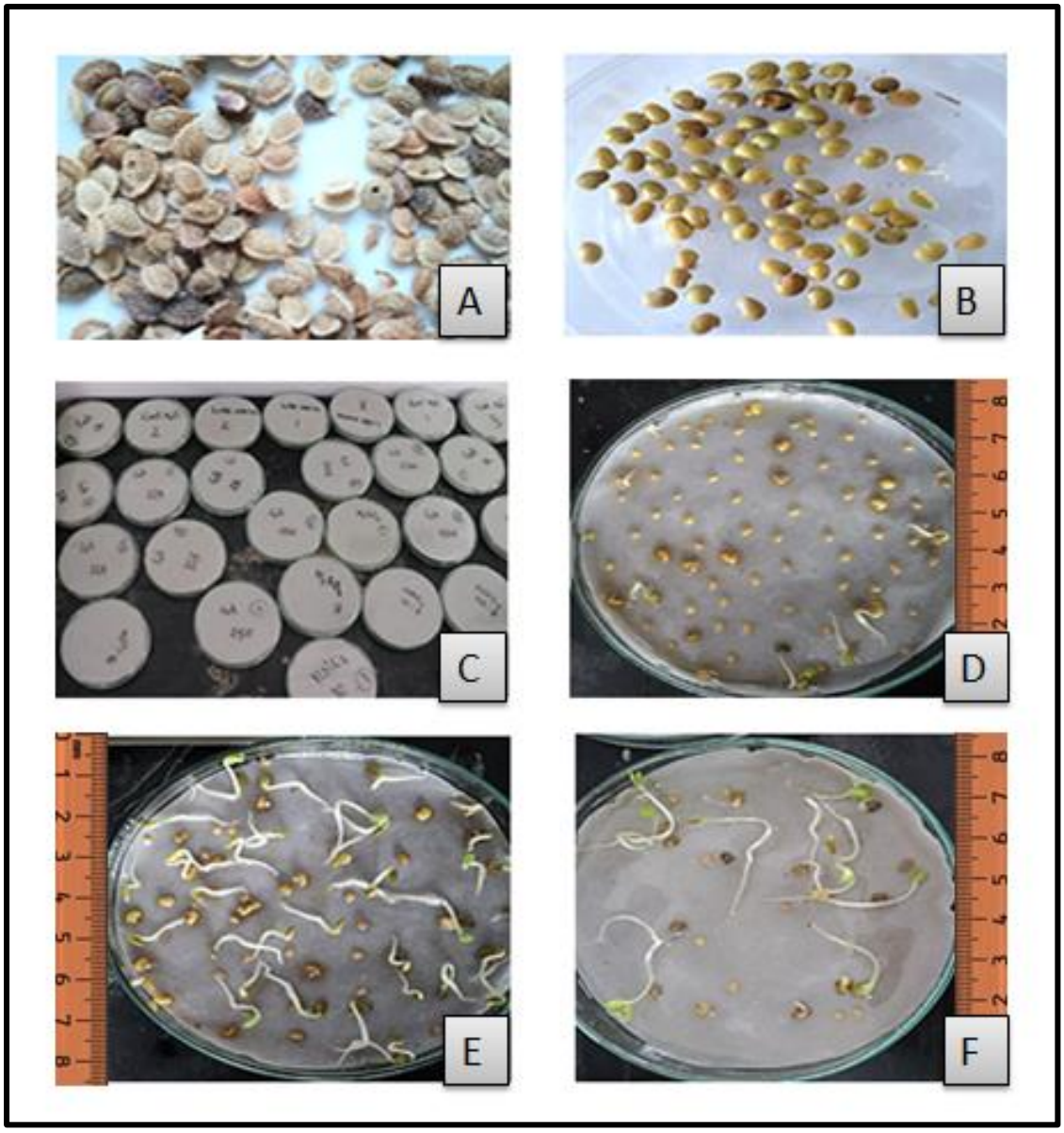


Figure A4.31: A. Fruits B. Seeds C. All concentrations D. Control E. Boiling water F. Lukewarm water

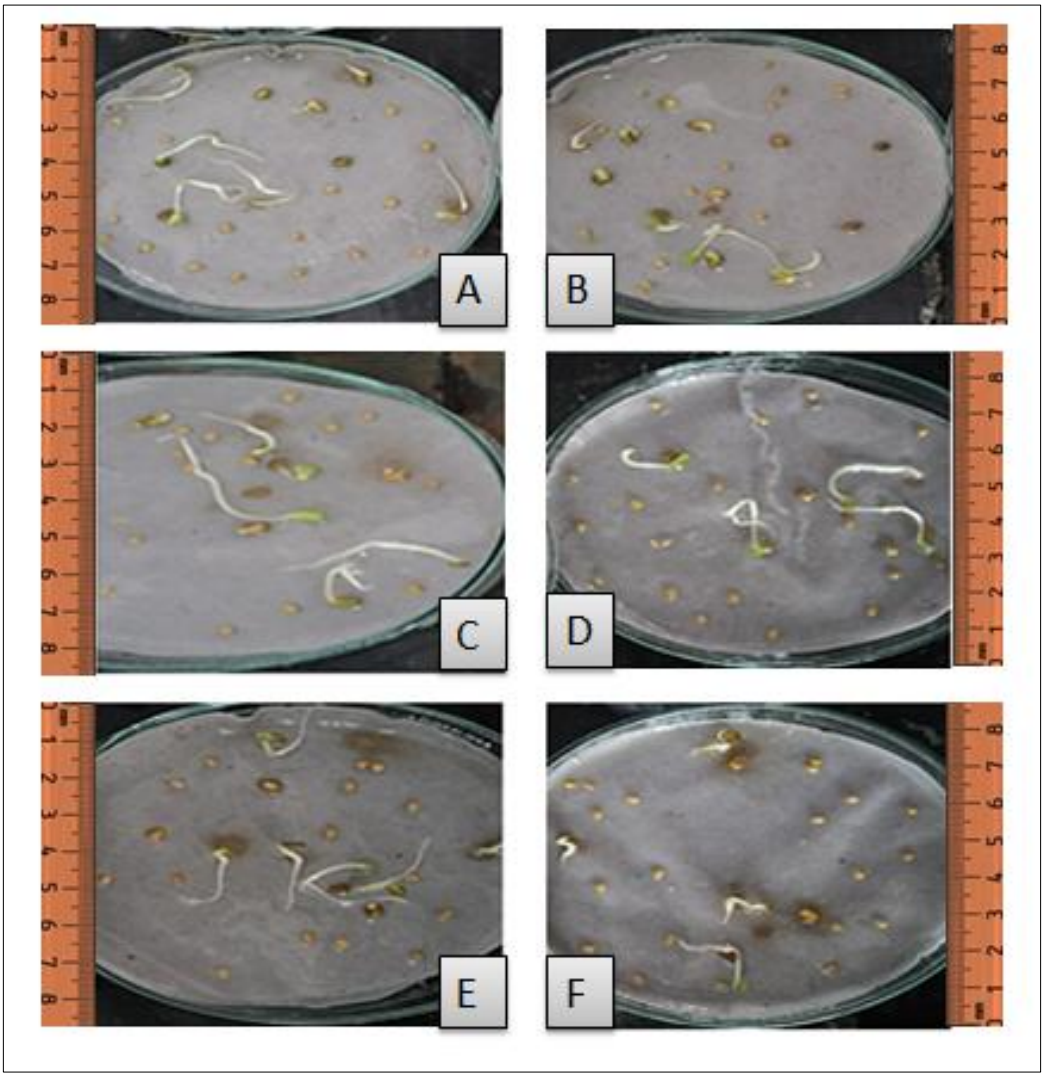


Figure A4.32: A. GA 125 B. GA 250 C. GA 500 D. GA 1K E. Cool water F. HNO_3

Section 1: Standardization of the herbs

Table 4.8: Different parameters of *T. cuneifolia* (underground and aerial parts) and *G. glabra* (rhizome)

Parameters	**Roots (underground parts)**		**Leaves and stem (aerial parts)**
	T. cuneifolia	*G. glabra* (rhizome) (Meena *et al*., 2010)	*T. cuneifolia*
Physicochemical Tests			
Water soluble extractive	17.19 %	25.69%	16.33 %
Alcohol soluble extractive	14.98 %	23.37%	8.49 %
Moisture content (By KF)	4.48 %	7.67%	5.56 %
Ash content	6.75 %	7.92%	14.71 %
Acid insoluble ash	1.23 %	0.62%	6.15 %
Water soluble ash	2.61 %		4.42 %
Sulphated ash	3.02 %		4.11 %
Swelling index	Absent		6.0
Heavy Metals			
Lead	Not Detected	0.021 ppm	Not Detected
Cadmium	0.1021 ppm	Less than 0.001ppm	Not Detected
Arsenic	Not Detected	Less than 0.001ppm	Not Detected
Mercury	Not Detected	0.703 ppm	0.093 ppm
Microbiological Tests			
Total plate count	178 x 10^2cfu/g	2075.1 x 10^2 cfu/g	125 x 10^2cfu/g
Total fungal count	17 x 10^2 cfu/g	197.62 x 10^2cfu/g	7 x 10^2cfu/g
Escherichia coli	Absent	Absent	Absent
Pseudomonas aeruginosa	Present	Absent	Present
Staphylococcus aureus	Present	Absent	Present
Salmonella Spp.	Absent	Absent	Absent

Initially the prime objective of the project was to detect glycyrrhizin in *T. cuneifolia* roots which is a saponin glycoside. Thus most of the extraction procedures were based on the existing literature on glycyrrhizin extraction. After extensive HPLC analysis glycyrrhizin (α

and β glycyrrhetinic acid) we could come to the conclusion that glycyrrhizin is not present in *T. cuneifolia* roots. However there might be other glycosides which are similar to glycyrrhetinic acid. Thus further studies were conducted for the detection of possible glycosides which could be possibly because of β-amyrin series as per the LCMS studies. However it has been further explained in conclusion chapter.

Alcohol soluble extractive and water soluble extractive were studied for the Quality evaluation as per the WHO guidelines.

Section 2: Phytochemistry

Table 4.9: The basis of various probable phytoconstituents found in *T. cuneifolia* by LC-MS studies are as follows:

Highest (longest) Peak in LC& Highest intensity in mass spectra						
Sr. no.	**Sample**	**Peak**	**MS in positive mode**	**minus 1**	**actual mol. wt.**	**Name of the compound**
1	S1	peak 9	300.4	299.4	300.3	4'-hydroxywogonin
2	S1	peak 10	299.3	298.3	298.3	Pinocembrin 7-acetate (oil)
3	S1	Peak 18	369.4	368.4	368.4	Eucalyptin acetate (pow.)
	S1				368.3	5,7-Diacetoxy-8-methoxyflavone (crys)
4	S1	Peak 19	136.9	135.9	137	P-hydroxybenzoic acid
5	S1	Peak 18	285.4	284.4	284.3	Acacetin (Pow.)
6	S2	Peak 5	431.4	430.4	430.4	Artemetin acetate
7	S3	Peak 8	455.3	454.3	454.4	Kaempferol tetracetate (pow)
8	S3	Peak 8	433.3	432.3	432.4	3',4',5',3,5,6,7- heptamethoxyflavone (crys)
	S3				432.4	3',4',5',3,5,7,8- heptamethoxyflavone (crys)
9	S3	Peak 10	315.4	314.4	314.3	3-O-Acetlypinobanskin (Oil)
	S3				314.3	Cirsimaritin (Pow)
	S3				314.3	Kumatakenin
	S3				314.3	Naringenin trimethyl ether (oil)
	S3				314.3	5,7-Dihydroxy-6,8-dimethoxy flavone (pow)
	S3				314.3	Pectolinaringenin (yellow powder)
10	S3	Peak 10	457.4	456.4	456.4	2',5,6',7-Tetrahydroxyflavone (pow.)
11	S3	Peak 11	439.5	438.5	438.5	Liachianone A (Pow)
12	S3	Peak 11	299	298	298.3	Pinocembrin 7-acetate (oil)
13	S4	Peak 10	274.5	273.5	274.3	(-)-Epiafzelechin (Pow)
	S4				274.3	(+)-Afzelechin
22	S5	Peak 6	299	298	298.3	Pinocembrin 7-acetate (oil)
23	S5	Peak 10	274.5	273.5	274.3	(-)-Epiafzelechin (Pow)
	S5	Peak 10				(+)-Afzelechin
24	S6	Peak 10	435.6	434.6	434.4	Avicularin (quercetin *O*-glycoside)
25	S6	Peak 9	439.6	438.6	438.5	Leachianone A (Pow)

Medium and Low Peaks in LC with Highest and medium intensity in mass spectra						
Sr. no.	**Sample**	**Peak**	**MS in positive mode**	**minus 1**	**actual mol. wt.**	**Name of the compound**
1	S1	peak 1	84.2	83.2	84.16	Cyclohexane (wikipedia)
2	S1	peak 3	215.3	214.3	214.3	3-[4-(sec-butyl)piperazin-1-yl]propanoic acid (http://www.molport.com/)
3	S1	Peak 4	181.1	180.1	181.1	1-N,1-N,2-N,2-N-tetrafluorobenzene-1,2-diamine(http://www.molport.com/)
4	S1	Peak 5	209.6	208.6	209.6	5-(2-chlorophenyl)-1,2,4-oxadiazole-3-carbaldehyde (http://www.molport.com/)
5	S1	Peak 5	453.6	452.6	452.6	Repaglinide
6	S2	Peak 6	299.0	298	298	Methyl stearate Ref: Seida et al., International Current Pharmaceutical Journal, May 2013, 2(6): 109-111 Investigation and isolation of the active constituents of petroleum ether fraction of Medicago sativa L. sprouts Ahmed A. Seida1,2, Hala El-Hefnawy1, Dina R. Abou-Hussein1,3, *Fatma Alzahraa Mokhtar1
7	S2	Peak 6	273.1	272.1	272.4	Przewalskin (diterpenoid) (rare phytochemicals)
8	S2	Peak 7	301.4	300.4	300.4	Coronarin A (diterpenoid) (rare phytochemicals)
9	S2	Peak 7	271.4	270.4	270.2	Apigenin (Flavonoid) (rare phytochemicals)
10	S1	Peak 6	395.4	394.4	394.4	2',4',5'-Trimethoxy-2",2"-dimethylpyrano[5",6":6,7]isoflavone (flavonoid) (rare phytochemicals)
11	S1	Peak 7	399.4	398.4	398.4	Naringenin triacetate (rare phytochemicals)
12	S1	Peak 11	255.4	254.4	254.2	4',5-Dihydroxyflavone(rare phytochemicals)
13	S1	Peak 18	369.4	368.4	368.3	5,7-Diacetoxy-8-methoxyflavone(rare phytochemicals)
14	S1	Peak 18	353.5	352.5	352.3	Erysubin B flavonoid (rare phytochemicals)
15	S1	Peak 22	318.5	317.5	316.3	3-O-Methylquercetin (rare phytochemicals)
16	S1	Peak 22	423.6	422.6	422.4	Catechin 7-xyloside (rare phytochemicals)
17	S2	Peak 4	287.0	286.0	286.2	Luteolin or 2'-Hydroxygenistein ((rare phytochemicals)
18	S2	Peak 9	271.3	270.3	270.2	Galangin[flavonoid] ((rare phytochemicals)
19	S2	Peak 10	285.3	284.3	284.4	Coronarin E [diterpenoid] (rare phytochemicals)
20	S3	Peak 4	361.3	360.3	360.3	Jaceidin (flavonoid) (rare phytochemicals)
21	S3	Peak 6	291	290.0	290.3	Catechin (flav.) (rare phytochemicals)
22	S3	Peak 7	455.1	454.1	454.4	Kaempferol tetracetate (fl.) (rare phytochemicals)
23	S3	Peak 9	457.4	456.4	456.4	2',5,6',7-Tetraacetoxyflavanone (rare phytochemicals)

24	S1	Peak10	461.4	460.4	460.7	Cabraleadiol (Triterpenoid)(rare phytochemicals)
25	S2	Peak 4	441.5	440.5	440.7	Ganoderol B (triterpenoid)(rare phytochemicals)
26	S2	Peak 5	431.4	430.4	430.6	Eichlerialactone (triterpenoid) (rare phytochemicals)
27	S2	Peak 10	427.5	426.5	426.7	Beta amyrin (triterpenoid) (rare phytochemicals)
28	S3	Peak 6	473.4	472.4	472.5	Limonol (triterpenoid) (rare phytochemicals)
29	S4	Peak 15	453.5	452.5	452.7	Ganoderic acid SZ (triterpenoid) (rare phytochemicals)
30	S3	Peak 6	439.5	438.5	438.7	Ganoderol A (triterpenoid) (rare phytochemicals)
31	S1	Peak 19	283	282	282.3	Isomagnolone ((Lignan)(rare phytochemicals)
32	S1	Peak 20	267.4	266.4	266.3	(+) – Conocarpan (Lignan)(rare phytochemicals)
33	S1	Peak 22	537.5	536.5	536.6	9-O-Feruloyllariciresinol (Lignan)(rare phytochemicals)
34	S4	Peak 10	421.5	420.5	420.5	Lyoniresinol (Lignan) (rare phytochemicals)
						Options 4
35	S4	Peak 5	455.3	454.3	454.7	Ganoderiol F (triterpenoid) 3-Hydroxy-11-ursen-28,13-olide Alstonic Acid B Coccinic Acid
36	S1	Peak 22	423.6	422.6	422.5	Cedrelone (triterpenoid) (rare phytochemicals)

The separation and plant constituents is mainly carried out using one or other, or a combination of different chromatographic techniques: paper chromatography (PC), thin layer chromatography (TLC), high performance thin layer chromatography (HPTLC), high performance liquid chromatography (HPLC), Liquid chromatography – Mass spectroscopy (LCMS) and Gas chromatography – Mass spectroscopy (GCMS). Various phytocomponents present in *T. cuneifolia* were analysed by the above stated chromatographic techniques and thus with these techniques the results obtained are as follows:

4.2.1 Preliminary Analysis

Based on the preliminary studies presence and absence of various class of phyto-components were analysed. The details of the various phytoconstituents are as follows:

Table 4.10: Preliminary analysis of different phytoconstituents

Sr.no.	Parameters	Root	Percentage w/w
1	Alkaloids	+	-
2	Flavonoids	+	1.19%
3	Tannin	+	12.44%

4	Saponin	+	2.70%
5	Resin	-	-
6	Phenol	-	-
7	Anthocyanin	+	-
8	Glycosides	+	-
9	Steroids and Phytosterols	+	-

4.2.2 Phenols and Phenolic acids

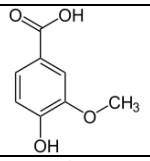
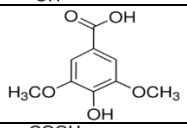
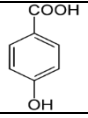
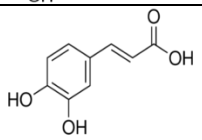
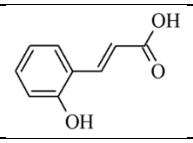
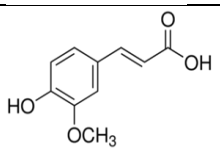
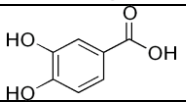
Phenolic compounds are all aromatic so that they all show intense absorption in the UV region of the spectrum. In addition, phenolic compound characteristically exhibit bathochromic shift in their spectra in presence of alkali. Spectral method are therefore, especially important for the identification and quantitative analysis of phenols. With this view **the phenolic components were analysed and the paper chromatographic studies showed the presence of p-hydroxybenzoic acid, protocatechuic acid, vanillic acid, syringic acid, o-coumaric acid (cis and trans), caffeic acid and ferulic acid (Figure 4.1). Even the roots showed the presence of p-hydroxybenzoic acid in LC-MS analysis.**

As per recent references the phenolic acids components like v**anillic acid is used for** Hepatoprotective effect (Itoh *et al*., 2009) and for Treating ulcerative colitis (Kim *et al*., 2010). **Syringic acid is used for** Hepatoprotective effect (Itoh *et al*., 2009) and for prevention from diabetic cataract pathogenesis (Xiaoyong, 2012). **p-hydroxy benzoic acid:** *p*-hydroxybenzoic acid esters are used as preservatives (Aalto *et al*., 1953). **Caffeic acid:** Plants containing chlorogenic acid and/or caffeic acid have been used as herbal remedies and possess some of the following pharmacological properties: antiarthritic, antidiarrheal, antiinflammatory, antirheumatic, antitumor, antiviral, astringent, cardiotonic, carminative, chloretic, coronary vasodilatory, diaphoretic, diuretic, gastric sedative, hypotensive, intestinal antiseptic, purgative, and spasmolytic effects. Medicinal plants containing chlorogenic and/or caffeic acid have also been used as remedies for the common cold, hematemesis, hematuria, hemorrhoids, lumbago, neuralgia, tinnitus, and toothache (Raymond, 1998). Antioxidant activity (Gulcin, 2006). It is used in the form of Caffeic acid Phenethyl ester - Neurological disorders and emergencies (Akyol, 2011).

Further, Ferulic acid has been used as Antioxidant, (Graf, 1992). It seems much effective for cosmetic use as whitening agent and sunscreen (Syaku *et al*., 1989). It has been reported that the ferulic acid ester of vitamin E has extremely decreased melanin generation (Funasaka *et al*., 1997).Prevention of food discoloration (Anonymous, 1999; Maoka *et al*.,

2008).Cosmetic Effect (Skin Whitening, Photo-protection) (Anonymous, 1980; Anonymous, 1991; Anonymous, 1997; Tournas *et al*., 2006; Murray *et al*., 2008). Ferulic acid inhibited growth of colon cancer cells in vitro (Mori *et al*., 1999). Further in vivo test confirmed the inhibitory effect on carcinogenesis of colon cancer in rats (Hudson *et al*., 2000).Blood sugar lowering effect (Anonymous, 2000).Blood pressure lowering effect (Ardiansyah *et al*., 2008).Brain function enhancing effect (Cheng *et al*., 2008; Mohammad and Butterfield, 2005; Cho *et al*., 2005; Jin *et al*., 2005; Perlugi *et al*., 2006). **Protocatechuic acid has been used in treatement of** Cancer chemopreventive activity (Takuji *et al*., 2011) and as an antioxidant (Xican *et al*., 2011).

Table 4.11: Phenolic acids and their structures

1	**Vanillic acid**	
2	**Syringic acid**	
3	**p-hydroxy benzoic acid**	
4	**Caffeic acid**	
5	**O-coumaric acid**	
6	**Ferulic acid**	
7	**Protocatechuic acid**	

Based on paper chromatographic studies the following phenolic acids were identified

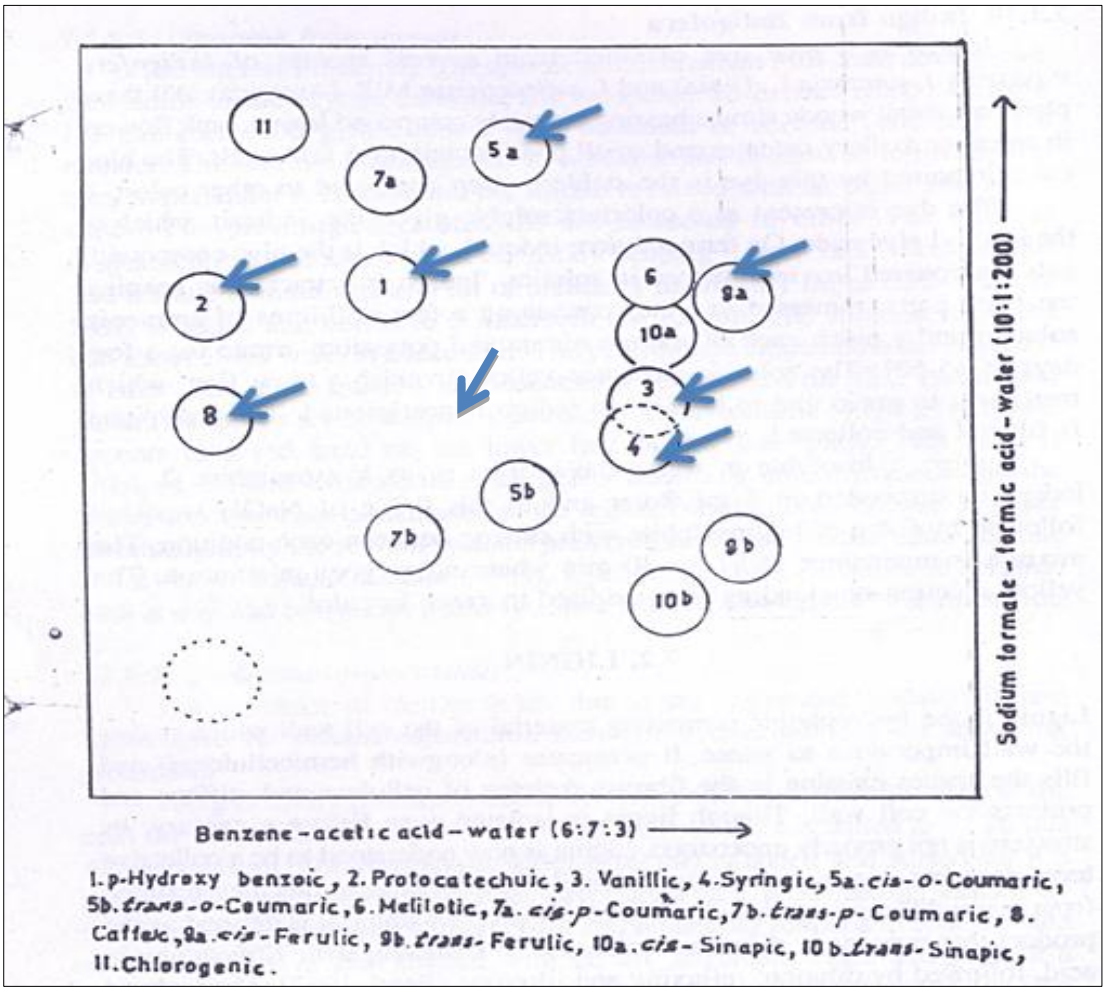


Figure 4.1: 2-D chromatogram showing the positions of the phenolic acids (Daniel, 1991)

Table 4.12: The colour reactions of phenolic acids: (Daniel, 1991)

Sr. no	**Phenolic acids**	**Colors**		
		UV	Diazotized p-nitraniline	Diazotised sulphanilic acid
1	p-Hydroxy benzoic acid	-	Pink	Yellow
2	Protocatechuic acid	-	Brownish violet	Pink
3	Vanillic acid		Purple	Orange
4	Syringic acid		Blue	Red
5	Cis-o-Coumaric acid	Bluish yellow	Purple	Orange
6	Trans-o-Coumaric acid	Bluish yellow	Purple	Orange
7	Caffeic acid	Blue	Brown	Pink
8	Ferulic acid	Blue	Bluish green	Purple

Table 4.13: Phenolic acids present in different parts of the plant

Sr.no.	Plant part used	Presence of Phenolic acids
1	Root	Vanillic acid, syringic acid, p-hydroxy benzoic acid, caffeic acid.
2	Stem	O-coumaric (*cis* and *trans isomers*), syringic acid, ferulic acid.
3	Leaf	Protocatechuic acid, caffeic acid, p- hydroxy benzoic acid, o-coumaric acid (*cis* and *trans isomers*), vanillic acid, ferulic acid.
4	Flower	Vanillic acid, syringic acid, p-hydroxy benzoic acid.

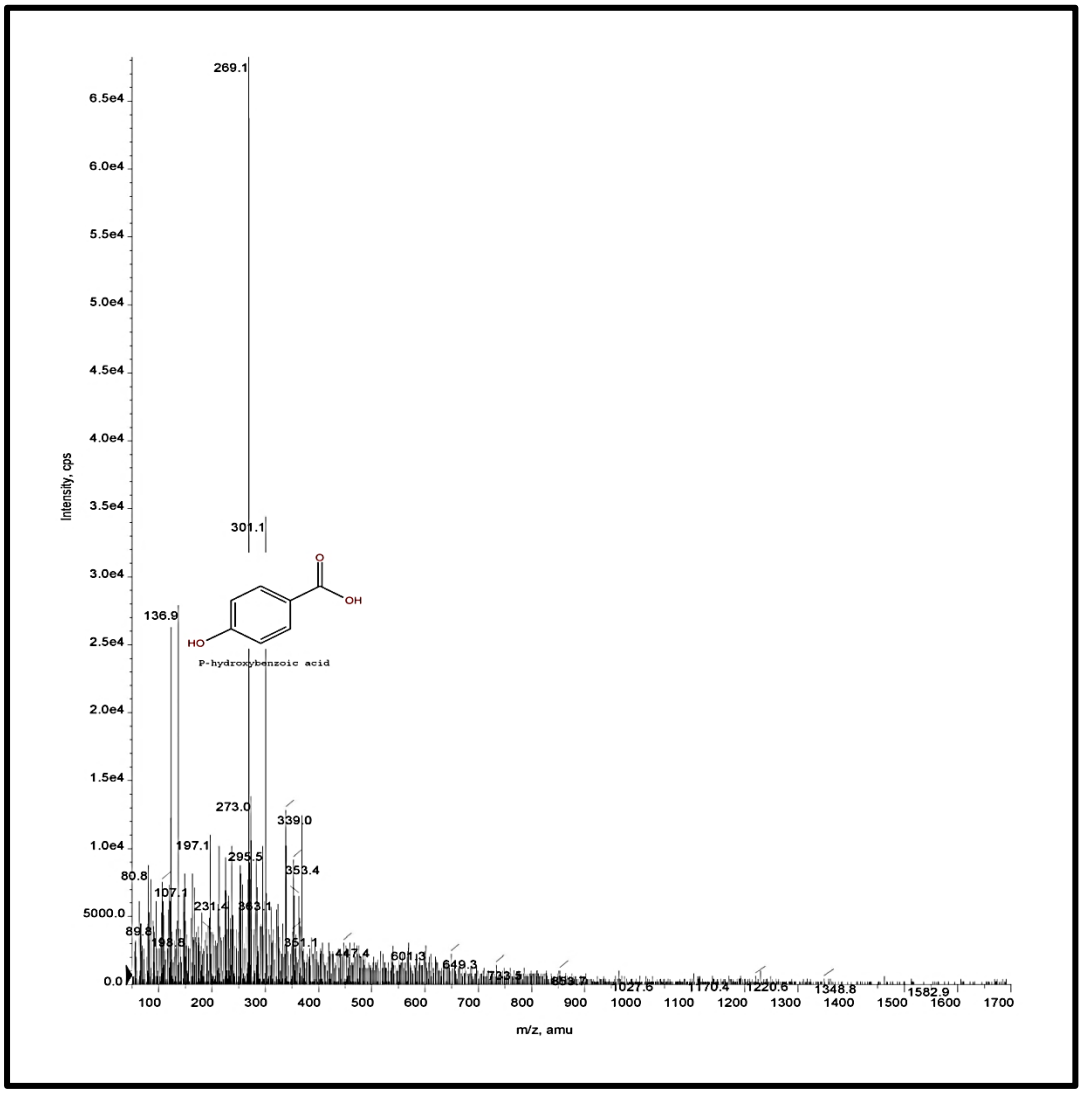


Figure 4.2: LCMS of p-hydroxy benzoic acid

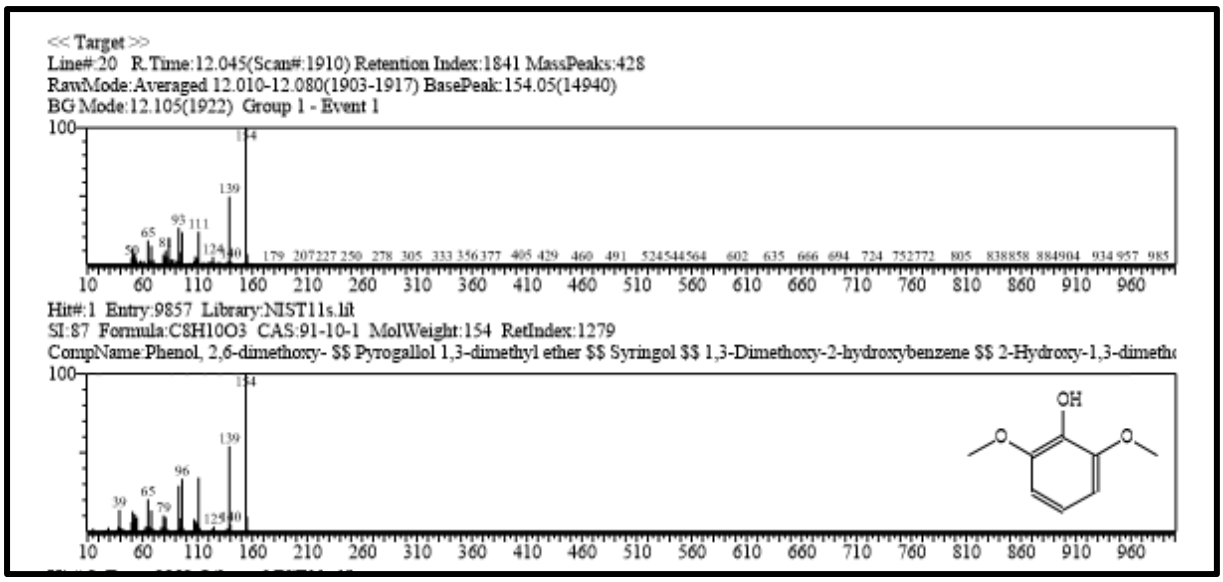


Figure 4.3: GCMS of syringol (Syringic acid)

4.2.3 Phenylpropanoids:

Phenypropanoids are naturally occurring phenolic compounds which have an aromatic ring to which a three carbon side chain is attached they are derived biosynthetically from the aromatic protein amino acid phenyl alanine and they may contain one or more C_6-C_3 residues. Lignans are dimers and is formed condensation of two cinnamic acid/cinnamic alcohol through beta carbon of their aliphatic side chains. **Two such probable lignans Conocarpan and 9-O-Feruloyllariciresinol were recorded in roots of *T. cuneifolia*** (Figure. 4.5). Lariciresinol has been recoded from *Linum usitatissimum* (Shiva, 2007) while Conocarpan has been recorded in Combretaceae family.

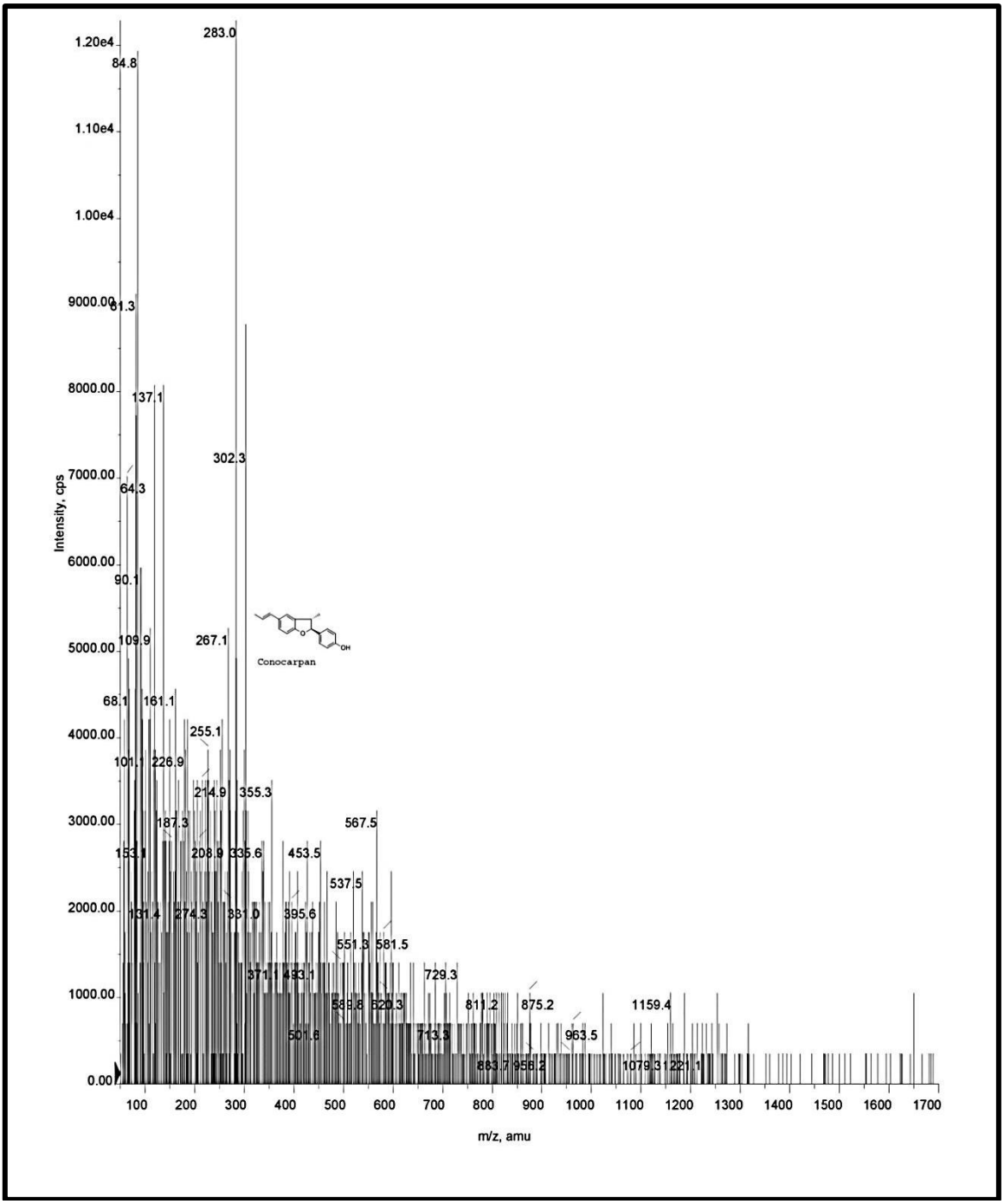


Figure 4.5: LCMS of Conocarpan

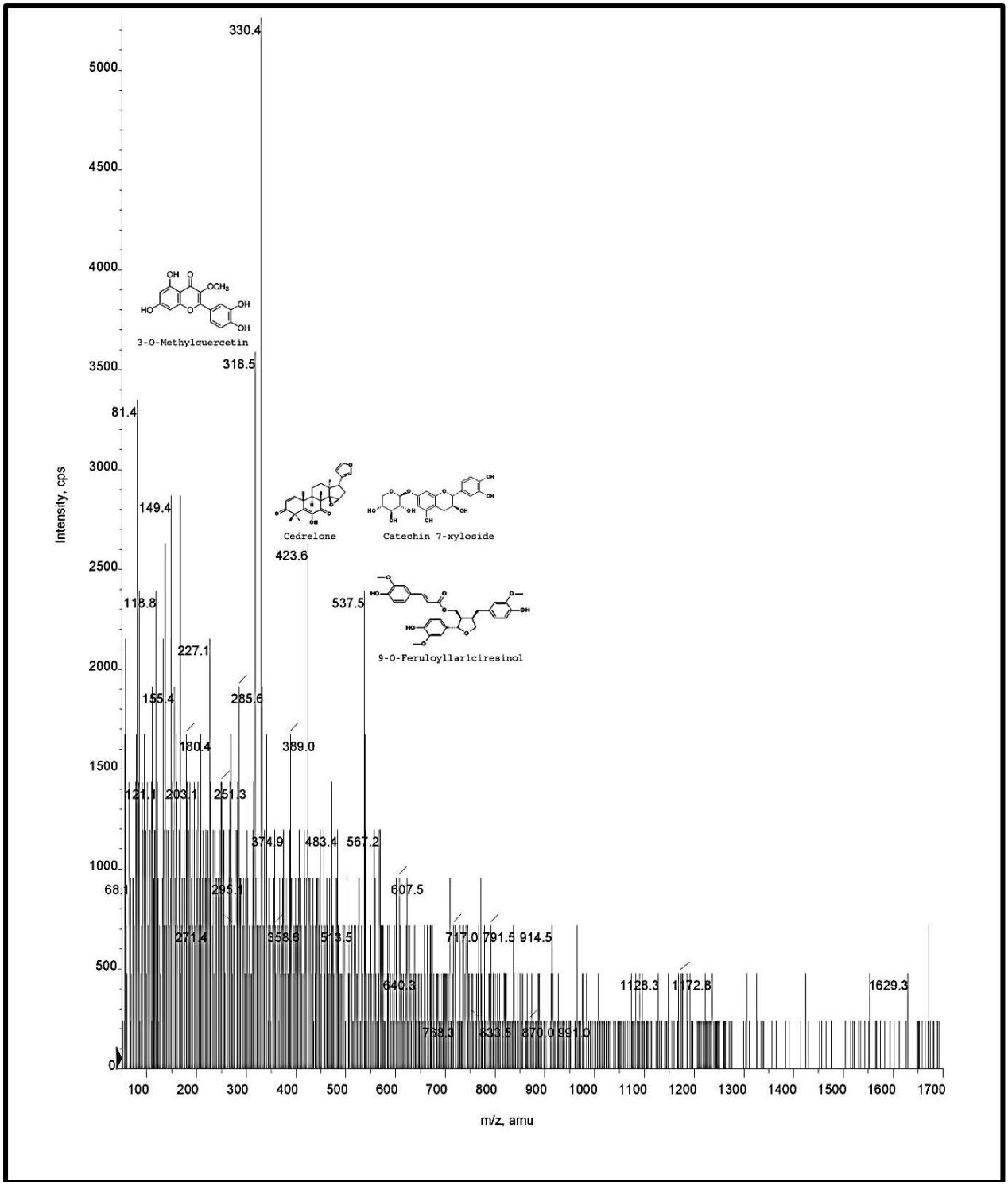


Figure 4.6: LCMS of 9-O-Feruloyllariciresinol (lignan)

4.2.4 Flavonoids:

Flavonoids are a large group of phenolic plant constituents. To date, almost 6500 different flavonoids have been identified these compounds consist of two benzene rings that are connected by an oxygen containing pyrene ring therefore, flavonoids can be regarded as chromane derrivatives with phenyl substituent in the C2 or C3 postion. Flavonoid are hydroxlated in position 3, 5, 7, 3-, 4- and/or 5-. Flavonoids are mainly present as *O*-or *C*-glycosides. Aglycones occur less frequently (Rijke *et al*., 2006). Atleast 8 different monosaccharides or combination of these (di-trisaccharides) can bind to the different hydroxyl groups of the flavonoid aglycone. Of the various flavonoids the anthocyanin are the most important and wide spread group of colouring matters in plants. These intensly coloured water soluble pigments are responsible for nearly all the pink, scarlet, red, mauve, violet and blue colours in petals, leaves and fruits of higher plants. The anthocyanin are all based chemically on a single aromatic structure that of cynadin, and all are derived from this pigment by addition or subtraction of hydroxyl group or by methylation or by glycoslylation (Harborne 1973). **The flowers of *T. cuneifolia* showed the presence of delphinidin (Figure 4.7).** Delphindin is a rare anthocyanin found in the nature (Harborne 1973).

Table 4.14: Flavonols present in different parts of the plant

Sr.no.	Plant Part Used	Flavonol Identified
1	Root	Isorhamnetin
2	Stem	Quercetin
3	Leaf	Isorhamnetin
4	Flower	Isorhamnetin

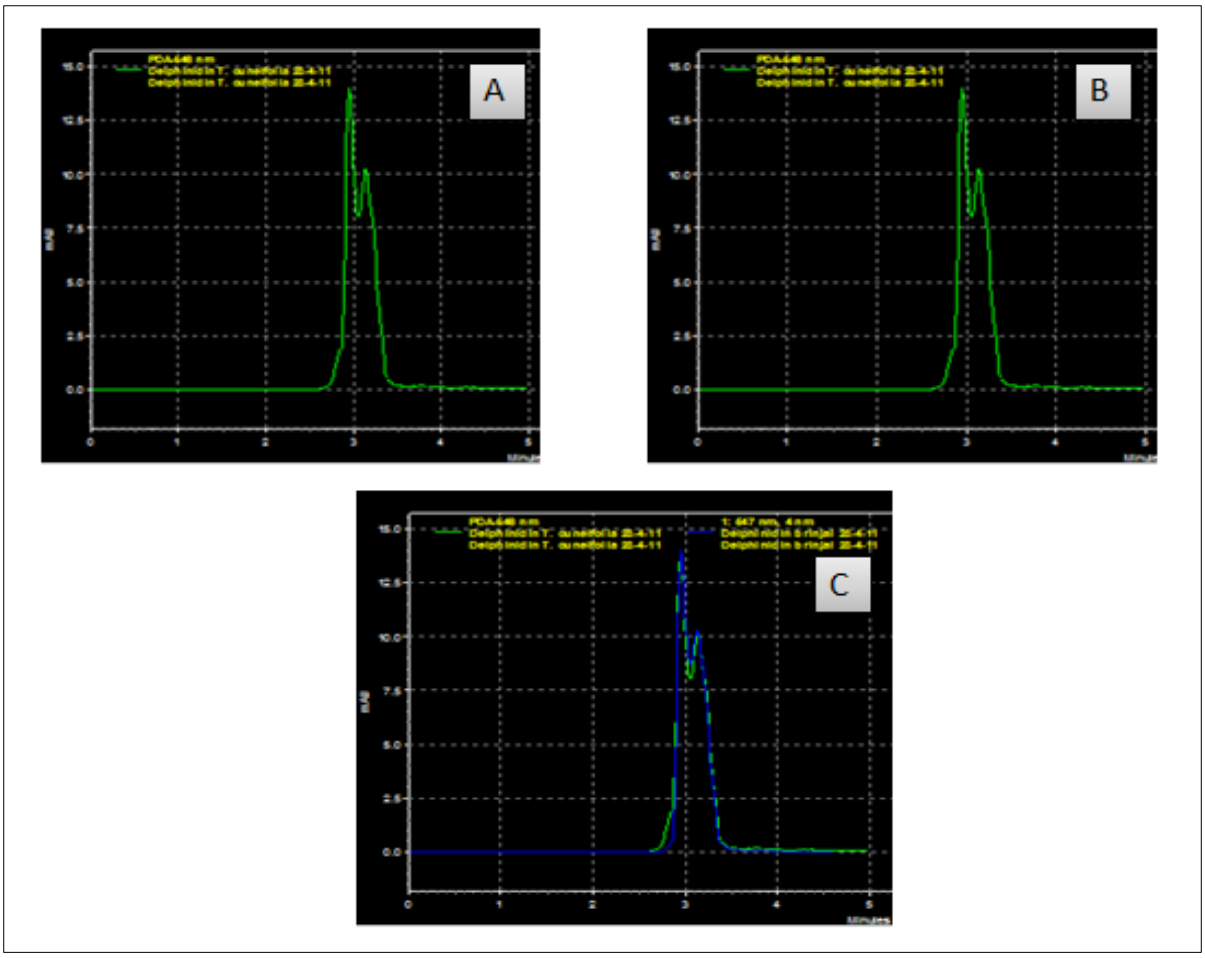


Figure 4.7: A) *Solanum melongena*– Delphinidin B) *Taverniera cuneifolia* – c) Delphinidin Overlapping of Both (*S. melongena* and *T. cuneifolia*) chromatograms

4.2.5 Flavonols

Flavonols are a class of flavonoids that have the 3-hydroxyflavone backbone (IUPAC name: 3-hydroxy-2-phenylchromen-4-one). Their diversity stems from the different positions the phenolic -OH groups. (Cermak and Wolffram, 2006). Flavonols are widely distributed in plants, both as co-pigments to anthocyanins in petals and also in leaves of higher plants. Like the anthocyanins, they occur most frequently in glycosidic combination. Although two or three hundred flavonoids are known, only three are common: kaempferol, quercetin and myricetin (Harborne, 1973). **Kaempferol (from roots) and Quercetin (from Stem) were even recorded in *T.cuenifolia* by UV spectroscopy (Fig 4.8) and LC-MS (fig 4.9.).** Quercetin is an aglycone form of a number of flavonoid glycosides (Juergenliemk *et al*., 2003). **Isorhamnetin was recorded in roots, leaves and flowers by UV spectroscopy. Galangin was another component which is probably present in the roots of *T. cuneifolia***

(Fig 4.13). However, till date this component has been recorded in the rhizome of *Alpinia galanga* belonging to family of Zingiberaceae (Kaur *et al*., 2010).

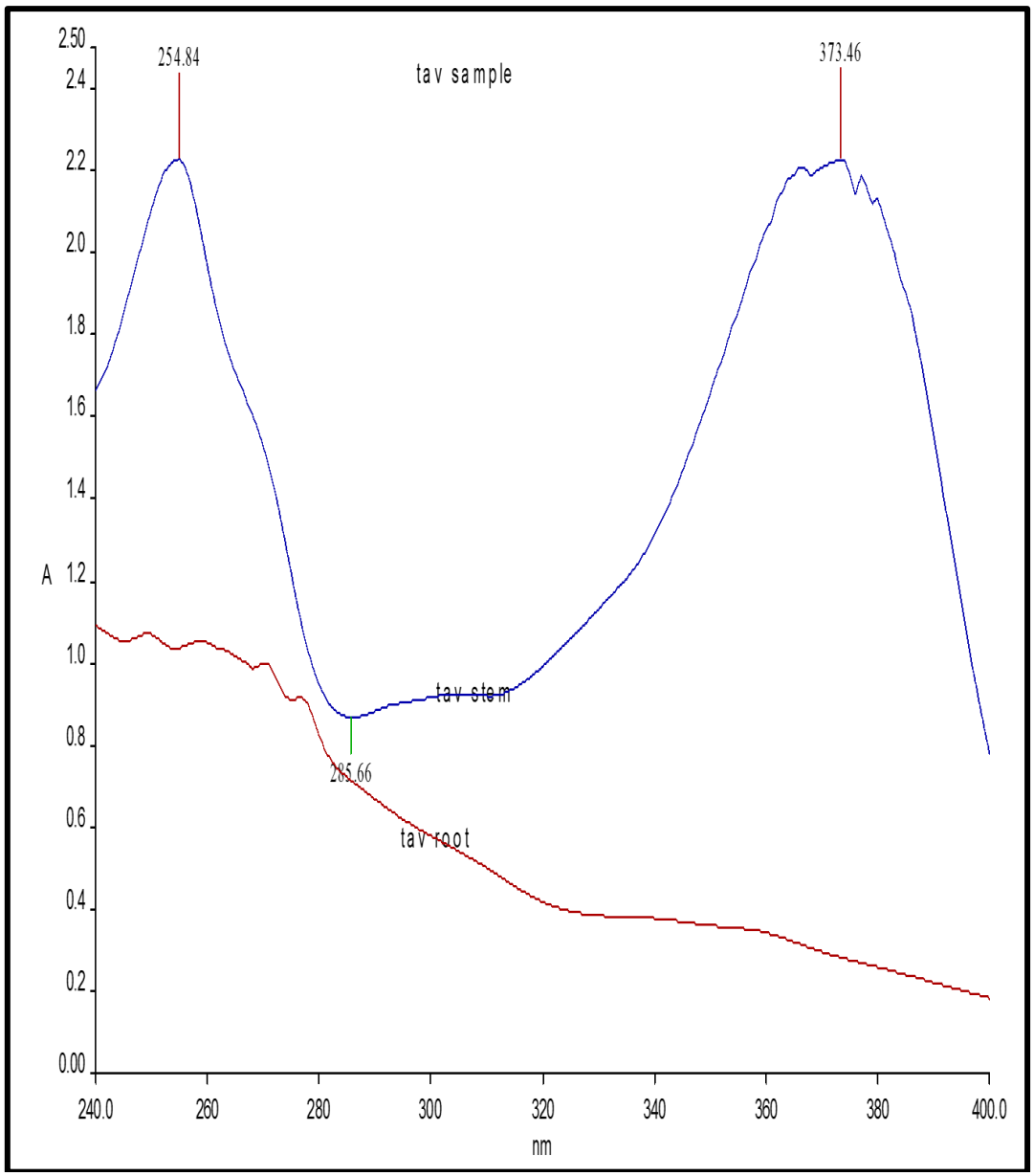


Figure 4.8 : UV spectrum of Quercetin in stem of *T.cuneifolia*
(Spectral max. 254.84, 373.46)

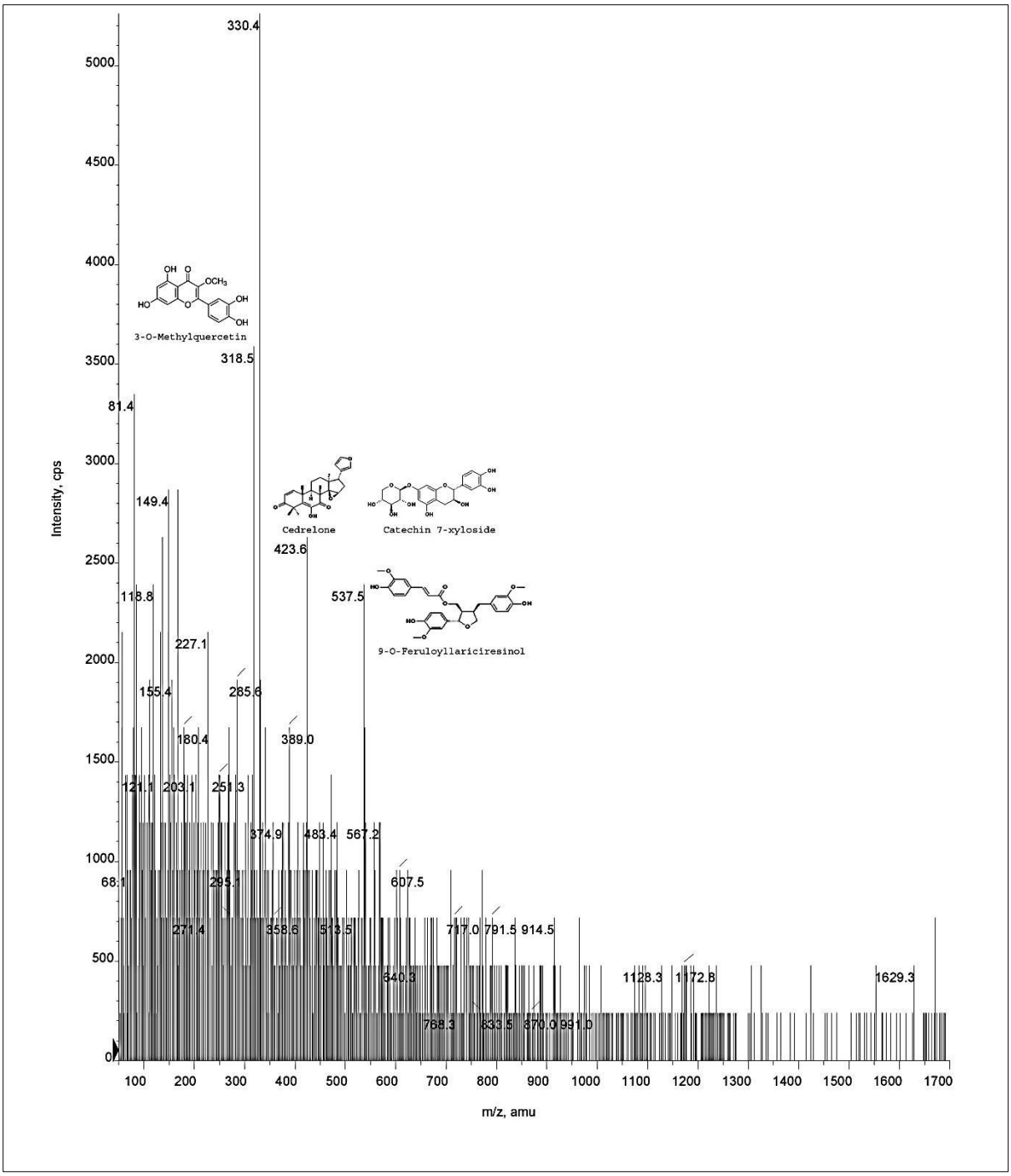


Figure 4.9: LCMS of 3-O-methyl quercetin in roots

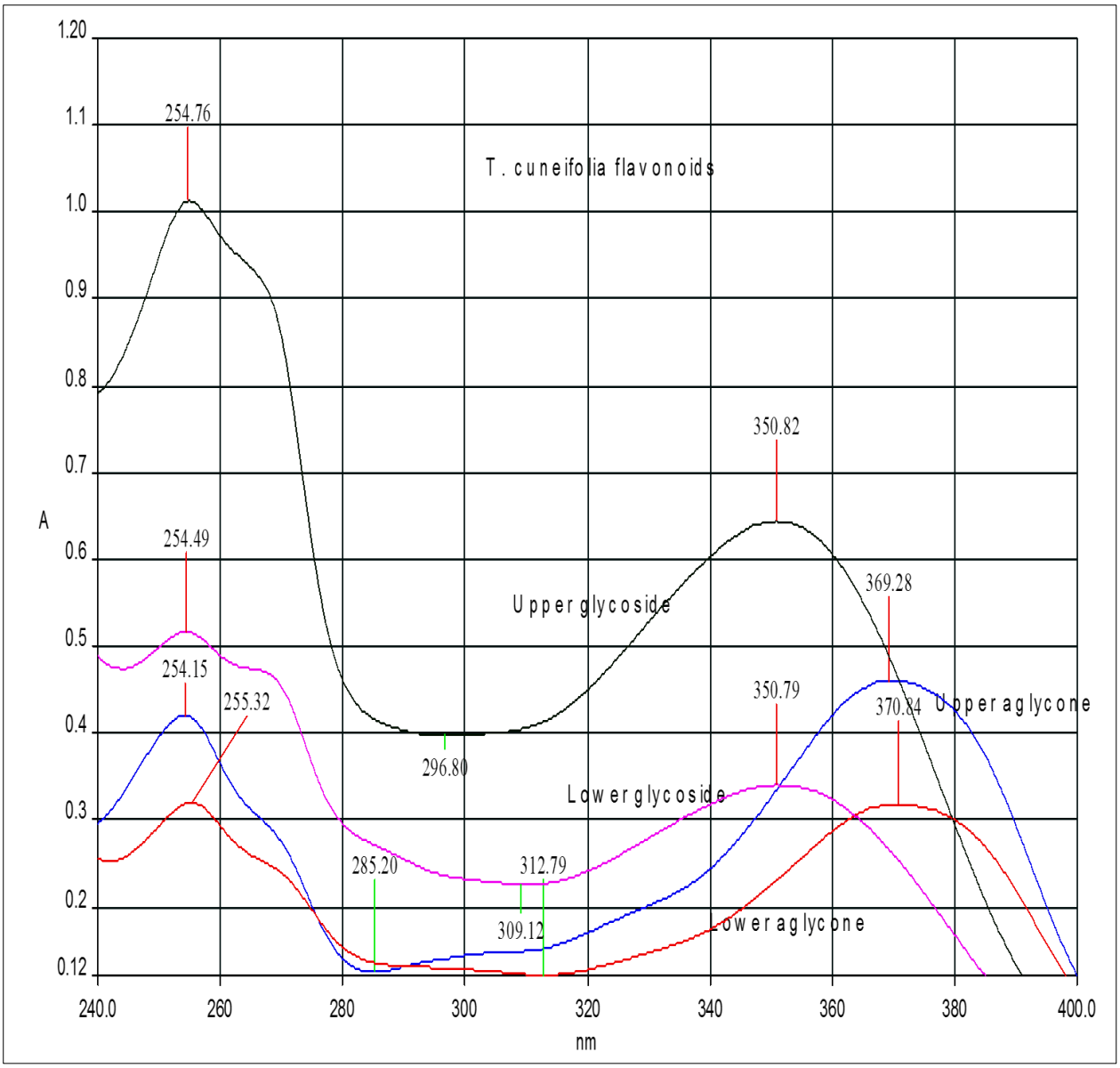


Figure 4.10: *T. cuneifolia* root marked blue (Upper aglycone) (UV spectrum-Spectral max. 254.15, 369.28)

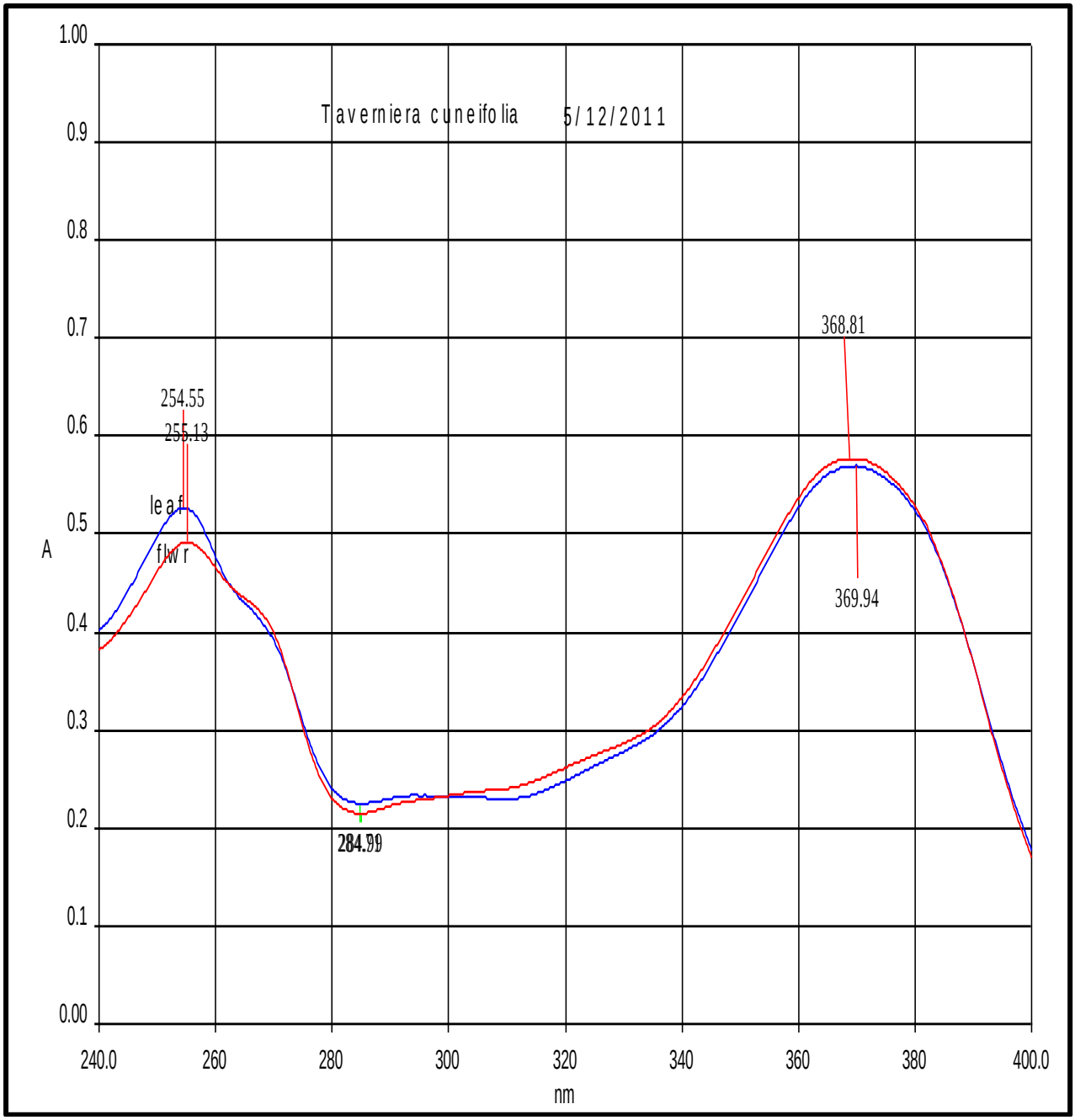


Figure 4.11: UV spectrum
Blue - *T. cuneifolia* leaf (Spectral max. 254.55, 369.94). Red - *T. cuneifolia* flower (Spectral max. 255.13, 368.81)

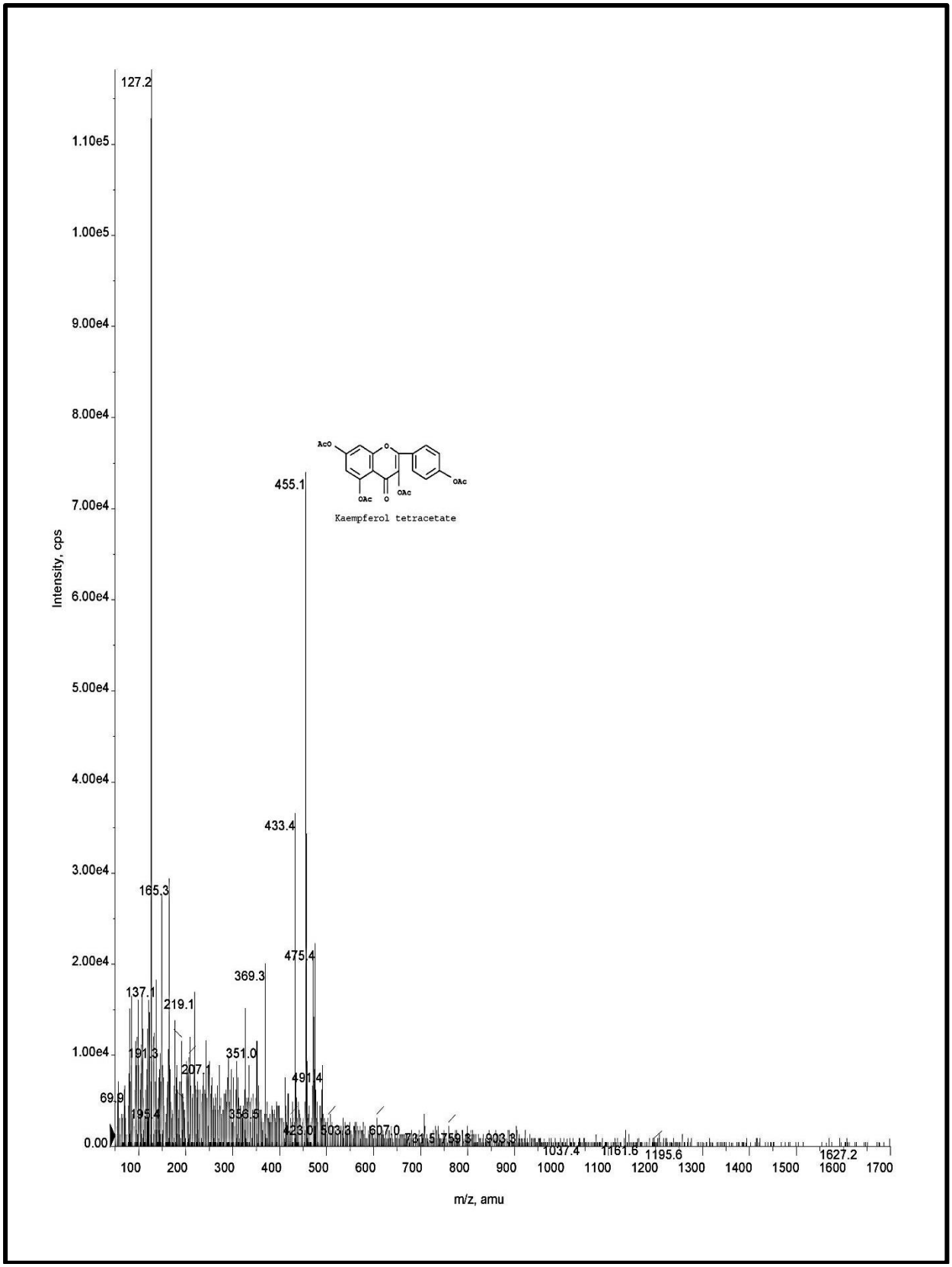


Figure 4.12: LC-MS of Kaempferol tetracetate

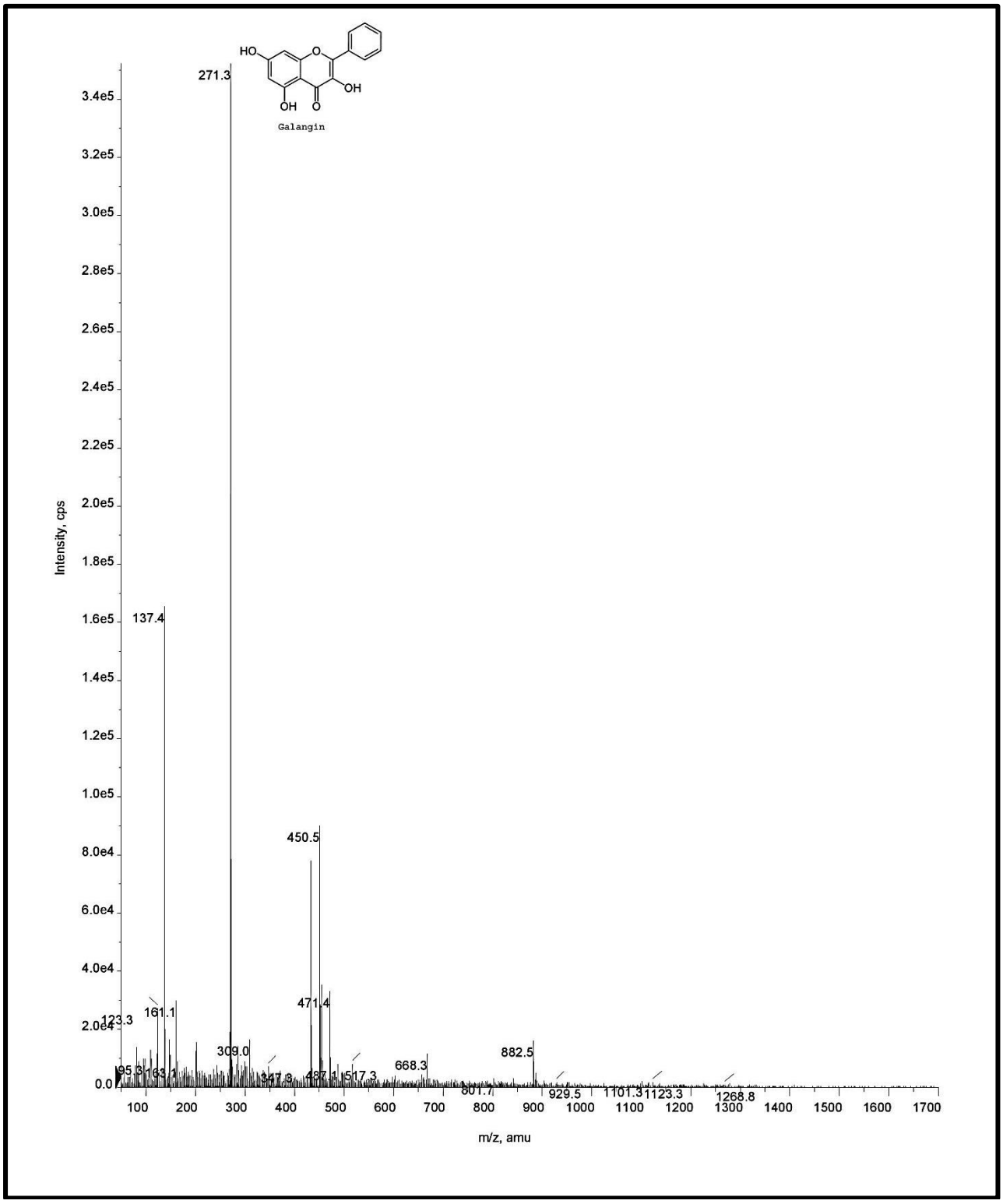


Figure 4.13: LCMS of Galangin

4.2.6 Flavones:

Flavones are a class of flavonoids based on the backbone of 2-phenylchromen-4-one (2-phenyl-1-benzopyran-4-one) shown on the right.Natural flavones include Apigenin (4',5,7-trihydroxyflavone), Luteolin (3',4',5,7-tetrahydroxyflavone) and Tangeritin (4',5,6,7,8-penta methoxyflavone),chrysin(5,7-OH), 6-hydroxyflavone, baicalein (5,6,7-trihydroxyflavone), scutellarein (5,6,7,4'-tetrahydroxyflavone), wogonin (5,7 -OH, 8 -OCH_3). Synthetic flavones are Diosmin and Flavoxate.

Seven different types of possible flavones were recorded in the roots of *T. cuneifolia* this includes, 5,7-diacetoxy,8-methoxy flavone, Acacetine, 3',4',5',3,5,7, 8 – heptamethoxy flavone, 3',4',5',3,5,6,7- heptamethoxyflavone, Luteolin, Apigenin, 2',5,6',7-Tetrahydroxyflavone. Eg. Fig. 4.14 - 5,7-diacetoxy,8-methoxy flavone

Of these Apigenin and luteolin are common Flavones found in other Fabaceae members. Apigenin has been also reported in *Glycyrrhiza glabra* (Singh *et al*., 2009) and *Taverniera lappacea* (Ibrahim and Mohammed, 2009) Flavonones have been extensively used as chemotaxonomic markers and are abundant in Polygonaceae, Rutaceae, Leguminosae, Umbelliferae and Compositae (Trease and Evans 2002).

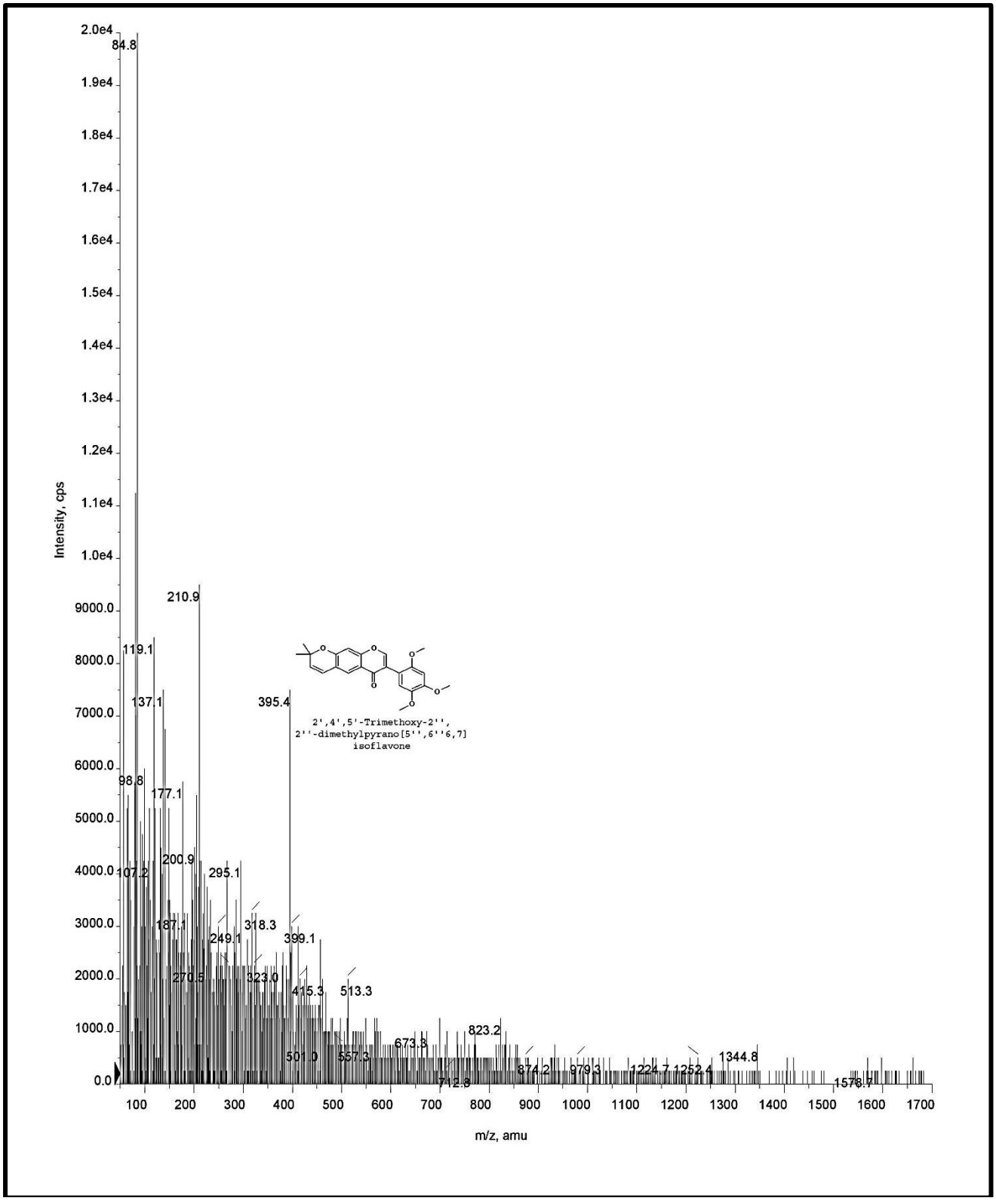


Figure 4.13: LCMS of 2’,4’,5’-trimethoxy-2",2"-dimethylpyrano[5",6":6,7] isoflavone

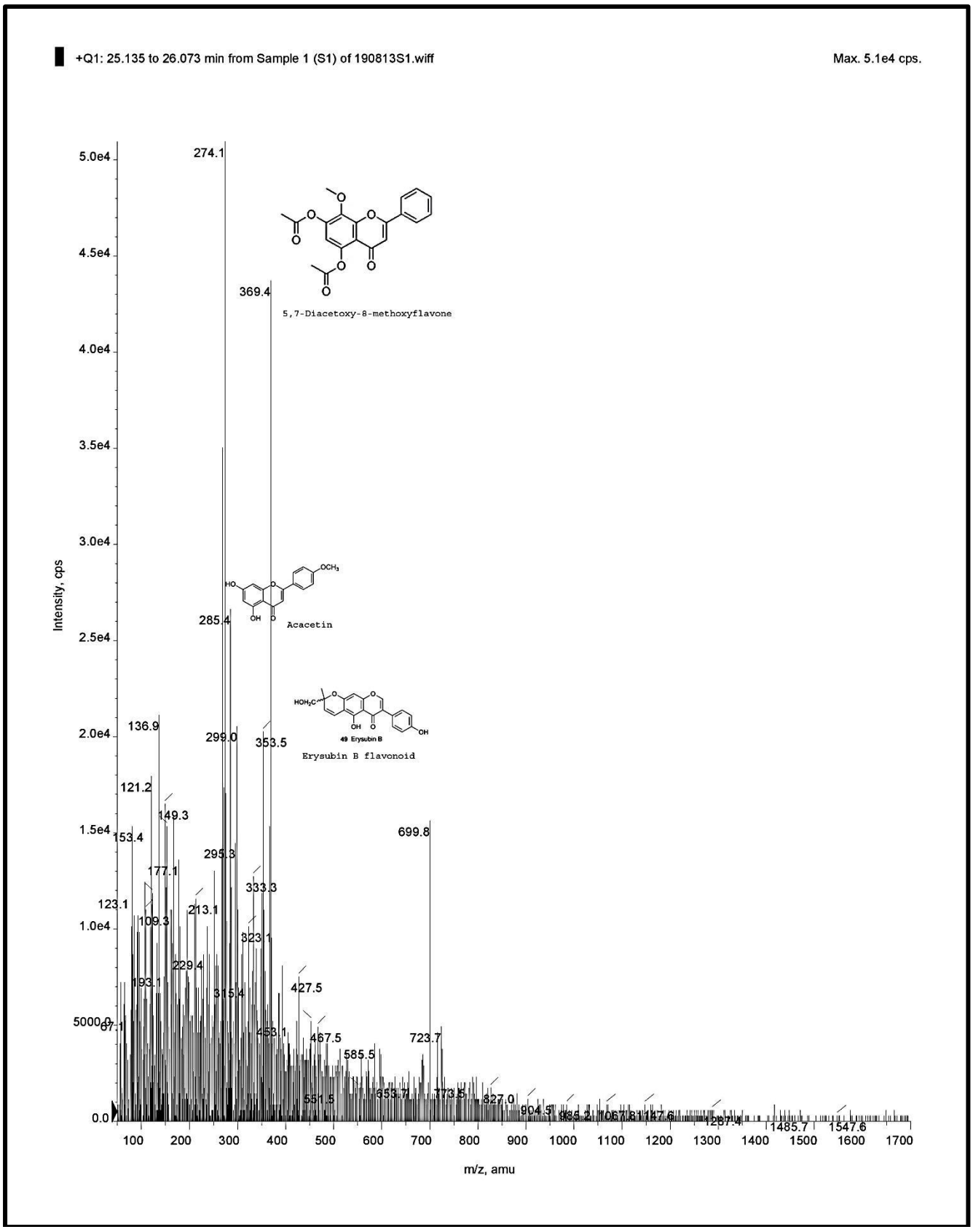


Figure 4.14: LC-MS of 5, 7-diacetoxy,8-methoxy flavone, Acacetine (flavones)

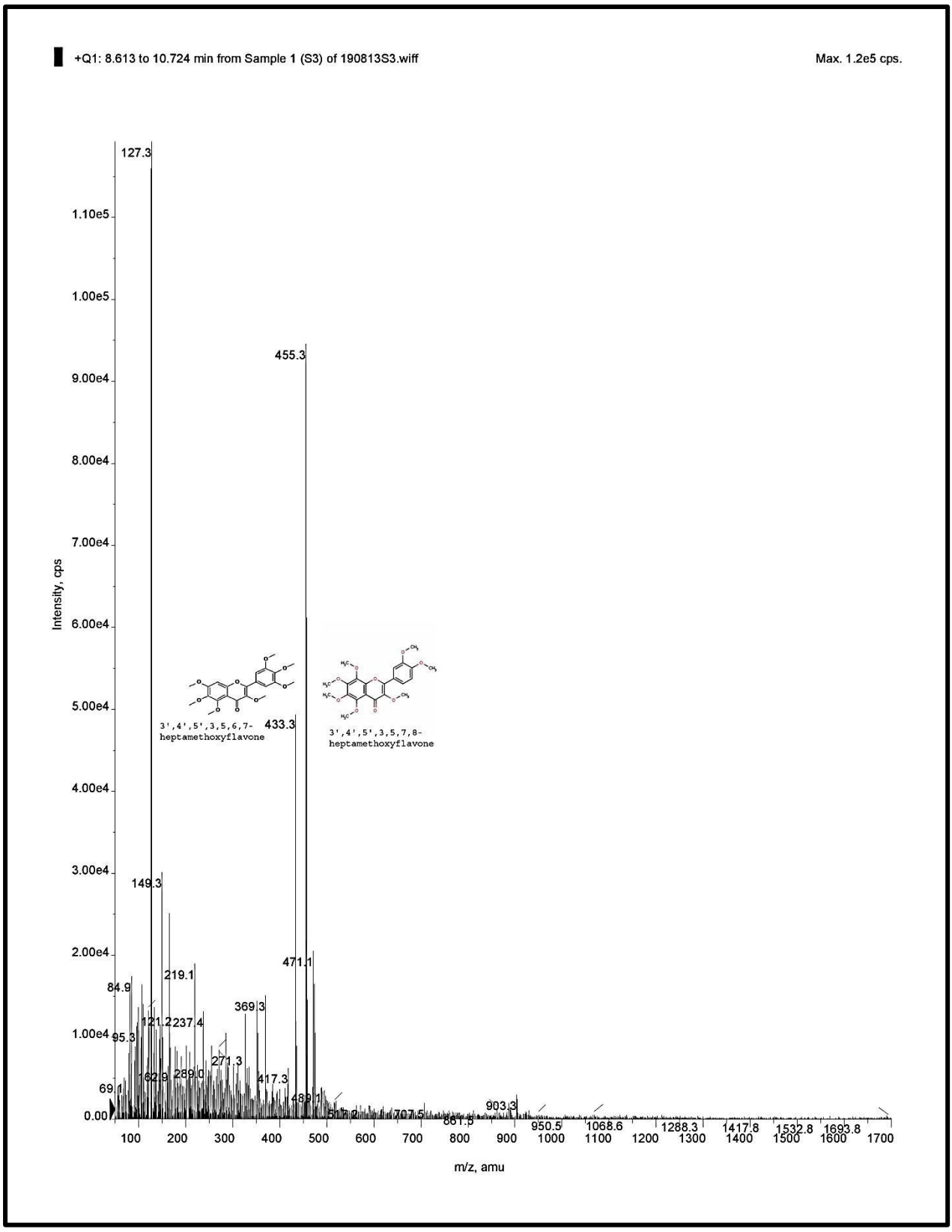


Figure 4.15: 3',4',5',3,5,7, 8 – heptamethoxyflavone , 3',4',5',3,5,6,7- heptamethoxyflavone

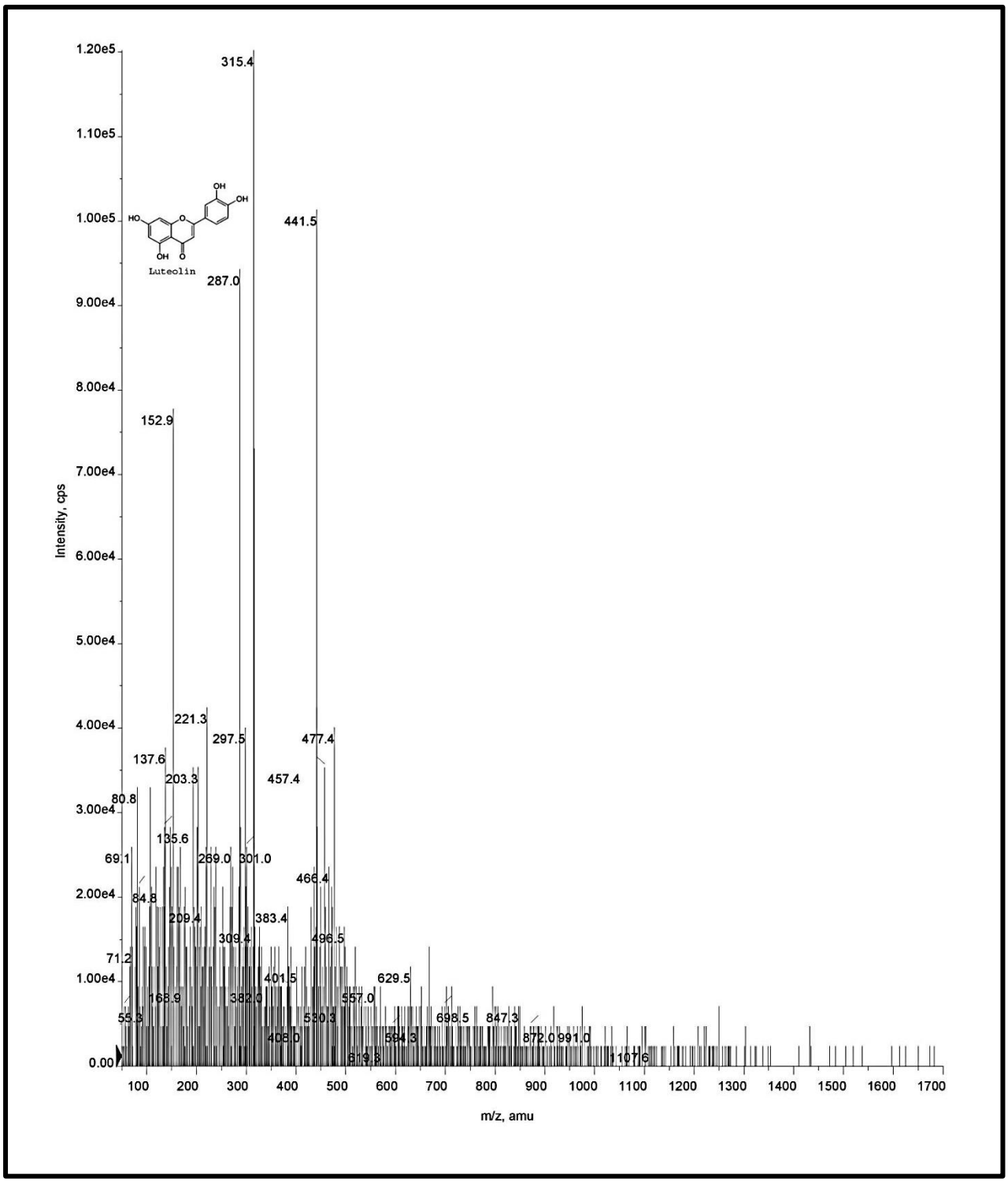


Figure 4.16: LCMS of Luteolin

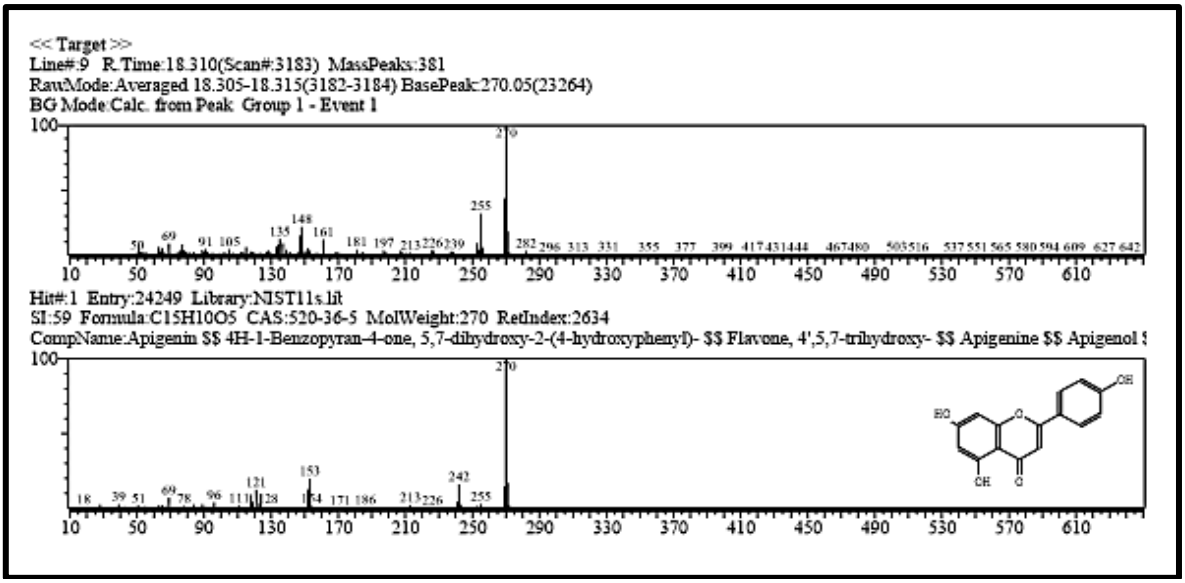


Figure 4.17: GCMS of Apigenin

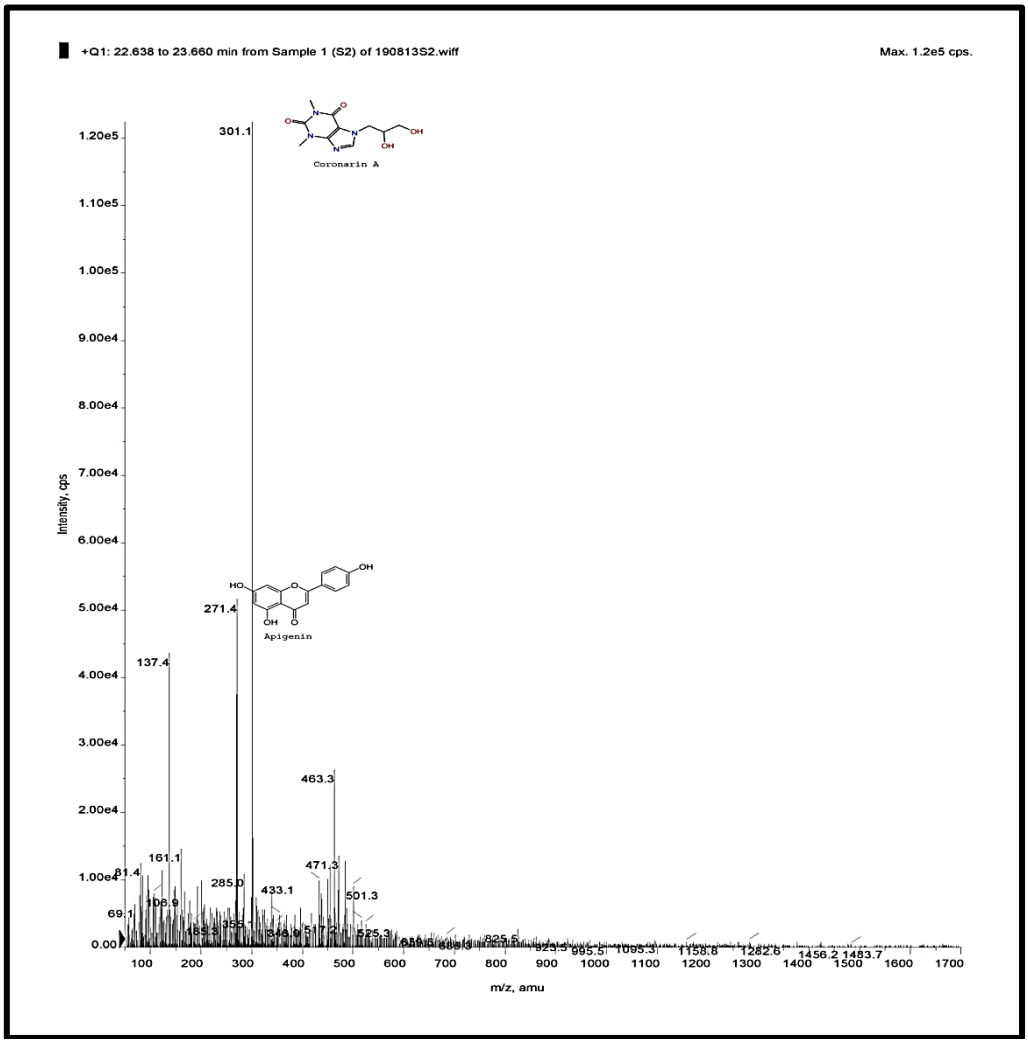


Figure 4.18: LC-MS of Apigenin

Flavanone: Flavanone found in the *T. cuneifolia* roots are 3-O-Acetyl pinobanskin

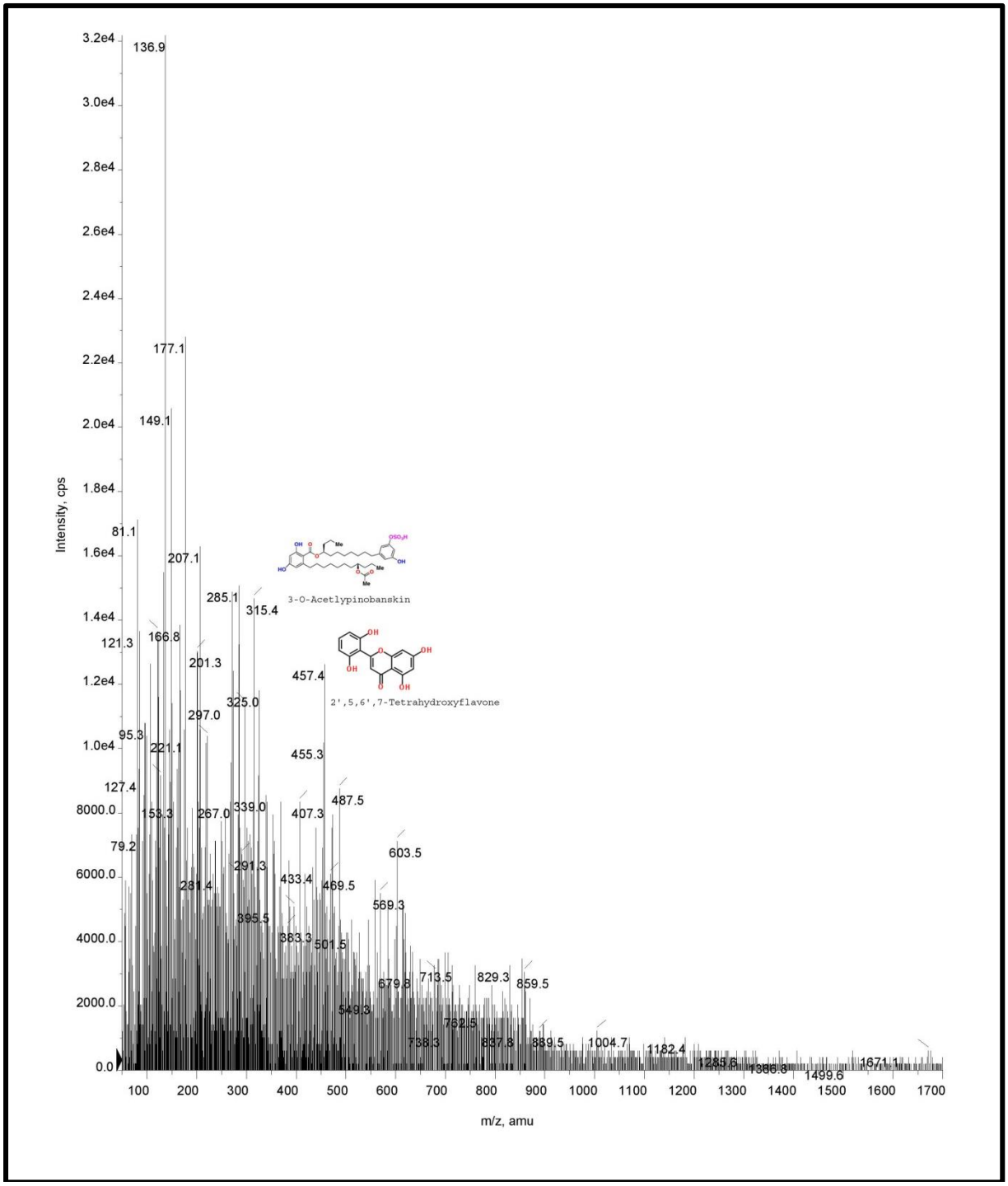


Figure 4.19: LC-MS of 3-O-Acetyl pinobanskin

4.2.7 Flavan 3-ol:

Flavan-3-ols and their polymeric condensation products, the proanthocyanidins, are regarded as functional ingredients in various beverages, whole and processed foods, herbal remedies and supplements. Their presence in food affects food quality parameters such as astringency, bitterness, sourness, sweetness, salivary viscosity, aroma, and color formation. The ability of flavan-3-ols to aid food functionality has also been established in terms of microbial stability, foamability, oxidative stability, and heat stability. While some foods only contain monomeric flavan-3-ols [(–)-epicatechin predominates] and dimeric proanthocyanidins, most foods contain oligomers of degree of polymerization values ranging from 1–10 or greater than 10. Flavan-3-ols have been reported to exhibit several health beneficial effects by acting as antioxidant, anticarcinogen, cardiopreventive, antimicrobial, anti-viral, and neuro-protective agents. This review summarizes the distribution and health effects of these compounds (Aron and Kennedy, 2008). **Catechin 7-xyloside and Epiafzelechin are the possible Flavan 3-ol found in *T.cuneifolia.***

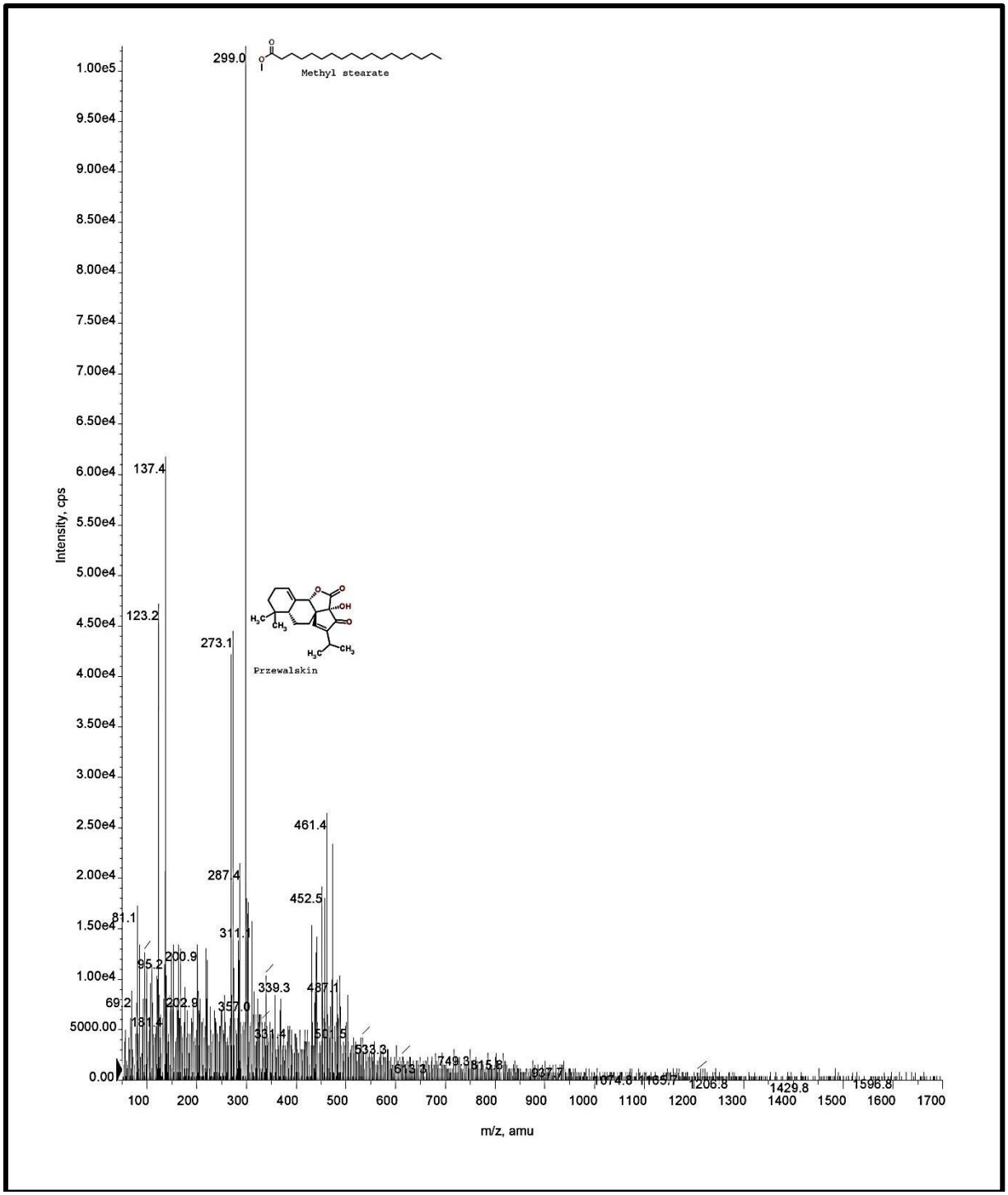


Figure 4.20: LC-MS of Przewalskin

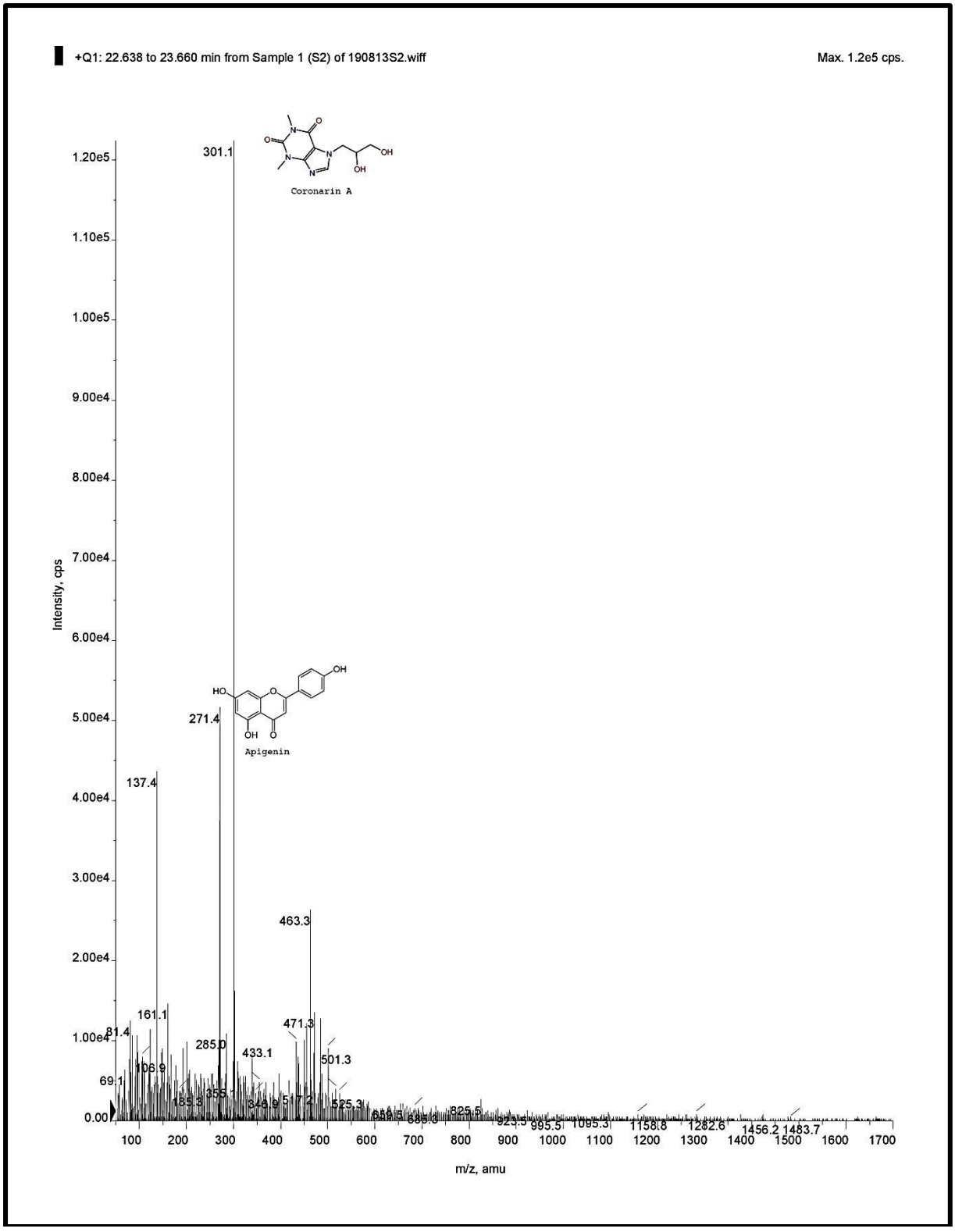


Figure 4.21: LC-MS of Coronarin A

4.2.8 Sterols:

Sterols, also known as steroid alcohols, are a subgroup of the steroids and an important class of organic molecules. They occur naturally in plants, animals, and fungi. Phytosterols, which encompass plant sterols and stanols, are steroid compounds similar to cholesterol which occur in plants and vary only in carbon side chains and/or presence or absence of a double bond. Stanols are saturated sterols, having no double bonds in the sterol ring structure. More than 200 sterols and related compounds have been identified (Akhisa and Kokke, 1991) Free phytosterols extracted from oils are insoluble in water, relatively insoluble in oil, and soluble in alcohols.Phytosterol-enriched foods and dietary supplements have been marketed for decades (Weingartner *et al*., 2008).

HPLC and GCMS analysis *T. cuneifolia* roots showed the presence of lupeol, β-sitosterol and Stigmasterol (Fig 4.22, 4.23, plate photo 4.1). Lupeol and β-sitosterol has also been also reported before by Khan *et al*., 2012 from the *T. cuneifolia* roots.

Lupeol is a pharmacologically active triterpenoid. It has several medicinal properties, one being anti-inflammatory. Lupeol has a complex pharmacology in humans, displaying antiprotozoal, antimicrobial, antiinflammatory, antitumor and chemopreventive properties. (Gallo and Sarachin, 2009) It is an effective inhibitor in laboratory models of prostate and skin cancers (Prasad *et al*., 2008; Nigam *et al*., 2007; Saleem *et al*., 2004). Lupeol has been reported in various fabaceae members (Pokle, 2012)

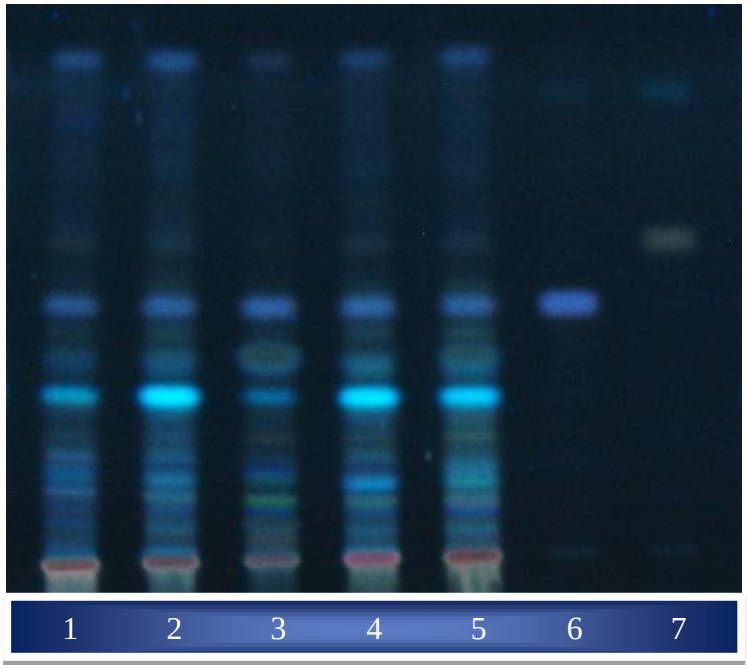
β-Sitosterol is one of several phytosterols (plant sterols) with chemical structures similar to that of cholesterol. β-sitosterol reduces blood levels of cholesterol, and is sometimes used in treating hypercholesterolemia β-Sitosterol inhibits cholesterol absorption in the intestine (Matsuoka *et al*., 2003).

Stigmasterol is an unsaturated plant sterol occurring in the plant fats or oils of soybean, calabar bean, and rape seed, and in a number of medicinal herbs, including the Chinese herbs *Ophiopogon japonicus* (Mai men dong), in *Mirabilis jalapa* (Siddiqui *et al*., 1990)

Lupeol and β-sitosterol (Plate 4.1)

HPTLC plate photo representing regional variation of roots of *T. cuneifolia* with standards β-sitosterol and Lupeol

Plate Photo at 336nm



Track 1 Kutch

Track 2 Jamnagar

Track 3 Andhra Pradesh

Track 4 Jodhpur

Track 5 Rajkot

Track 6 Beta-sitosterol

Track 7 Lupeol

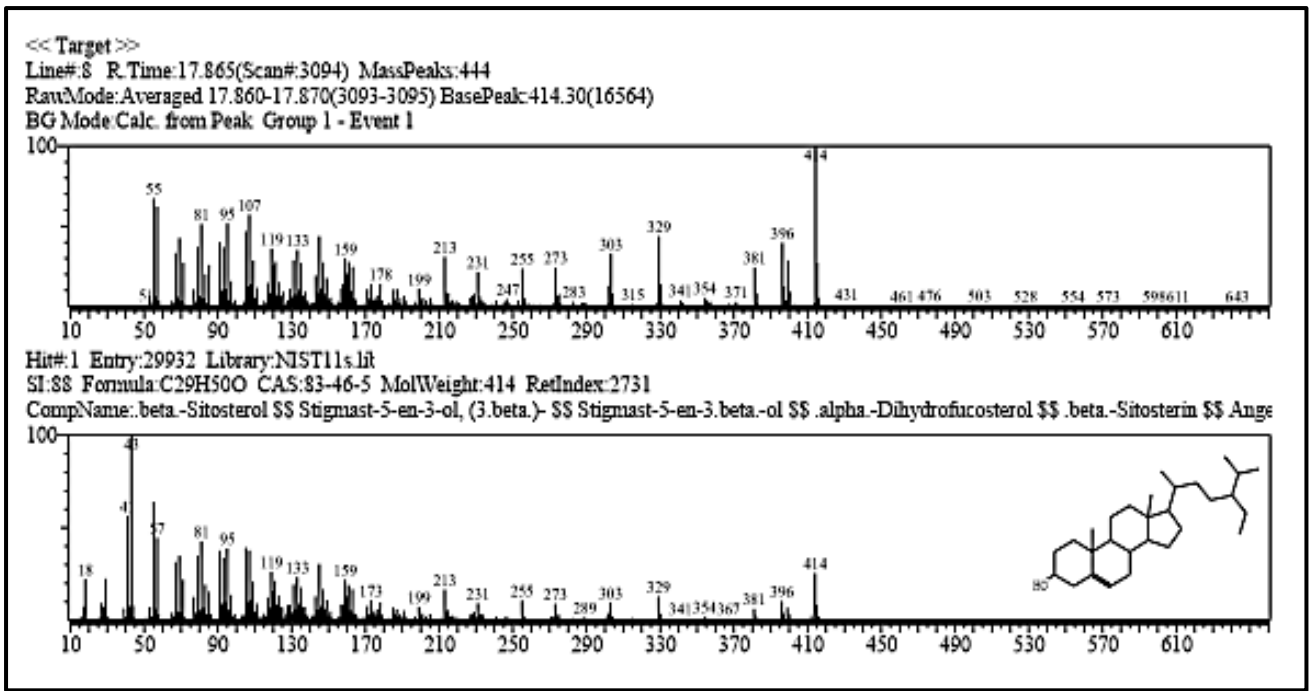


Figure 4.22 : GC-MS data for β-sitosterol

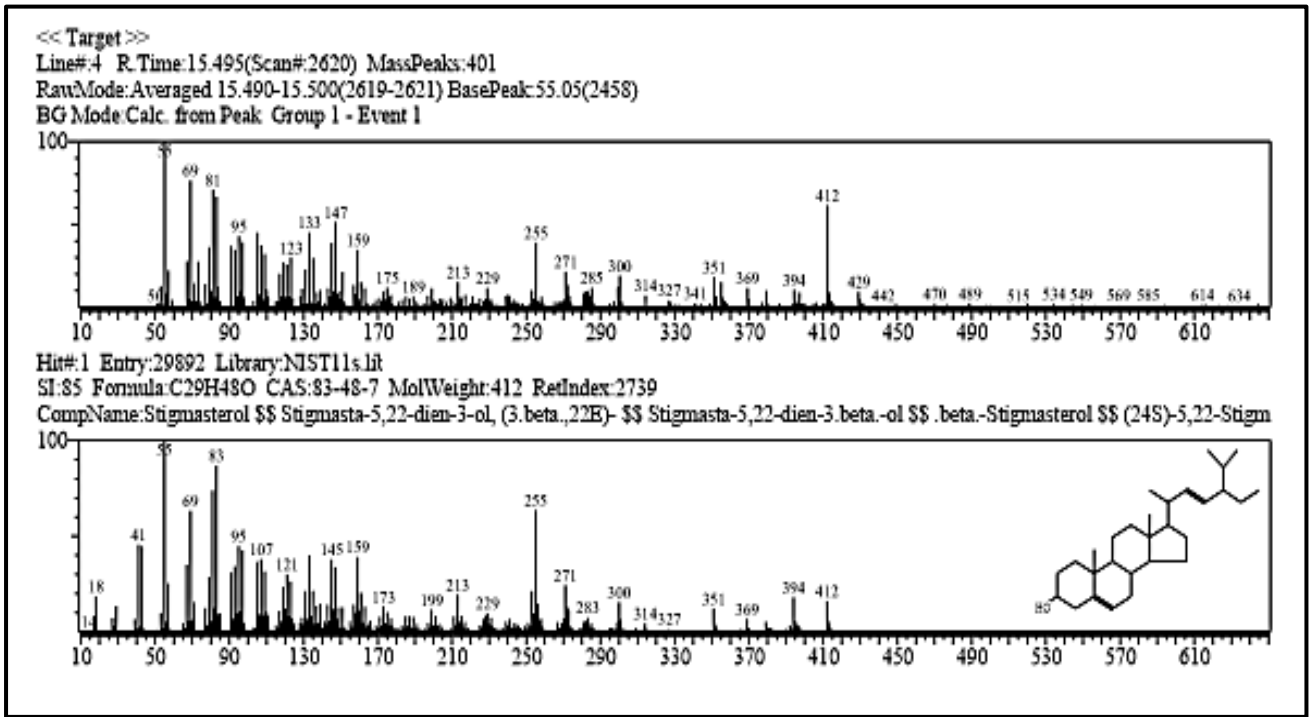


Figure 4.23: GC-MS data for Stigmasterol

4.2.9 Triterpenes:

Triterpenes are terpenes consisting of six isoprene units and have the molecular formula $C_{30}H_{48}$. A notable pentacyclic triterpene is Boswellic acid. Animals, plants, and fungi, create triterpenes, like, squalene, ambrein, and ganoderic acid (Melanie, 2009).

***T. cuneifolia* roots showed the presence of α-amyrin and β-amyrin both by LCMS and GCMS analysis. Eichlerialactone might be also present in the roots as depicted in LCMS peaks (Fig 4.25, 4.26).**

Amyrins are widely distributed in nature and have been isolated from a variety of plant sources. It has been reported from *Glycyrrhiza glabra* and other fabaceae members. Mixture of both α-amyrin and β-amyrin have shown antinociceptive property (Otuki *et al*., 2005), Antihyperglycemic and hypolipidemic properties (Almeida *et al*., 2012**).** Eichlerialactone found in the members of Meliaceae family **(Anonymous, 2012)**

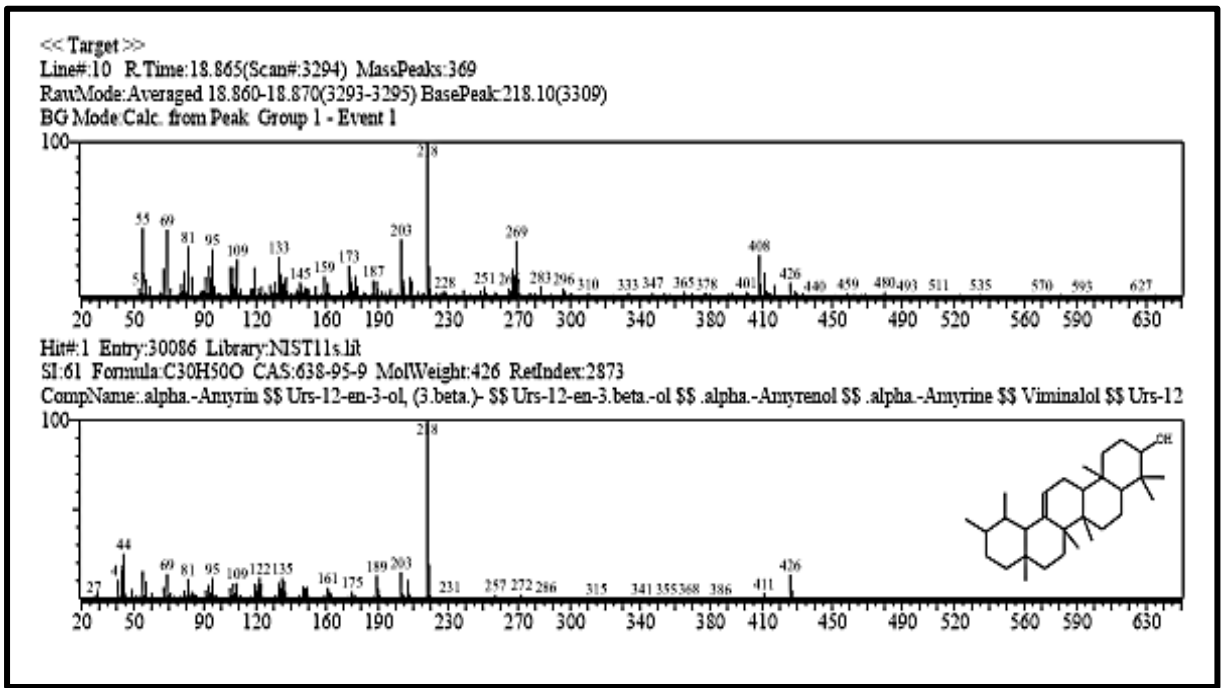


Figure 4.24: GC-MS data for α-amyrin

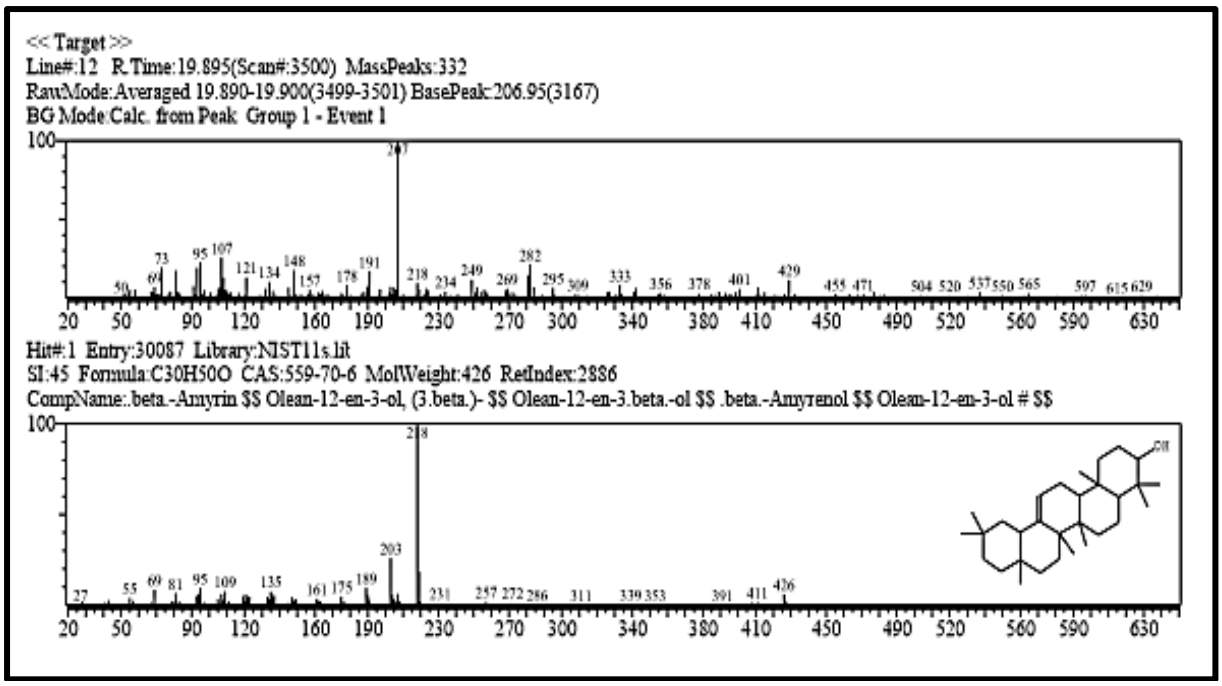


Figure 4.25: GC-MS data for β-Amyrin

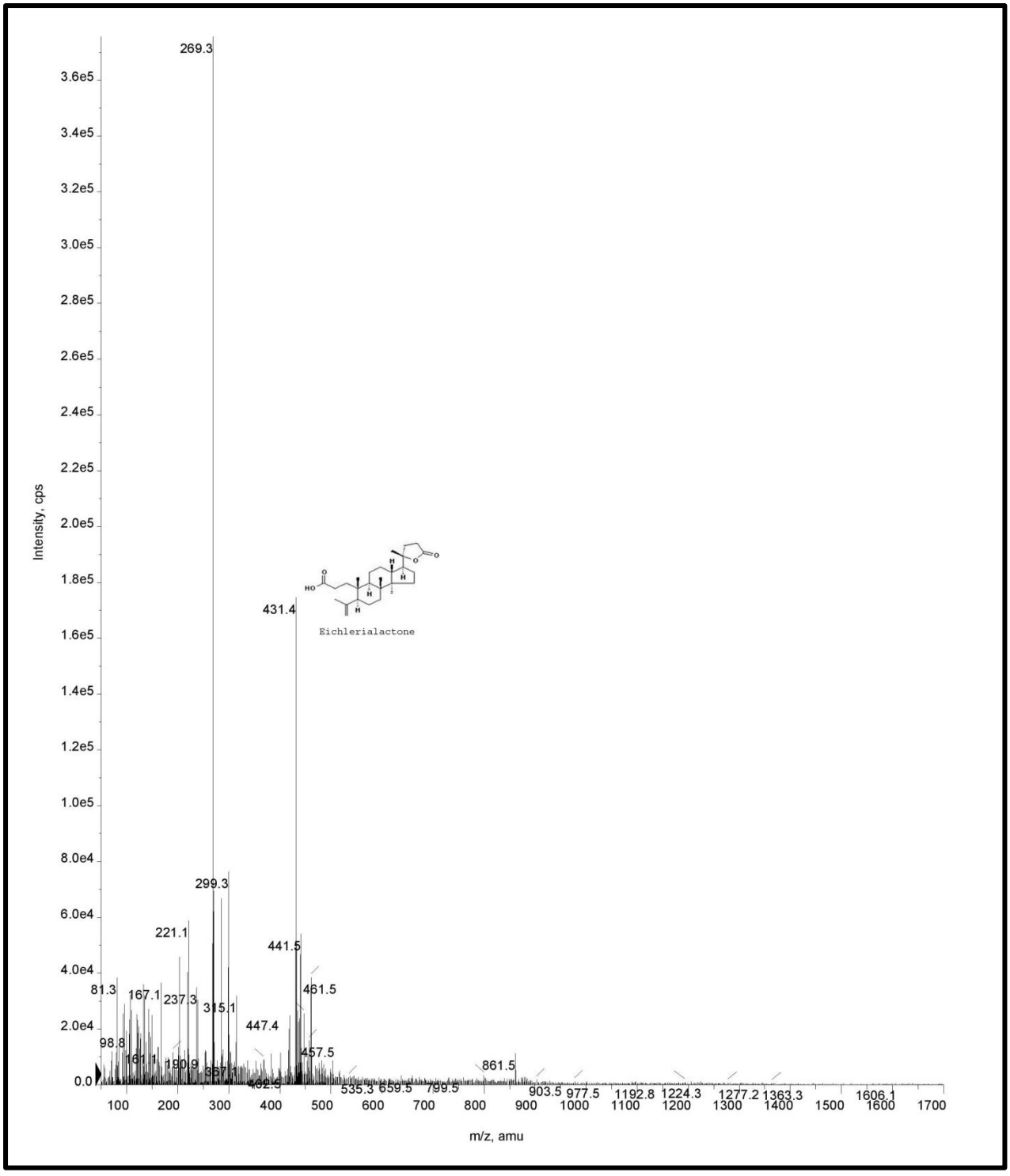


Figure 4.26: Eichlerialactone

4.2.10 FATTY ACIDS

Fatty acid is a carboxylic acid with a long aliphatic tail (chain), which is either saturated or unsaturated. Most naturally occurring fatty acids have a chain of an even number of carbon atoms, from 4 to 28 (Anonymous, 1997). Fatty acids are usually derived from triglycerides or phospholipids. When they are not attached to other molecules, they are known as "free" fatty acids. Fatty acids are important sources of fuel because, when metabolized, they yield large quantities of ATP. Many cell types can use either glucose or fatty acids for this purpose. In particular, heart and skeletal muscle prefer fatty acids. Despite long-standing assertions to the contrary, the brain can use fatty acids as a source of fuel (Ebert *et al*., 2003; Valencia, 2012) in addition to glucose and ketone bodies. Fatty acids that have carbon–carbon double bonds are known as unsaturated. Fatty acids without double bonds are known as saturated. They differ in length as well.

Essential fatty acids

Fatty acids that are required by the human body but cannot be made in sufficient quantity from other substrates, and therefore must be obtained from food, are called essential fatty acids. There are two series of essential fatty acids linoleic acid (LA) and alpha-linolenic acid (ALA). They are widely distributed in plant oils.

GCMS analysis of *T. cuneifolia* roots showed the presence of saturated fatty acids such as Caproic acid, Myristic acid, Lauric acid, Palmitic acid, Stearic acid and unsaturated fatty acid - Linoleic acid and Oleic acid (Fig. 4.27 – 4.37). The presence of fatty acids in roots was also been reflected by the froth formation**. The fruits also showed the presence of Stearic acid, Oleic acid, Linoleic acid and Palmitic acid.**

Hexanoic acid (**caproic acid**) is a fatty acid found naturally in various animal fats and oils, and is one of the chemicals that give the decomposing fleshy seed coat of the ginkgo its characteristic unpleasant odor. (http://scidiv.bellevuecollege.edu/rkr/Ginkgo/Ginkgo.html). It is also one of the components of vanilla. The oil of *T.cunefolia* too has a characteristic odor. The primary use of hexanoic acid is in the manufacture of its esters for artificial flavors, and in the manufacture of hexylderivatives, such as hexylphenols **(Anonymous, 1989)**

Myristic acid is named after the nutmeg *Myristica fragrans*. Nutmeg butter is 75% trimyristin, the triglyceride of myristic acid. It has also been found in palm kernel oil, coconut oil, butter fat and is a minor component of many other animal fats. The

ester isopropyl myristate is used in cosmetic and topical medicinal preparations where good absorption through the skin is desired. Reduction of myristic acid yields myristyl aldehyde and myristyl alcohol (Anonymous, 2001).

Lauric acid, as a component of triglycerides, comprises about half of the fatty acid content in coconut oil, laurel oil, and in palm kernel oil (Beare-Rogers, 2001; Anneken et al., 2006)

It has shown antimicrobial properties (Hoffman et al., 2001; Ouattar et al., 2000; Dawson et al., 2002; Ruzin and Novick, 2000).

Palmitic acid, or **hexadecanoic acid** as its name indicates, it is a major component of the oil from palm trees (palm oil,palm kernel, and palm kernel oil). It is mainly used to produce soaps, cosmetics, and release agents. Sodium palmitate is permitted as a natural additive in organic products (US Soil Association standard 50.5.3).

Stearic acid is one of the most common saturated fatty acids found in nature following palmitic acid. Stearic acid is mainly used in the production of detergents, soaps, and cosmetics such as shampoos and shaving cream products.

Linoleic acid (LA) is an unsaturated omega-6 fatty acid. It belongs to one of the two families of essential fatty acids. The body cannot synthesize linoleic acid from other food components (Burr et al., 1930). It has become increasingly popular in the beauty products industry because of its beneficial properties on the skin. Research points to linoleic acid's antioxidant, anti-inflammatory, acne reductive, and moisture retentive properties when applied topically on the skin. (Anonymous, 1993, Letawe, 1998; Darmstadt, 2002, Peyrat-Maillard, 2003). Linoleic acid may be linked to obesity by promoting overeating and damaging the arcuate nucleus in the brain's hypothalamus (Raloff, 2012).

Oleic acid is a fatty acid that occurs naturally in various animal and vegetable fats and oils. Oleic acid (in triglyceride form) is included in normal human diet as part of animal fats and vegetable oil. Oleic acid as its sodium salt is a major component of soap as an emulsifying agent. It is also used as emollient (Carrasco, 2009).

Fatty Acid Compostion Of Seed

T. cuneifolia fruits were extracted which resulted in 6.24 ± 0.41% oil. The study shows that the oil is composed of four fatty acids with 25.73% saturated fatty acids and 68.69%

unsaturated fatty acids. The saturated fatty acids are palmitic acid (20.72%) and stearic acid (5.01%) while unsaturated fatty acid includes oleic acid (16.99%) and linoleic acid (6-omega FA - 51.70%). Even the fatty acid composition of *Glycyrrhiza uralensis* showed a composition of 61.84% consisting of linoleic acid 24.3%, α-linolenic acid 25.51%, stearic acid 3.02% and palmitic acid 7.98%. The oil composition of *T. cuneifolia* is having quite similar Fatty acid composition as that of *Sesamum indicum Madhuca indica*, *Carthamus tinctorius* and *Prunus amygdalus*. The oil composition of *T. cuneifolia* varies with that of fabaceae members like, *Crotolaria juncea, Medicagospps, Arachis hypogea, Glycine max.* The study provided the evidence that the *T. cuneifolia* seed oil has the presence of PUFA, MUFA and SFA. Omega-6 fatty acids (Linoleic acid) are reported from sunflower oil and safflower oil which are known to reduce the risk of cardiovascular disease (Chaiyasit *et al*., 2007). Among n-3 fatty acids [Omega-3], neither long-chain nor short-chain forms were consistently associated with breast cancer risk.

Table 4.15: Fatty acid compostion of the fruits of *T.cuneifolia*

Fatty acid	Isomers	% Composition
Saturated fats		
Palmitic	(16:0)	20.72 ± 5.95
Stearic	(18:0)	5.01 ± 0.96
Monounsaturated fats		
Oleic	(18:1n9c)	16.99 ± 1.90
Polyunsaturated fats		
Linoleic	(18:2n6c)	51.70 ± 3.69
Saturated		25.73 ± 6.91
Unsaturated		68.69 ± 5.59

Table 4.16: Chemical properties of *Taverniera cuneifolia* seed oil

Determination	Present study
Acid value	32.66 ± 3.16
Saponification value	162.55 ± 2.33
Iodine value	88.31 ± 10.96

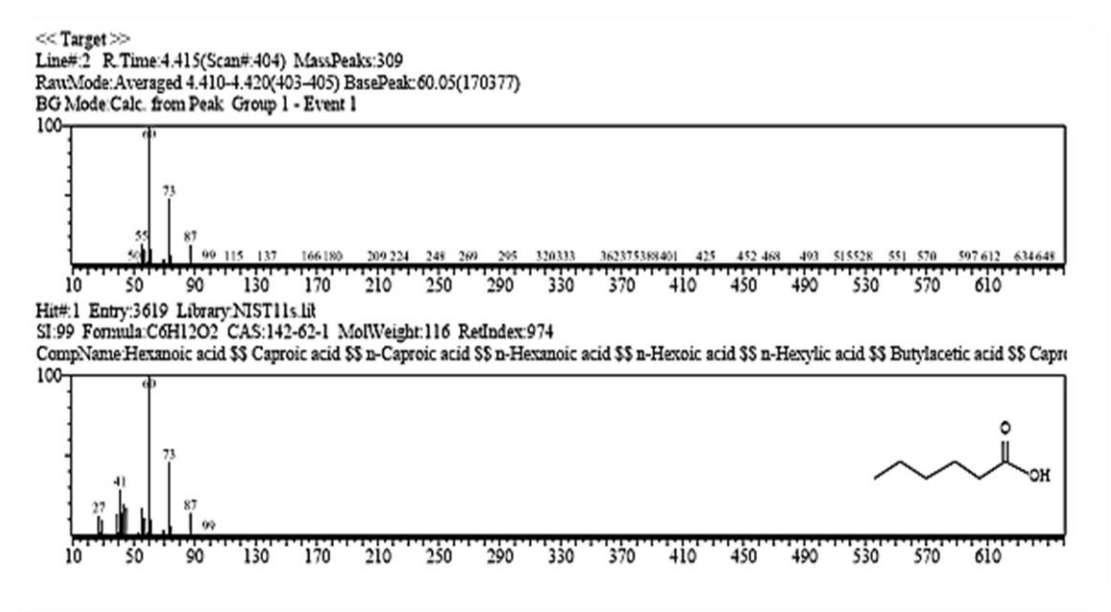


Figure 4.27: GCMS of caproic acid

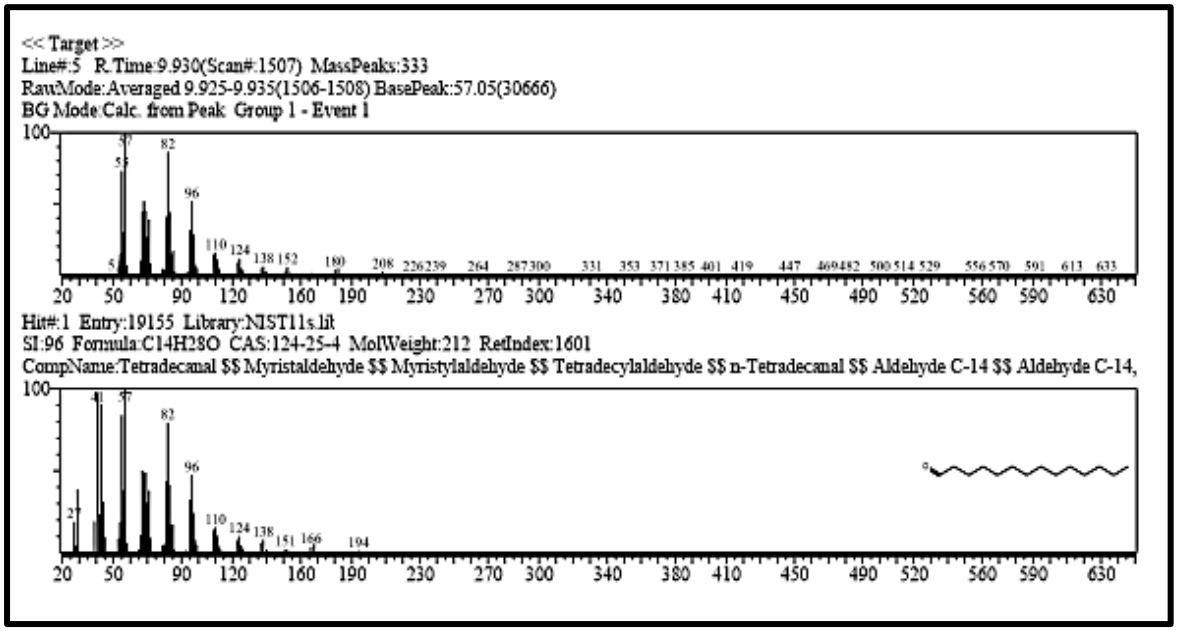


Figure 4.28: GCMS of Myristaldehyde

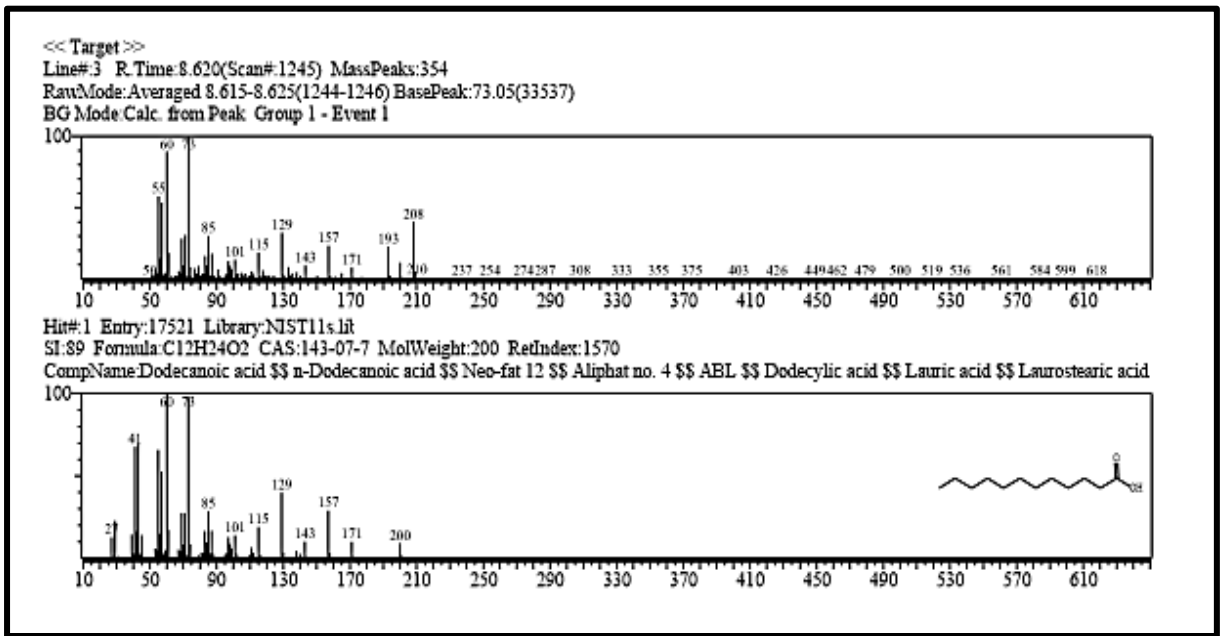


Figure 4.29: GCMS of Lauric acid (Saturated Fatty Acid)

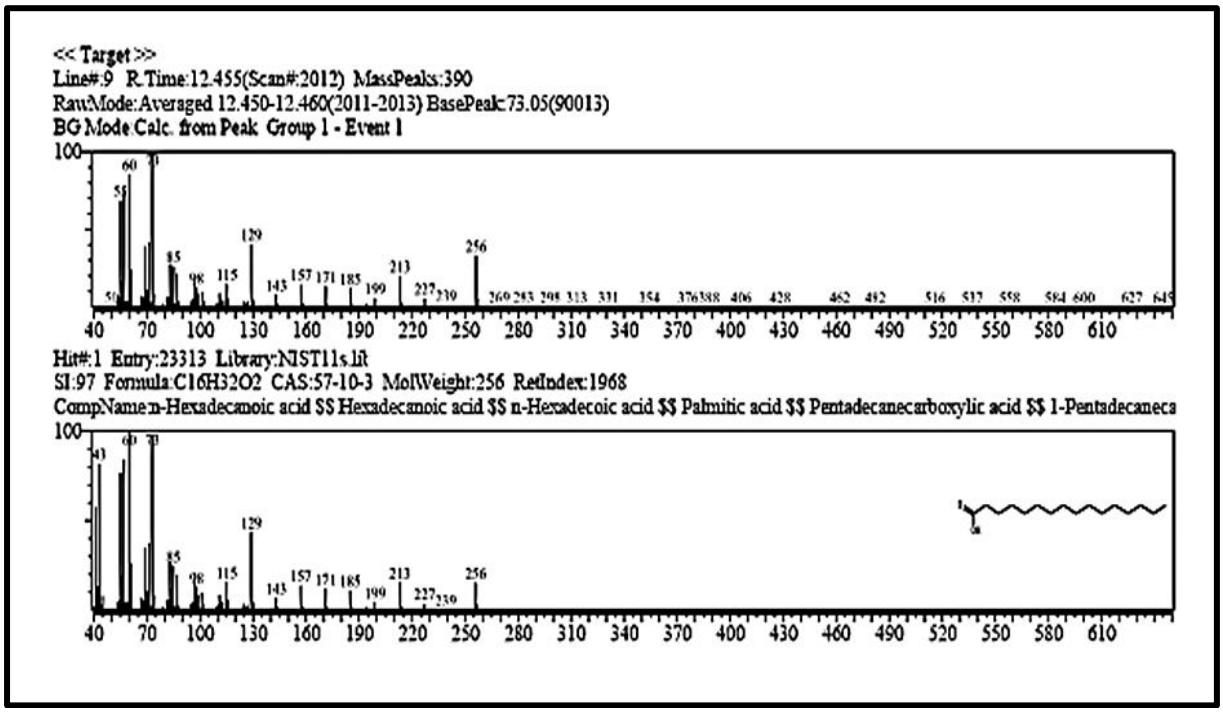


Figure 4.30 GCMS of palmitic Acid

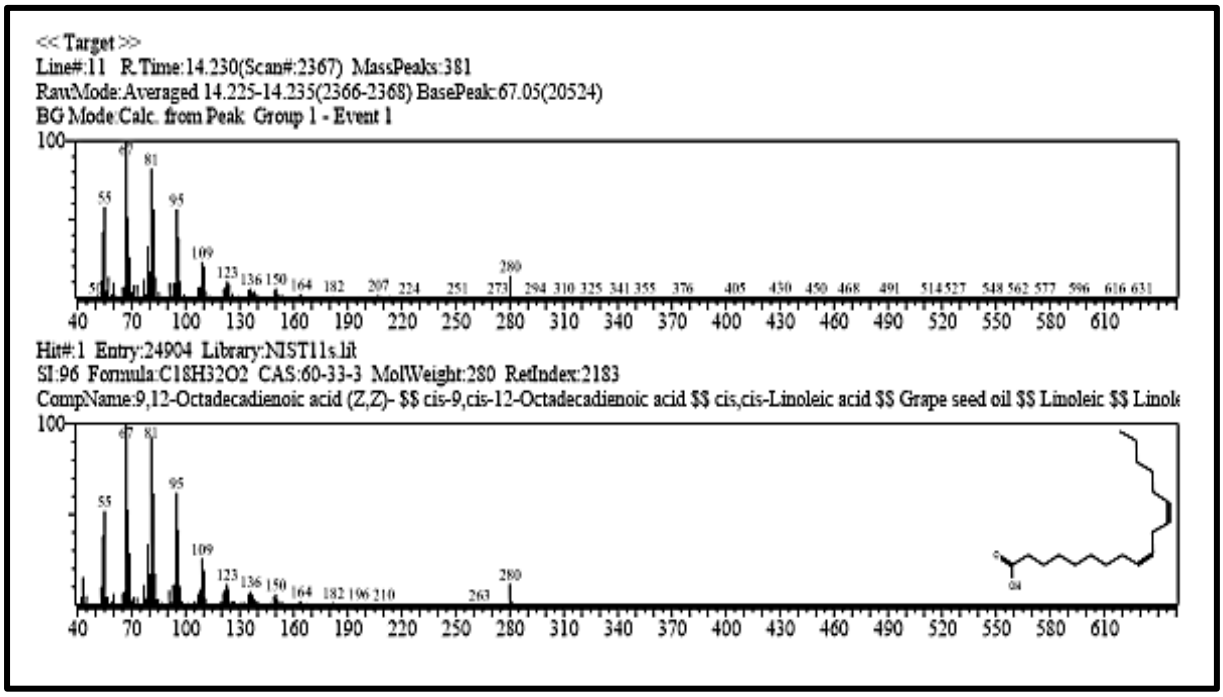


Figure 4.31: GCMS of cis, cis linoleic acid

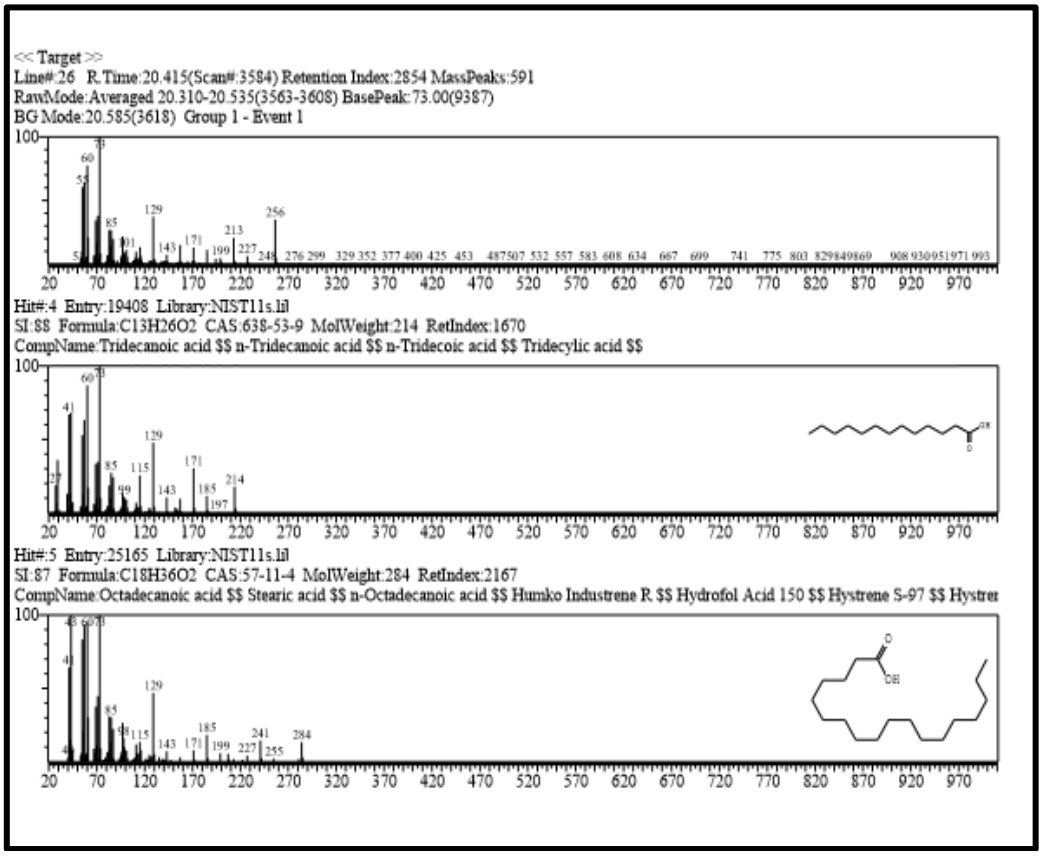


Figure 4.32: GCMS of stearic acid

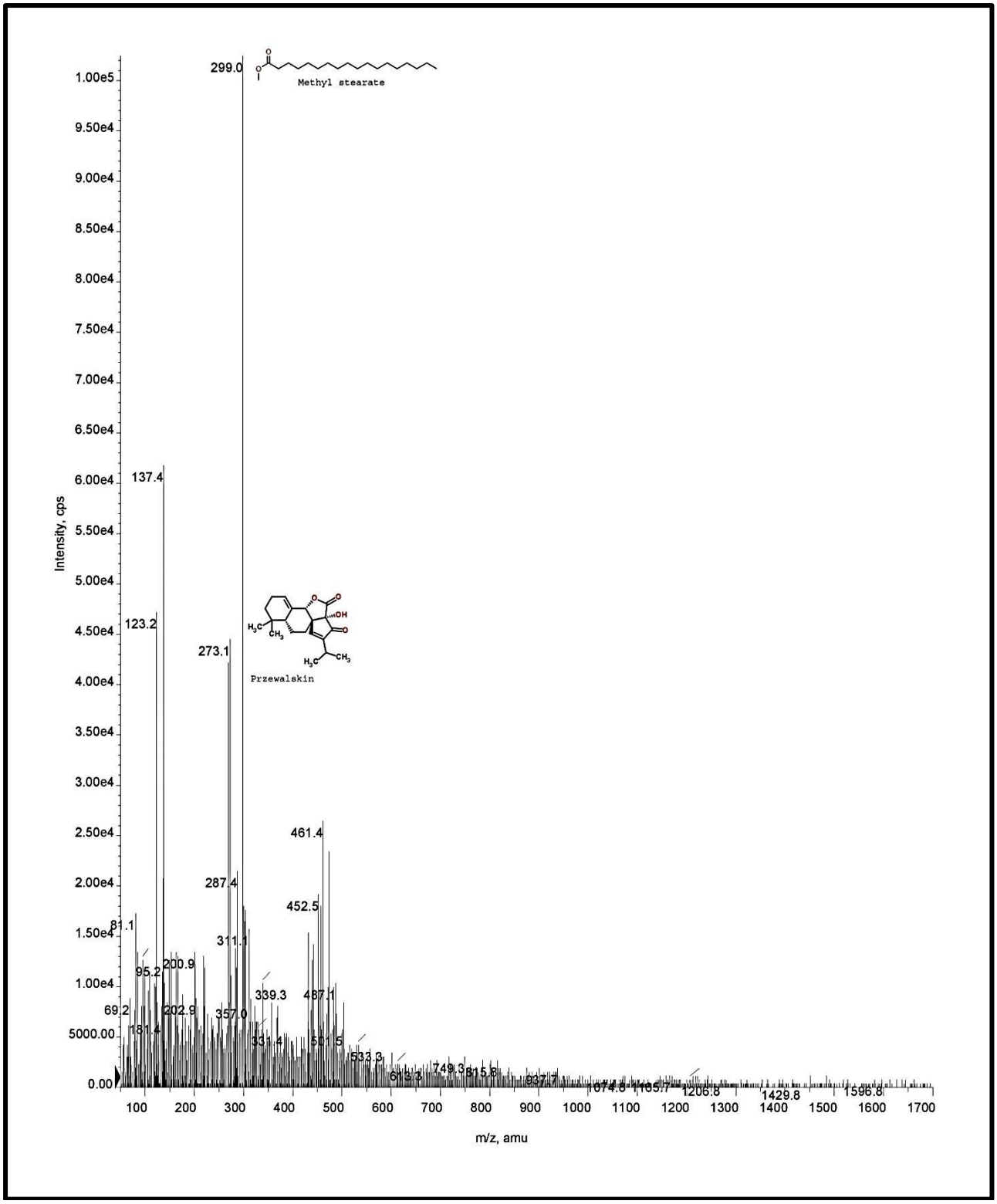


Figure 4.33: LCMS of Methyl stearate (Stearic acid)

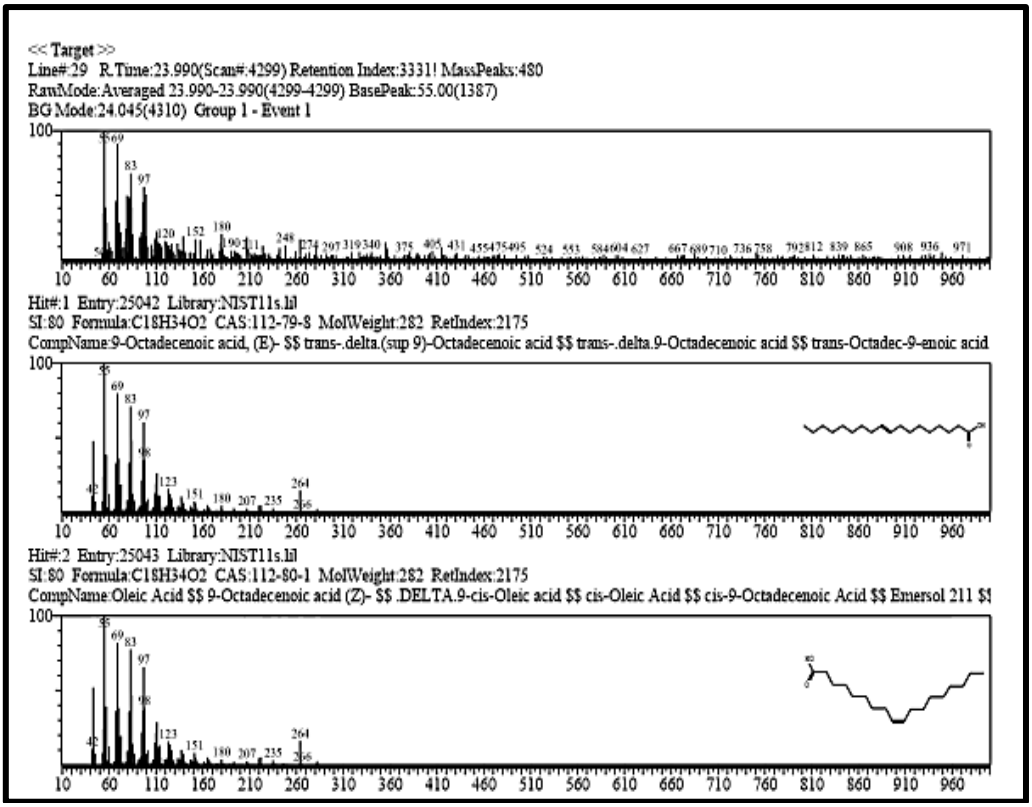


Figure 4.34: GCMS of oleic acid

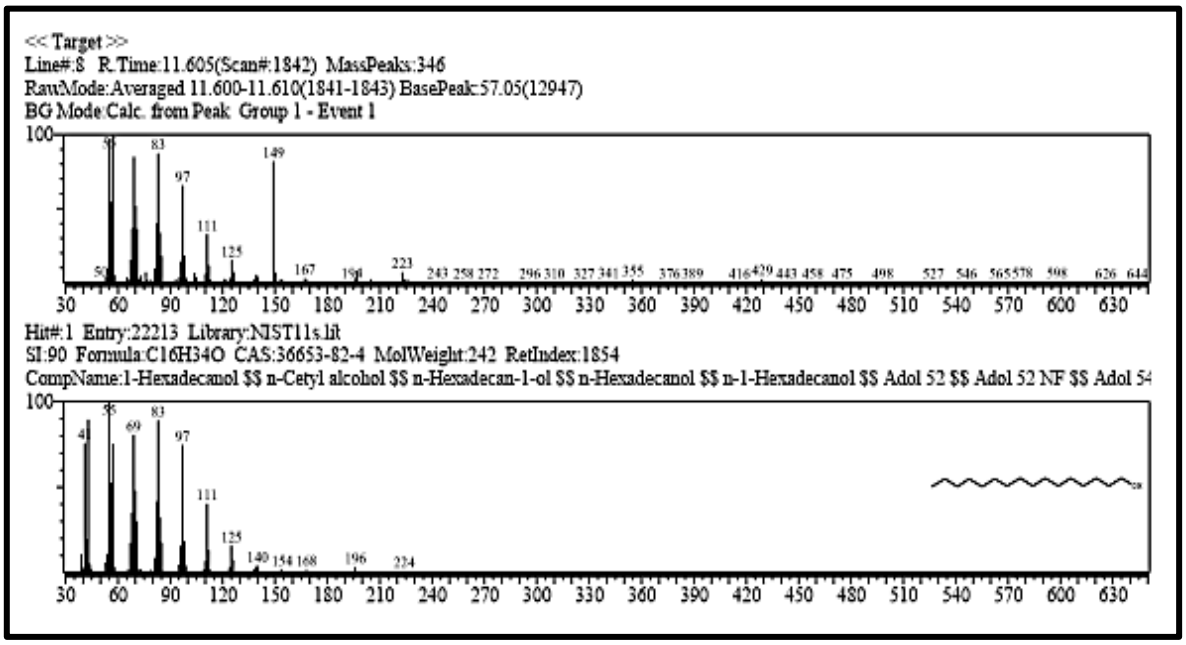


Figure 4.35: GCMS of palmityl alcohol/ hexadecanol

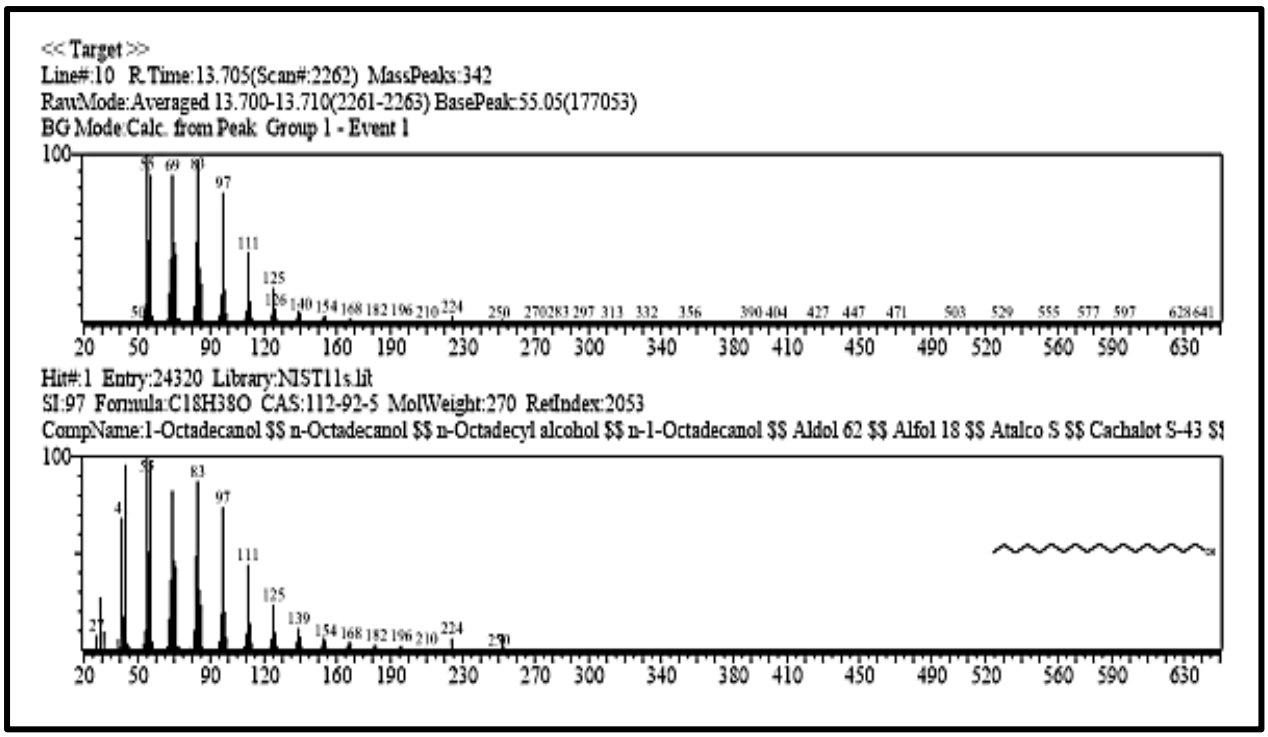


Figure 4.36: GCMS of octadecanol (stearyl alcohol) (fatty alcohol)

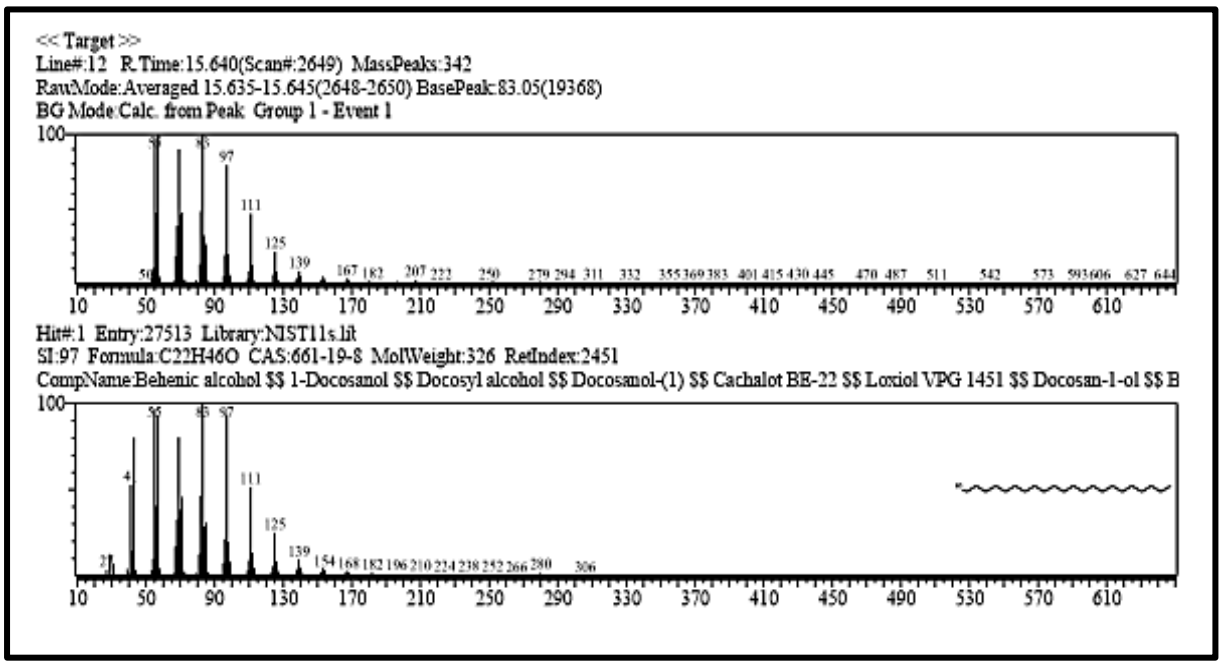


Figure 4.37: GCMS of behenic alcohol

4.2.11 Amino acids

Amino acids are biologically important organic compounds made from amine ($-NH_2$) and carboxylic acid (-COOH) functional groups, along with a side-chain specific to each amino acid. The key elements of an amino acid are carbon, hydrogen, oxygen, and nitrogen, though other elements are found in the side-chains of certain amino acids. About 500 amino acids are known (Wagner *et al*., 1983).

Amino acids occur in plants both in the free State and as the basic units of proteins and other metabolites. They are compounds containing one or more amino groups and one or more carboxylic acid groups. Most of those found in nature are α-amino acids with an asymmetric carbon atom and the general formula R-CH (NH2)COOH. Some 20 different ones have been isolated from proteins. (Trease and Evans, 2002).

Of the 22 amino acids present, 15 amino acids were recorded in seeds of *T. cuneifolia*, 13 amino acids in roots, 13 amino acids in leaves and 14 amino acids in seeds with fruit cover. The Essential amino acids were Arginine, Methionine, Phenylalanine, Tryptophan, lysine, Histidine, Isoleucine, Leucine, Valine and Threonine and Non essential amino acids: Alanine, Asparagine, Cystein, Glutamine, Glutamic acid, Glycine, Proline, Serine, and Tyrosine.

The amino acids regarded as essential for humans are phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, leucine, lysine and histidine (Young, 1994). Additionally, cysteine (or sulphur-containing amino acids), tyrosine (or aromatic amino acids), and arginine are required by infants and growing children (Imura and Okada, 1998; Anonymous, 2007). Essential amino acids are "essential" not because they are more important to life than the others, but because the body does not synthesize them. They must be present in the diet or they will not be present in the body. In addition, the amino acids arginine, cysteine, glycine, glutamine, histidine, proline, serine and tyrosine are considered conditionally essential, meaning they are not normally required in the diet, but must be supplied exogenously to specific populations that do not synthesize them in adequate amounts (Furst, Stehle 2004).

Paper chromatographic analysis of amino acids

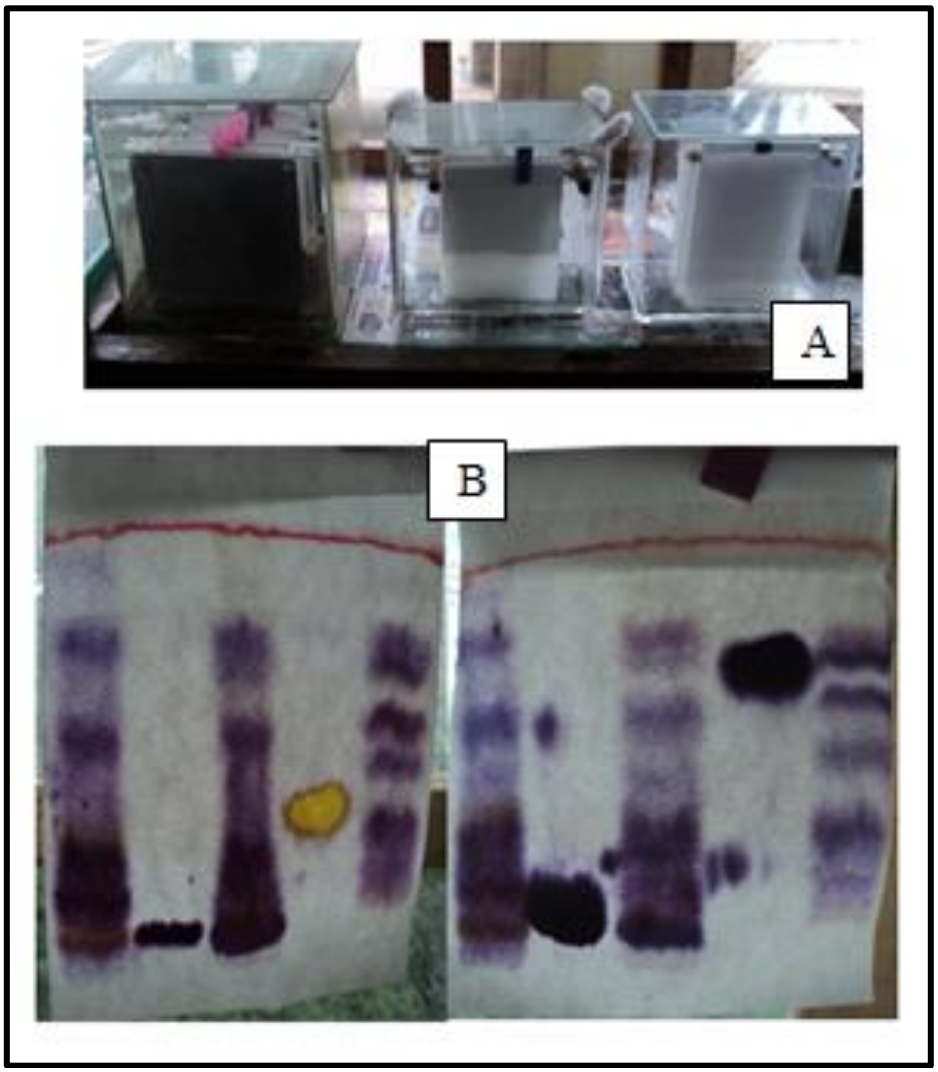


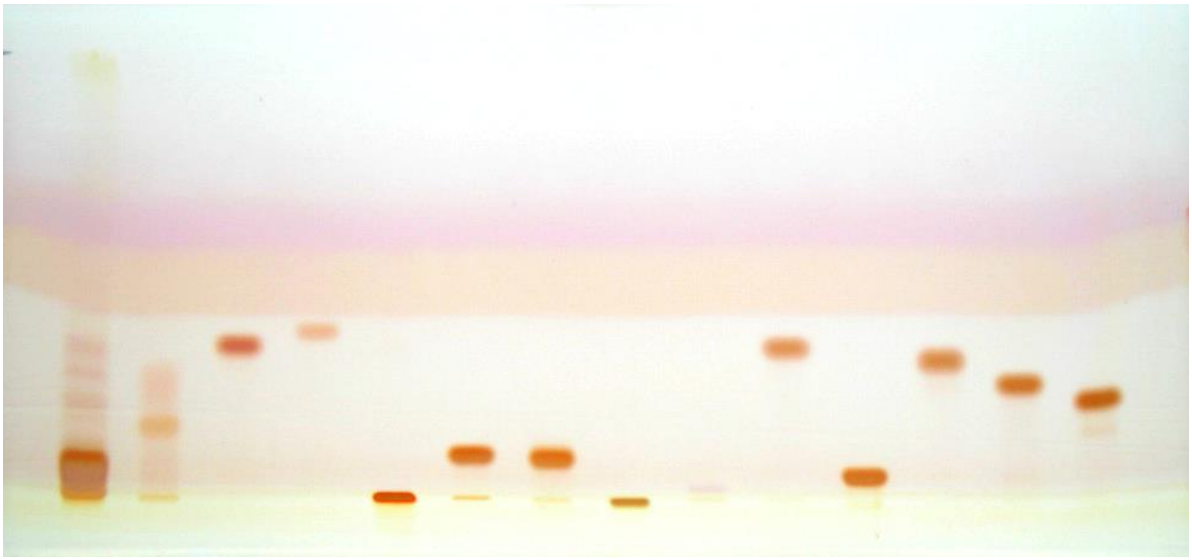
Figure 4.38: A) Chromatographic chambers B) Paper chromatography of amino acids

Table 4.17: Amino acids in *T. cuneifolia*

Sr no.	Amino acids Standards	Seed	Roots	Leaves	Seeds with fruit cover
1	Methionine	+	+	+	+
2	Glycine	+	+	+	+
3	Proline	-	-	-	-
4	Phenylalanine	+	+	+	+
5	Serine	+	+	+	+
6	Tryptophan	+	-	-	-
7	Arginine	+	+	+	+
8	Lysine	+	+	+	+
9	Alanine	+	+	+	+
10	Asparagine	-	+	-	-
11	Histidine	+	+	+	+
12	Glutamic acid	+	+	+	+
13	Isoleucine	+	+	+	+
14	Tyrosine	-	+	+	-
15	Glutamine	+	+	+	+
16	Leucine	+	+	+	+
17	Crystine	-	-	-	-
18	Valine	+	+	+	+
19	Threonine	+	+	+	+

Present (+), Absent (-)

Amino acids (Plate 4.2)



Track details:
Track 1: Plant Sample (10 µl)
Track 2: Cysteine 250 ppm (5µl)
Track 3: L- Phenyl alanine 250 ppm (5µl)
Track 4: Tryptophan 250 ppm (5µl)
Track 5: Arginine 250 ppm (5µl)
Track 6: Alanine 250 ppm (5µl)
Track 7: Threonine 250 ppm (5µl)
Track 8: L- Histamine 250 ppm (5µl)
Track 9: Aspartic acid 250 ppm (5µl)
Track 10: L- Lucine 250 ppm (5µl)
Track 11: L- Serine 250 ppm (5µl)
Track 12: Isoleucine 250 ppm (5µl)
Track 13: L- Methionine 250 ppm (5µl)
Track 14: Valine 250 ppm (5µl)

4.2.12 Sugars:

A **carbohydrate** is an organic compound comprising only carbon, hydrogen, and oxygen, usually with a hydrogen:oxygen atom ratio of 2:1 (as in water); in other words, with the empirical formula $C_m(H_2O)_n$ (where *m* could be different from *n*) (Anonymous, 2013). Some exceptions exist; for example, deoxyribose, a sugar component of DNA, (Solomon, 2004) has the empirical formula $C_5H_{10}O_4$ (Anonymous, 2011) Carbohydrates are technically hydrates of carbon; structurally it is more accurate to view them as polyhydroxy aldehydes and ketones (Anonymous, 2013).

The carbohydrates (saccharides) are divided into four chemical groupings: monosaccharides, disaccharides, oligosaccharides and polysaccharides. In general, the monosaccharides and disaccharides, which are smaller (lower molecular weight) carbohydrates, are commonly referred to as sugars (Flitsch et al., 2003). The word *saccharide* comes from the Greek word sákkharon, meaning "sugar."

In chemistry, a **glycoside** is a molecule in which a sugar is bound to another functional group via a glycosidic bond. Glycosides play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides. These can be activated by enzymehydrolysis (Brito and Marco, 2007) which causes the sugar part to be broken off, making the chemical available for use. Many such plant glycosides are used as medications. In animals and humans, poisons are often bound to sugar molecules as part of their elimination from the body.In formal terms; a glycoside is any molecule in which a sugar group is bonded through its anomeric carbon to another group via a glycosidic bond. Glycosides can be linked by an O- (an *O-glycoside*), N- (a *glycosylamine*), S (a *thioglycoside*), or C- (a *C-glycoside*) glycosidic bond. The given definition is the one used by IUPAC, which recommends the Haworth projection to correctly assign stereochemical configurations (Lindhorst, 2007). Many authors require in addition that the sugar be bonded to a *non-sugar* for the molecule to qualify as a glycoside, thus excluding polysaccharides. The sugar group is then known as the **glycone** and the non-sugar group as the **aglycone** or **genin** part of the glycoside. The glycone can consist of a single sugar group (monosaccharide) or several sugar groups (oligosaccharide).

GC MS analysis of roots showed presence of 5-Hydroxymethylfurfural, Furfuryl alcohol, Maltose, Beta-D-glucopyranose Maltose and Beta-D-glucopyranose (fig 4.39 – 4.42). HPTLC analysis of Sugars in roots with the standards showed presence of

Mannose, Fructose, Arabinose, Ribose, Glucose, Lactose Mohohydrate, Maltose Monohydrate, Xylose, Galactose and Sucrose (Plate 4.3).

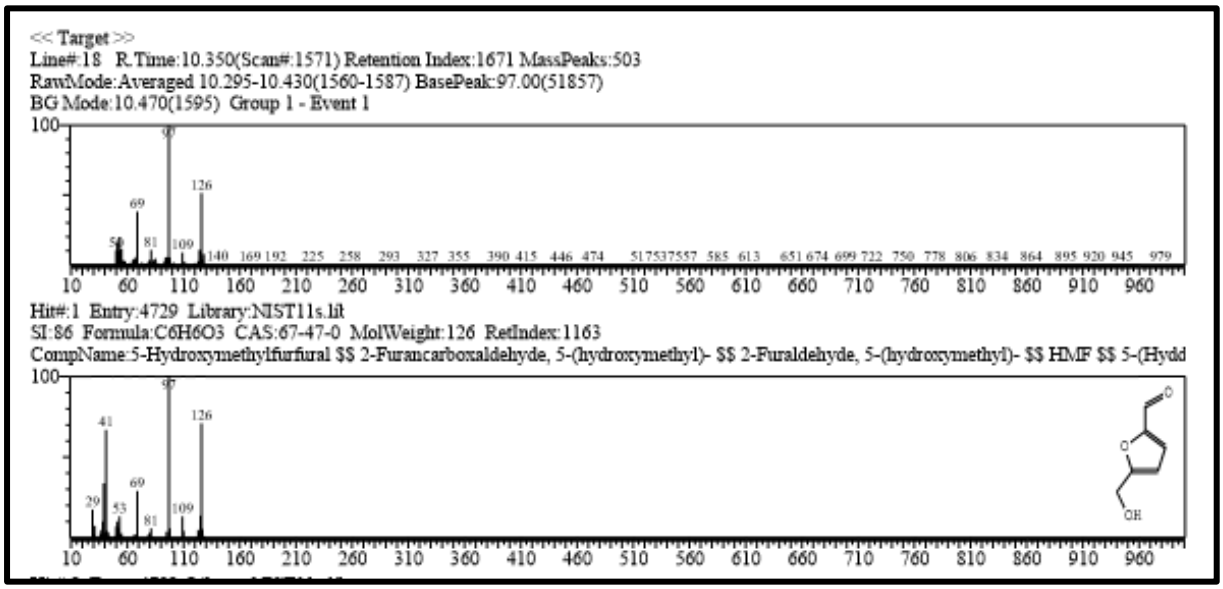


Figure 4.39: 5-Hydroxymethylfurfural

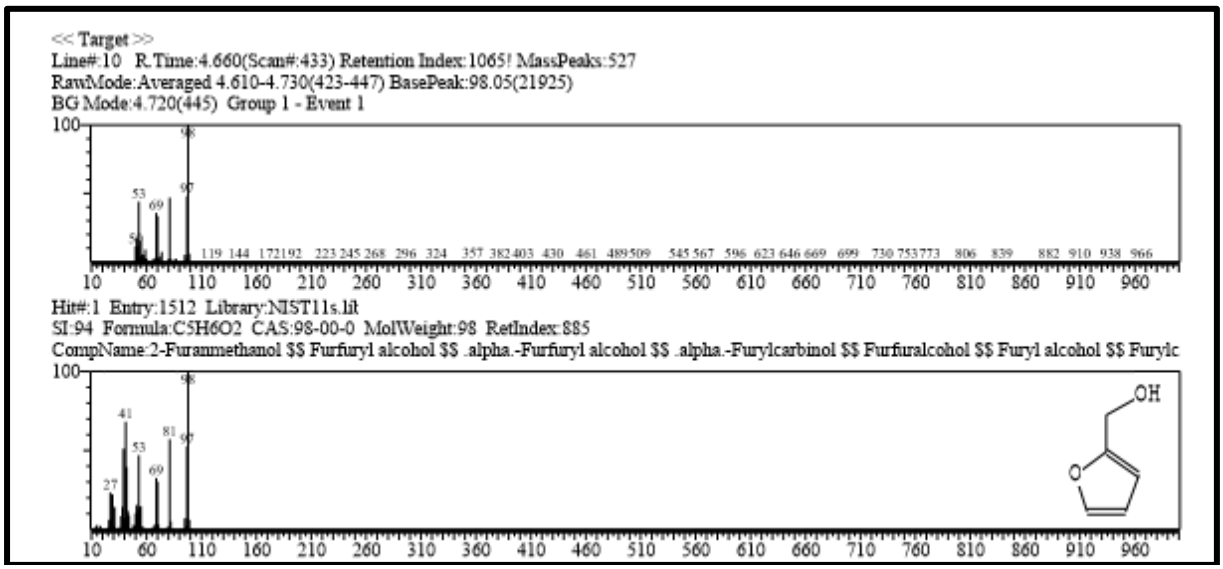


Figure 4.40: Furfuryl alcohol

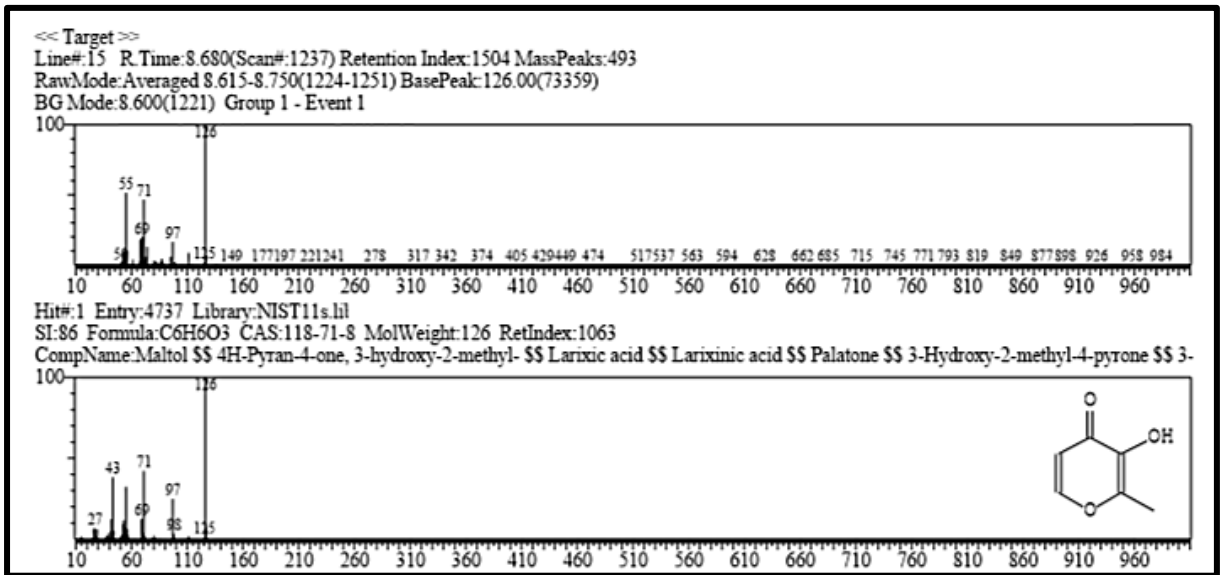


Figure 4.41: Maltol (Maltose)

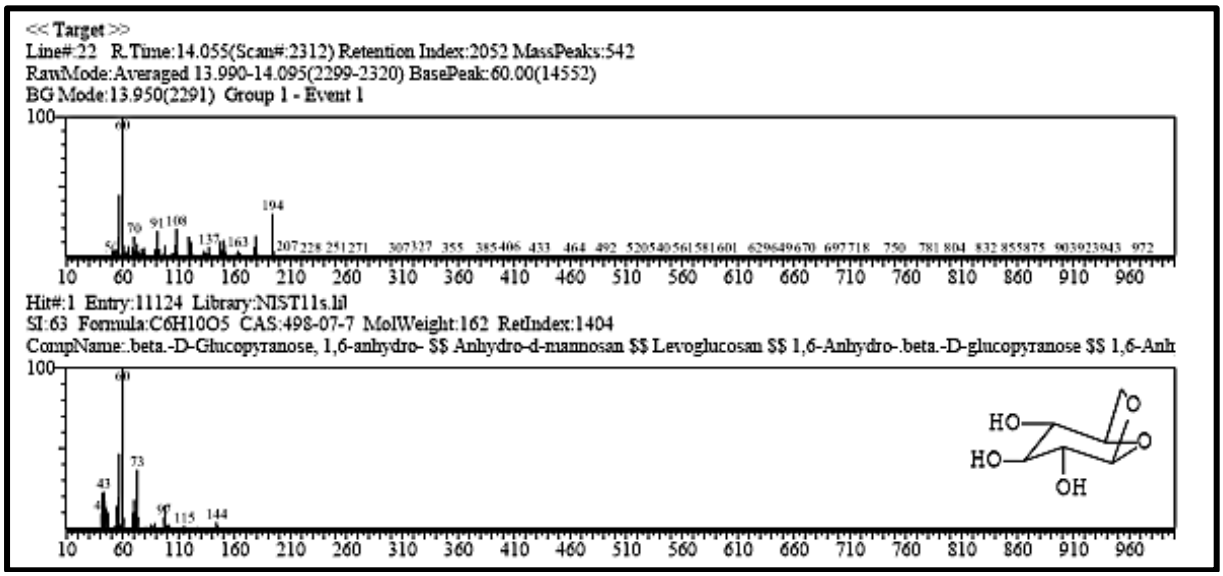
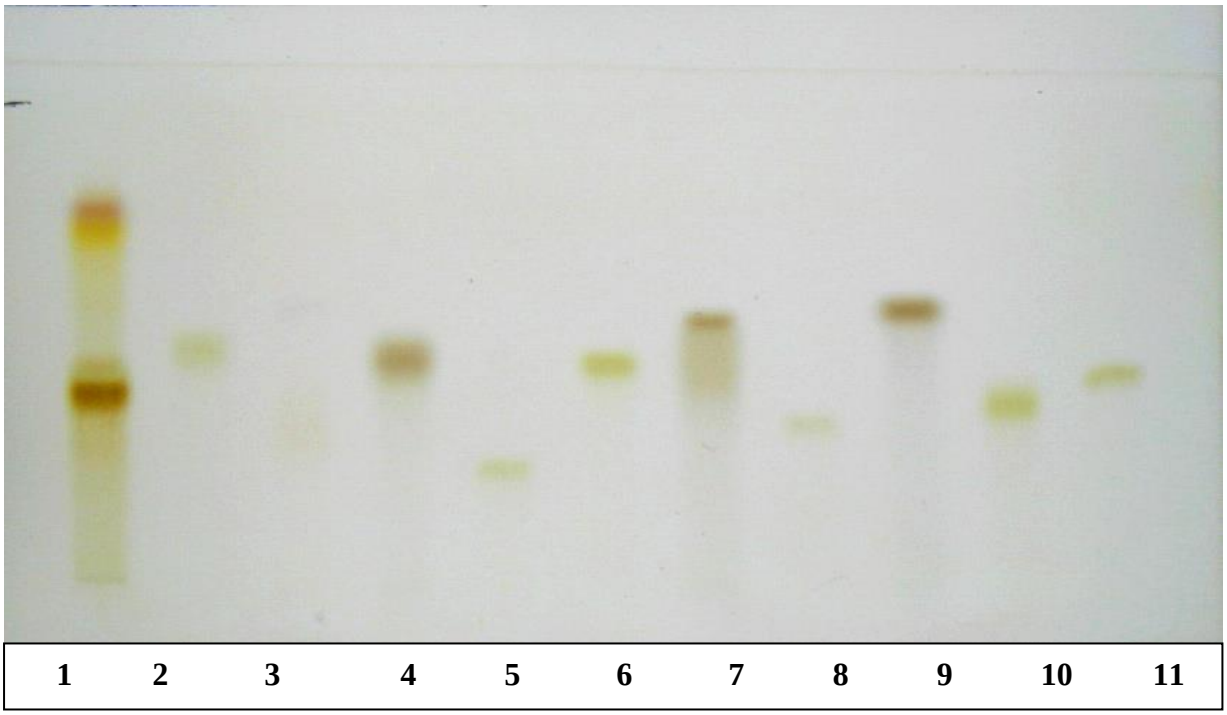
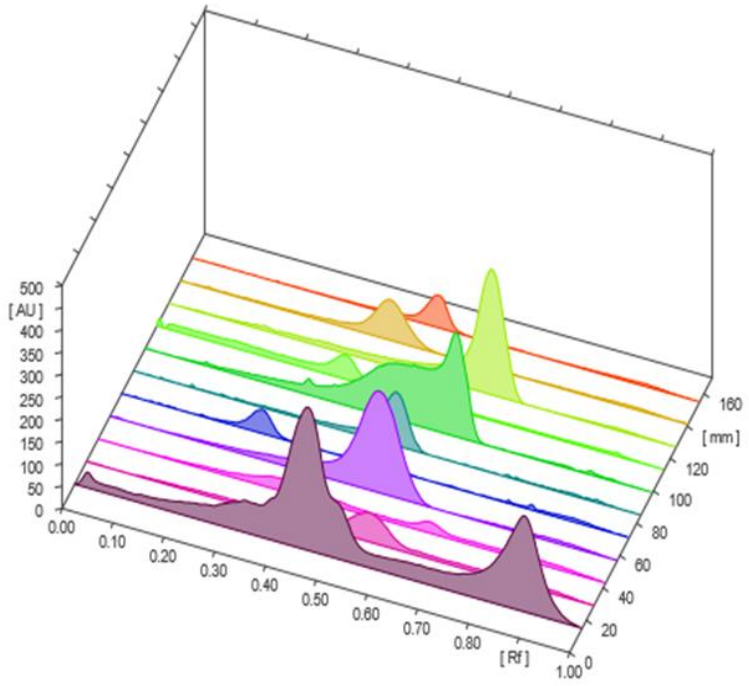


Figure 4.42: Maltose Beta-D-glucopyranose

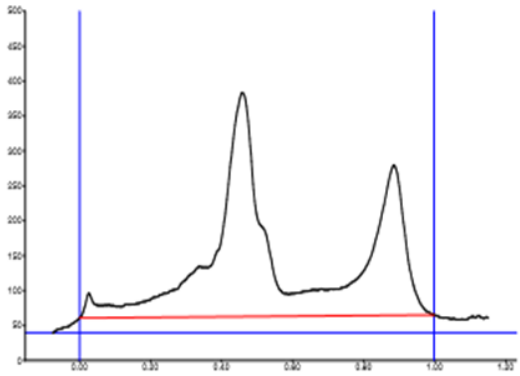
HPTLC analysis of Sugars (Plate 4.3)

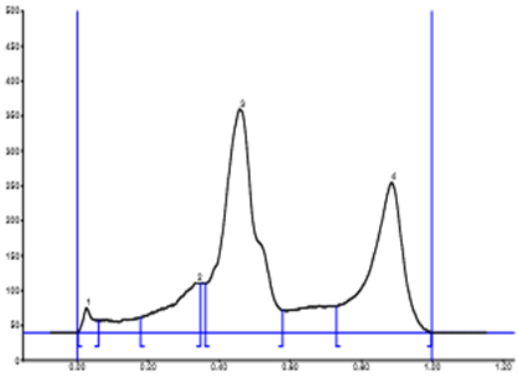


Track details:
Track 1: Plant Sample (10 µl)
Track 2: Mannose 250 ppm (5µl)
Track 3: Fructose 250 ppm (5µl)
Track 4: Arabinose 250 ppm (5µl)
Track 5: Ribose 250 ppm (5µl)
Track 6: Glucose 250 ppm (5µl)
Track 7: Lactose Mohohydrate 250 ppm (5µl)
Track 8: Maltose Monohydrate 250 ppm (5µl)
Track 9: Xylose 250 ppm (5µl)
Track 10: Galactose 250 ppm (5µl)
Track 11: Sucrose 250 ppm (5µl)



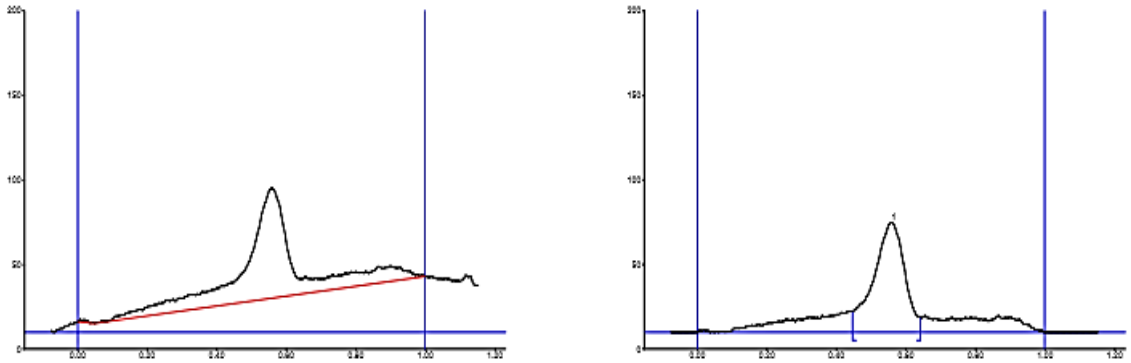
Track 1, ID: SAMPLE





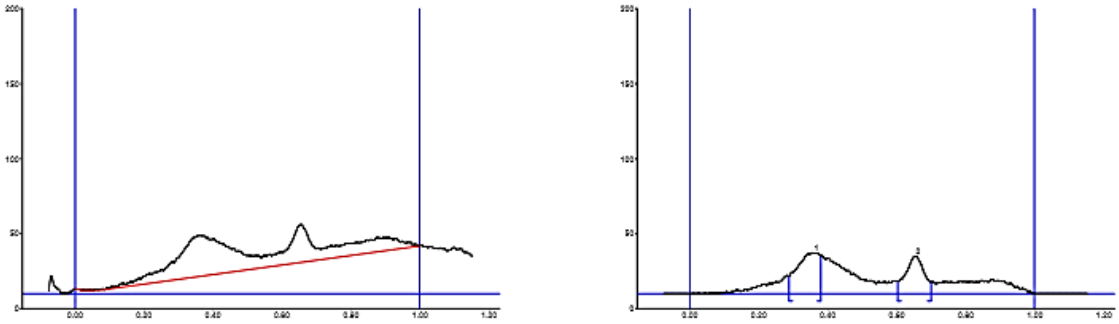
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.00	0.8	0.03	35.4	5.51	0.06	17.5	768.8	1.82	unknown *
2	0.18	22.2	0.34	72.1	11.22	0.35	70.9	4808.0	11.41	unknown *
3	0.36	70.6	0.46	320.5	49.88	0.58	32.4	21809.9	51.77	unknown *
4	0.73	37.9	0.89	214.6	33.39	1.00	0.3	14742.3	34.99	unknown *

Track 2, ID: MANNOSE



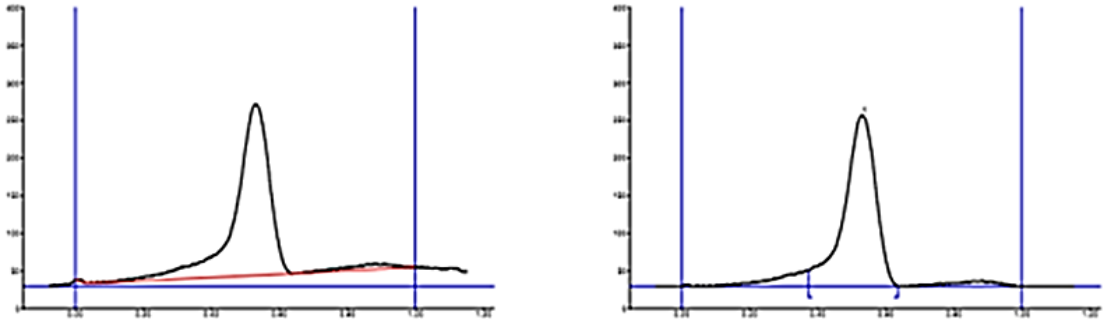
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.45	12.4	0.56	65.2	100.00	0.64	8.8	4346.0	100.00	unknown *

Track 3, ID: FRUCTOSE



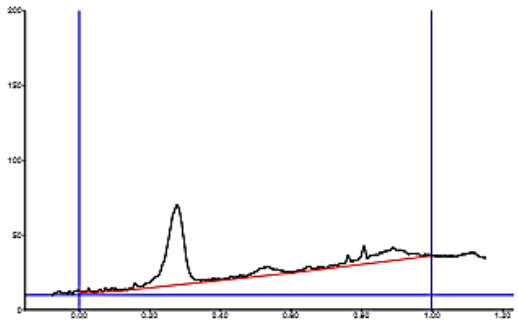
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.29	11.7	0.36	27.5	52.15	0.38	25.4	1347.0	57.12	unknown *
2	0.60	8.4	0.66	25.2	47.85	0.70	7.7	1011.1	42.88	unknown *

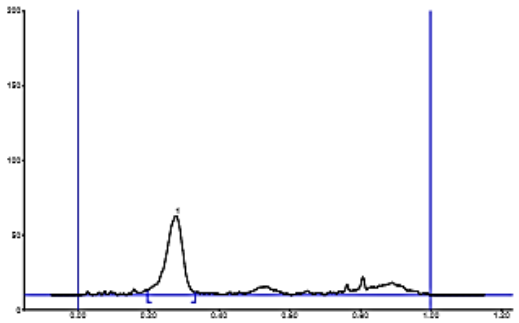
Track 4, ID: ARABINOSE



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.37	21.3	0.53	227.2	100.00	0.64	0.1	15697.4	100.00	unknown *

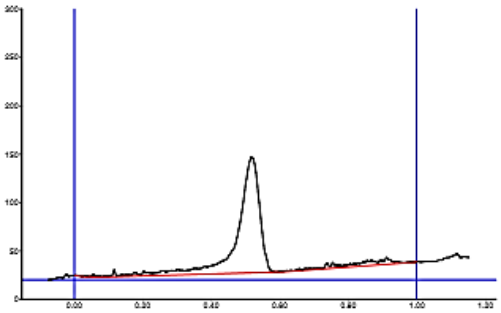
Track 5, ID: RIBOSE

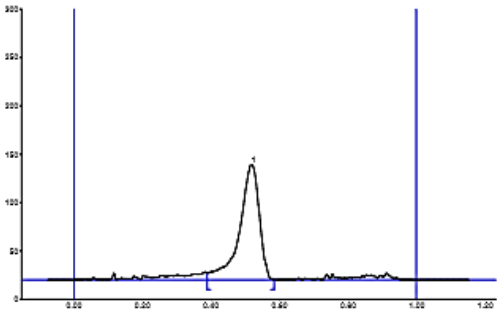




Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.20	3.1	0.28	53.3	100.00	0.33	1.8	1910.5	100.00	unknown *

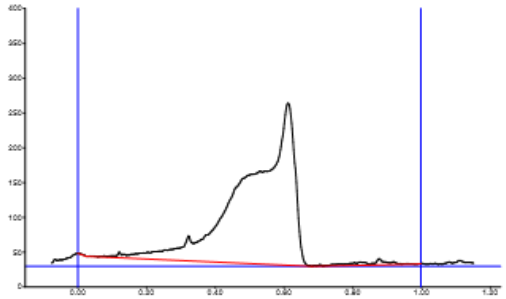
Track 6, ID: GLUCOSE

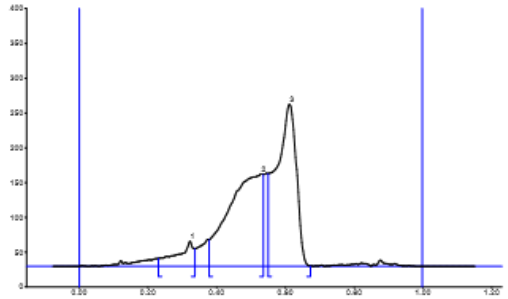




Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.39	7.0	0.52	119.9	100.00	0.58	0.1	5179.2	100.00	unknown *

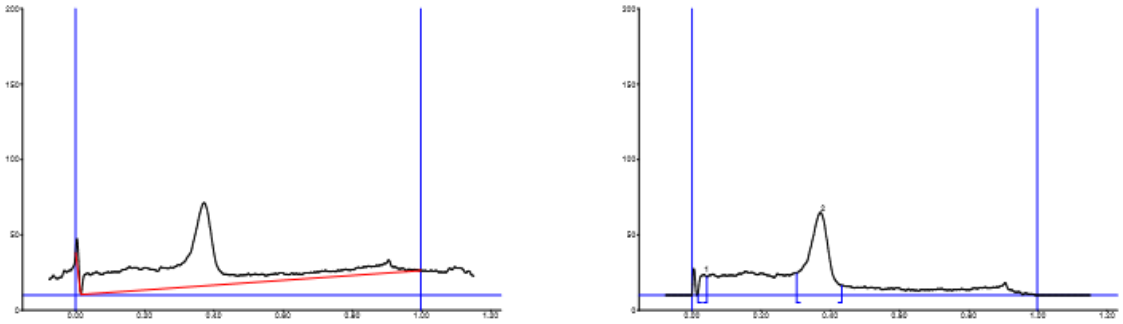
Track 7, ID: LACTOSE MONOHYDRATE





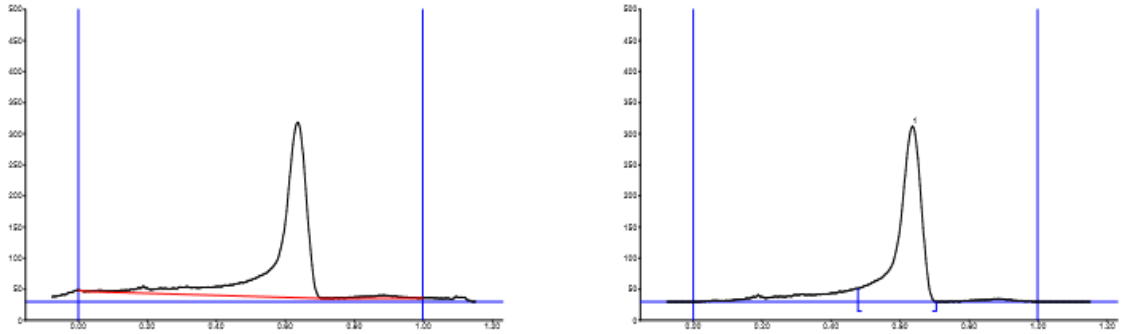
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.23	11.2	0.32	36.0	8.96	0.34	25.2	1307.4	6.07	unknown *
2	0.38	38.1	0.53	133.8	33.25	0.54	132.0	9915.9	46.03	unknown *
3	0.55	133.3	0.61	232.5	57.79	0.67	0.6	10318.9	47.90	unknown *

Track 8, ID: MALTOSE MONOHYDRATE



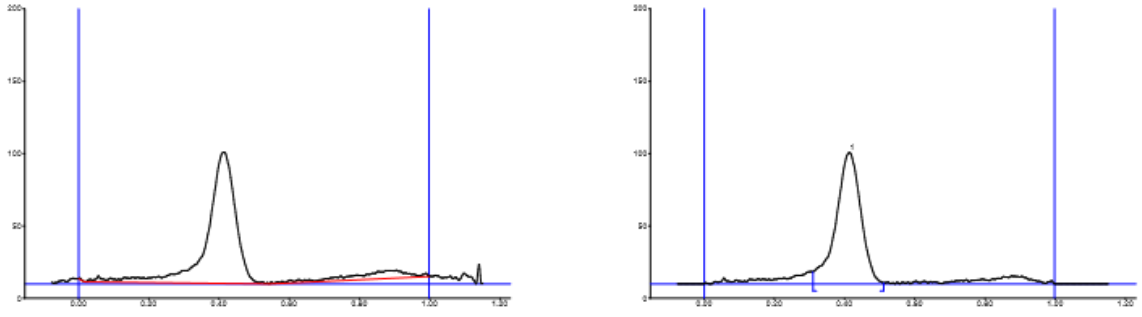
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.02	0.5	0.04	13.7	19.91	0.04	12.3	201.4	7.78	unknown *
2	0.30	14.7	0.37	54.9	80.09	0.43	6.5	2386.2	92.22	unknown *

Track 9, ID: XYLOSE

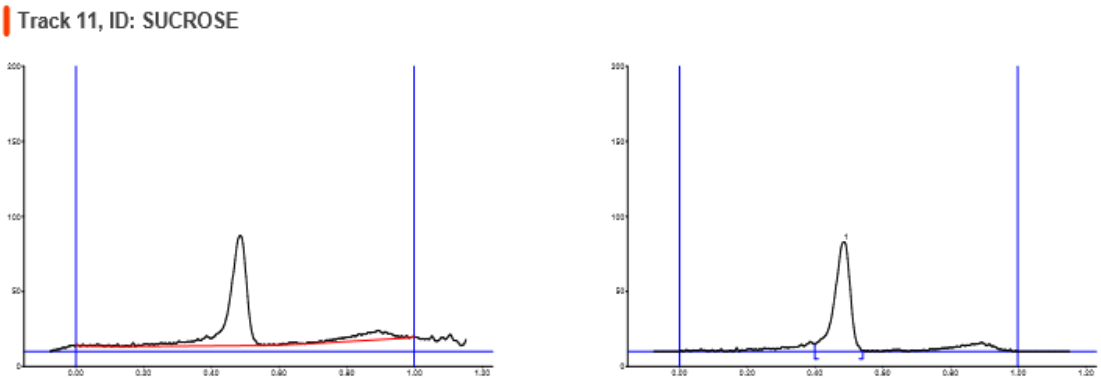


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.48	21.4	0.64	282.1	100.00	0.71	0.0	13854.5	100.00	unknown *

Track 10, ID: GALACTOSE



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.31	8.4	0.42	90.7	100.00	0.51	0.8	4844.8	100.00	unknown *



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.40	4.9	0.49	73.2	100.00	0.54	0.7	2600.8	100.00	unknown *

4.2.13 Macromolecules:

A **macromolecule** is a very large molecule commonly created by polymerization of smaller subunits. This includes biopolymers (nucleic acids, proteins, and carbohydrates as well as non-polymeric molecules with large molecular mass such as lipids and macrocycles. The individual constituent molecules of macromolecules are called monomers. Glycoproteins are proteins that contain oligosaccharide chains (glycans) covalently attached to polypeptide side-chains. The carbohydrate is attached to the protein in a cotranslational or posttranslational modification. This process is known as glycosylation. Secreted extracellular proteins are often glycosylated. In proteins that have segments extending extracellularly, the extracellular segments are also glycosylated. Glycoproteins are often important integral membrane proteins, where they play a role in cell–cell interactions. Glycoproteins are also formed in the cytosol, but their functions and the pathways producing these modifications in this compartment are less well understood. Owing to the presence of sugars in the root the glycoproteins and other proteins were analysed to understand the component responsible for sweetness.

Protein analysis was done by electrophoresis. The electrophoresis of proteins conducted on *T. cuneifolia* roots, *T. cuneifolia* seeds and *T. abyssinica* roots shows that all the three have good amount of protein content in it. All the three samples showed the presence of three types of molecular weight i.e. higher molecular weight proteins, medium molecular weight proteins and low molecular weight proteins. These were run with protein biomarkeras per section. *T. cuneifolia* roots, seeds and *T. abyssinica* showed a band with the molecular weight of 97.4kDa. All the three had 66kDa weight proteins. All the three showed similarly for 43kDa molecular weight. All three showed presence

of 29kDa weight proteins. Only *T. cuneifolia* roots and *T. abyssinica* roots showed 20.1kDa weight. The low molecular weight protein (14.3kDa) showed presence in *T. cuneifolia* roots and *T. abyssinica* roots (Fig. 4.43 A & 4.43 B).

To further confirm the presence of Glycoproteins all the fractions which were run in CBB dye were further run with Schiff's reagent. Here the blue arrows show the presence of glycoprotein in all the three parts. i.e. *T. cuneifolia* roots, *T. cuneifolia* seeds and *T. abyssinica* roots. *T. cuneifolia* root showed the highest amount of glycoprotein as compared to others.

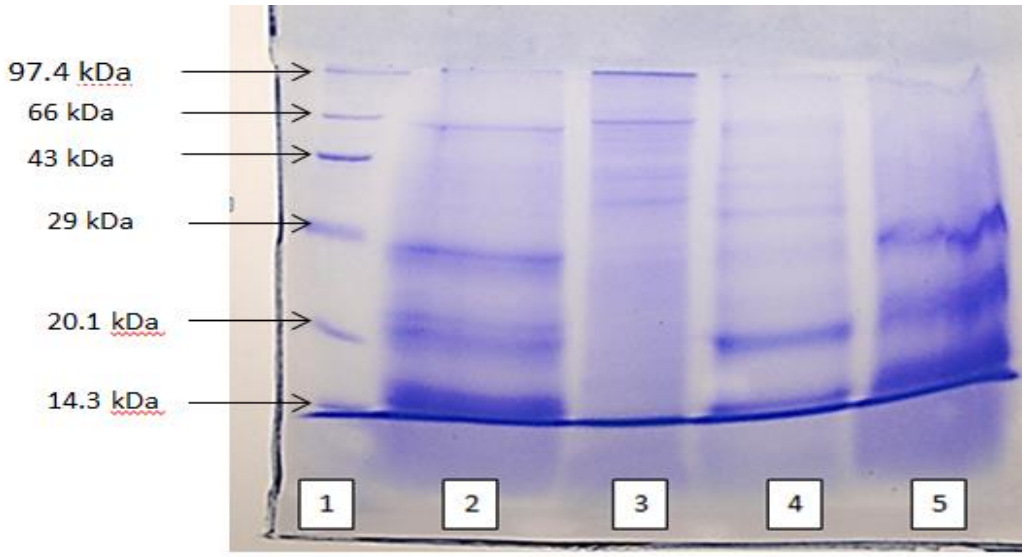


Figure 4.43 A: Gel stained in CBB (Coomassie Brilliant Blue) dye

Track 1: Molecular Mass Standard
Track 2: *Taverniera cuneifolia* roots (T1)
Track 3: *Taverniera cuneifolia* seeds
Track 4: *Taverniera abyssinica* roots
Track 5: *Taverniera cuneifolia* roots (T2)

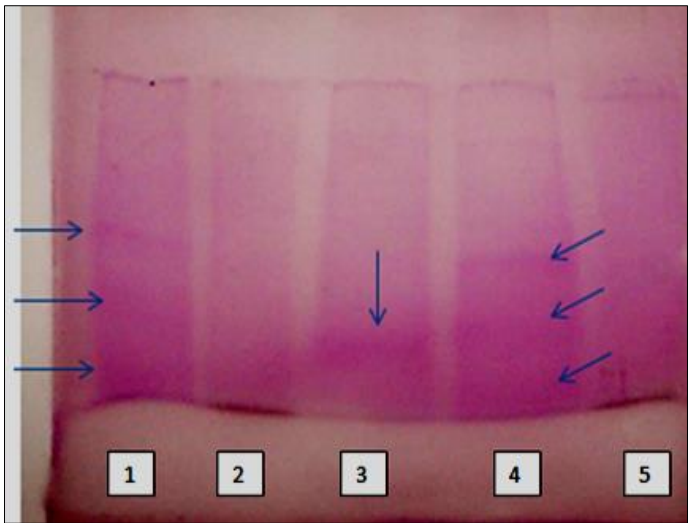


Figure 4.43A.: Gel stained in Periodic Acid Schiff Reagent for Glycoproteins
Track 1: *Taverniera cuneifolia* roots
Track 2: *Taverniera cuneifolia* seeds
Track 3: *Taverniera abyssinica* roots
Track 4: *Taverniera cuneifolia* roots
Track 5: *Taverniera abyssinica* roots

4.2.14 Vitamins:

A **vitamin** is an organic compound required by an organism as a vital nutrient in limited amounts. An organic chemical compound (or related set of compounds) is called a vitamin when it cannot be synthesized in sufficient quantities by an organism, and must be obtained from the diet.

A treatment modality for minor recurrent aphthous stomatitis 0.2% Chlorhexidine gluconate mouthwash in combination with vitamin B-complex were statistically significant ($p<0.01$) in reducing duration of ulcers, recurrence of ulcers and increasing the gap between successive ulcer free days (Nabikhan et al., 2012). The roots of *T.cuneifolia* are locally used for the treatment of Ulcers. Probably the presence of these vitamins in *Taverniera cuneifolia* are playing important role in treatment of ulcers.

The HPLC analysis of vitamins shows that there is presence of Riboflavin, thiamine, pyridoxine and nicotinamide in the roots of *T. cuneifolia*.

Riboflavin:

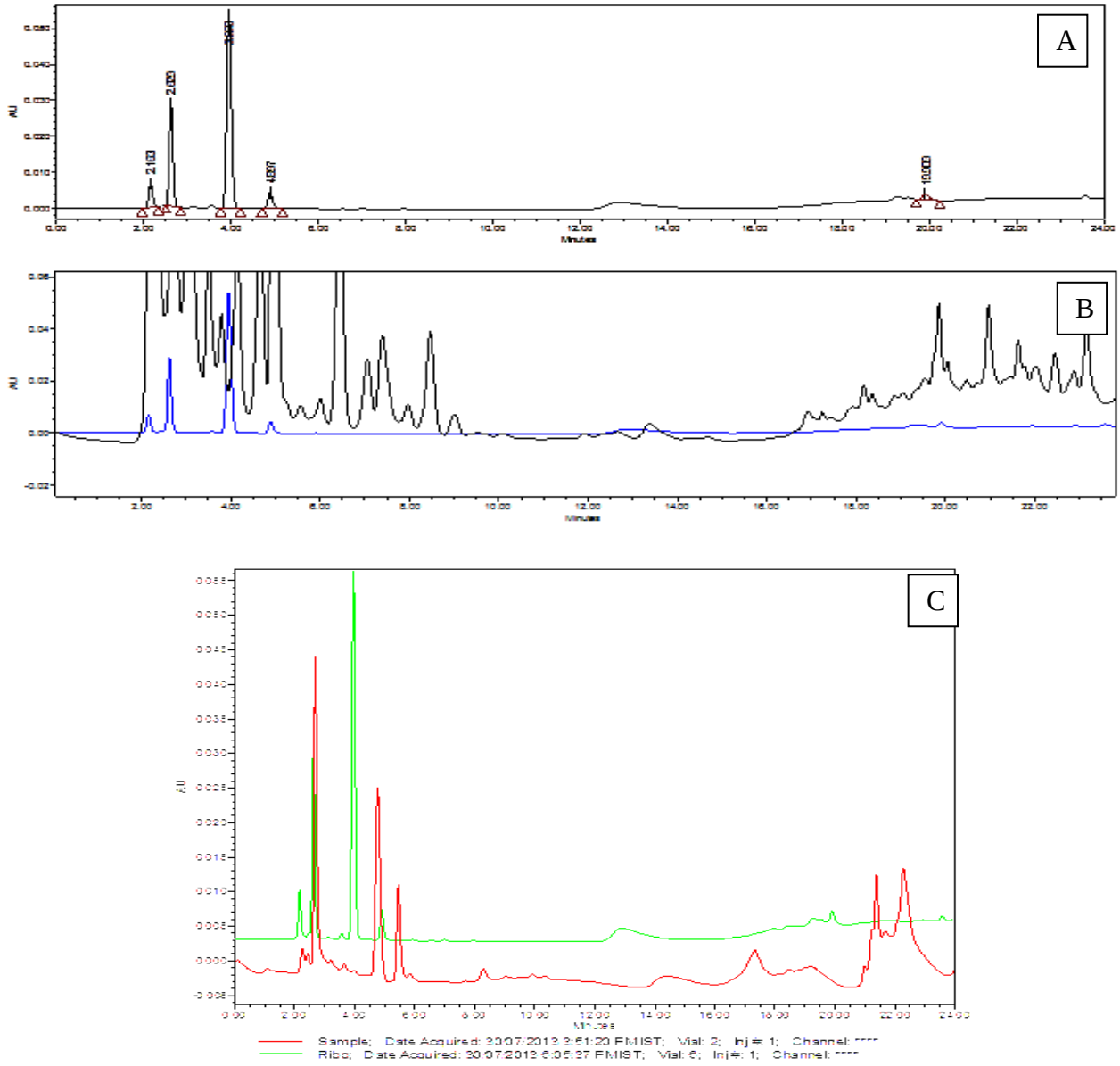


Figure: sample (Black) + Riboflavin standard (Blue)

Figure 4.43.: (A) Riboflavin standard
(B) sample (Black) + Riboflavin standard (Blue) sample (Zoomed form of C)
(C) sample (Black) + Riboflavin standard (Blue)

	Name	Retention Time	Area	% Area	Height
1	Riboflavin	2.163	42206	6.30	6824
2		2.629	168515	25.14	28704
3		3.958	406366	60.62	54337
4		4.897	34528	5.15	4454
5		19.909	18702	2.79	1768

Pyridoxine:

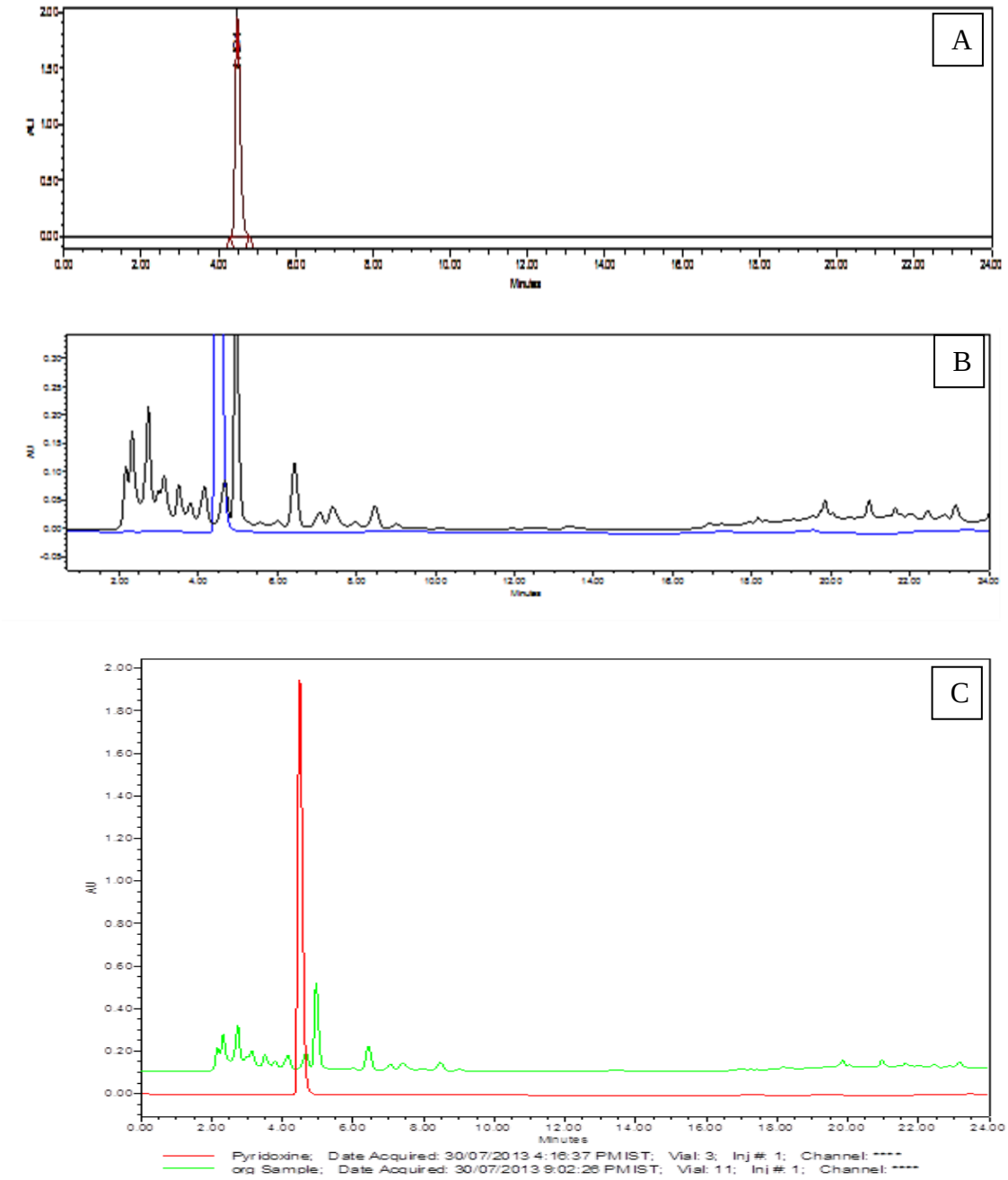


Figure 4.44 (A) Pyridoxine standard (Blue)
(B) sample (Black) + Pyridoxine standard (Blue) sample (Zoomed form of C)
(C) sample (Black) + Pyridoxine standard (Blue)

	Name	Retention Time	Area	% Area	Height
1	Pyridoxine	4.491	17794888	100.00	1960856

Nicotinamide

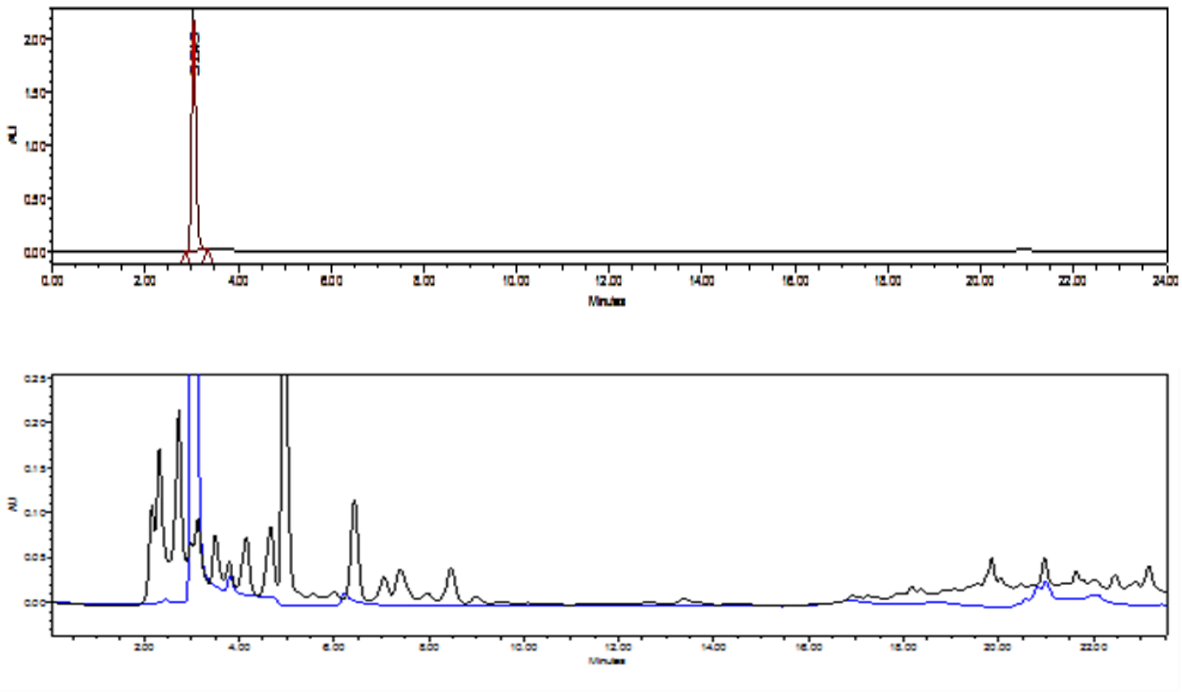


Figure 4.45 : Sample (Black) + Nicotinamide standard (Blue)

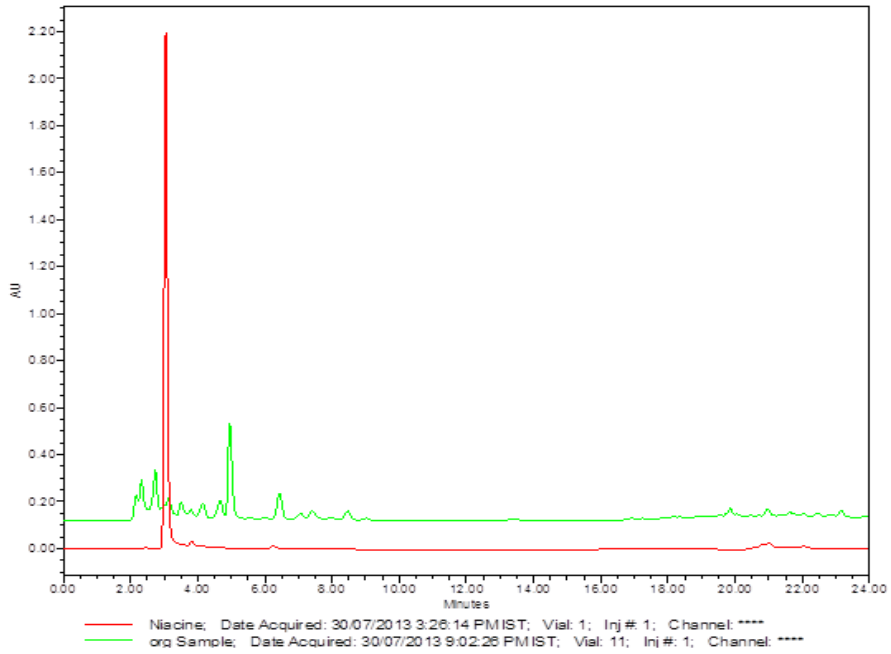


Figure 4.46: (A) Nicotinamide standard (Blue)
(B) sample (Black) + Nicotinamide standard (Blue) (Zoomed form of C)
(C) sample (Black) + Nicotinamide standard

	Name	Retention Time	Area	% Area	Height
1	Nicotinamide	3.045	14037901	100.00	2181910

Thiamine:

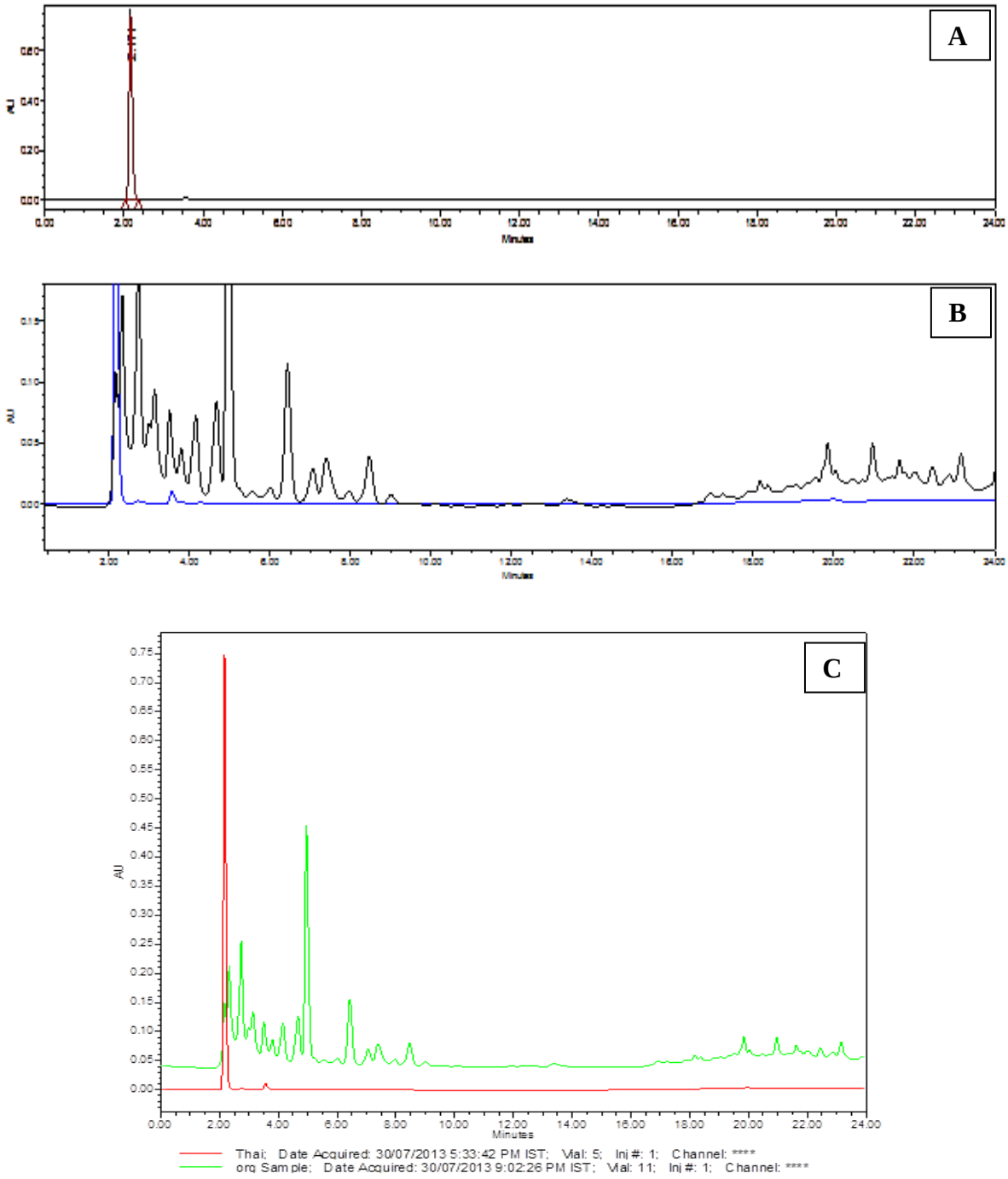


Figure 4.47: (A) Thiamine standard
(B) Thiamine standard (Blue) with plant sample (Zoomed form of C)
(C) Thiamine standard (Red) with plant sample

	Name	Retention Time	Area	% Area	Height
1	Thiamine	2.171	4361223	100.00	744733

:

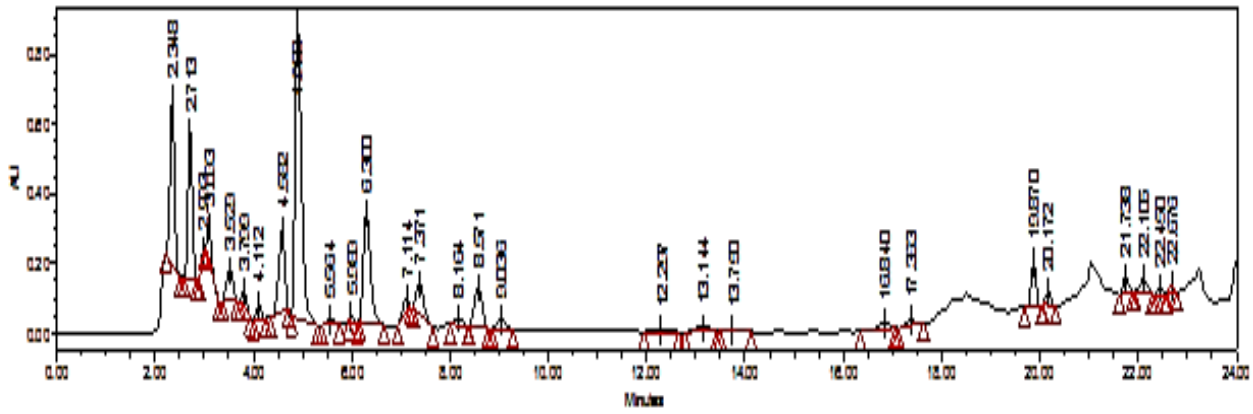


Figure 4.48: Integrated vitamins chromatogram in *T. cuneifolia* root sample

Table 4.11: Retention time and percentage area of vitamins

	Name	Retention Time	Area	% Area	Height
	Conc. Sample				
1	Thiamine	2.348	2951114	10.93	476190
2		2.713	2643012	9.79	418489
3		2.993	67253	0.25	6847
4	Nicotinamide	3.093	531998	1.97	95167
5		3.529	834495	3.09	89060
6	Riboflavin	3.799	281845	1.04	42720
7		4.112	268735	1.00	41986
8	Pyridoxine	4.582	1996304	7.40	235881
9		4.900	7717571	28.59	847903
10		5.564	135411	0.50	13433
11		5.989	78673	0.29	13843
12		6.300	3283949	12.17	314875
13		7.114	298446	1.11	38477
14		7.371	877510	3.25	86076
15		8.164	233599	0.87	22971
16		8.571	1218520	4.51	113101
17		9.036	352711	1.31	28144
18		12.297	132994	0.49	7424
19		13.144	241283	0.89	13980
20		13.750	94987	0.35	4627
21		16.840	310390	1.15	23160
22		17.363	193137	0.72	15949
23		19.870	895512	3.32	132861
24		20.172	286920	1.06	37415
25		21.738	365412	1.35	48408
26		22.105	421552	1.56	42629
27		22.450	142744	0.53	20378
28		22.676	133994	0.50	20459

Fasciolin and furfural are also found in *Glycyrrhiza glabra.*

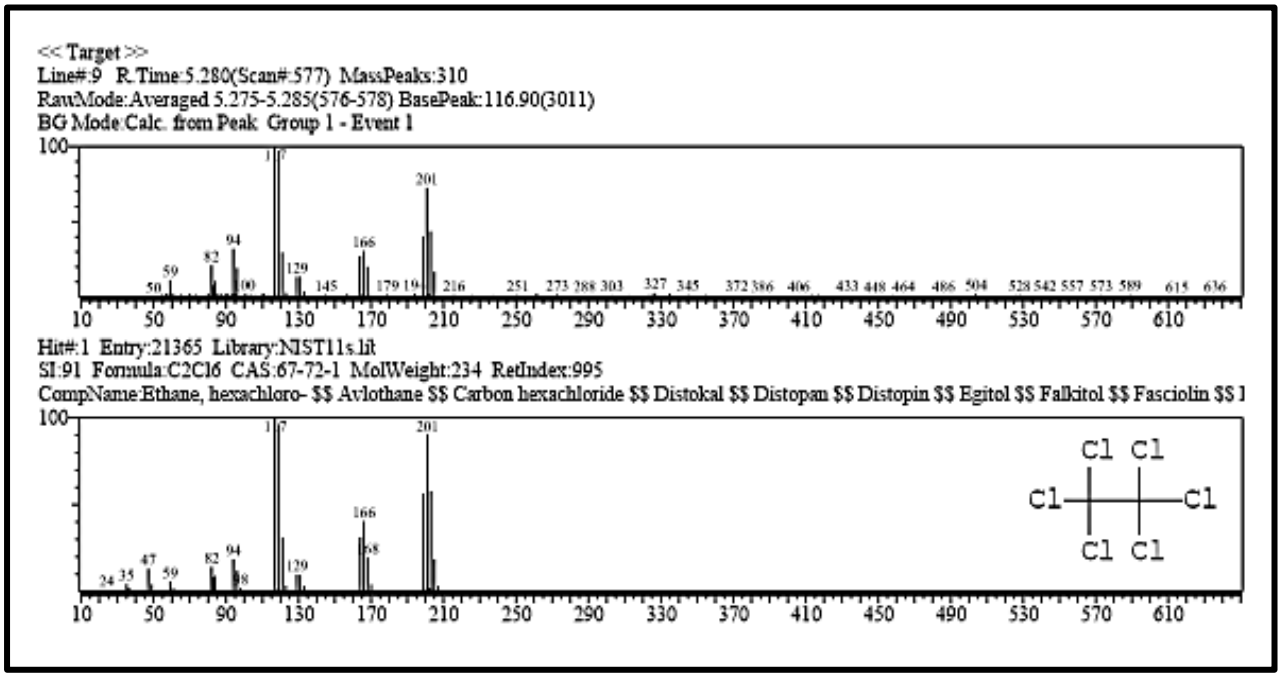


Figure 4.49: GCMS of Fasciolin

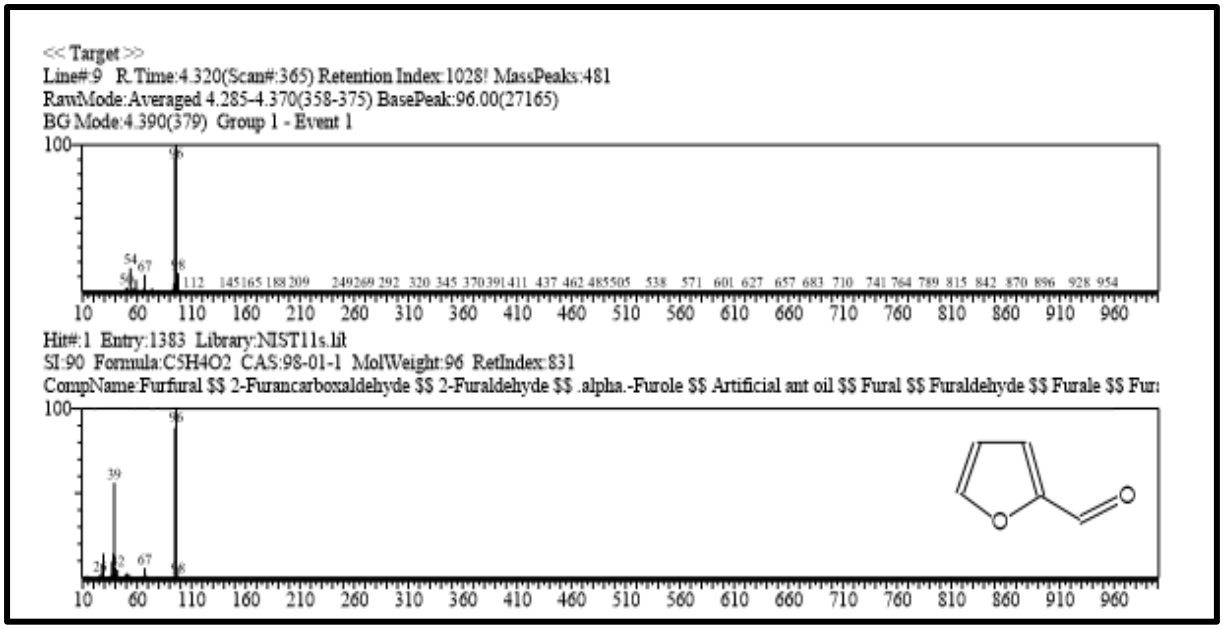


Figure 4.50: GCMS of furfural

4.2.15 SEASONAL, REGIONAL, SPECIES AND ELUTROPIC HPTLC FINGERPRINTING

The quality control methods underwent many developments since ancient times, from the botany shapes, microscopy structure identification to the physical and chemical properties based on the major components identification to almost all components identification depending on fingerprint spectra (FPS). The classification of herbs based on fingerprint spectra of herbs is now becoming the platform in identifying herbs. Currently, fingerprint spectra analysis includes two major aspects which are based on spectroscopy fingerprint spectra such as infrared spectroscopy and ultraviolet spectroscopy. And the second is based on the HPTLC and HPLC fingerprint spectra. The spectral fingerprinting techniques are quick, easy, and accurate while the HPTLC method is able to determine the partial components in herbs, and can determine their concentrations quantitatively. For this reason, HPTLC FPS is currently considered a quick method in evaluating quality of herbal medicine, regional variation, seasonal variation etc.

Regional variation, species variation and seasonal parameters play a very important role in the phytoconstituents compostion of a plant. There are references stating that *T. cuneifolia* has glycyrrhizin (Saluja 1969, Zore 2008 & Notani 2012), an effort was made made study the regional and seasonal variation in possible phytoconstituents. Khan *et al*., 2012 has reported β-sitosterol and Lupeol from *T. cuneifolia* roots, stem and leaves of Maharashtra region.

4.2.16 Regional Variation

In this section the roots collected from different regions i.e. Kutch, Rajkot, Jamnagar, jodhpur and Andhra Pradesh were compared with the standards of β-sitosterol and lupeol content. . In Gujarat, only Kutch showed presence of lupeol both the other regions (Rajkot and Jamnagar) showed presence of both β-sitosterol and lupeol. Outside Gujarat Jodhpur had presence of β-sitosterol and lupeol but Andhra Pradesh showed presence of only β-sitosterol. Andhra Pradesh has the highest amount of area percentage of β-sitosterol with 4.45% and least is in Jodhpur with 1.90%. Kutch has highest amount of lupeol (2.99%) and lowest is in Jodhpur with 2.01%.

Table 4.12: Regional variation of T. cuneifolia roots with β-sitosterol and lupeol

	Jamnagar	Rajkot	Kutch	Andhra pradesh	Jodhpur
β-sitosterol –	1.98%	2.64%	-	**4.45%**	1.90%
lupeol –	2.44%	2.21%	**2.99%**	-	2.01%.

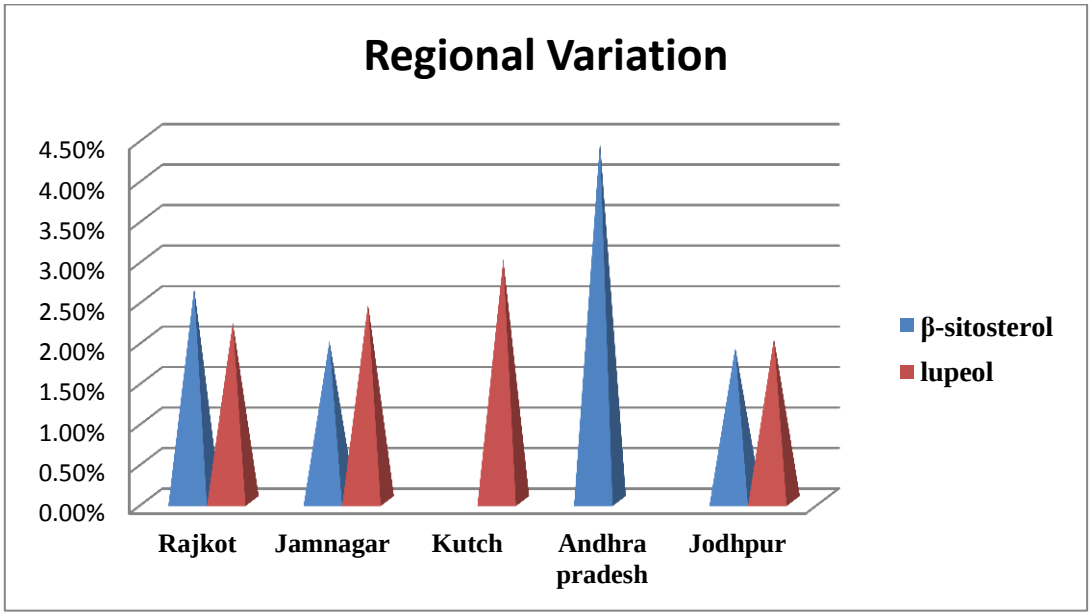
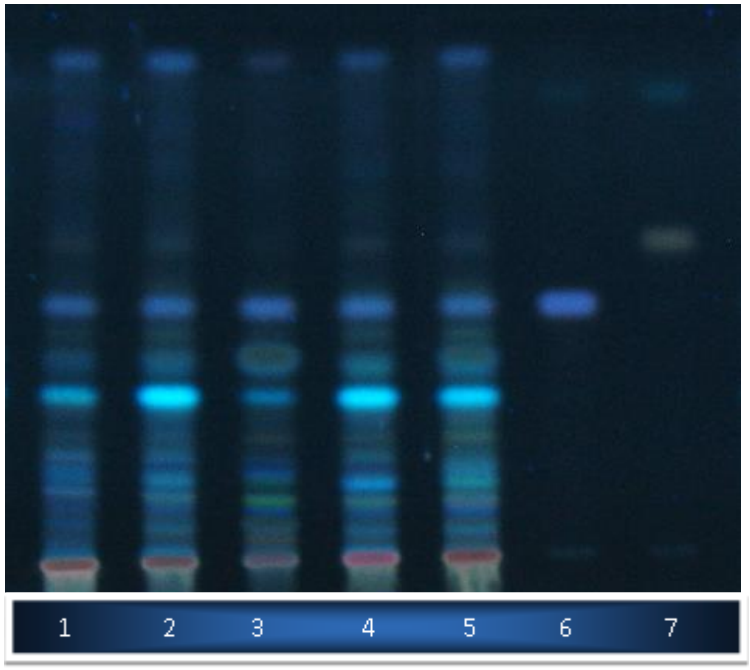


Figure 4.50: Graph showing HPTLC data of regional variation

(plate 4.4) **HPTLC plate photo representing regional variation of roots of *T. cuneifolia* with standards β-sitosterol and Lupeol**

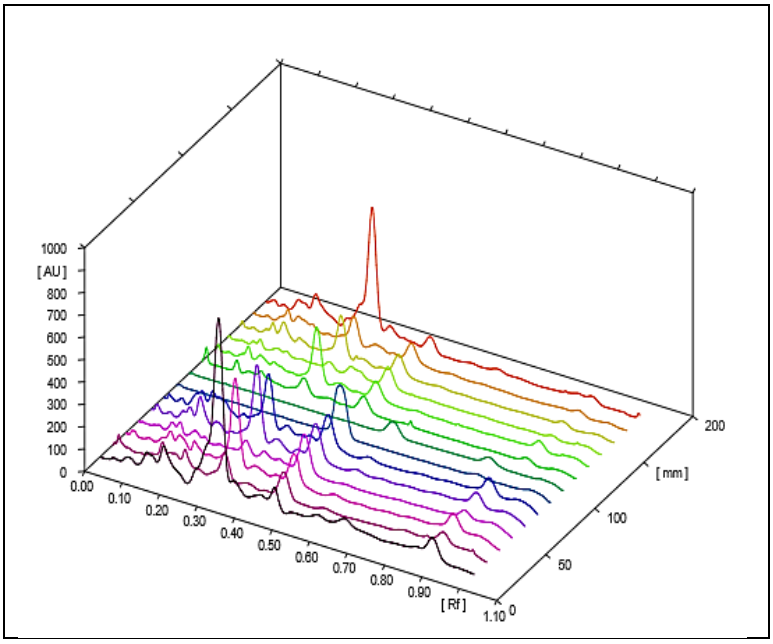
Plate Photo at 336nm



Order of spotting:

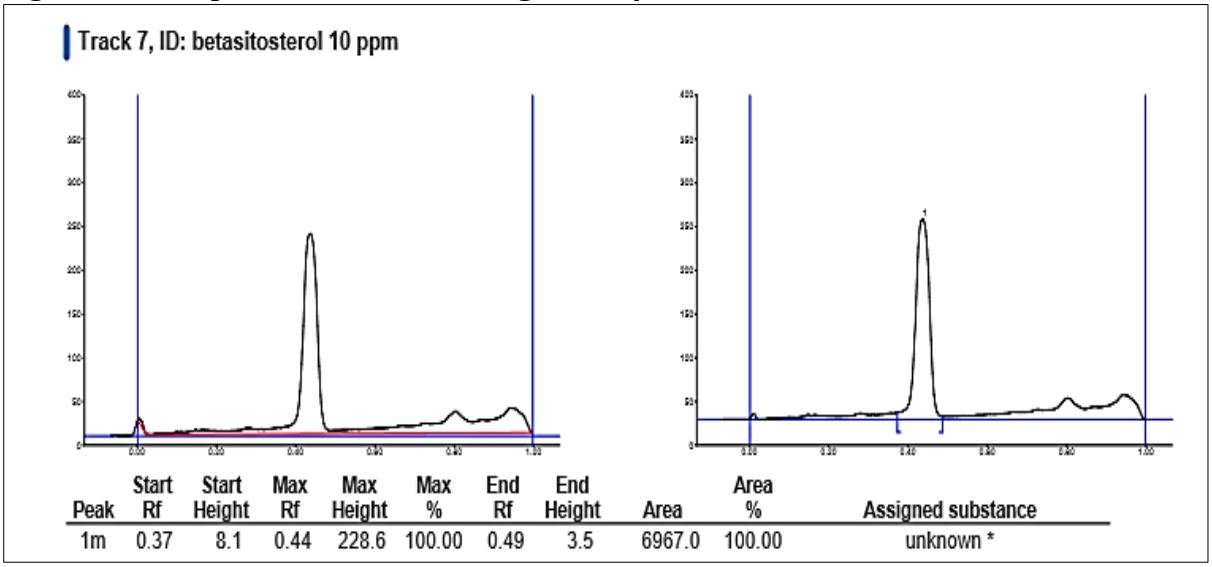
Track 1 Kutch Track 2 Jamnagar Track 3 Andhra Pradesh Track 4 Jodhpur

Track 5 Rajkot Track 6 Beta-sitosterol Track 7 Lupeol



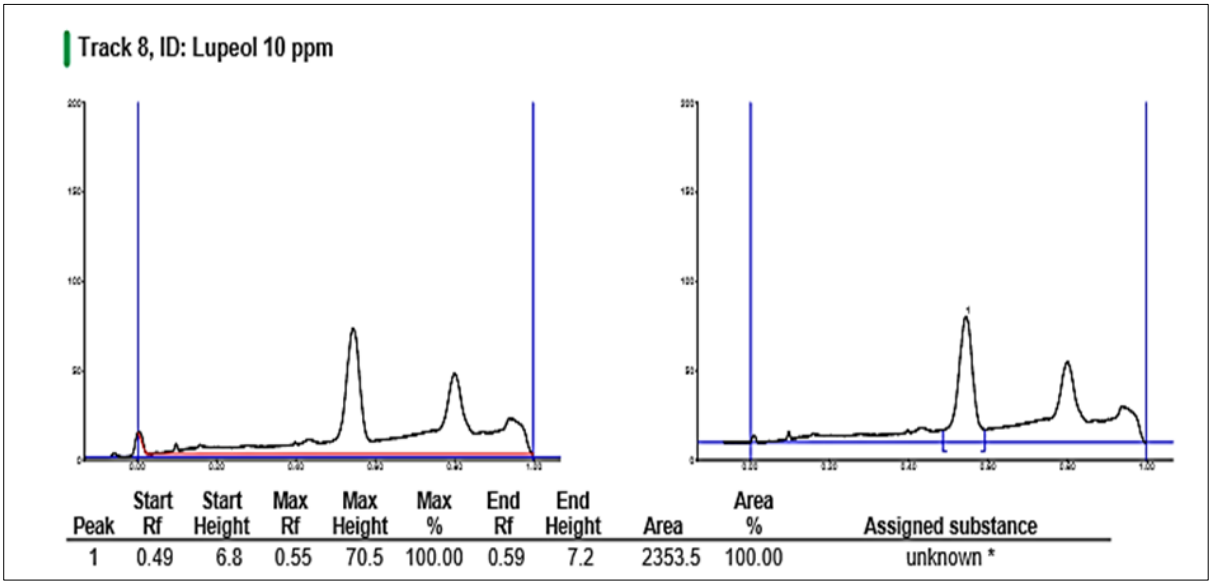
Sample ID	Sample ID
T.absyssi 1	Lupeol 10 ppm
Kutch 1	Tc Kutch 2
Jamnagar 1	Tc Jamnagar 2
TC AP 1	Tc AP 2
Tc Jodhpur 1	Tc Jodhpur 2
Tc Rajkot 1	Tc Rajkot 2
betasitosterol 10 ppm	T. absyssinica 2

Figure 4.51: Representative chromatogram of β-sitosterol



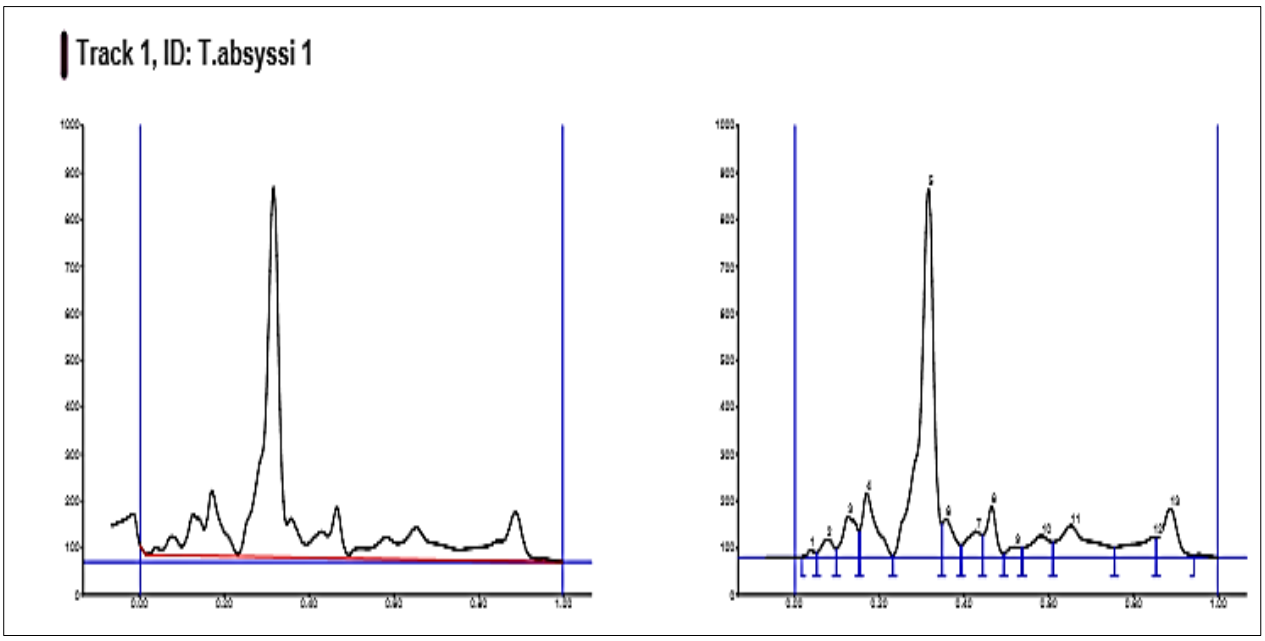
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1m	0.37	8.1	0.44	228.6	100.00	0.49	3.5	6967.0	100.00	unknown *

Figure 4.52: Representative chromatogram of Lupeol



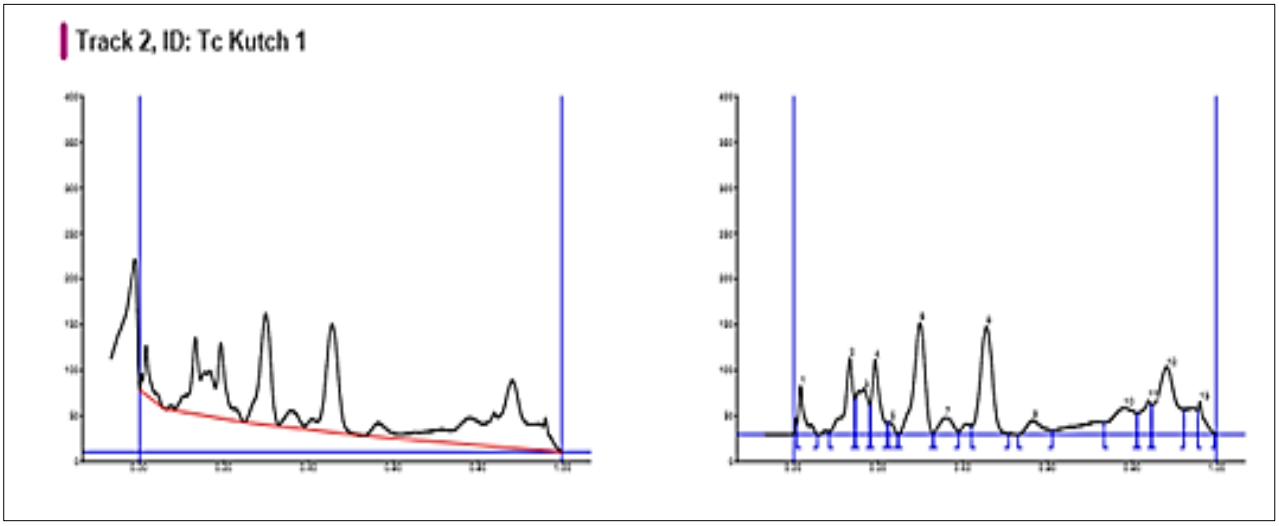
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.49	6.8	0.55	70.5	100.00	0.59	7.2	2353.5	100.00	unknown *

Figure 4.53: Representative chromatogram of *Taverniera abyssinica*



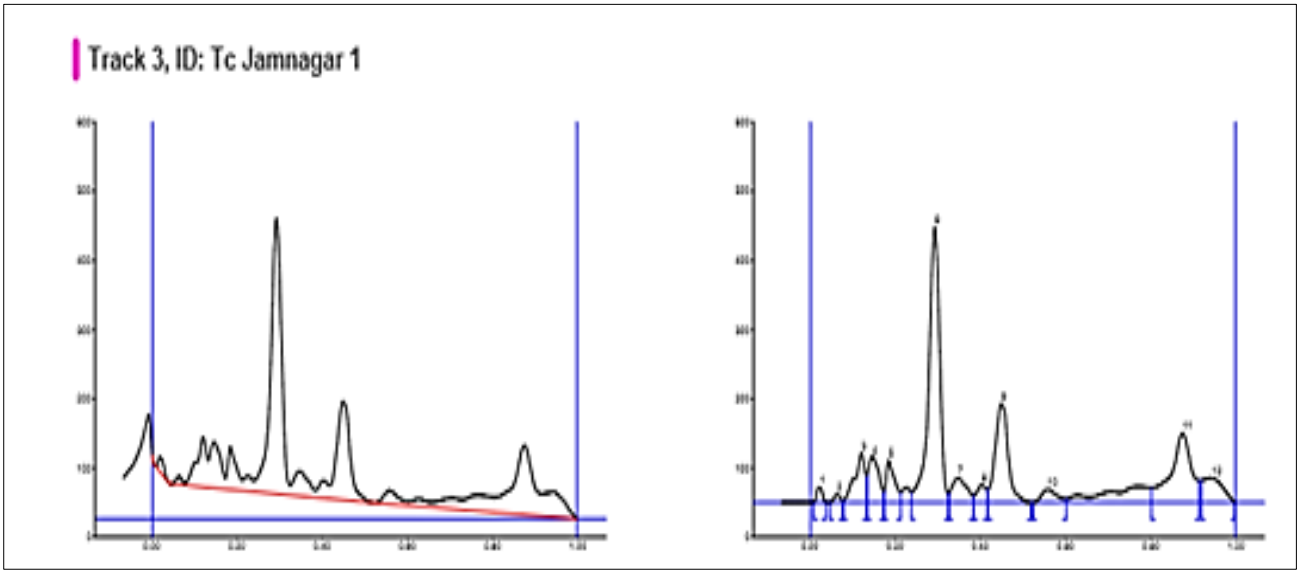
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.02	0.1	0.04	15.9	0.99	0.05	11.3	259.6	0.53	unknown *
2	0.05	11.4	0.08	40.0	2.49	0.10	18.9	983.5	2.03	unknown *
3	0.10	19.1	0.13	87.9	5.48	0.15	57.0	2522.0	5.20	unknown *
4	0.15	58.4	0.17	138.8	8.65	0.23	4.8	3960.8	8.16	unknown *
5	0.23	5.0	0.32	787.3	49.06	0.35	71.3	22488.7	46.35	unknown *
6	0.35	71.9	0.36	82.4	5.14	0.39	27.6	1952.5	4.02	unknown *
7	0.39	27.7	0.43	56.1	3.50	0.44	47.5	1714.0	3.53	unknown *
8	0.45	47.6	0.47	109.0	6.79	0.49	7.9	2196.4	4.53	unknown *
9	0.50	8.1	0.52	22.7	1.42	0.54	22.0	597.8	1.23	unknown *
10	0.54	22.0	0.58	46.7	2.91	0.61	33.3	1901.2	3.92	unknown *
11	0.61	33.4	0.65	68.8	4.29	0.76	22.1	4378.7	9.02	unknown *
12	0.76	22.2	0.85	43.5	2.71	0.85	42.6	2293.7	4.73	unknown *
13	0.86	42.6	0.89	105.4	6.57	0.94	5.2	3275.3	6.75	unknown *

Figure 4.54: Representative chromatogram of *T. cuneifolia* (Kutch)



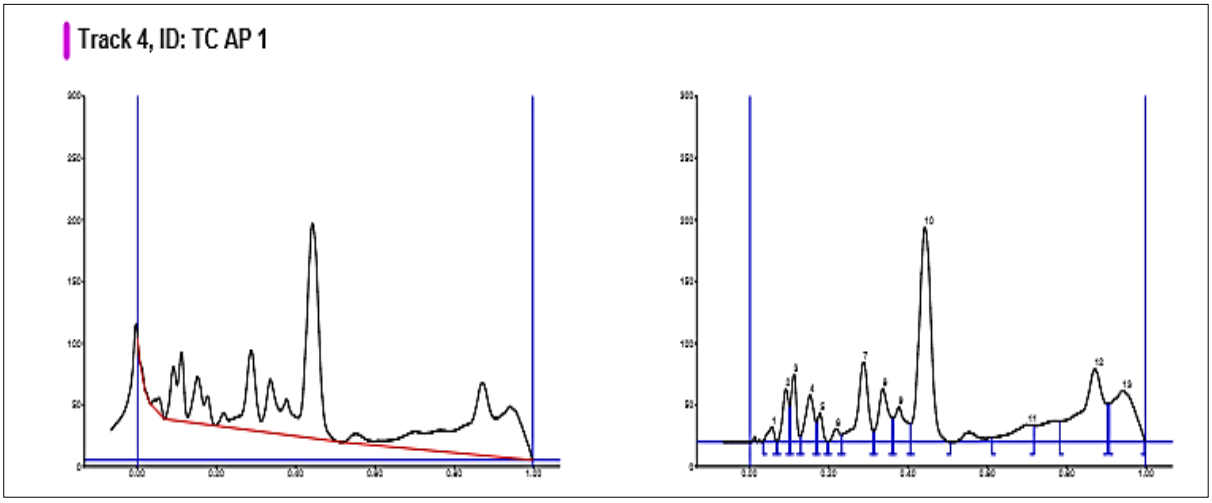
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	12.4	0.02	53.9	7.35	0.06	0.0	631.7	3.78	unknown *
2	0.09	2.8	0.13	83.6	11.41	0.15	38.5	1430.1	8.56	unknown *
3	0.15	39.0	0.17	49.8	6.79	0.18	32.4	1188.0	7.11	unknown *
4	0.18	33.5	0.19	82.3	11.23	0.22	12.8	1260.7	7.54	unknown *
5	0.23	13.1	0.23	13.3	1.81	0.25	0.3	144.1	0.86	unknown *
6	0.25	0.1	0.30	122.1	16.65	0.33	1.9	2821.8	16.88	unknown *
7	0.33	2.2	0.36	18.6	2.54	0.39	3.3	515.7	3.09	unknown *
8	0.42	9.6	0.46	117.7	16.06	0.51	0.8	2929.2	17.53	unknown *
9	0.53	0.0	0.57	15.1	2.06	0.61	4.8	500.5	2.99	unknown *
10	0.73	13.3	0.78	29.3	4.00	0.81	23.7	1342.9	8.04	unknown *
11	0.81	23.7	0.84	37.3	5.09	0.85	32.4	752.9	4.51	unknown *
12	0.85	32.6	0.88	74.0	10.09	0.92	27.5	2723.5	16.30	unknown *
13	0.96	26.1	0.96	36.0	4.91	1.00	1.1	471.4	2.82	unknown *

Figure 4.55: Representative chromatogram of *T. cuneifolia* (Jamnagar)



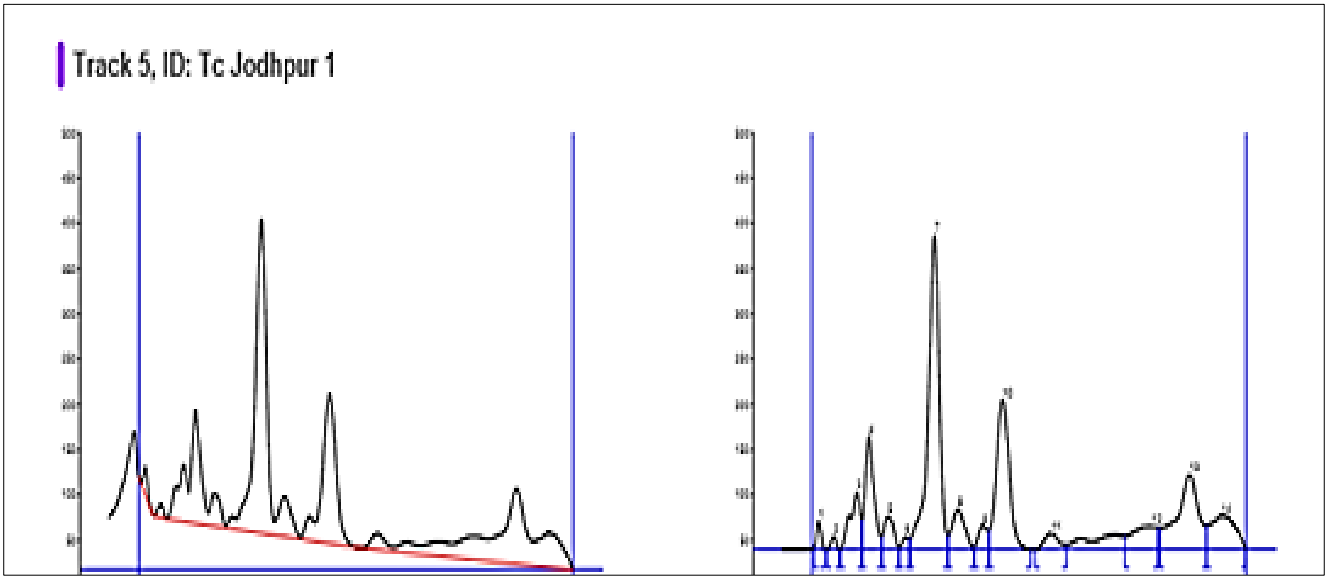
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	0.8	0.02	22.0	2.22	0.04	0.2	258.5	1.04	unknown *
2	0.05	0.0	0.06	12.1	1.22	0.08	3.2	149.5	0.60	unknown *
3	0.08	3.4	0.12	71.7	7.23	0.13	38.4	1512.5	6.06	unknown *
4	0.13	40.0	0.15	66.6	6.72	0.17	15.8	1417.0	5.68	unknown *
5	0.17	16.9	0.19	61.2	6.17	0.21	15.4	1068.5	4.28	unknown *
6	0.24	16.1	0.29	398.0	40.12	0.33	13.5	8907.6	35.69	unknown *
7	0.33	13.9	0.35	35.8	3.61	0.38	10.1	1071.3	4.29	unknown *
8	0.39	10.2	0.40	25.3	2.55	0.42	19.3	494.6	1.98	unknown *
9	0.42	19.3	0.45	142.8	14.40	0.52	0.1	3795.9	15.21	unknown *
10	0.52	0.0	0.56	19.5	1.96	0.60	6.2	609.1	2.44	unknown *
11	0.80	21.8	0.88	100.2	10.10	0.91	32.4	4184.1	16.76	unknown *
12	0.92	32.7	0.95	36.7	3.70	1.00	0.8	1489.0	5.97	unknown *

Figure 4.56: Representative chromatogram of *T. cuneifolia* (Andhra Pradesh)



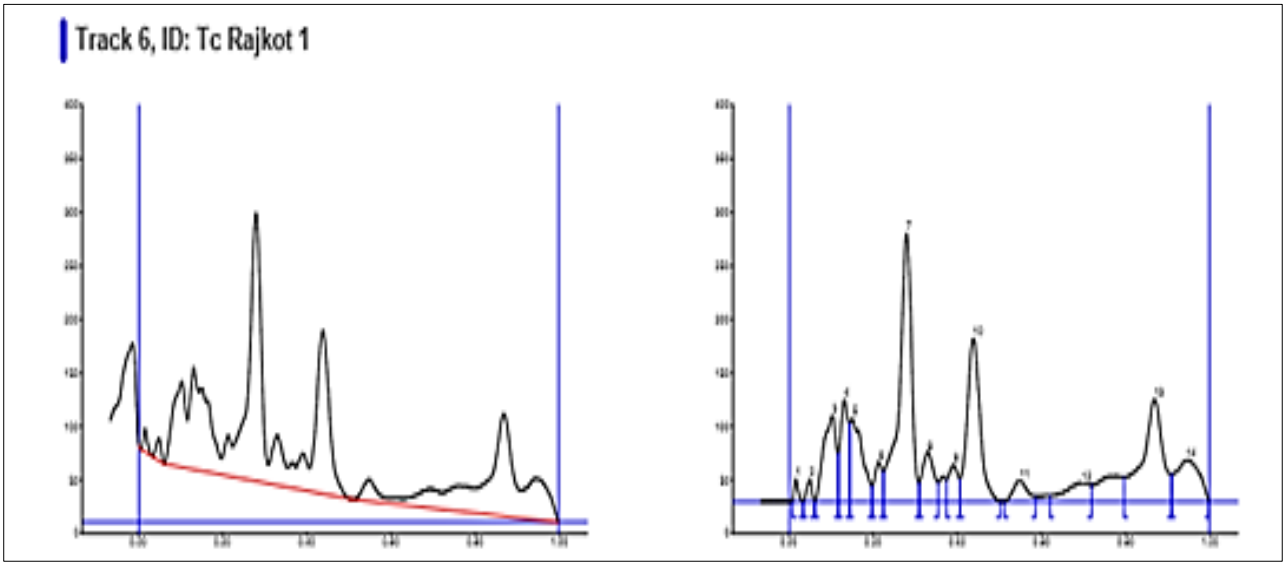
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.03	0.3	0.06	12.5	2.05	0.07	0.2	169.6	1.07	unknown *
2	0.07	0.0	0.09	43.2	7.08	0.10	28.8	567.1	3.59	unknown *
3	0.10	29.6	0.11	55.5	9.10	0.13	4.3	640.3	4.05	unknown *
4	0.13	4.7	0.15	38.1	6.24	0.17	16.0	711.2	4.50	unknown *
5	0.17	16.4	0.18	23.1	3.79	0.20	0.5	261.4	1.65	unknown *
6	0.20	0.5	0.22	11.0	1.80	0.23	5.8	164.2	1.04	unknown *
7	0.23	5.8	0.29	65.0	10.66	0.31	8.4	1507.4	9.53	unknown *
8	0.31	8.6	0.34	43.2	7.08	0.36	19.3	957.9	6.06	unknown *
9	0.36	19.4	0.38	29.0	4.76	0.41	15.1	704.5	4.45	unknown *
10	0.41	15.2	0.44	173.9	28.51	0.51	0.0	4537.8	28.69	unknown *
11	0.61	3.9	0.70	14.4	2.35	0.72	13.6	705.0	4.46	unknown *
12	0.78	16.9	0.87	59.2	9.70	0.90	31.0	2877.3	18.19	unknown *
13	0.91	31.4	0.94	41.9	6.87	1.00	1.2	2010.7	12.71	unknown *

Figure 4.57: Representative chromatogram of *T. cuneifolia* (Jodhpur)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	2.8	0.02	31.2	3.02	0.03	0.6	276.7	1.08	unknown *
2	0.04	0.2	0.05	14.5	1.41	0.07	0.1	155.8	0.61	unknown *
3	0.07	0.3	0.11	62.9	6.10	0.12	32.9	1290.2	5.02	unknown *
4	0.12	33.2	0.13	125.1	12.12	0.16	13.5	2209.8	8.60	unknown *
5	0.16	13.8	0.18	35.9	3.48	0.20	1.7	690.1	2.69	unknown *
6	0.20	1.7	0.22	13.8	1.34	0.23	11.8	176.9	0.69	unknown *
7	0.23	11.9	0.28	346.9	33.60	0.31	15.4	7801.4	30.36	unknown *
8	0.31	15.4	0.34	44.1	4.27	0.37	2.2	1139.9	4.44	unknown *
9	0.37	2.5	0.39	26.8	2.60	0.41	20.8	487.8	1.90	unknown *
10	0.41	21.3	0.44	166.0	16.08	0.50	0.1	4290.3	16.69	unknown *
11	0.51	0.0	0.55	18.3	1.77	0.59	4.9	515.7	2.01	unknown *
12	0.72	14.8	0.78	25.8	2.50	0.80	24.3	1258.1	4.90	unknown *
13	0.80	23.8	0.87	82.9	8.03	0.91	27.0	3536.2	13.76	unknown *
14	0.91	27.0	0.94	37.9	3.67	1.00	1.2	1870.8	7.28	unknown *

Figure 4.58: Representative chromatogram of *T. cuneifolia* (Rajkot)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	0.6	0.02	21.4	2.16	0.03	0.1	164.1	0.63	unknown *
2	0.03	0.5	0.05	20.9	2.11	0.06	0.8	215.7	0.83	unknown *
3	0.06	0.1	0.10	80.3	8.12	0.12	45.2	2071.7	7.93	unknown *
4	0.12	46.7	0.13	95.2	9.64	0.14	73.5	1538.2	5.89	unknown *
5	0.15	73.6	0.15	77.2	7.82	0.20	14.9	1989.0	7.61	unknown *
6	0.20	15.1	0.21	37.6	3.80	0.22	28.3	577.1	2.21	unknown *
7	0.23	28.3	0.28	250.4	25.34	0.31	17.5	6361.2	24.35	unknown *
8	0.31	18.0	0.33	46.6	4.71	0.35	18.5	1099.1	4.21	unknown *
9	0.37	20.5	0.39	34.2	3.46	0.41	21.7	690.7	2.64	unknown *
10	0.41	22.0	0.44	152.4	15.43	0.50	0.0	4072.3	15.59	unknown *
11	0.51	0.3	0.55	20.2	2.04	0.59	5.2	578.4	2.21	unknown *
12	0.62	5.9	0.70	17.9	1.81	0.72	15.5	924.6	3.54	unknown *
13	0.80	23.0	0.87	95.5	9.66	0.91	25.7	3947.6	15.11	unknown *
14	0.91	25.7	0.95	38.5	3.90	1.00	1.2	1891.8	7.24	unknown *

4.2.17 SEASONAL VARIATION

In this section of seasonal variation of roots, the study was done on summer, monsoon, and winter. All seasons showed presence of β-sitosterol but only summer season showed presence of lupeol. Monsoon season showed highest amount of β-sitosterol (7.08%) and least was found in summer season with 2.64%. Winter showed moderate amount.

Table 4.13: Seasonal variation of *T. cuneifolia* roots with β-sitosterol and lupeol

	Winter	Summer	Monsoon
β-sitosterol	3.89%	2.64%	**7.08%**
Lupeol	-	1.42%	-

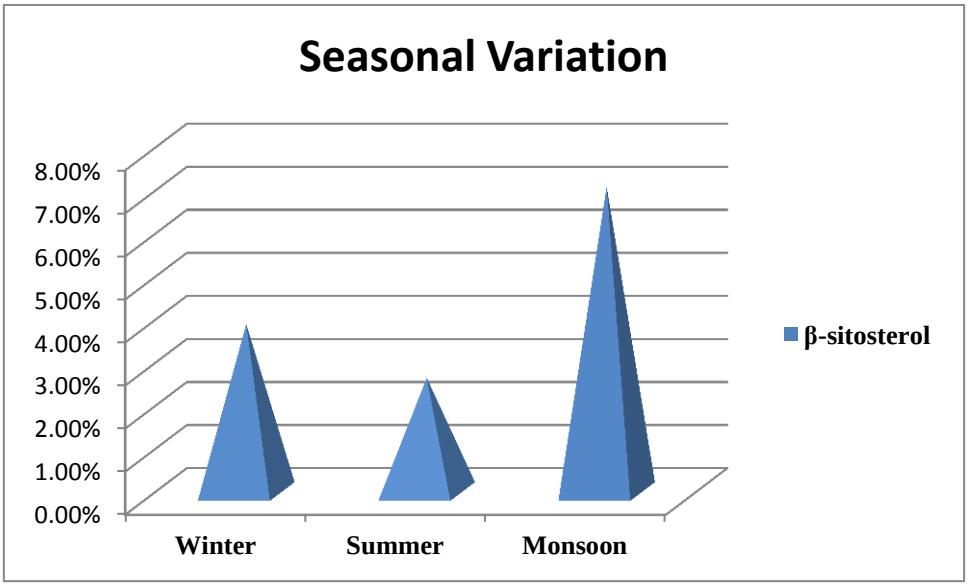
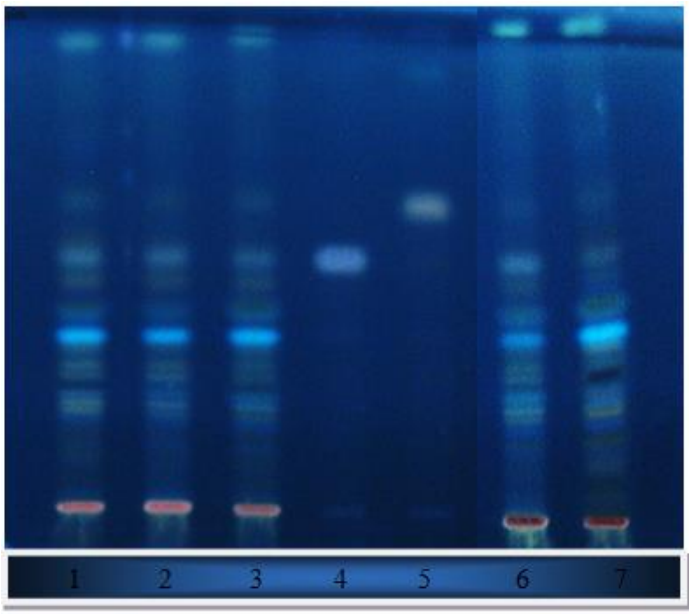


Figure 4.59: Graph showing Seasonal variation by HPTLC

Plate 4.5 HPTLC plate photo representing seasonal variation of *T. cuneifolia* and species
variation of roots the roots of genus *Taverniera*

Plate Photo at 336nm



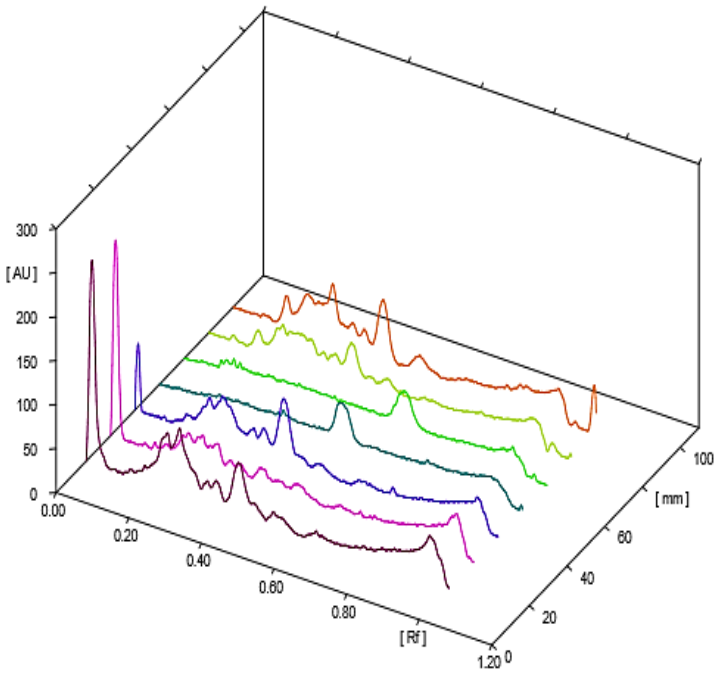
Order of spotting:

Track 1 *T.cuneifolia* summer (1:1) Track 2 *T.cuneifolia* monsoon (1:1)

Track 3 *T.cuneifolia* winter (1:1) Track 4 betasitosterol 10 ppm

Track 5 lupeol 10 ppm Track 6 Rajkot (1:1)

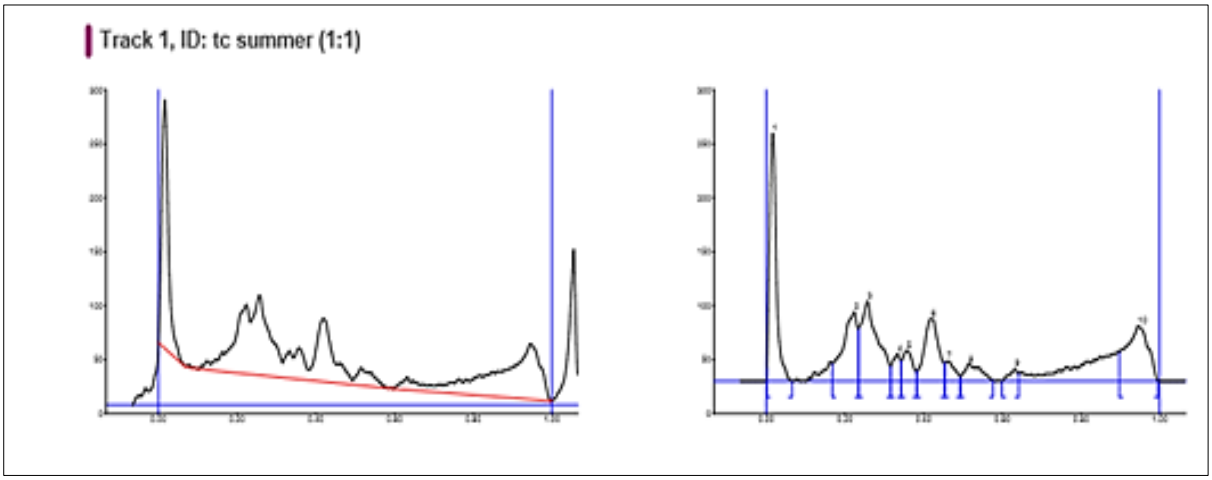
Track 7 *T. abyssinica* (1:1)



Sample ID

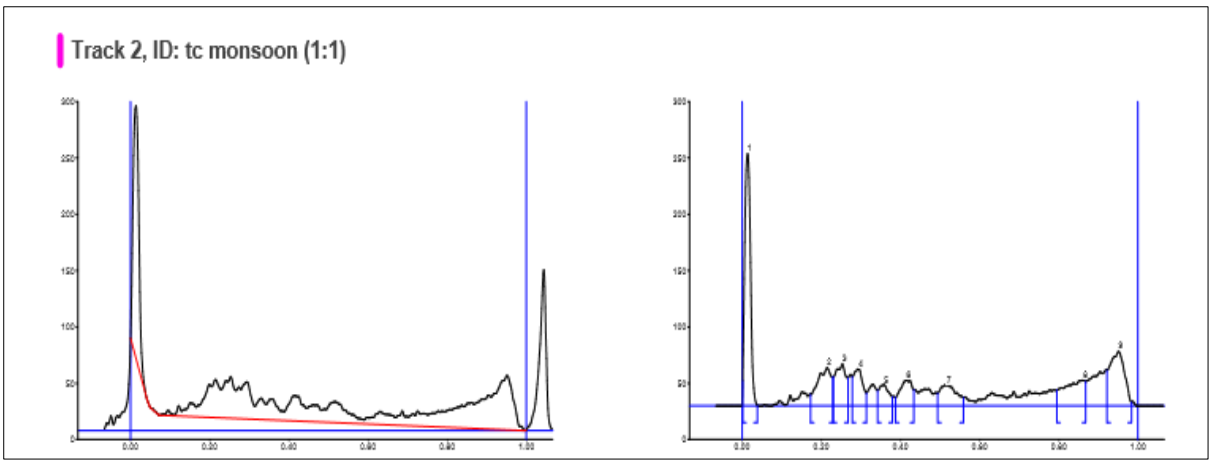
***T. cuneifolia* summer**
***T. cuneifolia* monsoon**
***T.cuneifolia* winter**
betasitosterol
lupeol
***T. cuneifolia* (Rajkot)**
T. abyssinica

Figure 4.60: Representative chromatogram of *T. cuneifolia* (Rajkot) Summer season



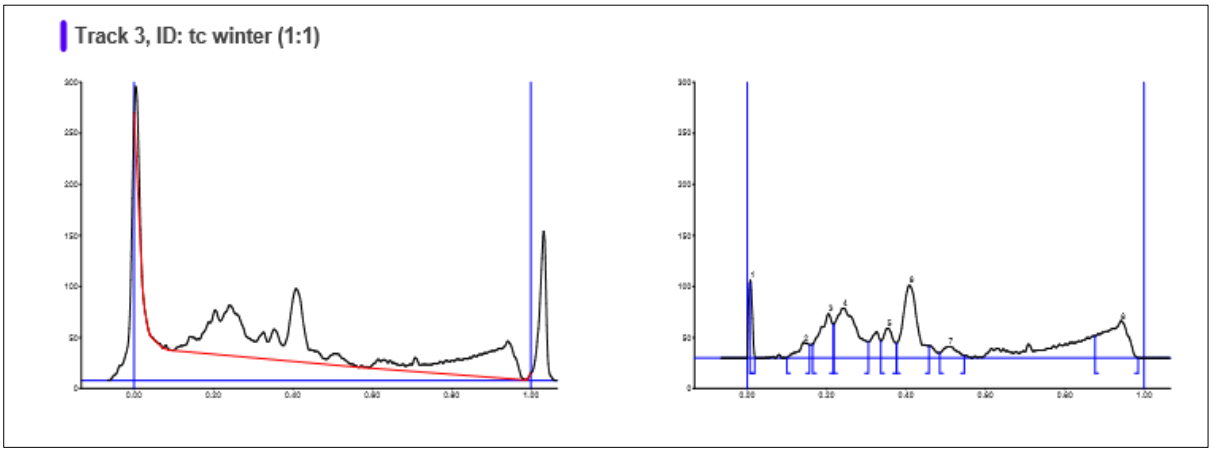
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.00	12.4	0.02	230.4	39.84	0.07	0.1	3451.3	23.45	unknown *
2	0.17	16.2	0.22	64.2	11.10	0.23	48.4	2036.0	13.83	unknown *
3	0.24	49.0	0.26	74.0	12.79	0.32	13.6	2713.4	18.43	unknown *
4	0.32	13.7	0.33	25.3	4.38	0.34	19.2	417.2	2.83	unknown *
5	0.34	19.3	0.36	28.8	4.99	0.38	9.3	625.6	4.25	unknown *
6	0.38	9.5	0.42	58.2	10.06	0.45	16.9	1830.5	12.44	unknown *
7	0.45	17.0	0.46	18.2	3.15	0.49	4.1	388.3	2.64	unknown *
8	0.49	4.1	0.52	16.4	2.83	0.58	1.1	585.2	3.98	unknown *
9	0.60	0.2	0.63	11.6	2.01	0.64	7.3	209.6	1.42	unknown *
10	0.90	27.2	0.95	51.3	8.87	1.00	0.1	2462.5	16.73	unknown *

Figure 4.61: Representative chromatogram of *T. cuneifolia* (Rajkot) monsoon season



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.00	22.4	0.01	223.9	48.80	0.04	0.3	2866.6	29.04	unknown *
2	0.17	10.3	0.21	33.7	7.34	0.23	25.4	1038.9	10.53	unknown *
3	0.23	25.7	0.25	37.0	8.07	0.27	25.0	886.3	8.98	unknown *
4	0.28	26.1	0.29	33.0	7.19	0.31	11.7	701.5	7.11	unknown *
5	0.34	13.9	0.36	18.8	4.09	0.38	8.3	424.8	4.30	unknown *
6	0.39	7.8	0.42	22.6	4.92	0.43	14.5	623.2	6.31	unknown *
7	0.49	11.3	0.52	18.6	4.05	0.56	7.6	698.8	7.08	unknown *
8	0.80	14.2	0.86	23.0	5.02	0.87	21.8	1053.0	10.67	unknown *
9	0.92	31.9	0.95	48.2	10.51	0.99	3.6	1576.5	15.97	unknown *

Figure 4.62: Representative chromatogram of *T. cuneifolia* (Rajkot) winter season



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	73.5	0.01	76.3	22.93	0.02	1.1	426.8	4.56	unknown *
2	0.10	0.5	0.14	15.2	4.57	0.16	12.7	360.0	3.84	unknown *
3	0.17	13.4	0.21	43.3	13.01	0.22	33.2	1188.3	12.68	unknown *
4	0.22	33.2	0.24	49.3	14.82	0.31	16.1	2209.8	23.59	unknown *
5	0.34	16.8	0.35	29.6	8.89	0.38	14.5	665.7	7.11	unknown *
6	0.38	14.6	0.41	71.2	21.41	0.46	11.9	2230.3	23.81	unknown *
7	0.49	5.1	0.51	11.2	3.38	0.55	2.4	364.5	3.89	unknown *
8	0.88	21.6	0.94	36.6	11.00	0.99	0.1	1923.5	20.53	unknown *

4.2.18 SPECIES VARIATION

In this roots of two species of *Taverniera* i.e. *T. cuneifolia* and *T. abyssinica* were compared for their β-sitosterol and lupeol content. B-sitosterol concentration is higher in T. cuneifolia compare to T. abyssinica while Lupeol was absent in both the species. Though the lupeol is present in T.cuneifolia in summer, the content of lupeol is not present in the plant material collected during monsoon period.

Table 4.14: Species variation of *Taverniera* roots with β-sitosterol and lupeol

	Taverniera abyssinica	*Taverniera cuneifolia*
β-sitosterol -	6.89%	**10.04%**
Lupeol	-	**-**

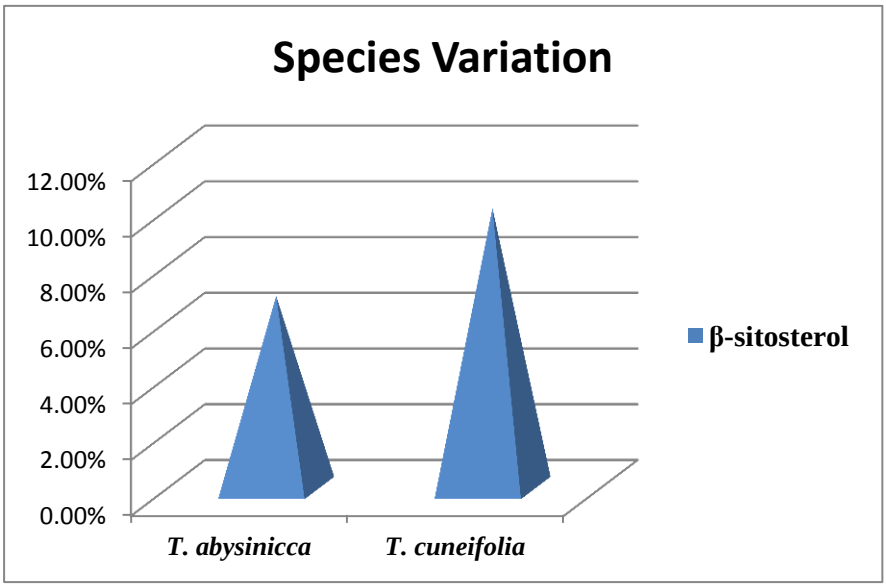
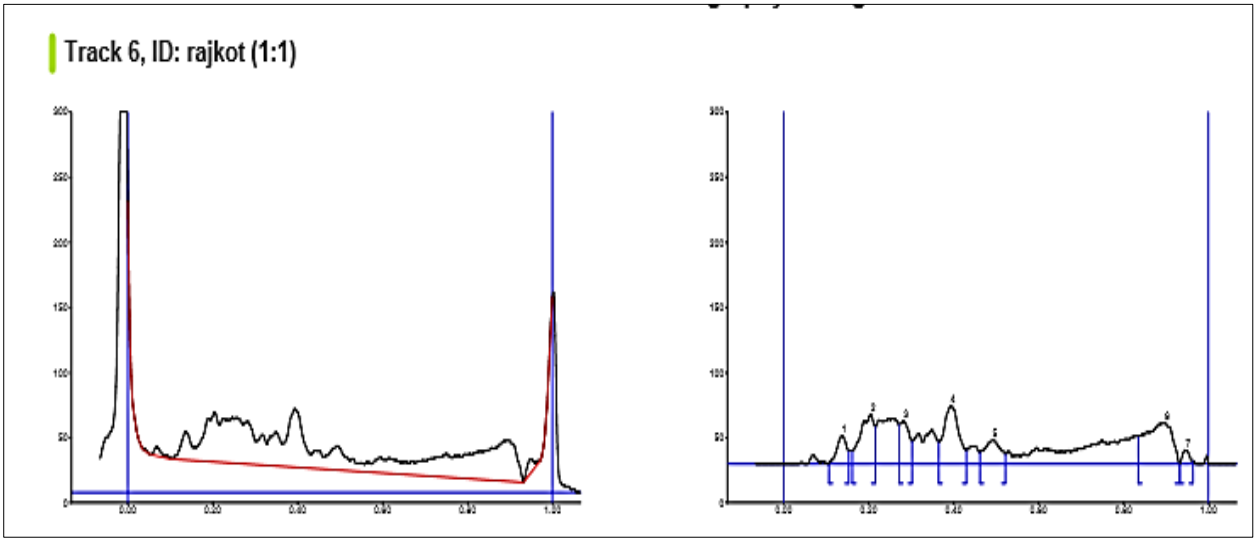


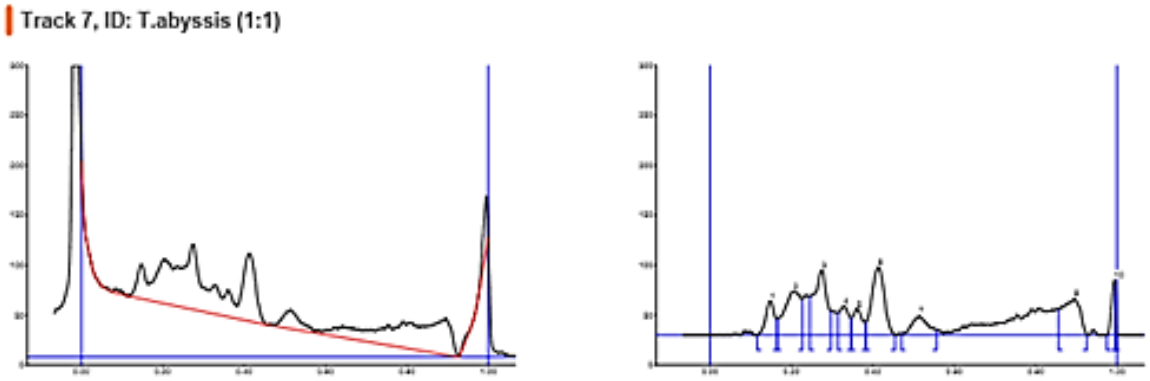
Figure 4.63: Graph showing species variation by HPTLC

Figure 4.64: Representative chromatogram of *T. cuneifolia* (Rajkot)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.11	0.3	0.14	22.0	11.06	0.15	10.2	429.4	7.03	unknown *
2	0.16	10.4	0.21	38.4	19.29	0.22	28.8	1095.5	17.93	unknown *
3	0.27	30.4	0.28	32.9	16.56	0.30	17.9	665.2	10.89	unknown *
4	0.37	17.1	0.39	44.8	22.52	0.43	10.8	1402.4	22.96	unknown *
5	0.46	9.3	0.49	18.5	9.31	0.52	7.9	613.1	10.04	unknown *
6	0.84	21.5	0.90	31.5	15.82	0.93	0.5	1748.9	28.63	unknown *
7	0.93	0.2	0.95	10.8	5.44	0.97	2.5	154.7	2.53	unknown *

Figure 4.65: Representative chromatogram of *T. abyssinica*

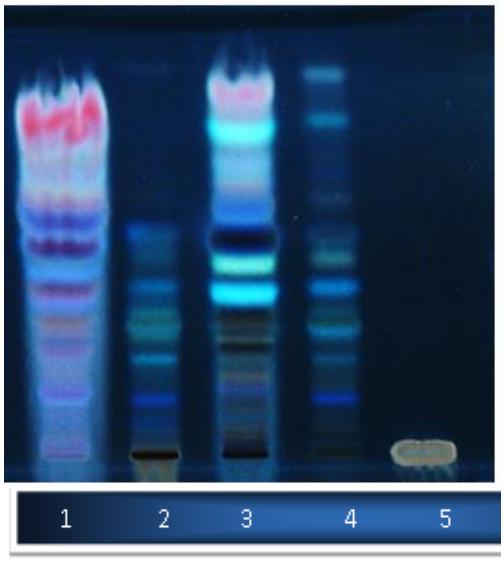


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.12	0.5	0.15	34.3	8.17	0.16	16.0	636.1	6.70	unknown *
2	0.17	16.2	0.21	44.4	10.59	0.23	36.1	1545.7	16.27	unknown *
3	0.25	38.9	0.27	65.7	15.68	0.30	24.0	1794.3	18.89	unknown *
4	0.31	22.4	0.33	29.4	7.01	0.35	17.2	604.7	6.37	unknown *
5	0.35	17.3	0.36	26.6	6.34	0.38	12.9	531.4	5.60	unknown *
6	0.38	13.0	0.41	67.9	16.20	0.45	0.0	1820.3	19.17	unknown *
7	0.47	1.3	0.51	18.8	4.49	0.56	5.1	654.6	6.89	unknown *
8	0.86	26.1	0.90	36.8	8.78	0.93	0.3	1387.8	14.61	unknown *
9	0.98	0.1	0.99	54.5	13.01	1.00	50.6	458.1	4.82	unknown *
10	1.00	0.1	0.99	40.8	9.73	1.00	50.6	64.5	0.68	unknown *

4.2.19 Elutropic series

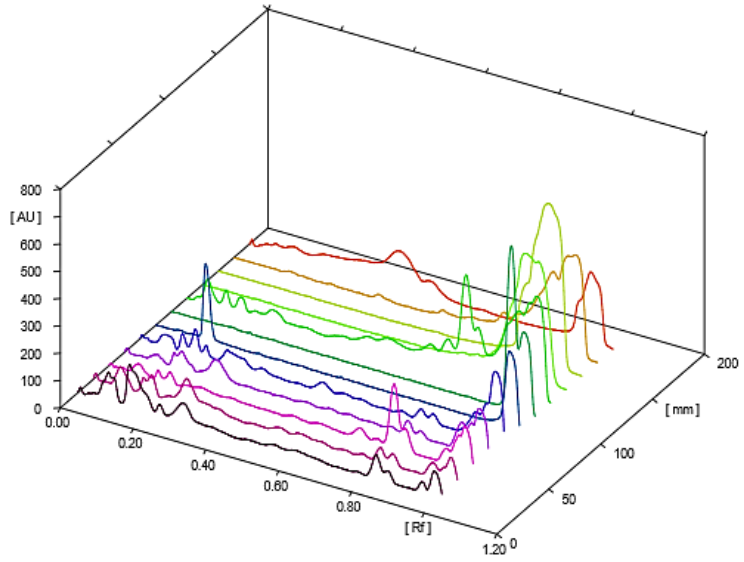
The elutropic series showed presence of 18β-glycyrrhetinic acid in all the solvents except in petroleum ether extract. Highest amount of 18β-glycyrrhetinic acid was found in ethyl acetate extract with 44.47% and next was water (42.14%) and least amount was found in methanol (23.10%). Very minute amount of Glycyrrhizin (1.65%) falling between the range of Rf 0.13-0.17 was seen which had not been assigned by the instrument.

Plate 4.6: .HPTLC plate photo representing elutropic series of the roots of *T. cuneifolia* (366nm)



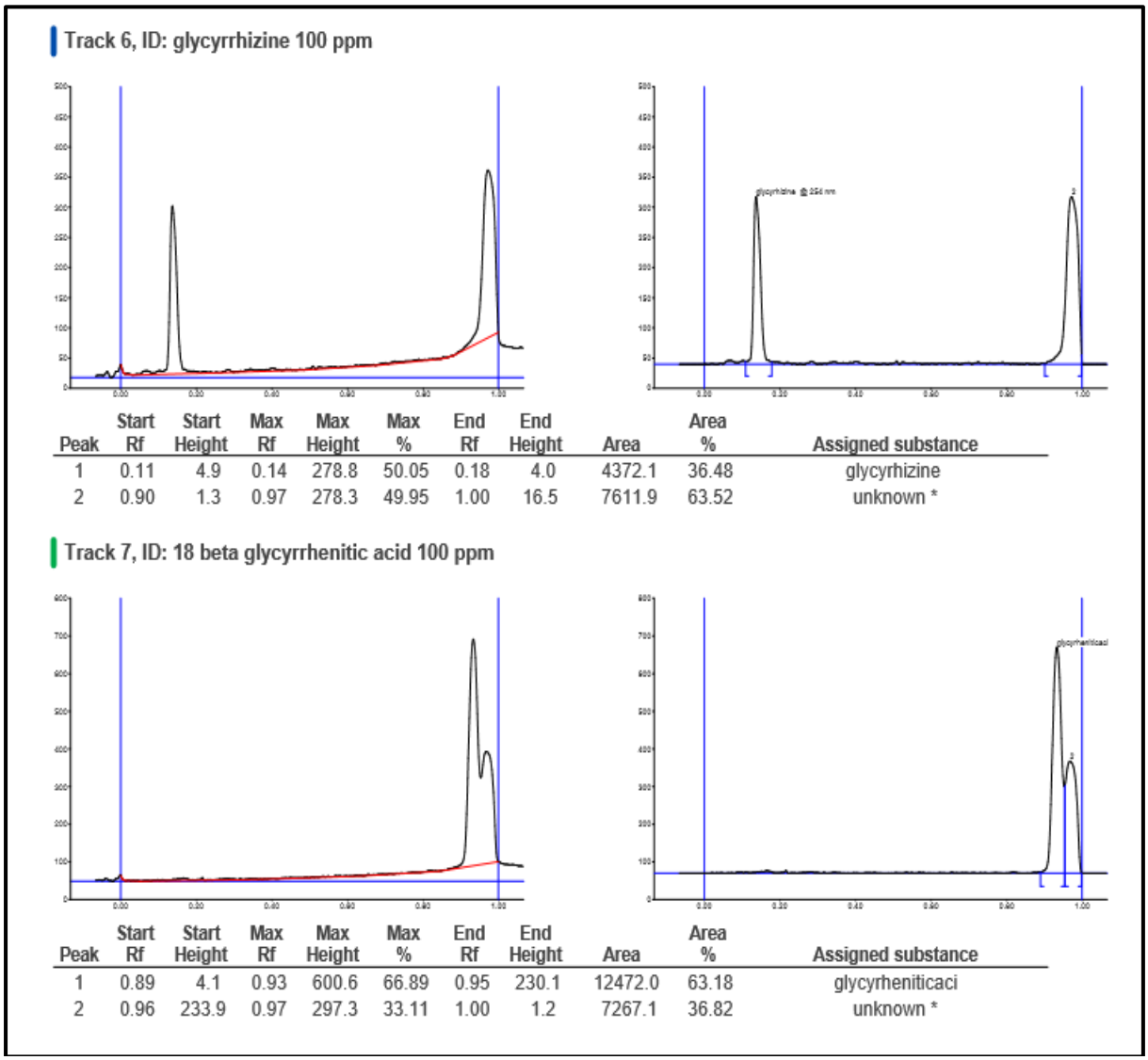
Order of spotting:

1. Petether extract (10 µl)
2. Methanolic extract (10 µl)
3. Dichloromethane extract (10 µl)
4. Ethylacetate extract (10 µl)
5. Water extract (10 µl)



Sample ID	Sample ID
Tc Rajkot	18 beta glycyrrhetinic acid
Tc Jamnagar	ES MeOH (Methanol)
Tc AP	ES DCM (Dichloromethane)
Tc Jodhpur 1	ES Petether (Petroleum ether)
Tc Kutch	ES EA (Ethyl acetate)
Glycyrrhizin	ES Water

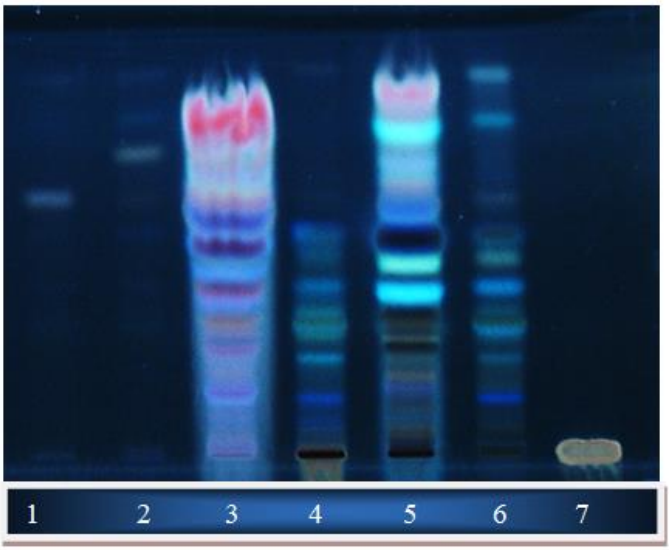
Figure 4.66: Representative chromatogram of standard glycyrrhizine and 18β-glycyrrhetinic acid



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.11	4.9	0.14	278.8	50.05	0.18	4.0	4372.1	36.48	glycyrhizine
2	0.90	1.3	0.97	278.3	49.95	1.00	16.5	7611.9	63.52	unknown *

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.89	4.1	0.93	600.6	66.89	0.95	230.1	12472.0	63.18	glycyrheniticaci
2	0.96	233.9	0.97	297.3	33.11	1.00	1.2	7267.1	36.82	unknown *

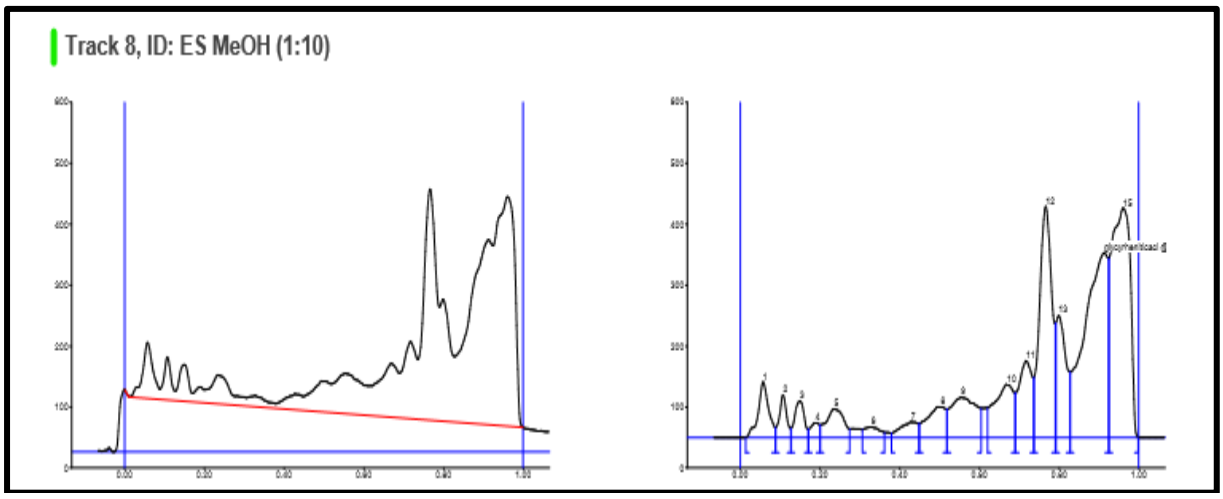
Fig 4.7: HPTLC plate photo representing elutropic series of the roots of *T. cuneifolia* (366nm)



Order of spotting:

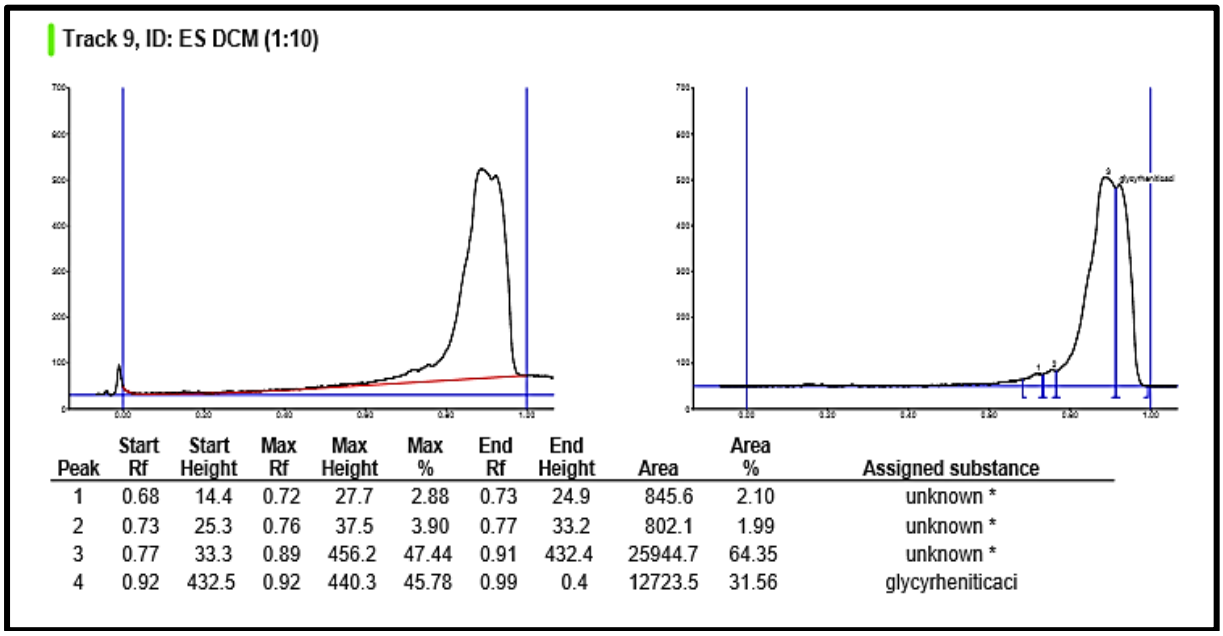
1. Betasitosterol 10ppm (10µl)
2. Lupeol 10ppm (20µl)
3. Petether extract (10 µl)
4. Methanolic extract (10 µl)
5. Dichloromethane extract (10 µl)
6. Ethylacetate extract (10 µl)
7. Water extract (10 µl)

Figure 4.67: Representative chromatogram of *T. cuneifolia* (Rajkot) Methanolic extract



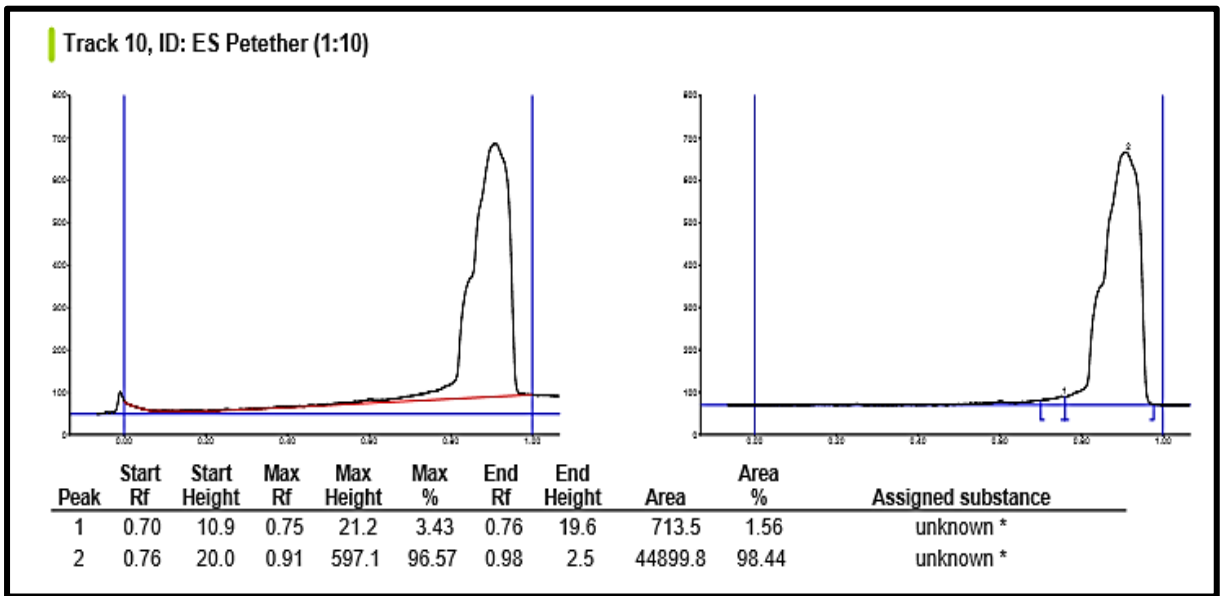
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	0.3	0.06	91.6	4.75	0.09	16.7	2118.0	3.16	unknown *
2	0.09	16.7	0.11	70.3	3.64	0.13	15.6	1109.2	1.65	unknown *
3	0.13	15.6	0.15	59.6	3.09	0.17	14.3	1254.6	1.87	unknown *
4	0.17	14.4	0.19	25.3	1.31	0.20	21.1	468.1	0.70	unknown *
5	0.20	21.2	0.24	47.3	2.45	0.27	14.6	1823.0	2.72	unknown *
6	0.31	13.4	0.33	17.9	0.93	0.36	8.9	622.3	0.93	unknown *
7	0.38	7.4	0.43	26.0	1.35	0.45	23.1	1013.4	1.51	unknown *
8	0.45	23.1	0.50	50.7	2.63	0.52	46.9	2081.9	3.10	unknown *
9	0.52	46.9	0.55	66.3	3.44	0.61	48.7	3728.1	5.55	unknown *
10	0.62	49.2	0.67	88.0	4.56	0.69	73.7	3707.2	5.52	unknown *
11	0.69	73.9	0.72	126.4	6.55	0.74	98.1	3655.4	5.45	unknown *
12	0.74	99.1	0.77	379.5	19.67	0.79	187.1	10353.7	15.43	unknown *
13	0.79	188.7	0.80	200.2	10.38	0.83	107.7	4327.5	6.45	unknown *
14	0.83	107.7	0.91	303.3	15.73	0.93	294.4	15504.0	23.10	glycyrheniticaci
15	0.93	294.9	0.96	376.6	19.52	1.00	0.8	15355.0	22.88	unknown *

Figure 4.68: Representative chromatogram of *T. cuneifolia* (Rajkot) Dichloromethane extract



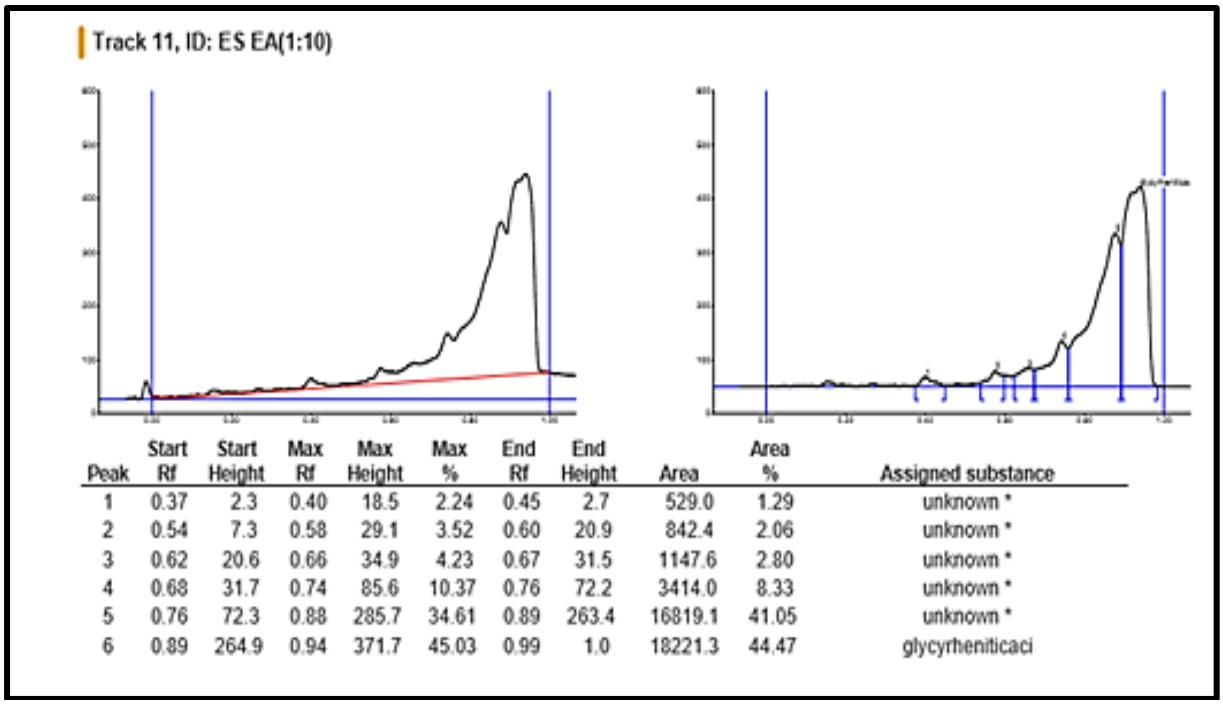
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.68	14.4	0.72	27.7	2.88	0.73	24.9	845.6	2.10	unknown *
2	0.73	25.3	0.76	37.5	3.90	0.77	33.2	802.1	1.99	unknown *
3	0.77	33.3	0.89	456.2	47.44	0.91	432.4	25944.7	64.35	unknown *
4	0.92	432.5	0.92	440.3	45.78	0.99	0.4	12723.5	31.56	glycyrheniticaci

Figure 4.69: Representative chromatogram of *T. cuneifolia* (Rajkot) Petroleum Ether extract



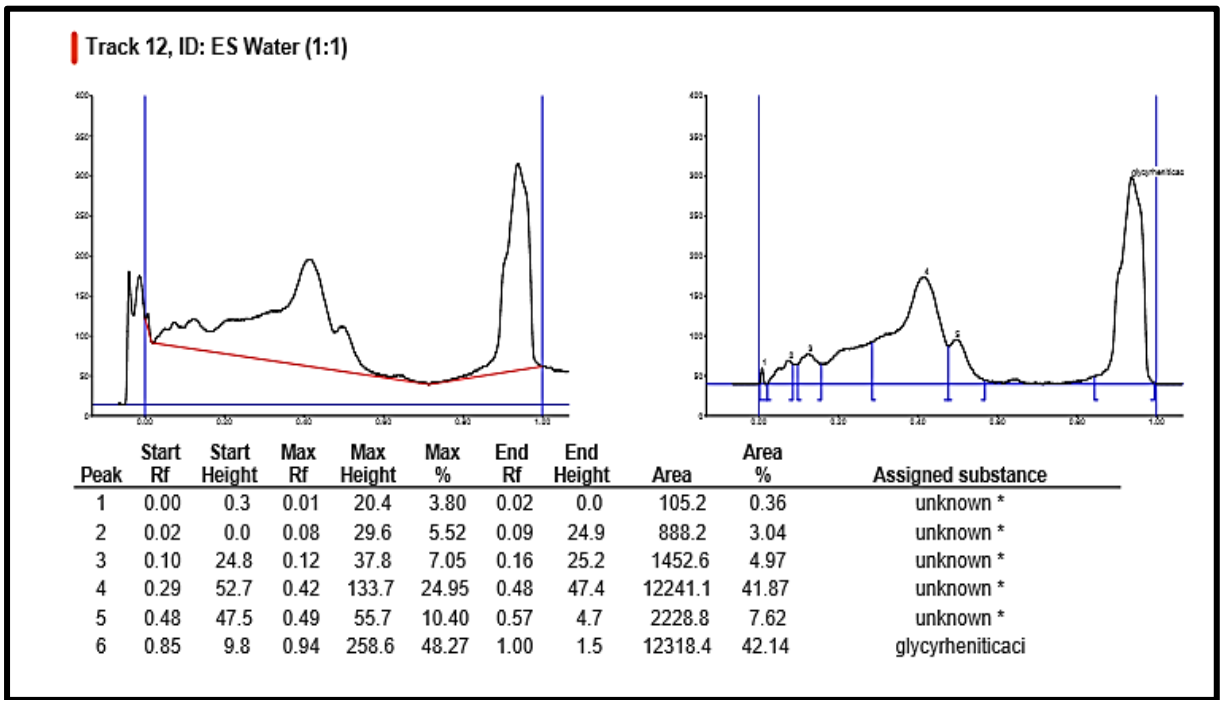
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.70	10.9	0.75	21.2	3.43	0.76	19.6	713.5	1.56	unknown *
2	0.76	20.0	0.91	597.1	96.57	0.98	2.5	44899.8	98.44	unknown *

Figure 4.70: Representative chromatogram of *T. cuneifolia* (Rajkot) Ethyl acetate extract



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.37	2.3	0.40	18.5	2.24	0.45	2.7	529.0	1.29	unknown *
2	0.54	7.3	0.58	29.1	3.52	0.60	20.9	842.4	2.06	unknown *
3	0.62	20.6	0.66	34.9	4.23	0.67	31.5	1147.6	2.80	unknown *
4	0.68	31.7	0.74	85.6	10.37	0.76	72.2	3414.0	8.33	unknown *
5	0.76	72.3	0.88	285.7	34.61	0.89	263.4	16819.1	41.05	unknown *
6	0.89	264.9	0.94	371.7	45.03	0.99	1.0	18221.3	44.47	glycyrheniticaci

Figure 4.71: Representative chromatogram of *T. cuneifolia* (Rajkot) water extract

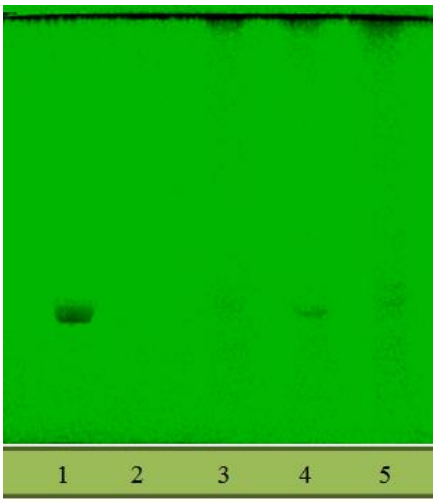


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.00	0.3	0.01	20.4	3.80	0.02	0.0	105.2	0.36	unknown *
2	0.02	0.0	0.08	29.6	5.52	0.09	24.9	888.2	3.04	unknown *
3	0.10	24.8	0.12	37.8	7.05	0.16	25.2	1452.6	4.97	unknown *
4	0.29	52.7	0.42	133.7	24.95	0.48	47.4	12241.1	41.87	unknown *
5	0.48	47.5	0.49	55.7	10.40	0.57	4.7	2228.8	7.62	unknown *
6	0.85	9.8	0.94	258.6	48.27	1.00	1.5	12318.4	42.14	glycyrheniticaci

Results and discussion:

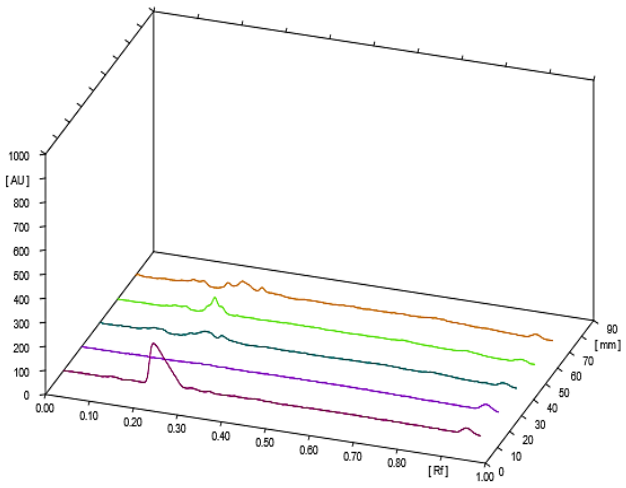
The seasonal variation showed presence of both glycyrrhizin and 18β-glycyrrhetinic acid. Highest amount of glycyrrhizin (59.14%) was seen in monsoon season and least (39.38%) was found in winter. 18β-glycyrrhetinic acid had no significant difference among all seasons. It ranged between 7.68%-8.50%.

Fig 4.7 HPTLC plate photo representing seasonal variation of roots of *T. cuneifolia* with Glycyrrhizin as standard (254nm)



Order of spotting:

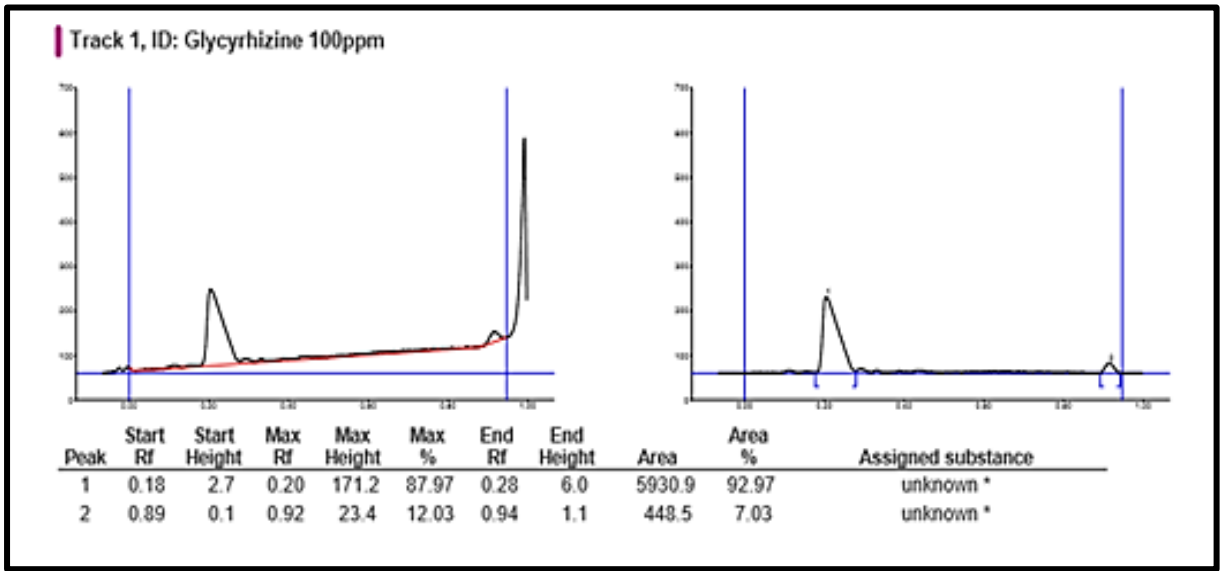
1. Glycyrrhizin 100 ppm (20 µl)
2. 18 β - Glycyrrhentinic acid 100 ppm (20 µl)
3. *T. cuneifolia* summer (10µl)
4. *T. cuneifolia* monsoon (10µl)
5. *T. cuneifolia* winter (10µl)



Sample ID

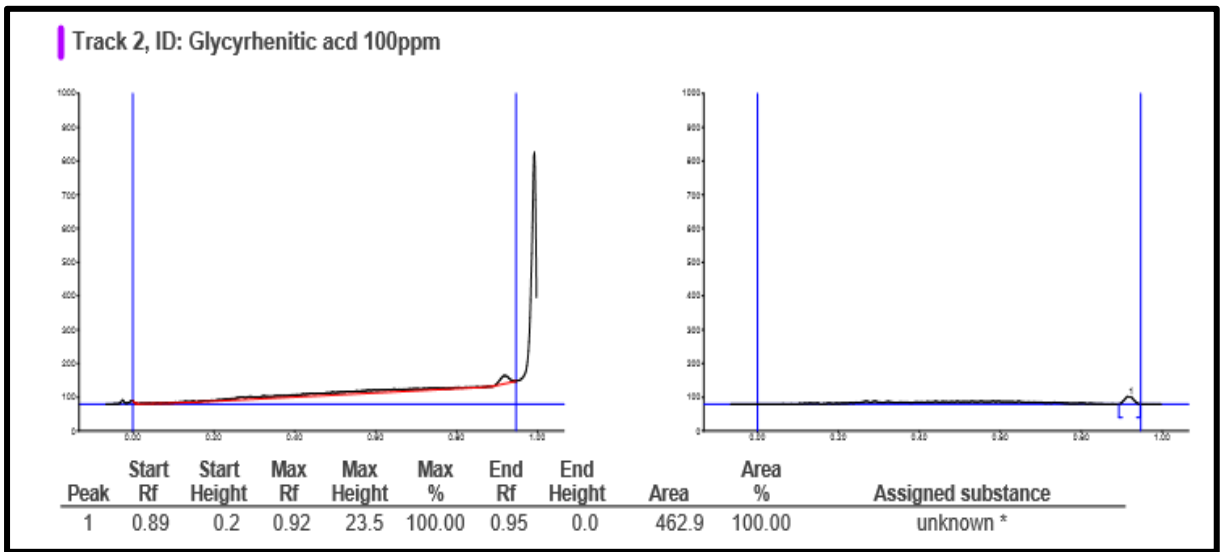
Glycyrrhizin 100 ppm
Glycyrrhentinic acid 100 ppm
Tc summer
Tc monsoon
Tc winter

Figure 4.72: Representative chromatogram of glycyrrhizine



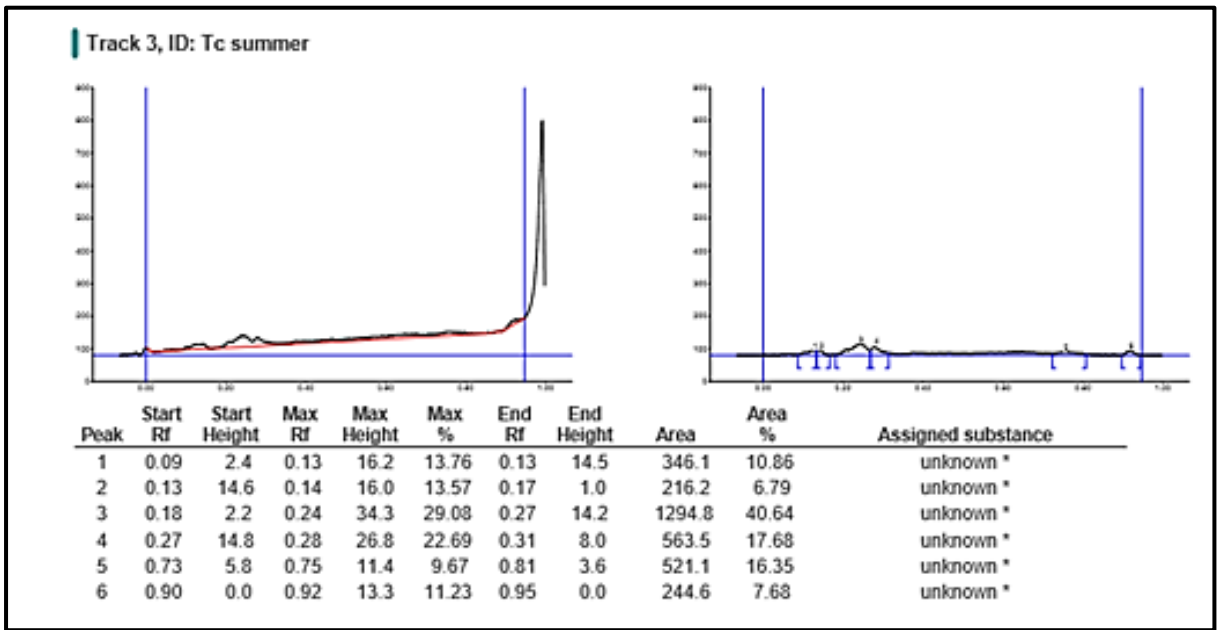
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.18	2.7	0.20	171.2	87.97	0.28	6.0	5930.9	92.97	unknown *
2	0.89	0.1	0.92	23.4	12.03	0.94	1.1	448.5	7.03	unknown *

Figure 4.73: Representative chromatogram of Glycyrhetinic acid



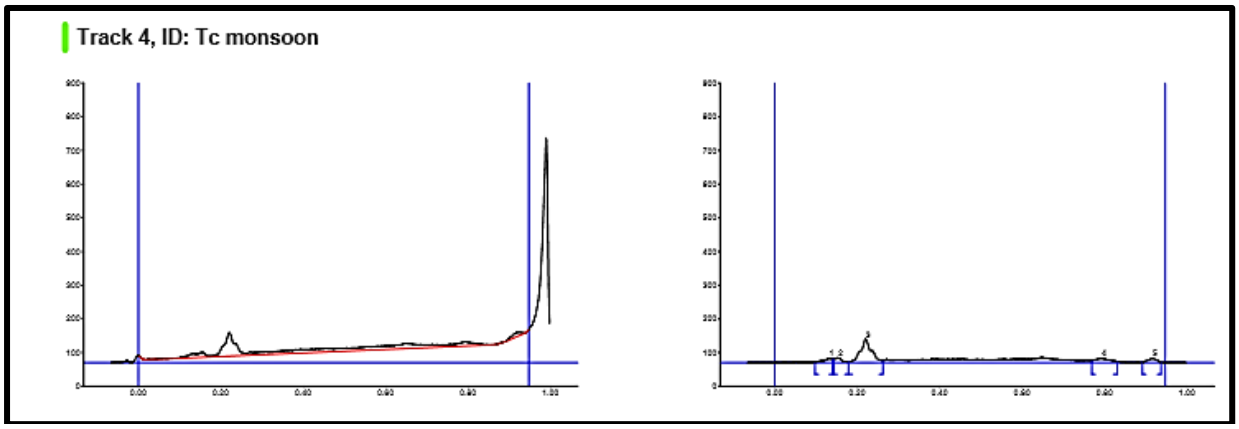
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.89	0.2	0.92	23.5	100.00	0.95	0.0	462.9	100.00	unknown *

Figure 4.74: Representative chromatogram of *T. cuneifolia* of summer season



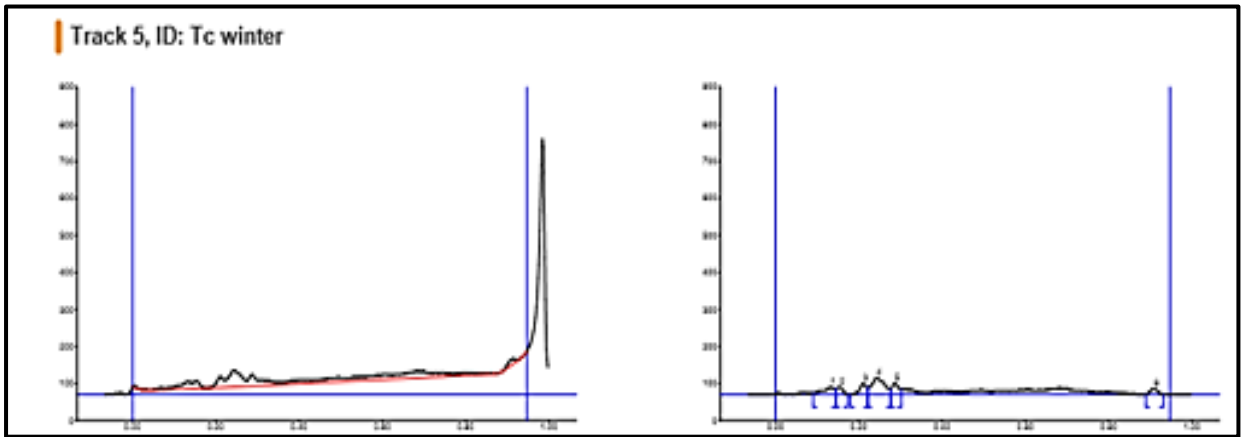
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.09	2.4	0.13	16.2	13.76	0.13	14.5	346.1	10.86	unknown *
2	0.13	14.6	0.14	16.0	13.57	0.17	1.0	216.2	6.79	unknown *
3	0.18	2.2	0.24	34.3	29.08	0.27	14.2	1294.8	40.64	unknown *
4	0.27	14.8	0.28	26.8	22.69	0.31	8.0	563.5	17.68	unknown *
5	0.73	5.8	0.75	11.4	9.67	0.81	3.6	521.1	16.35	unknown *
6	0.90	0.0	0.92	13.3	11.23	0.95	0.0	244.6	7.68	unknown *

Figure 4.75: Representative chromatogram of *T. cuneifolia* monsoon season



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.10	1.1	0.13	12.3	10.08	0.14	10.6	245.0	8.79	unknown *
2	0.14	10.6	0.15	15.1	12.33	0.18	2.7	242.9	8.71	unknown *
3	0.18	2.7	0.22	70.2	57.43	0.26	6.6	1648.3	59.14	unknown *
4	0.77	6.9	0.79	12.5	10.19	0.83	3.5	414.0	14.85	unknown *
5	0.89	0.3	0.92	12.2	9.96	0.94	0.0	236.9	8.50	unknown *

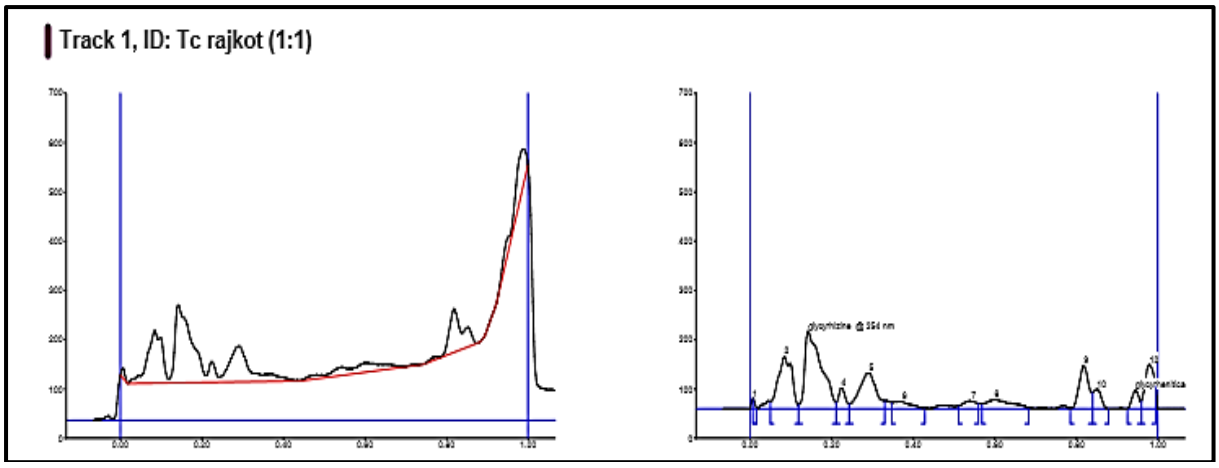
Figure 4.76: Representative chromatogram of *T. cuneifolia* winter season



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.09	4.8	0.13	20.0	12.40	0.14	15.8	498.8	16.02	unknown *
2	0.15	15.9	0.15	21.1	13.06	0.17	1.3	274.6	8.82	unknown *
3	0.18	0.0	0.21	29.2	18.10	0.22	19.1	423.7	13.61	unknown *
4	0.22	19.2	0.24	45.2	27.95	0.28	14.9	1225.9	39.38	unknown *
5	0.28	15.3	0.29	31.0	19.20	0.30	14.3	435.4	13.99	unknown *
6	0.89	0.2	0.91	15.0	9.28	0.93	0.0	254.4	8.17	unknown *

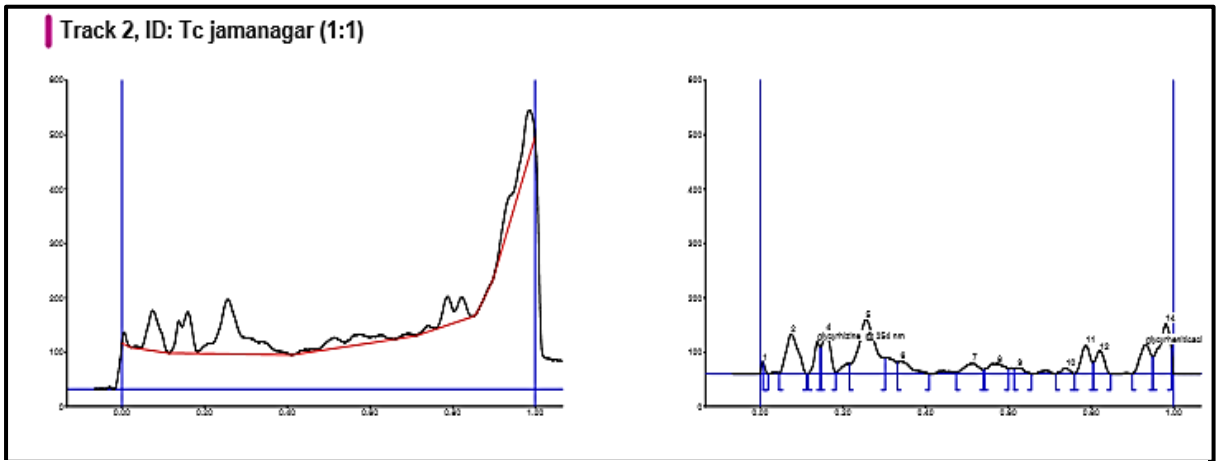
In the regional variation only Rajkot and Jamnagar showed presence of glycyrrhizin and all 5 regions showed presence of 18β-glycyrrhetinic acid. The content of glycyrrhizin was highest in Rajkot (29.45%) and Jamnagar with 4.66%. Jodhpur showed highest amount of 18β-glycyrrhetinic acid (15.96%) and least was in Rajkot with 2.95%.

Figure 4.77: Representative chromatogram of *T. cuneifolia* Rajkot



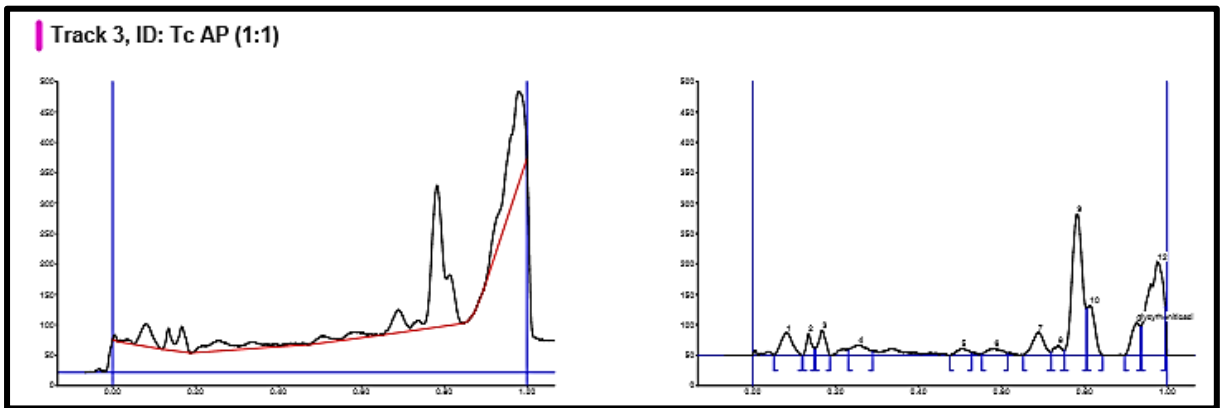
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	21.1	0.01	21.1	2.99	0.02	0.7	93.6	0.50	unknown *
2	0.05	14.8	0.08	106.3	15.08	0.12	8.0	3149.2	16.72	unknown *
3	0.12	8.3	0.14	157.5	22.34	0.21	13.4	5546.4	29.45	glycyrhizine
4	0.21	13.7	0.22	42.2	5.98	0.24	10.1	620.4	3.29	unknown *
5	0.24	10.3	0.29	72.1	10.23	0.33	16.3	2597.8	13.79	unknown *
6	0.35	12.9	0.37	15.1	2.14	0.43	0.7	575.2	3.05	unknown *
7	0.51	6.2	0.54	16.5	2.34	0.56	11.1	471.6	2.50	unknown *
8	0.57	10.3	0.60	19.0	2.70	0.68	1.8	1047.8	5.56	unknown *
9	0.79	1.2	0.82	88.7	12.58	0.84	32.6	1792.4	9.52	unknown *
10	0.84	33.1	0.85	40.0	5.67	0.88	0.0	674.9	3.58	unknown *
11	0.93	0.4	0.95	37.1	5.26	0.96	14.4	555.4	2.95	glycyrheniticaci
12	0.96	16.0	0.98	89.4	12.69	1.00	44.4	1707.3	9.07	unknown *

Figure 4.78: Representative chromatogram of *T. cuneifolia* Jamnagar



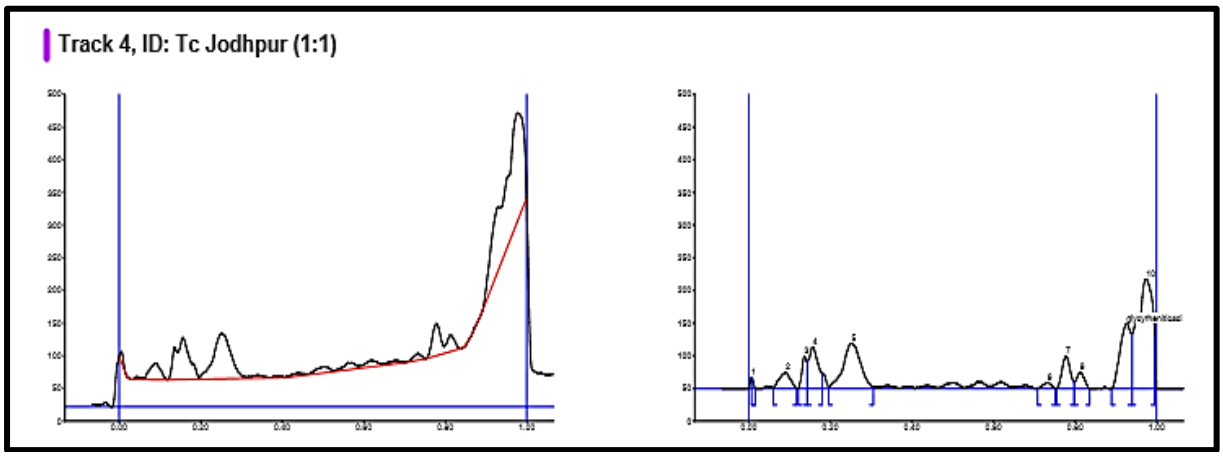
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	22.3	0.01	22.3	3.41	0.02	0.1	91.8	0.61	unknown *
2	0.05	1.9	0.07	73.3	11.20	0.11	0.5	1843.0	12.27	unknown *
3	0.12	0.5	0.14	58.8	8.98	0.15	48.8	699.1	4.66	glycyrhizine
4	0.15	49.2	0.16	75.8	11.59	0.18	3.8	1324.6	8.82	unknown *
5	0.22	18.4	0.26	99.4	15.20	0.30	29.7	3574.8	23.80	unknown *
6	0.33	21.5	0.34	23.3	3.56	0.41	0.1	663.1	4.42	unknown *
7	0.47	3.3	0.51	19.6	3.00	0.54	7.6	630.1	4.20	unknown *
8	0.54	8.0	0.57	19.1	2.92	0.60	9.6	663.3	4.42	unknown *
9	0.61	9.7	0.62	11.7	1.79	0.66	0.1	235.5	1.57	unknown *
10	0.72	0.2	0.74	11.1	1.70	0.76	1.0	194.1	1.29	unknown *
11	0.76	1.1	0.79	52.5	8.03	0.81	21.0	1010.3	6.73	unknown *
12	0.81	21.8	0.82	42.3	6.47	0.85	0.6	776.8	5.17	unknown *
13	0.90	0.1	0.93	53.5	8.18	0.95	32.1	1205.8	8.03	glycyrheniticaci
14	0.95	32.3	0.98	91.5	13.98	1.00	48.0	2106.4	14.03	unknown *

Figure 4.79: Representative chromatogram of *T. cuneifolia* Andhra Pradesh



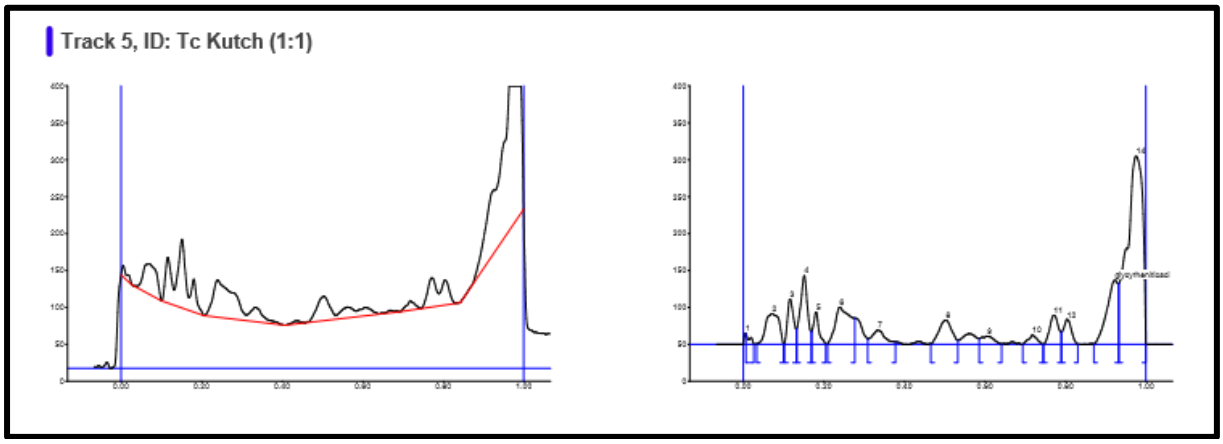
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.05	0.8	0.08	37.3	5.11	0.12	0.1	899.1	5.44	unknown *
2	0.12	0.4	0.13	35.6	4.87	0.15	11.1	396.5	2.40	unknown *
3	0.15	11.1	0.17	41.4	5.66	0.19	0.4	616.1	3.73	unknown *
4	0.23	10.0	0.26	17.1	2.34	0.29	7.4	573.8	3.47	unknown *
5	0.48	0.2	0.50	10.9	1.50	0.53	4.3	277.7	1.68	unknown *
6	0.55	2.6	0.58	11.0	1.50	0.62	3.4	370.8	2.24	unknown *
7	0.65	0.4	0.69	37.3	5.11	0.72	5.0	929.1	5.62	unknown *
8	0.72	5.5	0.74	15.8	2.16	0.75	8.3	280.1	1.69	unknown *
9	0.75	8.5	0.78	233.4	31.94	0.81	77.2	5098.8	30.83	unknown *
10	0.81	77.4	0.81	82.7	11.32	0.85	0.1	1296.7	7.84	unknown *
11	0.90	0.3	0.93	53.7	7.35	0.94	48.2	973.9	5.89	glycyrheniticaci
12	0.94	48.4	0.98	154.4	21.13	1.00	80.9	4824.1	29.17	unknown *

Figure 4.80: Representative chromatogram of *T. cuneifolia* Jodhpur



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	17.2	0.01	17.2	2.97	0.02	0.1	72.4	0.51	unknown *
2	0.06	1.3	0.09	25.5	4.41	0.12	0.4	590.8	4.16	unknown *
3	0.12	0.5	0.14	49.6	8.57	0.14	42.0	497.9	3.51	unknown *
4	0.14	42.2	0.16	64.3	11.11	0.18	23.4	1276.8	8.99	unknown *
5	0.20	3.3	0.25	69.4	11.98	0.31	3.2	2594.1	18.27	unknown *
6	0.71	1.1	0.73	10.1	1.74	0.75	0.1	170.6	1.20	unknown *
7	0.76	0.3	0.78	49.4	8.53	0.80	10.7	865.8	6.10	unknown *
8	0.80	10.9	0.81	25.5	4.39	0.84	0.1	418.6	2.95	unknown *
9	0.89	0.1	0.93	100.3	17.31	0.94	84.1	2265.6	15.96	glycyrheniticaci
10	0.94	84.3	0.98	168.0	29.00	1.00	79.5	5447.2	38.36	unknown *

Figure 4.81: Representative chromatogram of *T. cuneifolia* Kutch



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	14.9	0.01	14.9	1.86	0.03	0.5	121.3	0.61	unknown *
2	0.03	0.7	0.07	41.4	5.19	0.10	0.2	1211.2	6.10	unknown *
3	0.10	1.2	0.12	62.0	7.76	0.13	20.5	818.3	4.12	unknown *
4	0.13	21.5	0.15	93.1	11.66	0.17	17.1	1444.8	7.28	unknown *
5	0.17	17.9	0.18	44.5	5.57	0.21	0.4	532.2	2.68	unknown *
6	0.21	0.6	0.24	50.2	6.29	0.28	35.3	1700.4	8.57	unknown *
7	0.31	7.6	0.33	19.4	2.43	0.38	3.4	555.9	2.80	unknown *
8	0.47	0.7	0.50	33.2	4.16	0.53	5.6	915.0	4.61	unknown *
9	0.59	8.0	0.61	11.3	1.41	0.64	0.4	309.6	1.56	unknown *
10	0.69	0.2	0.72	13.0	1.63	0.75	0.1	234.0	1.18	unknown *
11	0.75	0.3	0.77	39.6	4.96	0.79	16.7	749.5	3.78	unknown *
12	0.79	16.8	0.81	33.9	4.25	0.83	0.3	575.6	2.90	unknown *
13	0.87	0.6	0.92	87.0	10.90	0.93	82.2	2113.9	10.65	glycyrheniticaci
14	0.93	82.8	0.98	254.9	31.93	1.00	0.0	8562.9	43.15	unknown *

Variation In Phytoconstituents Based On The Root Girth

As per the girth classification of roots given in Chapter 2. The Glycyrrhizin content was measured in different girth class. Maximum Glycyrrhizin was recorded in Thick roots (Figure-4.82).

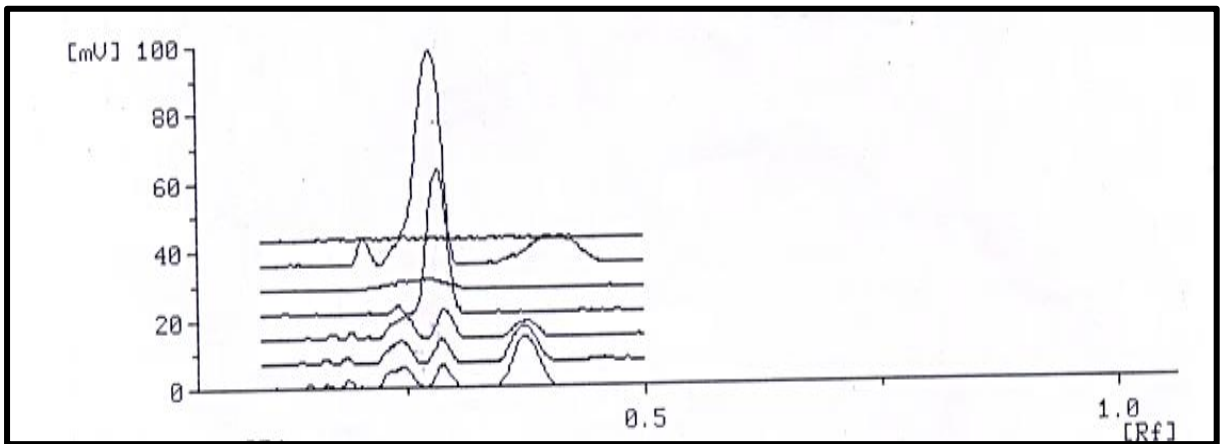


Figure 4.82: HPTLC: Comparision Of Standard Glycyrrhin, *G. glabra* With *Taverniera cuneifolia* Roots (3 Samples Of Different Girth)

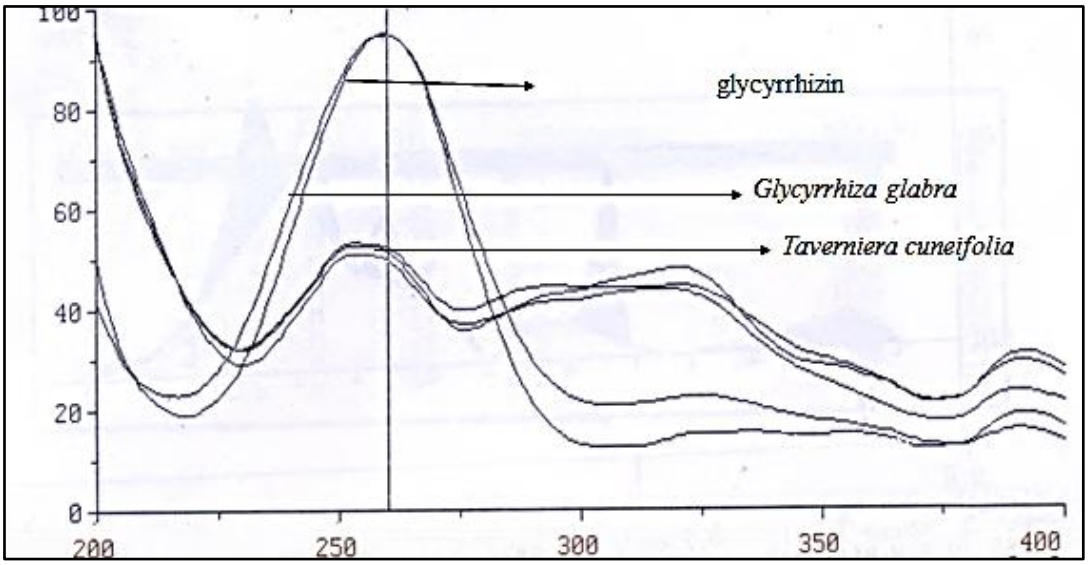
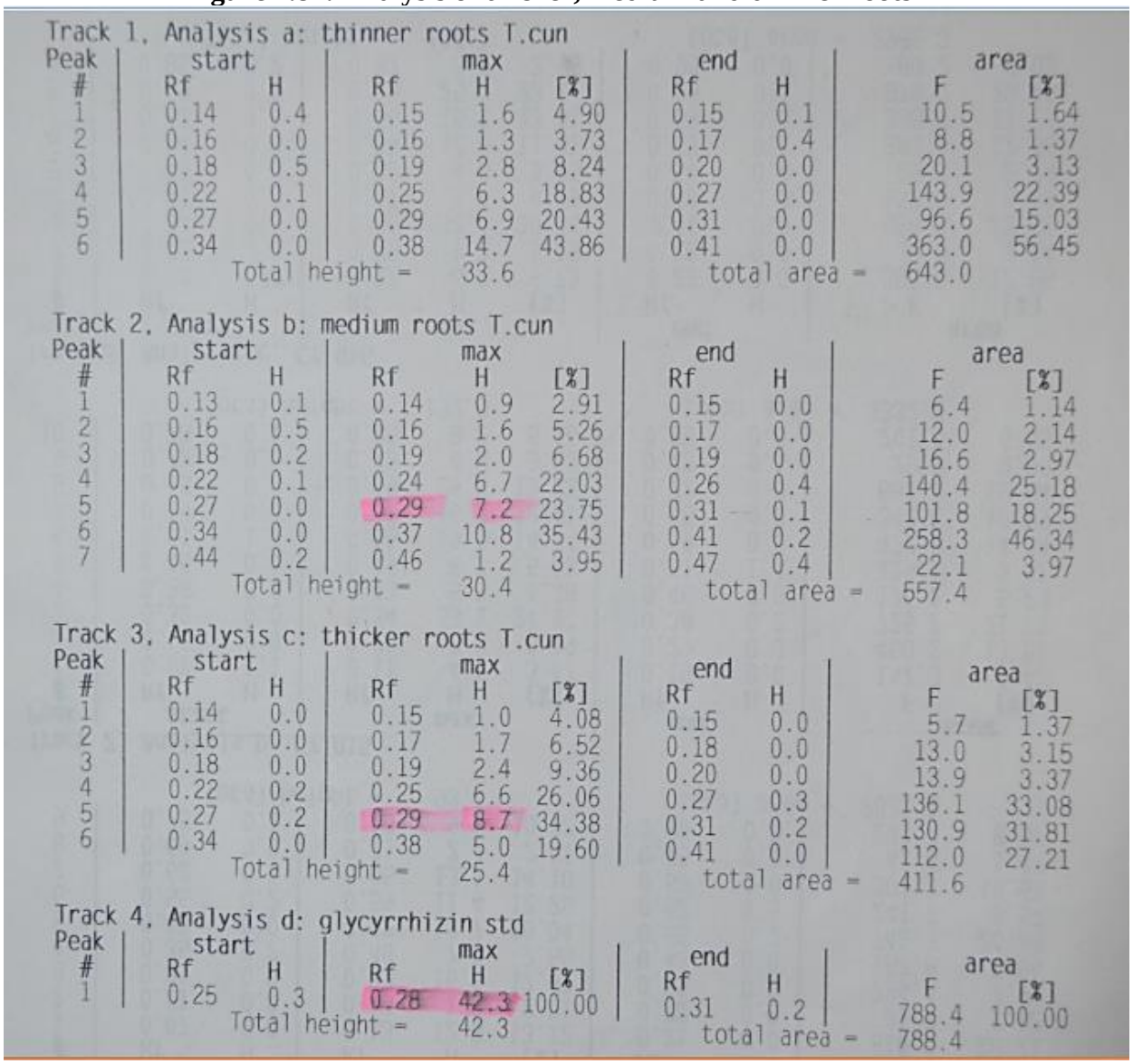


Figure 4.83 HPTLC: Chromatographic spectrum of Standard Glycyrrhin, *G. glabra* With *Taverniera cuneifolia* Roots (3 Samples of Different Girth)

Figure 4.84: Analysis of thicker, medium and thinner roots



Track 1, Analysis a: thinner roots T.cun

Peak #	start Rf	start H	max Rf	max H	max [%]	end Rf	end H	area F	area [%]
1	0.14	0.4	0.15	1.6	4.90	0.15	0.1	10.5	1.64
2	0.16	0.0	0.16	1.3	3.73	0.17	0.4	8.8	1.37
3	0.18	0.5	0.19	2.8	8.24	0.20	0.0	20.1	3.13
4	0.22	0.1	0.25	6.3	18.83	0.27	0.0	143.9	22.39
5	0.27	0.0	0.29	6.9	20.43	0.31	0.0	96.6	15.03
6	0.34	0.0	0.38	14.7	43.86	0.41	0.0	363.0	56.45
		Total height =		33.6			total area =	643.0	

Track 2, Analysis b: medium roots T.cun

Peak #	start Rf	start H	max Rf	max H	max [%]	end Rf	end H	area F	area [%]
1	0.13	0.1	0.14	0.9	2.91	0.15	0.0	6.4	1.14
2	0.16	0.5	0.16	1.6	5.26	0.17	0.0	12.0	2.14
3	0.18	0.2	0.19	2.0	6.68	0.19	0.0	16.6	2.97
4	0.22	0.1	0.24	6.7	22.03	0.26	0.4	140.4	25.18
5	0.27	0.0	0.29	7.2	23.75	0.31	0.1	101.8	18.25
6	0.34	0.0	0.37	10.8	35.43	0.41	0.2	258.3	46.34
7	0.44	0.2	0.46	1.2	3.95	0.47	0.4	22.1	3.97
		Total height =		30.4			total area =	557.4	

Track 3, Analysis c: thicker roots T.cun

Peak #	start Rf	start H	max Rf	max H	max [%]	end Rf	end H	area F	area [%]
1	0.14	0.0	0.15	1.0	4.08	0.15	0.0	5.7	1.37
2	0.16	0.0	0.17	1.7	6.52	0.18	0.0	13.0	3.15
3	0.18	0.0	0.19	2.4	9.36	0.20	0.0	13.9	3.37
4	0.22	0.2	0.25	6.6	26.06	0.27	0.3	136.1	33.08
5	0.27	0.2	0.29	8.7	34.38	0.31	0.2	130.9	31.81
6	0.34	0.0	0.38	5.0	19.60	0.41	0.0	112.0	27.21
		Total height =		25.4			total area =	411.6	

Track 4, Analysis d: glycyrrhizin std

Peak #	start Rf	start H	max Rf	max H	max [%]	end Rf	end H	area F	area [%]
1	0.25	0.3	0.28	42.3	100.00	0.31	0.2	788.4	100.00
		Total height =		42.3			total area =	788.4	

On the basis of HPTLC analysis, maximum amount of concentration of glycyrrhizin was seen in thicker roots with 34.38% and thin roots had 20.43%.

Prescence Of Glycyrrhizin In *T. cuneifolia* By Hplc Method

HPLC method was employed for the presence of glycyrrhizin in *Taverniera cuneifolia,* in comparison with the glycyrrhizin standard. Presence of glycyrrhizin was analysed by two different methods from the plant matrix. In the earlier reports it has been mentioned that *T. cuneifolia* contains glycyrrhizin (Pompei, 1979).

However, the HPLC analysis done using Method 1 showed absence of glycyrrhizin in *T. cuneifolia*, eventhough glycyrrhizin in *G. glabra* eluted out at the retention time of 8.4 minutes. Since the methanolic extract of *T. cuneifolia* showed complete absence of glycyrrhizin using Method 1, another method (Method 2) which is more specific for extraction of glycyrrhizin was used. Thus, Method 2 could be considered more appropriate for the extraction of glycyrrhizin as compared to the conventional Method 1 as could be noticed in Figure 4.85. Both the methods showed absence of glycyrrhizin in *T. cuneifolia* in comparision to glycyrrhizin standard.

HPLC Results

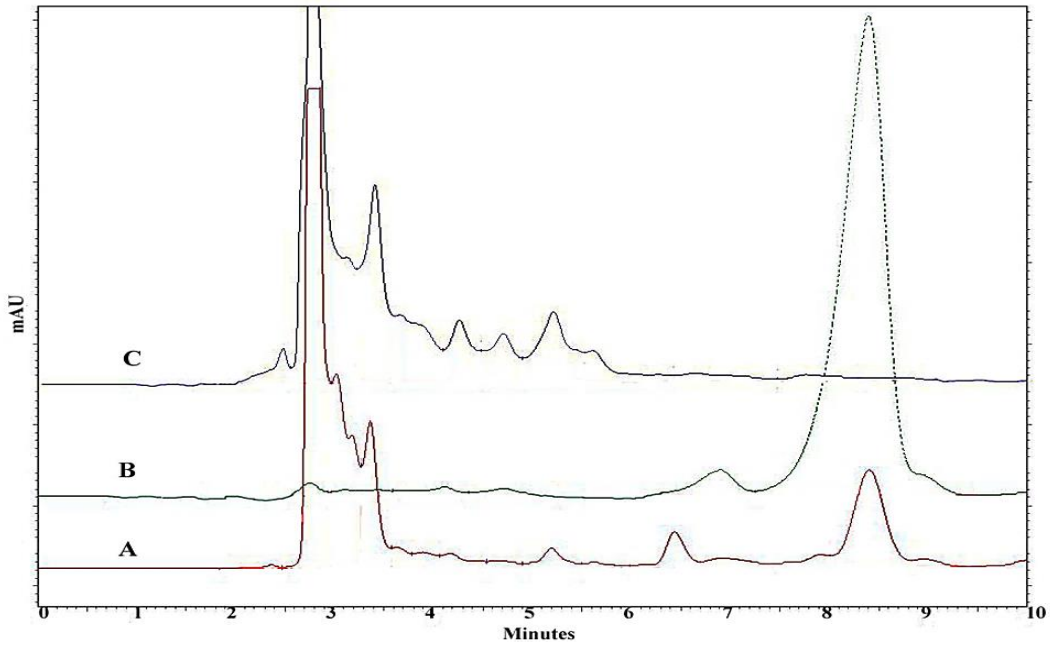


Figure 4.85 A) *G. glabra* extracts B) Glycyrrhizin standard C) *T. cuneifolia* when extracted using Method 1 have been shown.

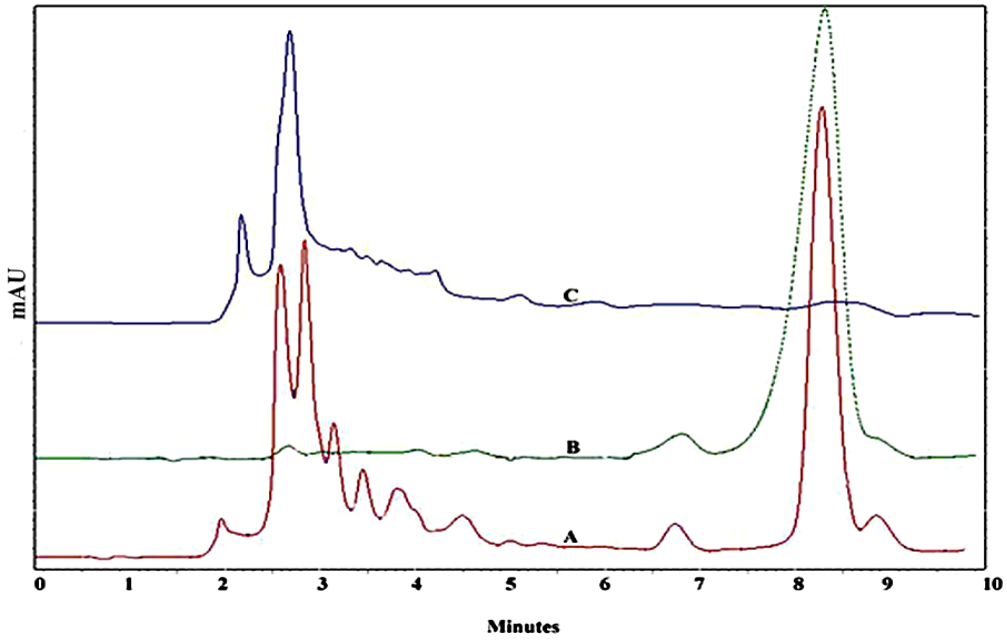


Figure 4.86 : A) *G. glabra* extracts B) Glycyrrhizin standard C) *T. cuneifolia* when extracted using Method 2 have been shown.

HPLC chromatograms are depicted in Fig no 4.87 , red colour shows the chromatogram of sephadex fraction (methanolic) of *T. cuneifolia* root, green chromatogram shows protein sample extracted from *T. cuneifolia* root and blue chromatogram shows spiking of both sephadex and protein fraction. From this observation it can be noted that sephadex fraction and protein samples show same peaks. Which further proves that the fractions obtained with Sephadex LH 20 could be glyco proteins.

The quantity used for extraction was based on the earlier reference on glycyrrhizin extraction (Wang *et al*., 2004; Wagner and Bladt, 1996) wherein we have followed the same procedure as stated by the author.

Glycyrrhizin and glycyrrhetinic acid is seen only in HPTLC analysis but in HPLC and other extensive studies there was absence of glycyrrhizin altogether. Its further discussed in conclusion chapter.

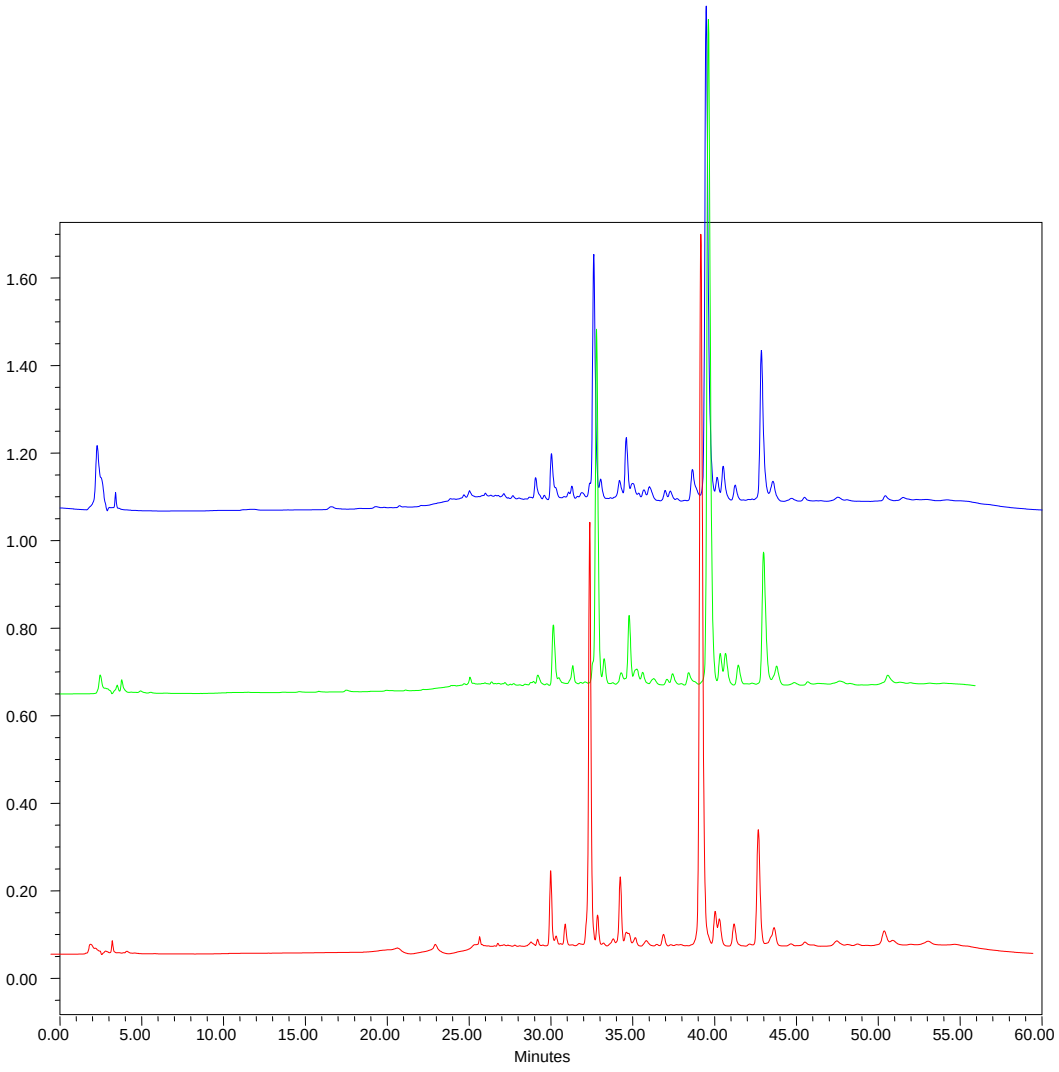


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Figure 4.87: A. Red colour depicts the chromatogram of sephadex fraction (methanolic)

B. Green chromatogram shows protein sample .

C. Blue depicts both sephadex (methanolic and protein)

COMPARATIVE HPLC ANALYSIS of *T. cuneifolia* and *T. abyssinica*

Methanolic extracts of *T. cuneifolia* and *T. abyssinica* were analysed by HPLC method. Most of the peaks were matching with each other indicating similarity in the phytoconstituents of *T. cuneifolia* and *T. abyssinica*. 20 phytoconstituents of these species had similar retension time (Table no 4.15). Its further comparision with the phytoconstituents showed nine peaks with similar retention times.

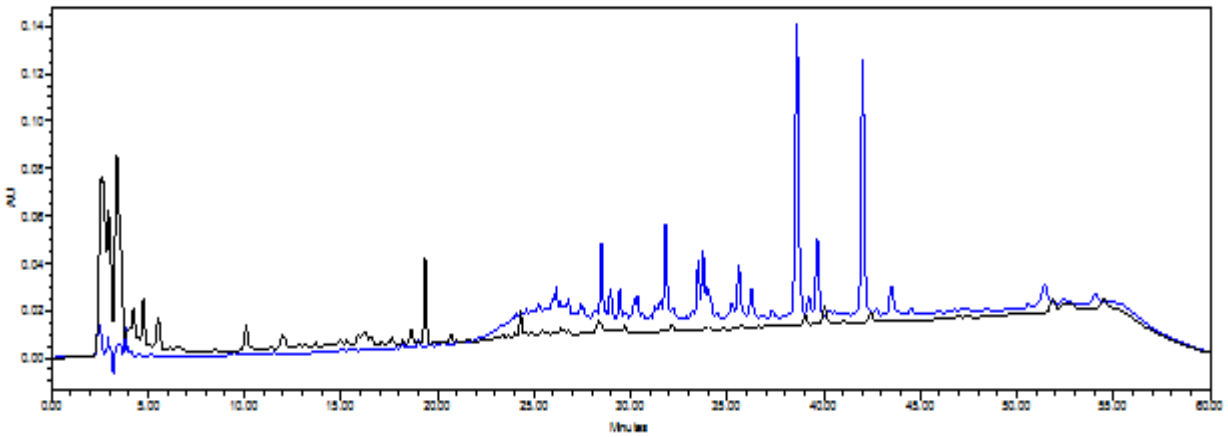


Figure 4.87: *T. cuneifolia* root and *T. cuneifolia* root superpernatant

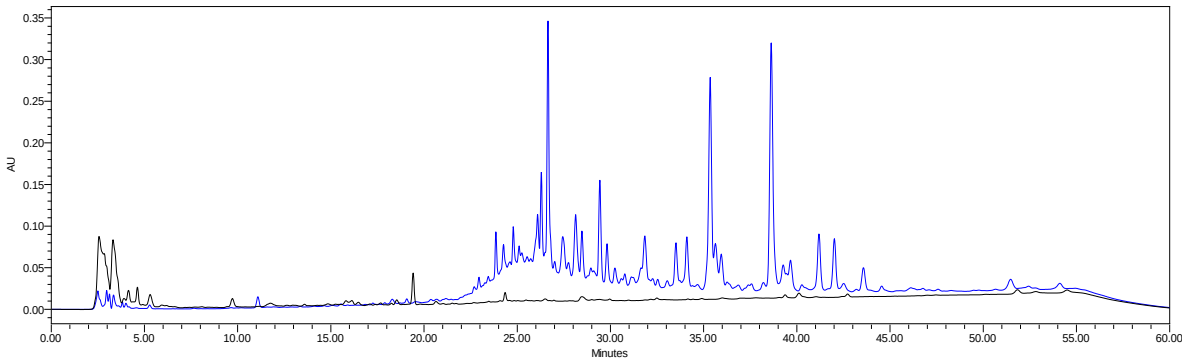


Figure 4.88: *T. abyssinica* root and *T. abyssinica* root superpernatant

Table 4.15: Comparison HPLC analysis of *T cuneifolia, T. abyssinica* roots and *T. cuneifolia* seeds.

	T. cuneifolia roots		***T. abyssinica roots***		***T. cuneifolia* seeds**	
Sr. no.	Retention Time	% Area	Retention Time	% Area	Retention Time	% Area
1	2.485	3.19	2.502	1.43	2.426	2.24
2	2.938	0.46	2.973	0.26	2.958	0.50
3	3.808	0.97	3.125	0.24	3.823	0.69
4	3.952	0.19	3.342	0.72	5.188	0.77
5	4.128	0.19	3.815	0.18	11.757	4.48
6	9.481	0.21	4.009	0.14	13.293	0.63
7	18.203	0.33	5.280	0.22	18.093	4.06
8	19.024	0.43	11.086	0.74	20.219	0.75
9	26.146	0.59	18.295	0.41	21.328	0.68
10	26.773	0.65	19.073	0.25	22.320	2.51
11	27.432	0.95	22.948	0.48	22.738	0.45
12	28.180	0.34	23.856	1.86	23.099	0.56
13	28.481	4.10	24.263	1.14	23.321	0.92
14	28.950	1.83	24.789	1.40	23.478	3.34
15	29.433	1.67	25.101	0.34	24.252	17.48
16	30.200	0.47	26.092	1.01	24.464	4.98
17	30.365	0.39	26.292	2.92	24.912	0.60
18	31.272	0.26	26.649	11.97	25.219	0.60
19	31.528	0.40	27.010	0.30	25.514	11.97
20	31.811	5.74	27.445	2.79	25.821	12.27
21	32.219	0.37	27.746	0.44	26.790	0.60
22	33.503	2.51	28.133	4.09	27.017	0.88
23	33.745	2.48	28.472	1.94	28.383	1.63
24	33.961	0.25	29.431	5.62	30.739	0.74
25	35.224	0.61	29.817	2.21	31.242	0.18
26	35.603	3.56	30.241	0.54	31.836	1.07
27	36.247	2.18	31.843	2.43	32.253	0.20
28	37.330	0.70	33.514	2.51	33.782	0.25
29	38.608	27.17	34.100	3.17	34.496	1.24
30	39.225	1.12	35.351	11.92	35.013	0.29
31	39.666	6.20	35.631	1.19	38.644	6.78
32	42.017	20.73	35.945	1.40	39.609	3.87
33	42.729	0.38	38.626	20.43	42.047	2.68
34	43.502	2.67	39.269	0.56	49.559	0.47
35	44.540	0.70	39.661	1.27	51.357	4.01
36	50.604	0.38	41.188	4.26	52.275	2.14
37	51.441	3.37	42.014	3.45	54.019	2.47
38	54.076	1.25	43.574	1.84		
			51.470	1.28		
			54.109	0.67		

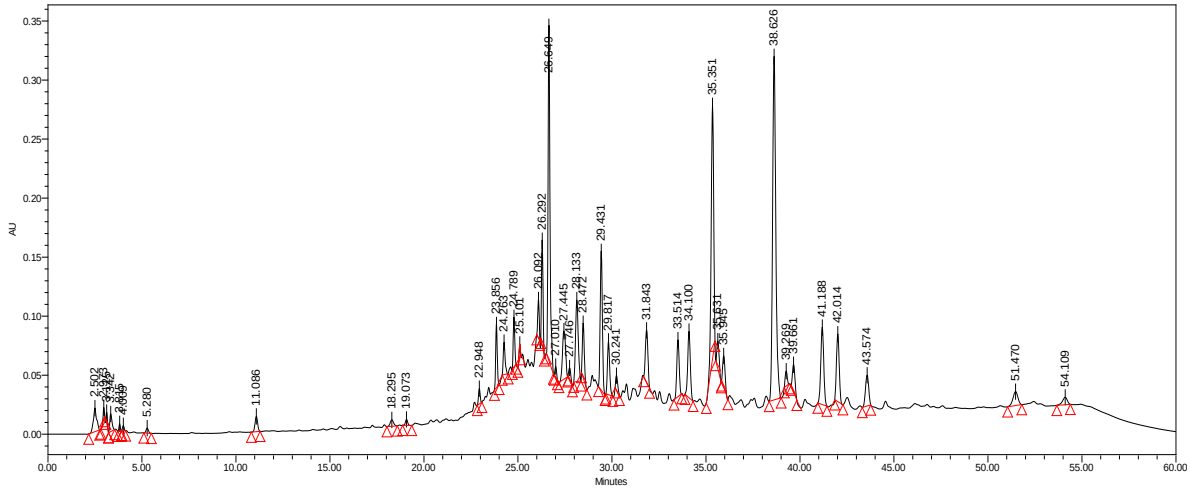


Figure 4.89: *T. cuneifolia* roots

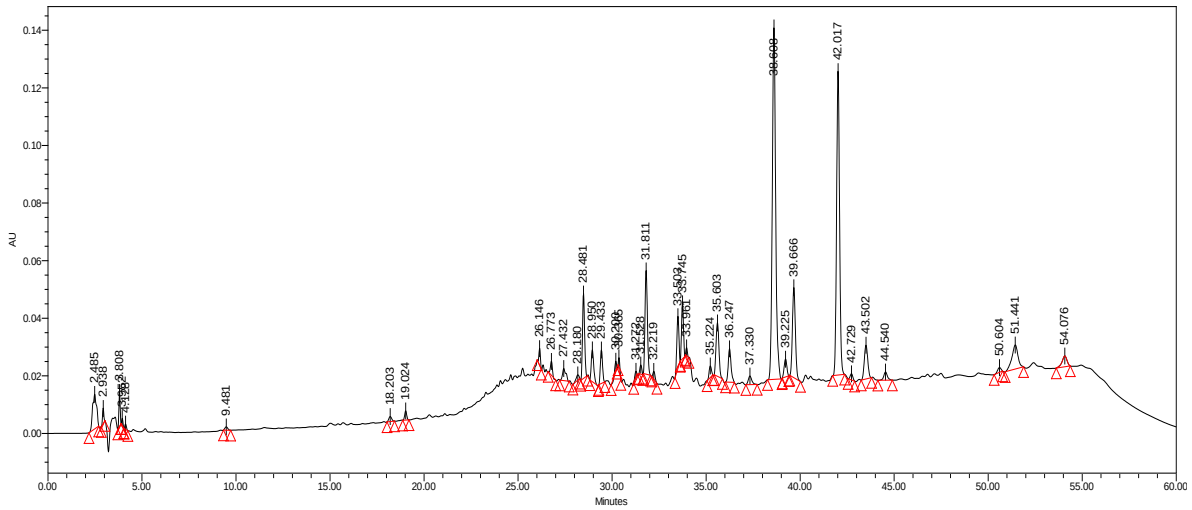


Figure 4.90: *Taverniera abyssinica* roots

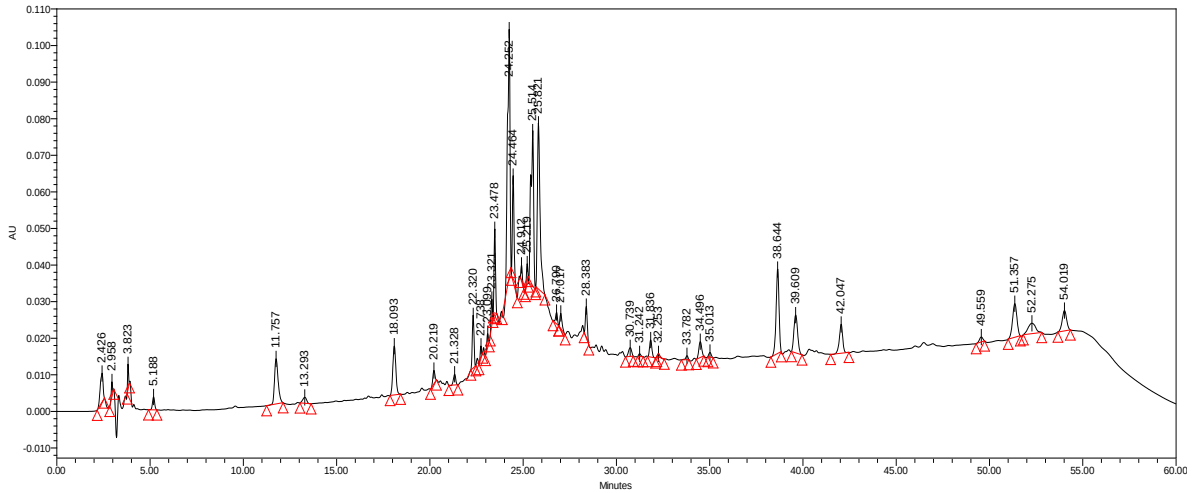


Figure 4.91: *T. cuneifolia* seeds

PROBABLE SIMILAR SAPONINS BETWEEN *T. aegyptiaca* and *T. cuneifolia.*

With refence to the work of Hassanean (1998, 2002) the LC-MS analysis of *T. cuneifolia was carried out.* LC-MS analysis showed that MS of 12 compounds of *T. aegyptiaca* are matching with the fractional components (of *T. cuneifolia*).

Table 4.16: MS Peaks of saponin peaks similar in both *T. aegyptiaca* and *T. cuneifolia*

	Name of the Compound	*T. aegyptiaca* (FAB-MS) (Further saponins)	*T. cuneifolia* (LC-MS)
1	28-methyl serratagenate-3-b-O-b-xylopyranosyl (1→2)-b-glucopyranoside	Compound 2 - 793,661,499 291 (M-H) -502	S4, Peak 12- 793.7 S6, Peak 6- 289.1 S6, Peak 6-793.7
2	28-methyl serratagenate 3-b-O-a-rhamnopyranosyl (1→2)-b-glucopyranoside	Compound 3 - 807, 661, 499 291 (M-H) -516	S5, Peak 17 – 805.7
3	3b-O-a-rhamno- pyranosyl (1→2) b-glucopyranosyl-olean-11,13(18)-dien-1b, 3b, 22b-triol	Compound 4 – 763, 617, 455	S6, Peak 8 – 615.7, 761.8, 453.8 S5, Peak 13 – 615.5, 453.8
4	3b-O-b-glucopyranosyl (1→2)-b-glucurono- pyranosylolean-11,13(18)-dien-1b,3b,22b-triol	Compound 5a – 807, 645, 455	S6, Peak 11 – 805.5, 645.7, 453.5
5	3b-O-b-xylopyranosyl(1→2)-b-glucuronopyranosylolean-11,13(18)-dien-1b,3b,22b- triol	Compound 6b – 777, 645, 455	S5, Peak 10 – 777.5, 455.5 S5, Peak 11 – 777.5, 455.5 S5, Peak 12 – 777.7
6	3b-O-a-rhamnopyranosyl (1→2)-b-glucuronopyranosylolean-11,13(18)-dien-1b, 3b, 22b-triol	Compound 7c – 791, 645, 455	S6, Peak 11 – 791.5, 453.5

		Saikosaponins	
7	3β-O-β-D-glucopyranosylolean-11, 13(18)-diene- 3β,22β-diol.	Compound 1- 603 (+ mode), 601 (-mode)	S1, Peak 7- 603.5
8	3β-O-β- D - glucopyranosylolean - 11,13(18) - diene - 1β,3β,22β-triol	Compound 2- 619 (+ mode), 439 (+ mode)	S1, Peak 9- 439.5
9	1β,3β,22β-trihydroxyolean-11, 13(18)-diene	Compound 4- 456.35965 235.16949 215	S1, Peak 12- 456.9 S2, Peak 2- 235.1 S1, Peak 21- 214.9
10		Triacetate 4a – 582 462 201	S1, Peak 20 – 581.5 S2, Peak 6 - 461.4 S2, Peak 6 - 200.9
11	1β,22β dihydroxyolean-11,13(18)- diene	Compound 5 – 440.3654 270 220	S2, Peak 4 – 441.5 S2, Peak 4 – 269 S2, Peak 4 – 221.3
12		Compound 5a - 464 368 273	S2, Peak 7 – 463.3 S2, Peak 10 –369 S2, Peak 7 – 271.4

GS-MS analysis of *T.cuneifolia*

The methanolic and ethyl acetate extract of *T.cuneifolia* was analysed. The analysis showed that 47 phytoconstituents in *T. cuneifolia.* Of these phytoconstituents 19 phytoconstituents are similar to *G.glabra* . The details are as follows:

Phytochemically, the *Taverniera cuneifolia* is composed of mono and polysaccharides. The most prominent monosaccharide in *T. cuneifolia* is mannose-6-phosphate, and most common polysaccharides are called gluco- mannans. Steroidal components in the roots of *T. cuneifolia* are given below in the table.. In order to study the nutritional potential of *T. cuneifolia*,

T. cuneifolia	*G. glabra*	*T. cuneifolia*	*G. glabra*
Lauric acid		Octadecane	
2',4'-Dimethoxypropiophenone		Octasanol	
Myristaldehyde		Benzaldehyde	+
Hexadecanol	+	Acrylic acid	
Palmitic acid	+	Propanoic acid, 2 hydroxy-2-methyl, methyl ester	+
n-Octadecanol	+	Furfural	+
cis, cis – Linoleic acid	+	3,5-Dimethylpyrazole	
Behenic alcohol		Furfuryl alcohol	
Thexyl alcohol		3-Furanmethanol	
Methyl diethyl carbinol		Furfural, 5-methyl	+
Methyl cyclopentanol		Maltol	
Cyclopentanol, 3-methyl		4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	
Oxetane		Hydroxymethylfurfural	
Triptene		Syringol	
Fasciolin	+	Beta-D-glucopyranose	
1-Nonadecene	+	Stearic acid	
Phenol, 2,4-bis (1,1-dimethylethyl)	+	Oleic acid	
Stigmasterol	+	alpha-Amyrin	+
Betasitosterol	+	beta-Amyrin	+
Apigenin	+	Hexylene glycol	
		1-Pentadecene	+

important parameters such as carbohydrates, protein, cholesterol, vitamin & iron content were

studied & compared with *Glycyrrhiza glabra*. Simultaneously as *T.cuneifolia* is known for its dietary properties the studies were also done on other parts of plant such as fruit cover, seed & seed cake to observe their potential from the prospective of nutraceutical sector. The various phytochemical components found in the *T.cuneifolia* are as follows:

Vitamins

T. cuneifolia contains water soluble vitamins like Vitamin B1 (Thiamine), Vitamin B2 (Riboflavin), Vitamin B3 (Niacin), Vitamin B6 (Pyridoxine)

20 - Amino Acids

Of the 22 amino acids present, 15 amino acids were recorded in seeds of *T. cuneifolia*, 13 amino acids in roots, 13 amino acids in leaves and 14 amino acids in seeds with fruit cover.

The human body requires 22 amino acids, the building blocks of proteins and *T. cuneifolia* provides twenty of them.

T. cuneifolia contains 19 of the 20 amino acids required by the human body for good health. The human body however produces only 12 of these. More importantly, it provides seven of the eight essential amino acids that cannot be manufactured by the body and which have to be consumed as food.

T. cuneifolia contains 10 essential amino acids:

Arginine, Methionine, Phenylalanine, Tryptophan, lysine, Histidine, Isoleucine, Leucine, Valine and Threonine

The 9 'non-essential' amino acids contained in *T. cuneifolia* are:

Alanine, Asparagine, Cystein, Glutamine, Glutamic acid, Glycine, Proline, Serine, and Tyrosine.

Sugars

These include the **important sugars (glucose fructose, arabinose, etc), maltol, furfuryl alcohol, β-d-glucopyranose.**

Lignan

Conocarpan and 9-O-Feruloyllariciresinol

Saponins

Saponins which are Glycosides form about 3% of what's in ***T. cuneifolia***. They are soapy substances which provide cleansing and antiseptic activity. They act powerfully as anti-microbials against bacteria, viruses, fungi and yeasts.

Sterols

These are anti-inflammatory agents. Lupeol also possesses antiseptic and analgesic properties. They act as **powerful anti-inflammatory agents**. In *T. cuneifolia* roots three sterols β-sitosterol, stigmasterol, lupeol have been identified.

Fatty acids

Fatty acids from roots obtained were Caproic acid, myristic acid, lauric acid, palmitic acid, linolenic acid, stearic acid, oleic acids, behenic acids. Fatty acids in seeds obtained were palmitic acid, stearic acid, oleic and linoleic acid.

Proteins

All types of proteins are present in *T. cuneifolia* High molecular weight proteins to low molecular weight proteins. Seeds contain higher level of proteins as compared to roots.

Phenolic acids

p-hydroxy benzoic acid, protocatechuic acid, vanillic acid, syringic acid, cis and trans *O*-coumaric acid, ferulic cid, caffeic acid

Flavonoids

Anthocyanins –Delphinidin was obtained from the flowers of *T. cuneifolia.*

Flavonols – quercetin, isorhamnetin., Kaempferol tetracetate,

Isoflavone- 2',4', 5'- trimethoxy- 2"2"- dimethyl pyrano[5",6", 6,7] isoflavone.

Flavones- 5,7- diacetoxy, 8-methoxy flavone, acacetine, 3',4,'5,'3,5,6,7-heptamethoxyflavone, luteolin, apigenin, 3',4,'5,'3,5,7,8- heptamethoxyflavone.

Flavan 3-ol – Catechin 7-glycoside, (-)-Epiafzelechin

Diterpenoid – Przewalskin, Coronarin A

Triterpenes- α and β amyrin

Section 3: Preclinical and toxicity studies

Taverniera cuneifolia is a potential substitute of licorice (*G. glabra*). However, there are no toxicological studies reported for use of the plant extract. Thus the evaluation of toxicological parameters – SGPT (Serum Glutamate Pyruvate Transaminase) and serum Creatinine in rats were done. *T. cuneifolia* root extract was orally given daily at different dosage regime -125 mg/kg, 500 mg/kg, 1g/kg, 2.5g/kg, 5g /kg body weight.

Figure 4.92: Demonstrates effect of plant extract on serum SGPT activity in dose dependent manner. A significant increase in activity was observed with the increase in dosage; wherein treatment of 5g/kg body weight demonstrated slightly elevated levels. However, the levels of enzyme activity in each case were within the normal range of 0-25 IU/dl. Since the dose dependent study was carried out for 30 days, it could be highly recommended that time dependent (in term long time treatment regime) evaluation would be important.

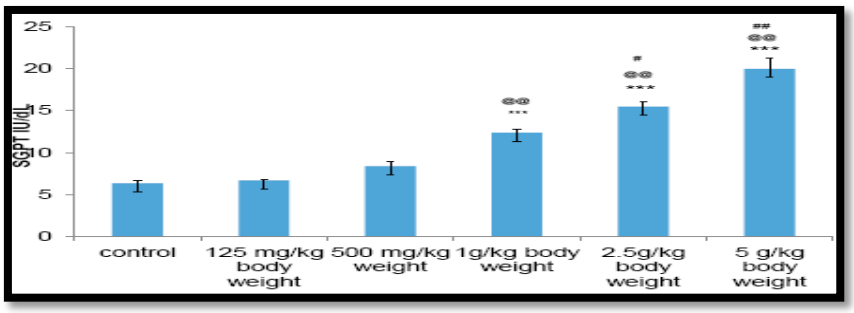


Figure 4.92: Dose dependent effect of *T. cuneifolia* extract on Serum Glutamate Pyruvate Transaminase (SGPT) Activity.N= 4-6, The values are represented as mean ± SEM,*******p<0.001, as compared with untreated control **@**p<0.01 as compared to 125mg/kg BW ;##p<0.01 as compared to 500 mg/kg BW

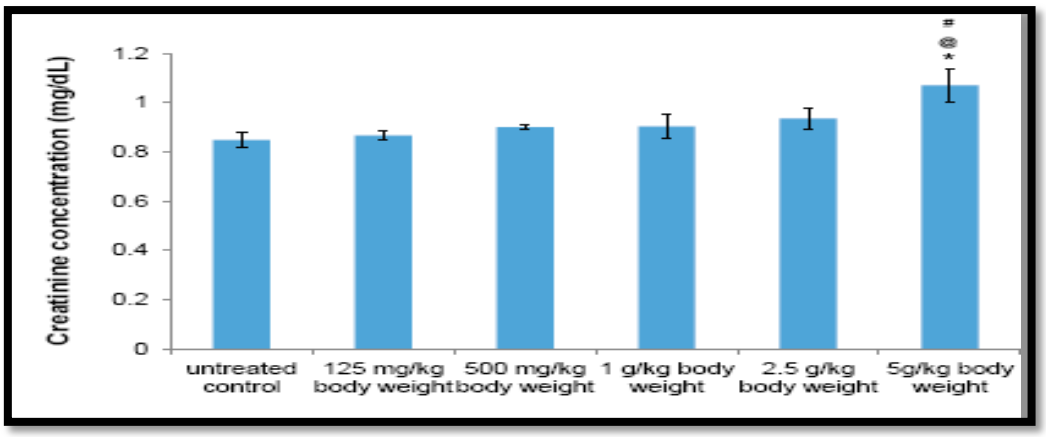


Figure 4.93: Dose dependent effect of T. cuneifolia extract on on Serum creatinine levels. N=4-6 *p<0.05 as compared to untreated control @p<0.05 as compared to 125mg/kg body weight #p<0.05 as compared to 500mg/kg body weight.

Figure 4.93: Demonstrates effect of plant extract on serum creatinine. Serum creatinine also did not show any significant elevations in its level and its level were observed in normal range of 0.7-1.2mg/dl. However, it was interesting to note that 5g/kg body weight dose demonstrated an increased SGPT activity & creatinine levels. Thereby it could be concluded that higher dose of plant extract treatment for more than 30 days may elevate the toxicity markers.

4.3.1 Discussion:

T. cuneifolia has not been scientifically studied for its toxicological effects. Therefore, initially the toxicological studies dealt with the dose dependent extract of the roots on animals. Five different doses (125 mg/kg, 500mg/kg, 1g/kg , 2.5g/kg & 5g/kg body weight) of plant root extract was orally fed to animals for 30 days and toxicological parameters like SGPT & Serum creatinine were monitored at the end of the treatment.

Serum SGPT activity demonstrated consistent increase in its activity in all doses of plant extract treated animals as compared to control. Similar observation were recorded in *Taverneira abyssinica* with the SGPT activity levels for the dosing group-125mg/kg and 500mg/kg body weight in extract when used to study its anti-ulcerative properties (Ibraham *et al.*, 1998). It has been previously reported that 1 to 5/.kg body weight dose group of *G. glabra*, a plant belonging to the same family of *T. cuneifolia*, is safe for daily consumption (Walker *et al.*, 1994).

Serum creatinine is a biomarker of kidney toxicity. Creatinine is produced in muscles due to action of enzyme creatinine kinase by using ATP. This creatinine is a waste product which is

excreted out of the body by kidney. In case of any damage to kidney (due to any injury or any drug use), there is increase in serum creatinine levels as creatinine is not excreted out properly. Our present plant study indicated that there was no significant alteration in creatinine levels. Only the higher dosing plant extract group demonstrated slight elevated levels of creatinine. Reports suggest that extract upto 5g/kg body weight for *G. glabra* is safe for consumption (Walker *et al.*, 1994). However, further studies on the same plant suggest that doses higher than 5g/kg body weight might show higher excretion of creatinine (Dewman *et al.*, 1998). There are reports that suggest higher doses of *G. glabra* can cause other complications like increased blood pressure (Rossum *et al.*, 2001), a side effect possibly due to renal toxicity. A recent review suggested that plants belonging to licorice can cause their effect on kidney filtration processes leading to nephrotoxicity (Allard *et al.*, 2013). However, exact active phyto- components causing toxicity needs to be explored especially before recommending for human consumption.

Section 4: Wound Healing

4.4.1 RESULTS:

Effect on wound contraction:

Wound healing process on excision wound was recorded for wound healing.

Table 4.17: During the early days (up to 3 days) moderately higher rate of contraction was observed in *T. cuneifolia* and *G. glabra* treated groups. At the end of 15 days treatment both the treated groups showed better wound contraction in comparison to control group. However there was no significant difference observed among the treated groups.

Table 4.17: Actual data of wound contraction (wound area in cubic mm) recorded during the study on excision wound

Group	0 day	3rd day	6th day	9th day	12th day	15th day	% change (0-15 days)
Control	246.69 ±24.18	208.15 ±4.33	177.81 ±5.14	146.93 ±8.77	82.16 ± 4.59	48.30 ±3.72	80.42 ↓
G glabra	278.42 ± 11.14	234.41 ± 8.30	179.68 ± 10.68	123.25 ± 13.87	71.47 ±10.83	37.68 ± 5.46	86.46 ↓
T cuneifolia	297.45 ± 18.43	248.30 ± 10.11	175.94 ± 11.80	113.68 ± 20.80	71.99 ±17.76	36.80 ± 5.28	87.62 ↓

Data: Mean ± SEM, ↓-Decrease

Data pertaining to the effect of test drugs on days taken complete epithelialisation have been provided in Table 4.18. An apparent and statistically significant decrease in days taken for complete healing was observed in both *G. glabra* and *T. cuneifolia* treated groups, among them the observed effect in *T. cuneifolia* treated group is found to be better.

Table 4.18: Days taken for complete epithelialization

Group	**Days taken for complete epithelialisation**	**% change**
Control	29.50 ± 0.71	---
G. glabra (positive control)	25.50 ± 1.02$^{\#}$	13.55↓
T. cuneifolia	23.50 ± 0.92$^{\#\#}$	20.33↓

Data: Mean ± SEM ↓ - Decrease $^{\#}P<0.05$, $^{\#\#}P<0.01$(One Way ANOVA)

Table 4.19: Consolidated statement of test drugs on wound contraction and days taken for complete epithelialization

Effect on wound contraction	*G. glabra*	*T. cuneifolia*
3rd day	NSE	NSE
6th day	NSE	NSE
9th day	NSE	NSE
12th day	NSI	NSI
15th day	NSI	NSI
Days taken for complete epithelialisation	SD	SD

NSE-No significant effect, NSI-Non significant increase, SD-Significant Decrease

4.4.2. Discussion:

Wound healing involves different phases such as contraction, epithelization, granulation and collagenation. Excision wound healing model is often use for wound healing evaluation because it represents a true wound that could be reproducibly analyzed in non-subjective, highly controlled manner. The most important component in this model is the wound closure. The efficacy of the medication is measured in terms of rate of wound contraction and duration required for complete epithelialization of the wound. *G. glabra* has shown better wound contraction and significant decrease in days taken for complete healing, which was

also reported earlier (Sam *et al.*, 2000; Oloumi *et al.*, 2007). Better wound contraction and significant healing of wound has been recorded in *T. cuneifolia* applied group in comparison to control as well as *G. glabra.*

The wound healing properties of *T.cuneifolia* could be owing to phenolic acids present (protocatechuic, **p-hydroxybenzoic**, p-coumaric and vanillic acids) and complex mixtures of lipophilic flavonoid aglycones (flavanones, flavonols, flavones and chalcones) are major and powerful antioxidants to protect cultured skin cells against oxidative damage. Similar features were even recorded in the leaves of *Chromolaena odorata* (Phan *et al.* 2001). In conclusion, the extract from *T.cuneifolia* contains a mixture of powerful antioxidant compounds that may be one of potential mechanism contributing to enhanced wound healing.

In addition, Ascorbic acid has shown to be involved in collagen gene expression. It was also found in some burn injury cases ointment containing superoxide dismutase (SOD), which stimulates wound healing. It was observed that asiaticoside enhanced induction of antioxidant levels at an initial stage of healing, which may be important contributory factor in the healing property (Shukla *et al.*, 1999). The antioxidant enzymes (superoxide dismutase and catalase) are known to quench the superoxide radical and thus prevent the damage of cells caused by free radicals (Shirwaikar*et al.*, 2003). So scavenging effect might be one of the most important components of wound healing. Also plant reported to have antioxidant activity (Beskina, 2006) may be responsible to support wound healing. Thus the enhanced wound healing may be due to the free radical scavenging action of the Glycyrrhetinic acid as well as enhanced antioxidant enzyme level in granuloma tissues. The Glycyrrhetinic acid was found to be powerful antioxidant to protect thermally injured mice oxidative damage (Utsunomiya, 1999). A recent report by Draelos (2012) suggested that skin creams containing licorice extracts can repair epidermal layer.

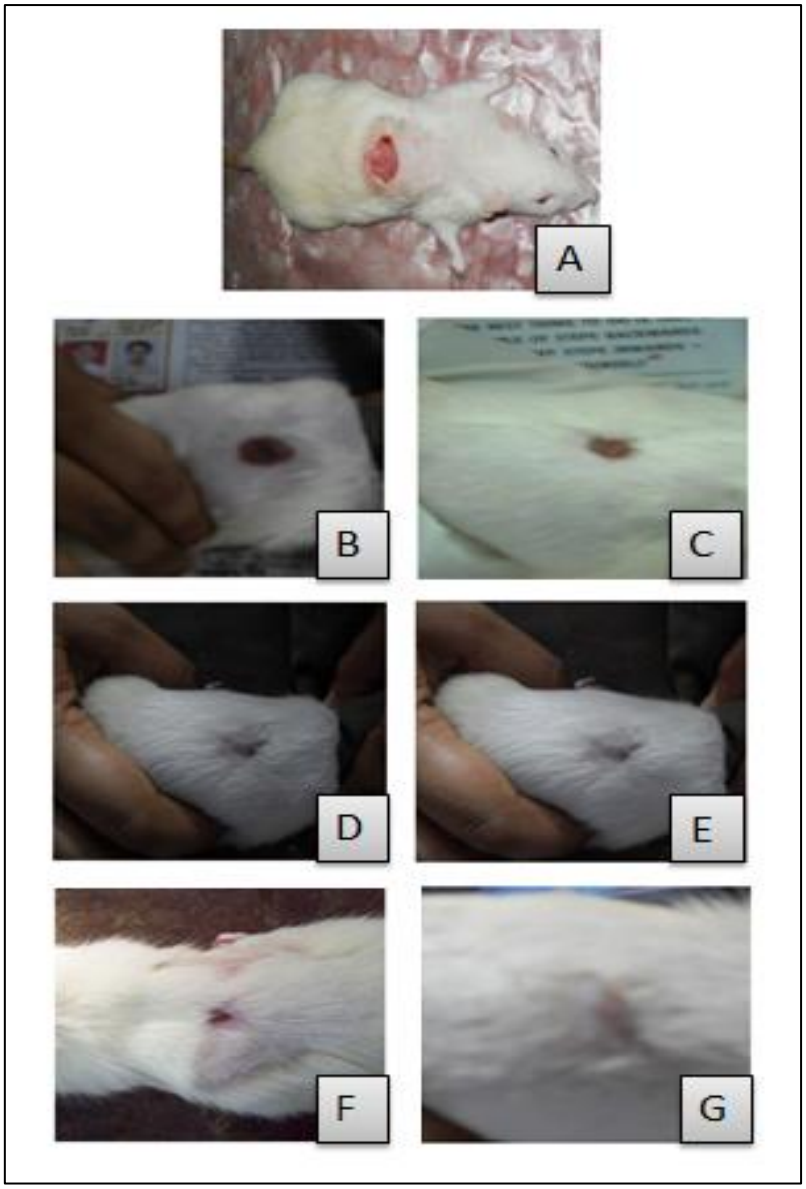


Figure 4.94: Different stages of excision wound in *T. cuneifolia*

A) Wound B) Day 0 C) Day 3 D) Day 6 E) Day 9 F) Day 12 G) Day 15

Section 5: Antitussive activity

The effect of *G. glabra* and *T. cuneifolia* on sulphur dioxide induced cough episodes has been presented in table –. Exposure of the mice to sulphur dioxide, the frequency of cough for control group was found to be 52.50 ± 2.24. Pre-treatment with *G. glabra* and *T. cuneifolia* remarkably inhibited the sulphur dioxide induced cough episodes in comparison to control group. Further among them, the observed inhibition in *T. cuneifolia* treated group is found to be better than that of *G. glabra.*

Table 4.20: Effect on SO_2 induced cough episodes

Groups	Number of cough episodes for 5 minutes	% inhibition
Control	52.50± 2.24	---
G. glabra (Positive Control)	35.67± 1.38[#]	32.05↓
T. cuneifolia	30.16±8.87[#]	42.55↓

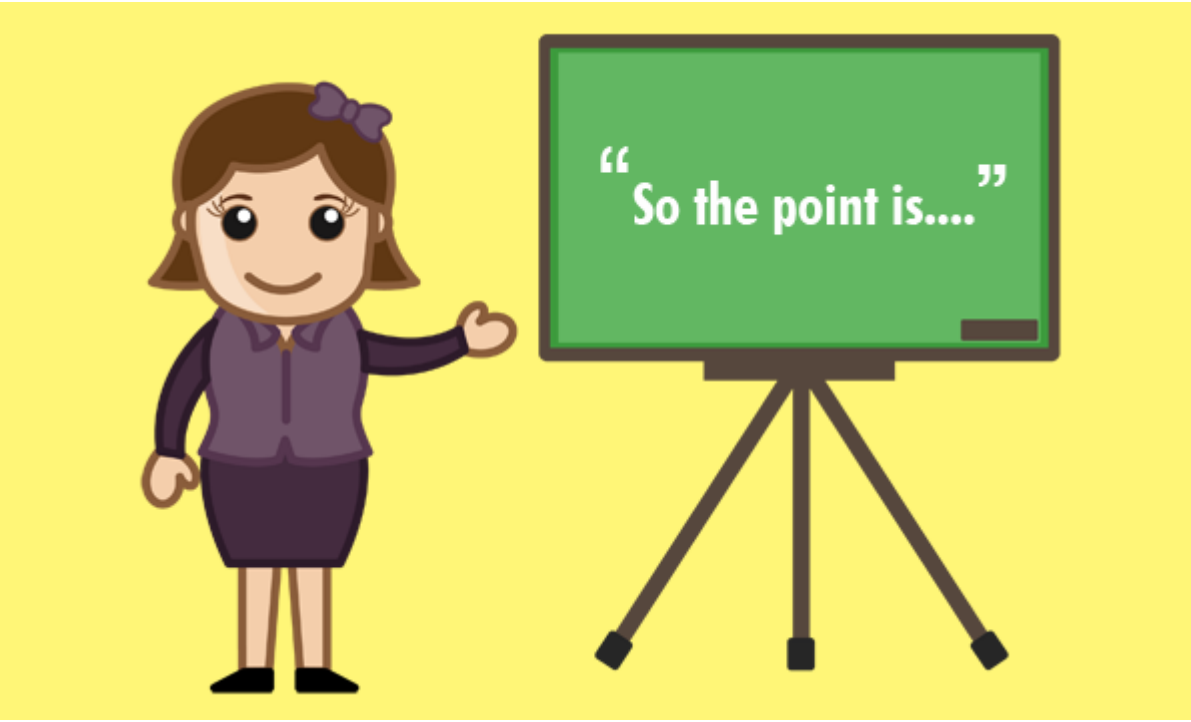
Data: Mean ± SEM ↓ - Decrease [#]P<0.05, (One Way ANOVA)

4.5.1 Discussion

Sulphur dioxide induced cough is widely used model for evaluating anti-tussive activity of a candidate compound. In the model employed for induction of cough in the present study the cough is caused by the irritation of the air way epithelium. The mechanism involved in observed activity may be suppressing the central cough centre or through the protective ciliary activity in the respiratory tract thus may form a protective and soothing layer over the respiratory mucosa and act as demulcents and in this way relieve the cough. As expected pre-treatment with *G. glabra* which has active phytoconstituent sapogenin glycyrrhizic acid would acts as potent anti-tussive and expectorant agent (Marjan and Hossein, 2008; Gupta *et al.,* 2009) which significantly inhibited the number of sulphur dioxide induced cough episodes. Whereas, when the *T. cuneifolia* pre-treated group having the sulphur based saponins could be responsible for antitussive activity, *T. cuneifolia extracts showed* better results in comparison to positive control *G. glabra.*

The phytoconstituents that have been reported for its antitussive properties are kaempferol, apigenin, 4'-hydroxywogonin, quercitrin, luteoiin, isorhamnetin, verbenalin, aucubin and β-sitosterol, and Isorhamnetinin in *Verbena officinalis* (Ren *et al.* 2013). Many of this phytoconstituents viz., apigenin, 4'-hydroxywogonin, quercitrin, luteoiin, isorhamnetin, verbenalin, aucubin and β-sitosterol, and Isorhamnetinin are even recorded in *T. cuneifolia*.

Chapter 5: Conclusion



CONCLUSION

- Ecologically *T. cuneifolia* grows well in black soil with poor rainfall.
- Pharmocogonistically the powder and plant material can be distinguished by vasicentric tracheids, oil globules, Prismatic crystals, medullary rays characters.
- Regarding propogation the seed viability is 68% and for propagation the seeds need to be treated with sulphuric acid.
- Many authors have shown presence of Glycyrrhizin in *T. cunefolia* however, the material is not showing presence of glycyrrhizin.
- Studies conducted previously have claimed that glycyrrhizin is the sweet principle of *Abrus precatorius* leaves (Hooper, 1894; Anonymous, 1985, Guignet, 1885; Akinloye, 1981). Later on it was reported that the perceived sweetness of *A. precatorius* was due to abrusosides and not due to glycyrrhizin (Choi *et al*., 1989). Even we had conducted studies on *Taverniera cuneifolia* root but could not find glycyrrhizin in it.
- Hydrolysis of the extract was done we got quercetin from the roots and other phenolic acids (vanillic acid, syringic acid, protocatechuic acid, ferulic acid, cis-*o*-coumaric acid, caffeic acid, p-hydroxy benzoic acid) but not 18-β-glycyrrhetinic acid.
- We have also used the standard of 18-α-glycyrrhetinic acid and 18-β-glycyrrhetinic acid. *Taverniera cuneifolia* which is considered to be as potential substitute showed absence of Glycyrrhizic acid, alpha and beta glycrrhetenic acid in extensive HPLC, GC-MS and LC-MS studies. However, there are 26 similar phytocomponents in both the species:

1	alpha-Amyrin
2	Apigenin
3	Apigenin
4	Benzaldehyde
5	beta-Amyrin
6	Betasitosterol
7	cis, cis – Linoleic acid
8	Dihydroxyflavone
9	Fasciolin
10	Furfural

11	Furfural, 5-methyl
12	Genistein
13	Hexadecanol
14	Kaempferol
15	Kumatakenin
16	Naringenin
17	1-Nonadecene
18	1-Pentadecene
19	n-Octadecanol
20	Palmitic acid

21	Phenol, 2,4-bis (1,1-dimethylethyl)
22	Pinocembrin
23	Propanoic acid, 2 hydroxy-2-methyl, methyl ester

24	Quercetin
25	Stigmasterol
26	β-amyrin

The active phytoconstituent of *G. glabra* is 18β-glycyrrhetinic acid which belongs to the beta amyrin series. The presence of β-amyrin in *G. glabra* and *T. cuneifolia* probably owes to the overlapping of the peaks in HPTLC between Glycyrrhizin and *T. cuneifolia* extracts. Indicative of glucopyranose group in *T. aegyptiaca* further states that *T. cuneifolia* coinciding with *G. glabra* owing to this group.

The LC-MS analysis of *T.cuneifolia* suggest that there are 10 probable saponins which are similar to *T. aegyptiaca* and are probably found even in *T. abyssinica* (based on the HPTLC finger print).

28-methyl serratagenate-3-b-O-b-xylopyranosyl (1→2)-b-glucopyranoside

28-methyl serratagenate 3-b-O-a-rhamnopyranosyl (1→2)-b-glucopyranoside

3b-O-a-rhamno- pyranosyl (1→2) b-glucopyranosyl-olean-11,13(18)-dien-1b, 3b, 22b-triol

3b-O-b-glucopyranosyl (1→2)-b-glucurono- pyranosylolean-11,13(18)-dien-1b,3b,22b-triol

3b-O-b-xylopyranosyl(1→2)-b-glucuronopyranosylolean-11,13(18)-dien-1b,3b,22b-triol

3b-O-a-rhamnopyranosyl (1→2)-b-glucuronopyranosylolean-11,13(18)-dien-1b, 3b, 22b-triol

3β-O-β-D-glucopyranosylolean- 11, 13(18)-diene- 3β,22β-diol.

3β-O-β- D - glucopyranosylolean - 11,13(18) - diene - 1β,3β,22β-triol

1β,3β,22β-trihydroxyolean-11, 13(18)-diene

1β,22β dihydroxyolean-11,13(18)- diene

T. cuneifolia roots which has sweet component as in *G.glabra* owes to the saccharides present in it. Phytochemically, the *Taverniera cuneifolia* is composed of mono and polysaccharides. Sterols components in the roots of *T. cuneifolia* are beta sitosterol, lupeol, stigmasterol. In

order to study the nutritional potential of *T. cuneifolia*, important parameters such as carbohydrates, protein, cholesterol, vitamin & iron content were studied & compared with *Glycyrrhiza glabra*. Simultaneously as *T.cuneifolia* is known for its dietary properties the studies were also done on other parts of plant such as fruit cover, seed & seed cake to observe their potential from the prospective of nutraceutical sector. The various phytochemical components and the nutritional properties of *T.cuneifolia* are owing to the following:

Vitamins

T. cuneifolia contains water soluble vitamins like Vitamin B1 (Thiamine), Vitamin B2 (Riboflavin), Vitamin B3 (Niacin), Vitamin B6 (Pyridoxine)

Amino Acids

Of the 22 amino acids present, 15 amino acids were recorded in seeds of *T. cuneifolia,* 13 amino acids in roots, 13 amino acids in leaves and 14 amino acids in seeds with fruit cover.

The human body requires 22 amino acids, the building blocks of proteins and *T. cuneifolia* provides twenty of them.

T. cuneifolia contains 19 of the 20 amino acids required by the human body for good health. The human body however produces only 12 of these. More importantly, it provides seven of the eight essential amino acids that cannot be manufactured by the body and which have to be consumed as food.

T. cuneifolia contains 10 essential amino acids:

Arginine, Methionine, Phenylalanine, Tryptophan, lysine, Histidine, Isoleucine, Leucine, Valine and Threonine

The 9 'non-essential' amino acids contained in *T. cuneifolia* are:
Alanine, Asparagine, Cystein, Glutamine, Glutamic acid, Glycine, Proline, Serine, and Tyrosine.

Sugars

These include the **important sugars (glucose fructose, arabinose, etc), maltol, furfuryl alcohol, β-d-glucopyranose.**

Lignan

Conocarpan and 9-O-Feruloyllariciresinol

Saponins

Saponins which are Glycosides form about 3% of what's in ***T. cuneifolia***. They are soapy substances which provide cleansing and antiseptic activity. They act powerfully as anti-microbials against bacteria, viruses, fungi and yeasts.

Sterols

These are anti-inflammatory agents. Lupeol also possesses antiseptic and analgesic properties. They act as **powerful anti-inflammatory agents**. In *T. cuneifolia* roots three sterols β-sitosterol, stigmasterol, lupeol have been identified.

Fatty acids:

Fatty acids from roots obtained were Caproic acid, myristic acid, lauric acid, palmitic acid, linolenic acid, stearic acid, oleic acids, behenic acids.

Fatty acids in seeds obtained were palmitic acid, stearic acid, oleic and linoleic acid.

Proteins

All types of proteins are present in *T. cuneifolia* High molecular weight proteins to low molecular weight proteins. Seeds contain higher level of proteins as compared to roots.

Phenolic acids

p-hydroxy benzoic acid, protocatechuic acid, vanillic acid, syringic acid, cis and trans *O*-coumaric acid, ferulic cid, caffeic acid

Flavonoids

Anthocyanins –Delphinidin was obtained from the flowers of *T. cuneifolia.*

Flavonols – quercetin, isorhamnetin., Kaempferol tetracetate,

Isoflavone- 2',4', 5'- trimethoxy- 2"2"- dimethyl pyrano[5",6", 6,7] isoflavone.

Flavones- 5,7- diacetoxy, 8-methoxy flavone, acacetine, 3',4,'5,'3,5,6,7-heptamethoxyflavone, luteolin, apigenin, 3',4,'5,'3,5,7,8- heptamethoxyflavone.

Flavan 3-ol – Catechin 7-glycoside, (-)-Epiafzeechin

Diterpenoid – Przewalskin, Coronarin A

Triterpenes- α and β amyrin

Chapter 6: References

CHAPTER 6 – REFERENCES

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Research Article

PHYSICOCHEMICAL AND PRELIMINARY PHYTOCHEMICAL STUDIES OF *TAVERNIERA CUNEIFOLIA* (ROTH.) ARN. – A POTENTIAL SUBSTITUTE OF *GLYCYRRIZHA GLABRA* L.

POONAM S. MANGALORKAR[1], SUNITA SHAILAJAN[2], BRIJESH NOTANI[3], AND PADAMNABHI S. NAGAR*[1]

[1]The Maharaja Sayajorao University of Baroda, Vadodara, Gujarat, India, [2]Ramnarain Ruia College, Matunga, Mumbai, Maharashtra, India, [3]Gujarat Ayurved University, Jamnagar, Gujarat, India.

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ABSTRACT

The present communication evaluates the physicochemical and preliminary phytochemical properties of *Taverniera cuneifolia* (Roth.) Arn. The roots of *T. cuneifolia* has been considered as a potential substitute of *Glycyrrhiza glabra*, popularly known as Indian Licorice. However, there is no detailed standardized work done so far. The results revealed that the concentration of all the heavy metals were below the WHO/FDA permissible limits. *Escherichia coli, Salmonella Spp.* were found to be absent. Total ash value content was 6.75 %, water soluble ash was 2.61% and sulphated ash was 3.02%. The water soluble extractive value indicated the presence of sugar, acids and inorganic compounds. The alcohol soluble extractive values indicated the presence of phenols, alkaloids, steroids, glycosides, flavonoids.

Keywords: *Taverniera cuneifolia*, *Glycyrrhiza glabra,* Licorice, Heavy metals, Physicochemical studies, Phytochemical qualitative test.

INTRODUCTION

Since origin of human's life plants continue to play a curative and therapeutic role in preserving human health against diseases[1]. Today we find a renewed interest in traditional medicines. Traditional ecological knowledge is associated with biodiversity research, bioprospecting, and cultural conservation[1,2,3,4]. In this context, India being a subtropical country is a good repository of plants that are widely used in the preparation of herbal therapies.

In this communication one such plant is selected which is traditionally used by the tribal's of Saurashtra region, in Gujarat. The genus *Taverniera* belongs to the family of Fabaceae and includes twelve species. It is endemic to the Northeast African and Southwest Asian countries[1]. *T. cuneifolia* is often referred to as Indian licorice owing to its sweet taste which is very similar to that of *G. glabra*[2]. *T. cuneifolia* is locally known as Jethimadh and it is used by the tribal's of Barda Hills of Jamnagar in Western India. It is used as a substitute of Licorice or in other words the plant itself is considered to be *G. glabra*[2]. It is traditionally known to be used as an expectorant, blood purifier, anti-inflammatory, wound healing, antiulcer and in treating spleen tumors[2].

T. cuneifolia is a much branched undershrub, 1-2 ft high. Leaves 1-3 foliate, stipules scarious, triangular, acute, deciduous one which is opposite the leaf. Leaflet obovate, thick glaucous, mucronulate. Flowers axillary, 2-6 flowered raceme longer than the leaves. Calyx finely pubescent, teeth triangular, acute. Corolla purplish pink in colour, standard obovate-orbicular, slightly longer than keel, glabrous, veined with dark purple parallel veins, emarginate, pods with 1-2 seeded joints, joints ovoid, echinate[2].

MATERIALS AND METHODS

Collection and processing of plant samples:

The plant material was collected from Munjka village, Near Saurashtra University Campus, Rajkot and Rosy port area, Jamnagar, Gujarat, India. The plant material was authenticated at BSI (Botanical Survey of India) Jodhpur, Rajasthan, India. Ref.no. BSI/AZC I.12012/ Tech./2011-12 (PL.ID)-55. The shade dried roots were used for the investigation of qualitative phytochemical test, physicochemical tests and microbial contamination.

Qualitative Phytochemical Tests

Procedure for HPTLC Analysis

1g of plant sample was accurately weighed, placed in a stoppered tube and 10 ml of methanol (solvent) was added, vortexed for 2 min and left to stand overnight at room temperature (28±2ºC). The contents of the tube were filtered through Whatmann No. 41 paper (E. Merck, Mumbai, India) and the filtrate was used as stock solution for further analysis. Same procedure was applied for other solvents like chloroform, ethyl acetate, toluene as well.

Sample Volume

10 µl of methanol, chloroform, ethyl acetate and toluene extract was spotted as track1 (T1), track 2 (T2), track 3 (T3) and track 4 (T4) respectively.

Chromatographic Conditions

Instrument

CAMAG HPTLC system comprising of Linomat IV Spotter, Scanner II, CAMAG CATS 3 software. TLC Plate used was Silica gel 60 F254. Mobile Phase employed was n-butanol: Acetic acid: Water (7: 1: 2). The saturation time was 30 min. Detection wavelength was UV-254 nm and UV-366 nm

Derivatising agent

Liebermann Burchard Reagent - 5 mL acetic anhydride + 5 mL Conc. H_2SO_4 + 50 mL Absolute Ethanol (prepared under cooling ice).

All the tests were performed according to the following test[2]:

Alkaloids	Dragendorff's test
Flavonoid	Shinoda test
Tannin	Neutral $FeCl_3$
Protein	Biuret test
Carbohydrate	Molisch's test
Reducing sugar	Fehling's test
Saponin	Foam test
Resin	Acetic anhydride test
Phenol	Neutral $FeCl_3$
Terpenoids	Leibermanburchard test

The physiochemical and microbiological tests were done as follows[3,4]:

Parameters	Test method
Physicochemical tests	
Water soluble extractive	IP 2007
Alcohol soluble extractive	IP 2007
Moisture content (By KF)	IP 2007
Ash content	IP 2007
Acid insoluble ach	IP 2007
Water soluble ash	IP 2007
Sulphated ash	IP 2007
Swelling index	IP 2007
Heavy metals	
Lead	API 2008
Cadmium	API 2008
Arsenic	API 2008
Mercury	API 2008
Microbiological tests	
Total plate count	API 2008
Total fungal count	API 2008
Escherichia coli	API 2008
Pseudomonas aeruginosa	API 2008
Staphylococcus aureus	API 2008
Salmonella Spp.	API 2008

(ABR.: IP – Indian Pharmacopoeia, **API** – Ayurvedic Pharmacopoeia of India.)

RESULTS AND DISCUSSION

Roots of *Taverniera cuneifolia* (Roth) Arn. were collected and analysed for the various parameters. Preliminary phytochemical results showed the presence or absence of certain phytochemicals in the drug. The tests were performed using methanol, water and chloroform extracts. Phytochemical test revealed the presence of alkaloid, flavonoid, tannin, protein, reducing sugar and saponin. The results are given in the Table 1.

The presence of four heavy metals namely Lead, Cadmium, Arsenic, Mercury and microbial contamination were analysed in the sample and the results are shown in the Table 2. The concentrations of all the heavy metals were below the WHO/FDA[2,3] permissible limits. In microbial contamination *Escherichia coli, Salmonella Spp.* were absent and *Pseudomonas aeruginosa, Staphylococcus aureus* were found to be present. Physicochemical parameters of the root of *T. cuneifolia* are depicted in Table 2. The moisture content of the root was found to be 4.48%. Total ash value of the plant material indicated the amount of minerals and earthy materials attached to the plant material. Analytical results showed total ash value content was 6.75%. The negligible amount of acid insoluble siliceous matter present in the root was 1.23%. The water soluble ash was 2.61% and sulphated ash was 3.02%. The water soluble extractive value indicated the presence of sugar, acids and inorganic compounds. The alcohol soluble extractive values indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids. The results are given in Table 2.

Table 1: Preliminary phytochemical qualitative test for roots of *T. cuneifolia*.

Natural Product	Test Performed	Result
Alkaloids	Dragendorff's test	+
Flavonoid	Shinoda test	+
Tannin	Neutral $FeCl_3$	+
Protein	Biuret test	+
Carbohydrate	Molisch's test	-
Reducing sugar	Fehling's test	+
Saponin	Foam test	+
Resin	Acetic anhydride test	-
Phenol	Neutral $FeCl_3$	-
Terpenoids	Leibermanburchard test	-

Table 2: Physicochemical parameters of roots of *T. cuneifolia*

Parameters	Result
Physicochemical Tests	
Water soluble extractive	17.19 %
Alcohol soluble extractive	14.98 %
Moisture content (By KF)	4.48 %
Ash content	6.75 %
Acid insoluble ach	1.23 %
Water soluble ash	2.61 %
Sulphated ash	3.02 %
Swelling index	Absent
Heavy Metals	
Lead	Not Detected
Cadmium	0.1021 ppm
Arsenic	Not Detected
Mercury	Not Detected

Microbiological Tests	
Total plate count	178 x 102 cfu/g
Total fungal count	17 x 102 cfu/g
Escherichia coli	Absent
Pseudomonas aeruginosa	Present
Staphylococcus aureus	Present
Salmonella Spp.	Absent

ABR.: IP – Indian Pharmacopoeia, **API** – Ayurvedic Pharmacopoeia of India, **ppm** – parts per million, cfu – colony forming unit.

Thin layer chromatographic technique was used to separate the chemical compounds present in the drug. Various solvent systems were used to separate the maximum number of chemical compounds in the drug.

TLC of the methanol extract developed in the mobile phase of Butanol: Acetic acid: Water (7: 1: 2) (Figure.1) and observed 9 spots at Rf value 0.18, 0.21, 0.26, 0.28, 0.32, 0.42, 0.53, 0.57, 0.76.

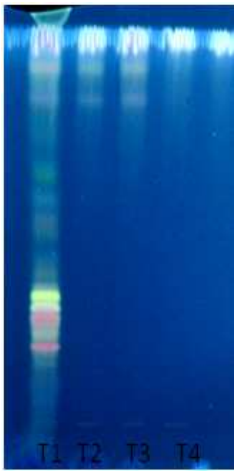

Fig. 1: TLC profile of the root of *T. cuneifolia* taken in different extracts:

T1- Methanolic extract, T2- Chloroform extract, T3- Ethyl acetate extract,

T4- Toluene extract (Under UV 366nm).

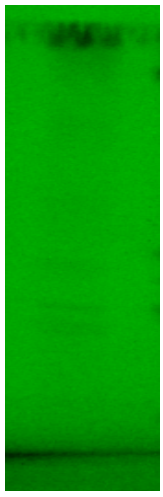

Fig. 2: TLC profile of the roots of *T.cuneifolia* (Under UV 254nm)

CONCLUSION

Phytochemical, physicochemical, toxic heavy metal analysis, microbial contaminants of the root sample along with TLC studies was done for the authentication of quality control of raw drugs. Roots of *Taverniera cuneifolia* exhibits a set of characters, which would help to identify the drug in dried condition.

It has been concluded from this study that estimation of heavy metals and microbial contamination is highly essential for raw drugs or plant parts used for the preparation of compound formulation drugs. The periodic assessment is essential for quality assurance and safer use of herbal drugs.

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ANATOMY AND PHARMACOGNOSY OF *TAVERNIERA CUNEIFOLIA* ROOT (ROTH) ARN., A POSSIBLE SUBSTITUTE OF *GLYCYRRHIZA GLABRA* L.

P. S. MANGALORKAR[1], B. R. PATEL[2] AND P. S. NAGAR*[1]

[1]Department of Botany, The Maharaja Sayajirao University of Baroda, Vadodara-390002, Gujarat, India.
[2]Department of Dravyaguna, Gujarat Ayurved University, Jamnagar, Gujarat, India.

ABSTRACT

Taverniera cuneifolia is an important traditional medicinal plant of India belonging to the family of Fabaceae. The roots of *Taverniera cuneifolia* is a potential substitute of *Glycyrrhiza glabra* (Licorice). In order to differentiate *G. glabra* from *T. cuneifolia*, macroscopic as well as microscopic studies of *T.cunefolia* were done. The present investigation deals with pharmacognostic characters, which includes anatomy, powder studies and histochemical test.

KEY WORDS:*Taverniera cuneifolia*, Licorice, pharmacognosy, roots.

P. S. NAGAR
Department of Botany, The Maharaja Sayajirao University of Baroda,
Vadodara-390002, Gujarat, India.

INTRODUCTION

The genus *Taverniera* belongs to the family of fabaceae and includes twelve species. It is endemic to the Northeast African and Southwest Asian countries [i]. *T. cuneifolia* is often referred to as Indian licorice owing to its sweet taste which is very similar to that of *G. glabra* [ii]. *T. cuneifolia* locally known as Jethimadh is used by the tribal's of Barda Hills of Jamnagar in Western India as a substitute of Licorice or in other words the plant itself is considered to be *G. glabra* [iii]. *T. cuneifolia* is known to have various properties like expectorant, blood purification, anti-inflammatory, wound healing, antiulcer and used in treating spleen tumors [iv]. *G. glabra* L., is popularly known as commercial licorice [ii]. The roots of *G. glabra* are very widely used in traditional systems of medicines all over the world [v]. *G. glabra* is rich in bioactivities like antiviral, anticancer, anti-ulcer, anti-diabetic, anti-inflammatory, anti-oxidant, anti-thrombic, anti-malarial, anti-fungal, anti-bacterial, estrogenic, immunostimulant, anti-allergenic and expectorant activities [vivii,-viiiix]. The commercial licorice has a huge demand in the Indian system of medicine, Ayurveda and majority of the requirement of the Ayurvedic drug industry in India is met through import from Afghanistan and Pakistan [ix]. Owing to huge demand plants like *A. precatorius* is used as adulterant or as a substitute for *G. glabra* [x,xi]. The anatomical and pharmacognostic features of the plant are used to determine the identity and the family of a species. Present study deals with the scientific pharmacognostic validity of indigenous alternative *T. cuneifolia* to identify the distinguishing biomarkers useful in quality control of this drug.

MATERIALS AND METHODS

i. Collection and authentication of the plant material

Roots of *Taverniera cuneifolia* were collected from Gondal road, Rajkot during June and October, 2009. The specimens collected were identified with the help of the "Flora of Gujarat State". Further the species was confirmed by comparison with the specimen available at in the Herbaria of Botanical Survey of India, Jodhpur, Rajasthan, India. (BSI/AZC I. 12012/ Tech./2011-12 (PI.ID.)-551.

ii. Anatomy

Roots were collected and cut into blocks of 4-5cm in length and were immediately fixed into Formalin: Acetic acid: Alcohol (90: 5: 5). (Berlyn & Miksche, 1986). Trimmed blocks were sectioned in transverse planes at a thickness of 12-15µm by using Leitz sliding microtome. After staining with Safranin : Toluidine blue sections were passed through alcohol : xylene series and mounted in dibutyl phthalate xylene (DPX). The sections were photographed under Leica DFC 295 Compound Research microscope connected to Digital Camera.

iii. Powder studies

Completely dried plant material was finely powdered. The fine powder obtained was stained with safranin and observed under a microscope to trace and identify the characters present. The characters observed were photographed under a Leica DME compound research microscope connected to its digital camera.

iv. Histochemical test

Slides were prepared using water, chloral hydrate as a clearing agent, stained with phloroglucinol and HCl for lignified tissues and mounted in glycerin.

Sr.no.	Test	Reagents
1	Test for lignin	Phloroglucinol + Conc. HCl
2	Test for tannin	$FeCl_3$ solution
3	Test for starch	Iodine solution

RESULTS

The results of the studies conducted on roots of T. cuneifolia are described below

The root was circular in outline, with the xylem forming the entire section. The outermost layers were quite disrupted and were composed of 5-7 layers of barrel shaped lignified cork cells. Underneath the cork was reduced cortex consisting of parenchyma cells loaded by starch grains. Pericyclic fibres were distributed all over the cortex zone. Phloem had presence of scattered bast fibres. Between xylem and phloem there were few strips of cambium. Next to cambium was xylem which was exarch in condition. Medullary rays were biseriate to multiseriate loaded with starch grains. The longitudinal sections of the root showed xylem vessel elements possessing simple pits. Medullary rays were 366.9µm – 884.3µm × 25.09µm – 100.3µm broad with polygonal ray cells having varying dimensions 95.3µm-173.4µm × 43.9µm-76.56µm. Prismatic crystals (17.25µm - 61.15µm × 15.68µm – 54.87µm) of calcium oxalate were found to be abundant in fibres.

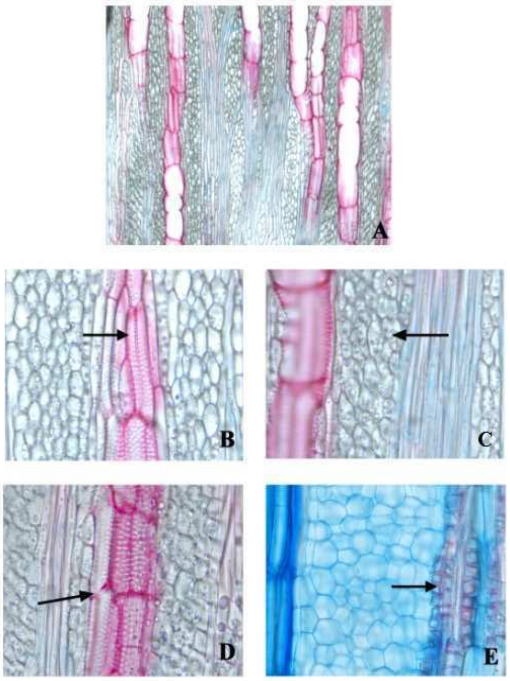

Figure 1

Longitudinal section of the root of Taverniera cuneifolia

A. Section of the root in 10x (200µm) B. Vessel elements C. Starch grains in ray cells D. Vessel elements and perforation plate in vessel elements E. Prismatic crystals. B-E 40x (200µm)

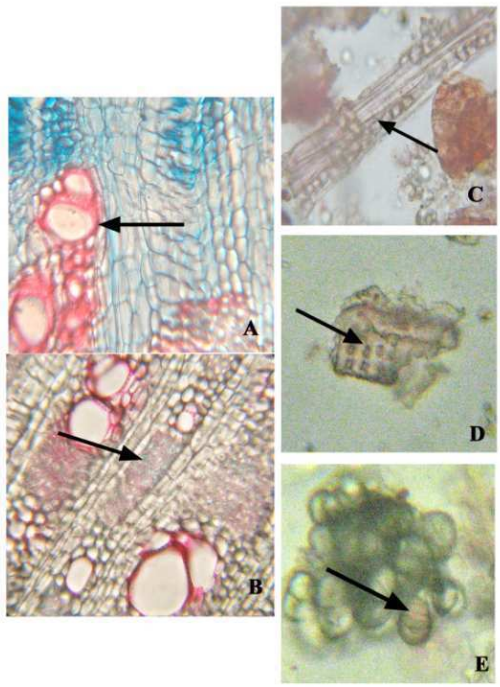

Figure 2

Transverse section and powder study of the root of Taverniera cuneifolia

A. Xylem vessel B. Fibres C. Prismatic crystal D. Pitted ray parenchyma E. Starch grains (100µm)

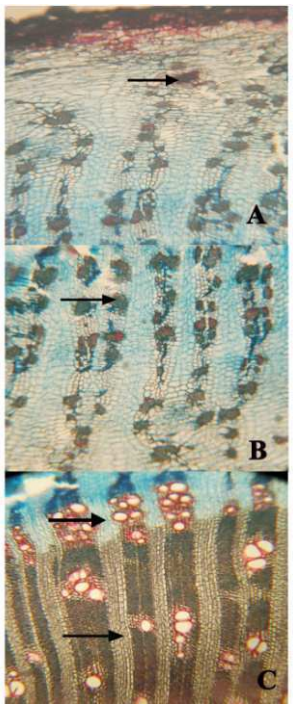

Figure 3

Transverse section of the root of Taverniera cuneifolia

A. Stone cells B. Bast fibres C. Xylem ray parenchyma and medullary rays (100µm)

Table 1
Histochemical tests

Sr. no.	Reagents	Observation	Characteristics
1	Phloroglucinol + Conc. HCl	Red	Lignified cells
2	$FeCl_3$ solution	Dark blue to black	Tannin cells
3	Iodine solution	Blue	Starch

DISCUSSION

The present study brings out a number of distinguishing features of the roots of this important medicinal plant. Pericyclic fibres, bast fibre, prismatic crystals, starch grains form key characters for identification.The multiseriately arranged medullary rays and the pitting in xylem elements are noteworthy. All these characters could be used as diagnostic biomarkers for *T. cuneifolia*, in both fresh material as well as in powder and also in checking adulteration of the same. However, there are no reports on the anatomy of the root, which is considered to be the most potent part of the plant. A detailed study of longitudinal sections of the root has been carried out in the present study, which is the first report of the plant. There are no reports of the measurements and dimensions of important characteristics in roots so far of the measurements and dimensions of important characteristics in root, which has been carried out in this work.

CONCLUSION

The present study shows important diagnostic biomarkers of the roots of *T. cuneifolia*, in both fresh materials as well in powder. This data can be of great value in the authentication of *Taverniera cuneifolia*, as well checking adulteration of the same while comparing with *Glycyrrhiza glabra*

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The authors wish to thank the University Grants Commission, New Delhi for providing research grant, Dr. Susy Albert, and The Head, Department of Botany, The Maharaja Sayajirao University of Baroda, Vadodara for providing necessary lab facility and accessibility to the instruments.

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Section B: Biological Sciences

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The Effect of Different Chemical Treatments and Salt Stresson the Germination Potential of *Tavernieracuneifolia* (Roth) Ali seeds

Poonam S. Mangalorkar*, Karan G. Rana, Rahul D. Parikh and Padamnabhi S. Nagar

Department of Botany, Faculty of Science, the Maharaja Sayajirao University of Baroda, Vadodara 390002, Gujarat, India

Received: 30December 2013; **Revised:** 15 January 2014; **Accepted:** 20 January 2014

Abstract:*Tavernieracuneifolia*is an ethnobotanically important traditional medicinal plant of Semi-Arid region of India belonging to the family of Fabaceae. As the plant is ecologically identified as glycophyte the seeds were treated with various salts. Effect of various salts (KCl, $MgCl_2$, NaCl, Na_2CO_3, $MgSO_4$,) on the germination of *T. cuneifolia* seed was studied. Germination decreased with increase in salinity. The inhibition of germination by salt solution was in the order of NaCl> $MgCl_2$> $MgSO_4$>KCl. Non-germinated seeds under various salt treatments when transferred to distilled water recovered completely, indicating little ionic effect of salinity on seed germination and viability. Germination rate was highest in those seeds which were treated with concentrated sulphuric acid with the germination percentage to be 80.

Keywords: Fabaceae, *Tavernieracuneifolia*, salts, treated.

INTRODUCTION

The genus *Taverniera*belongs to the family of fabaceae and includes twelve species. It is endemic to the Northeast African and Southwest Asian countries[1]. *T. cuneifolia* is often referred to as Indian licorice owing to its sweet taste which is very similar to that of *Glycyrrhiza glabra*[2]. *T. cuneifolia* locally known as Jethimadh is used by the tribal's of Barda Hills of Jamnagar in Western India as a substitute of Licorice or in other words the plant itself is considered to be *G. glabra*[3]. *T. cuneifolia* is known to have various properties like expectorant, blood purification, anti-inflammatory, wound healing, antiulcer, used

in treating spleen tumors[4].*G. glabra* L., is popularly known as commercial licorice[2]. The roots of *G. glabra* are very widely used in traditional systems of medicines all over the world[5]. *G. glabra* is rich in bioactivities like antiviral, anticancer, anti-ulcer, anti-diabetic, anti-inflammatory, anti-oxidant, anti-thrombic, anti-malarial, anti-fungal, anti-bacterial, estrogenic, immunostimulant, anti-allergenic and expectorant activities[6-10]. The commercial licorice has a huge demand in the Indian system of medicine, Ayurveda and majority of the requirement of the Ayurvedic drug industry in India is met through import from Afghanistan and Pakistan[9]. Owing to huge demand plants like *Abrusprecatorius* is used as adulterant or as a substitute for *G. glabra*[11, 12]. *G. glabra*inspite of being one of the most utilized species inHerbal medicines (Ayurvedic medicines) we are still dependent on its raw material to Iran, Italy, Afghanistan, Pakistan, Russia, and China. Propagation of *Glycyrrhizaglabra* is usually carried through stolons, cuttings of about 10-15 cm. Seed can be used, but seed-set is poor in India and seed germination is low[13].Thus, there is urgent need of the hour or to search for a suitable substitute. *T. cuneifolia* could be a possible substitute.

MATERIALS AND METHODS

Seeds of *T. cuneifolia* were collected from Munjka region, Rajkot, Gujarat, India in April 2012. Seeds were separated from pod in the laboratory and surface sterilized by treating with sodium hypochlorite. Seeds were germinated on 3 sheets of 9 cm diameter filter paper in10 cm diameter. Petri dishes with 10 ml of distilled water. The Petri dishes were sealed in plastic bags. Twenty five seeds were used for each treatment with three replicates each. Effect of GA_3: Seeds were soaked in 100, 250, 500 ppm GA_3 for 48 hours and 1000, 2000 ppm GA_3 for 72 hours [14,15]. The germination rate was calculated as follows (based on Wiese and Binning, 1987):

$$\text{Germination rate} = \sum_{n=1}^{5} (\text{number germinating since } n-1)/n$$

Where, *n* is the days of incubation.

The effect of different salts (KCl, $MgCl_2$, NaCl, Na_2CO_3, and MgSO4) was tested with different concentration each. The experiment was carried out at 31ºC. Germination was recorded at every alternate day and after 9 days un-germinated seeds were transferred to distilled water to determine the recovery of germination. The percent recovery was calculated using the following index:

Percent recovery = a-b / b-c x 100

Where, a = total number of seed germinated after being transferred to distilled water, b = total number of seed germinated in saline solution and c = total number of seeds[16].

RESULTS AND DISCUSSION

Salinity is a major environmental stress factor that affects seed germination in coastal salt marshes[16] where salinity ranges from 0.8% to 2.4%. Seeds of *T. cuneifolia* showed 56% germination in non-saline control. Seed germination decreased with increase in KCl, $MgCl_2$, NaCl, $MgSO_4$, Na_2CO_3 salts. Na_2CO_3 inhibited germination more than other salts and 0% seeds germinated, whereas 26.67% seeds germinated at 300mM NaCl solution (**Table 1**).Seed germination of *Chenopodiumglaucum* L. of the family Chenopodiaceae decreased with the increase in NaCl, Na_2CO_3, Na_2SO_4, $MgSO_4$, $MgCl_2$salts[19].The study showed that the inhibition to seed germination of *T. cuneifolia* is in the order of NaCl> $MgCl_2$> $MgSO_4$>KCl. Recovery of germination of *T. cuneifolia* seeds after treating it with different salt solutions was observed. Here, Na_2CO_3 treated seeds also showed recovery with 11% at 300mM solution. Maximum recovery germination was seen in $MgSO_4$ at 100mM with 70.45% (**Table 2**).*T. cuneifolia* belongs to the life form - Chaemophyte and its adaptability class is Glycophyte and salinity limit is 14 dSm^{-1}, as per Raunchier classification system[17]. It shows that the plant has the adaptation to grow in

saline and terrestrial zones. Inthis experiment, application of GA_3 stimulated thegermination of *T. cuneifolia*. This response was dependent on the concentration of applied GA_3. At lower concentrations, germination of these species was higher. In this species, increasing the concentration of GA_3 above 500ppm decreased the germination percentage (**Table 3**). In *Ferula gummosa* (Apiaceae), the highest germination percentage rate was obtained in the concentration range of 1000–2500 ppm. For *Teucriumpolium* (Labiatae) highest germination percentage was obtained at GA_3 at 500 ppm. GA_3 has a positive response across all applied GA_3 concentrations. Increasing GA_3 concentration increased both germination rate and percentage in *F. gummosa* and *T. polium*. *F.gummosa*didnot respond toGA_3concentrationsof lessthan 500ppm[18]. In*T. cuneifolia*germination rate was higher in those seeds which were treated with concentrated sulphuric acid with the germination percentage to be 80. The second highest germination percentage was 62.67% seen in boiling water and the third highest percentage germination was seen in lukewarm water with 57.33 %(**Table 3**).

Table 1: Seed germination of *Tavernieracuneifolia* when treated with different salt solutions

	Germination (%)				
Salt concentration (M)	KCl	$MgCl_2$	NaCl	$MgSO_4$	Na_2CO_3
0.10	78.67	50.67	24.00	73.33	0.0
0.15	65.30	64.00	50.67	46.67	0.0
0.20	69.33	62.67	17.33	60.00	0.0
0.25	58.67	25.33	26.67	30.67	0.0
0.30	09.33	21.33	26.67	21.33	0.0

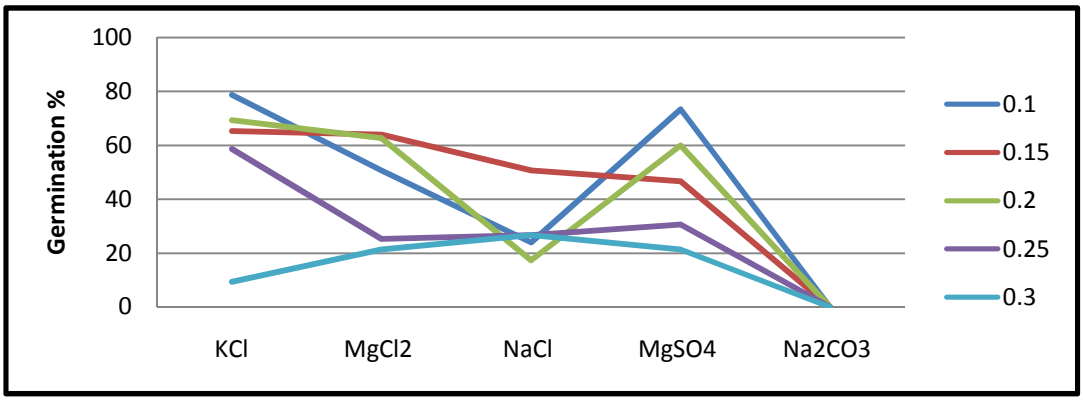

Figure 1: Seed germination when treated with different salt concentration

Table 2: Recovery of germination of *T. cuneifolia* when treated with different salt solutions

Salt concentration (mM)	Germination (%)				
	KCl	$MgCl_2$	NaCl	$MgSO_4$	Na_2CO_3
100	50	27.03	5.26	70.45	4.17
150	23.08	22.22	27.03	62.5	7.14
200	21.74	21.43	9.68	80	5.63
250	45.16	21.43	25.45	32.69	7.14
300	35.29	32.2	0	10.17	11.94

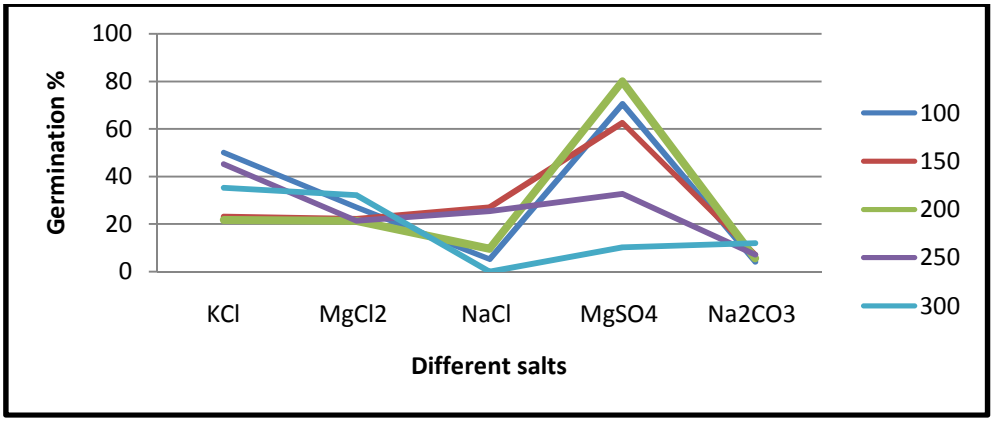

Figure 2: Recovery of germination after the removal from different salt concentrations

Table 3: Germination rate with different chemical treatments

Sr.No.	Chemical treatments	Germination rate (Seeds/day)	Germination %
1	GA_3 2000	0.19	18.67
2	$GA_3$1000	0.33	33.33
3	GA_3 500	0.47	46.67
4	GA_3 250	0.51	50.67
5	GA_3 125	0.44	44.00
6	Lukewarm water	0.57	57.33
7	Cool water	0.47	46.67
8	HNO_3 (10 minutes)	0.32	32.00
9	HNO_3 (30 minutes)	0.51	50.67
10	H_2SO_4	0.80	80.00
11	Boiling water	0.63	62.67
12	Control	0.39	38.67

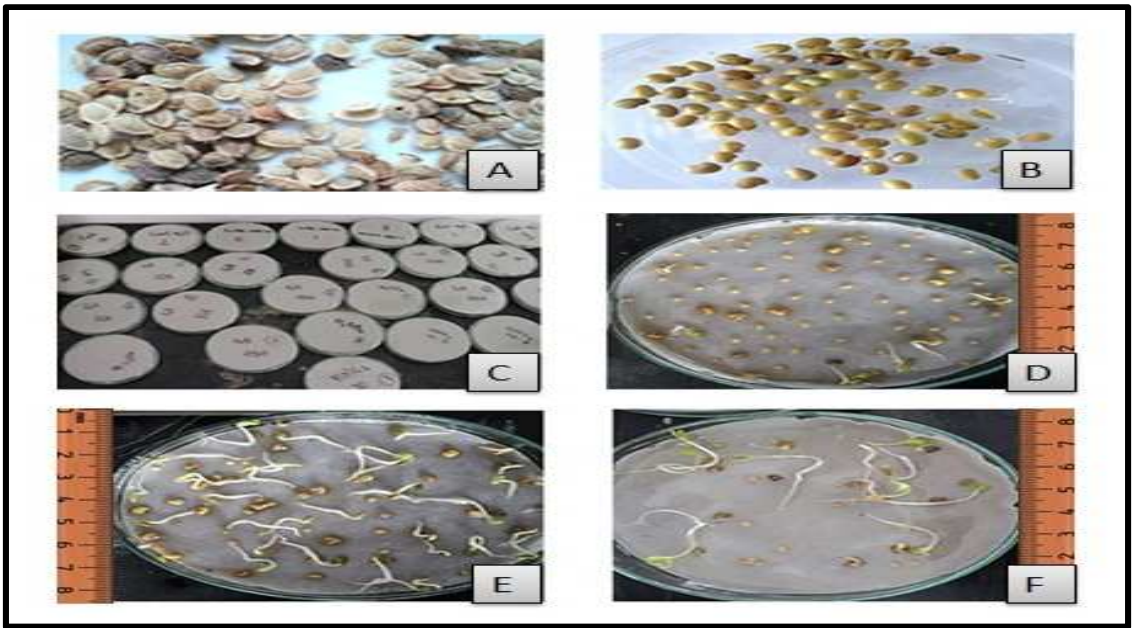

Figure 3: A. Fruits B. Seeds C. All concentrations D. Control E. Boiling water F. Lukewarm water

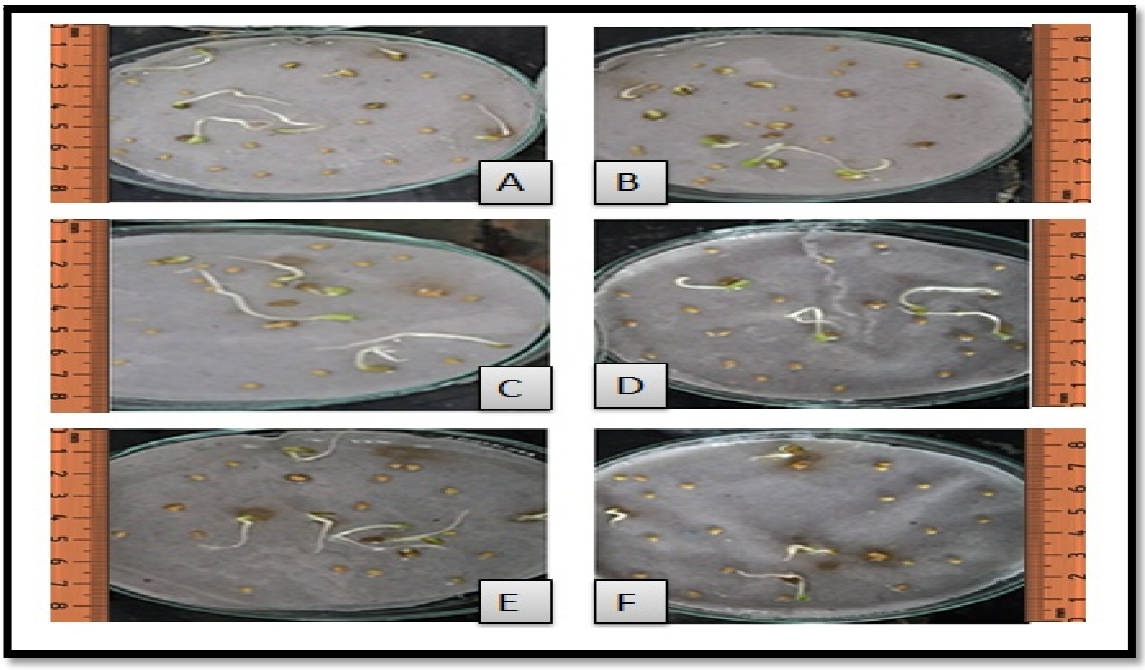

Figure 4: A. GA 125 B. GA 250 C. GA 500 D. GA 1K E. Cool water F. HNO_3

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Corresponding author:Poonam S. Mangalorkar; Department of Botany, Faculty of Science, the Maharaja Sayajirao University of Baroda, Vadodara 390002, Gujarat, India

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Research Article

HPTLC ANALYSIS OF THE ROOTS OF *TAVERNIERA CUNEIFOLIA* (ROTH) ALI

Poonam Mangalorkar[1]*, Sunita Shailajan[2] and Padamnabhi Nagar[1]
[1]Department of Botany, Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India
[2]Herbal Research Lab, Ramnarain Ruia College, Matunga, Mumbai, Maharashtra, India
*Corresponding Author Email: poonammangalorkar000@gmail.com
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ABSTRACT
Taverniera cuneifolia (Roth) Ali is commonly called as jethimadh (Licorice). High-Performance Thin-Layer Chromatographic analysis was carried out for the regional variation of different regions of India, seasonal variation and species variation. The root powder was extracted with Methanol and further analysis was performed. Quantitation of β-sitosterol and lupeol was carried out for *T. cuneifolia* roots collected from different regions of India like Kutch, Rajkot, Jamnagar (Gujarat), Jodhpur (Rajasthan), Kurnool (Andhra Pradesh), India. The concentrations of β-sitosterol were found to be 1.98 % in Jamnagar, India, 2.64 % in Rajkot, India, 4.45 % in Andhra Pradesh, India and 1.90 % in Jodhpur, India. The concentrations of lupeol were found to be 2.44 % in Jamnagar, India, 2.21 % in Rajkot, India, 2.29 % in Kutch, India and 2.01 % in Jodhpur, India. Seasonal variation of β-sitosterol was found to be highest in monsoon (7.08 %), winter (3.89 %) and summer (2.64 %). Lupeol was found only in summer (1.42 %). Species variation of *Taverniera* roots showed only presence of β-sitosterol where *Taverniera cuneifolia* (10.04 %) and *Taverniera abyssinica* (6.89 %). Quantitation was carried out on HPTLC silica gel 60 F254 pre-coated plates with the mobile phase Toluene: Methanol: 8: 1 (v/v). A TLC scanner set at 366 nm in fluorescence / reflectance mode was used for quantitation.
Keywords: Licorice, *Taverniera cuneifolia*, HPTLC, Regional, Seasonal.

INTRODUCTION

The genus *Taverniera* belongs to the family of Fabaceae and includes twelve species. It is endemic to the Northeast African and Southwest Asian countries[1]. *T. cuneifolia* is often referred to as Indian licorice owing to its sweet taste which is very similar to that of *G. glabra*[2]. *T. cuneifolia* is locally known as Jethimadh and it is used by the tribal's of Barda Hills of Jamnagar in Western India. It is used as a substitute of Licorice or in other words the plant itself is considered to be *G. glabra*[2]. It is traditionally known to be used as an expectorant, blood purifier, anti- inflammatory, wound healing, anti ulcer and in treating spleen tumors[2]. The commercial licorice has a huge demand in the Indian system of medicine, Ayurveda and majority of the requirement of the Ayurvedic drug industry in India is met through import from Afghanistan and Pakistan[3]. Owing to huge demand plants like *A. precatorius* is used as adulterant or as a substitute for *G. glabra*[4,5]. Literature reveals the presence of β-sitosterol and lupeol in *T. cuneifolia*[6]. Hence, geographical variation in different parts of India, seasonal variation and species variation were analysed using β-sitosterol and lupeol.

MATERIALS AND METHODS

Collection, drying and processing of plant samples

The plant material was collected from Munjka village, Near Saurashtra University Campus, Rajkot and Rosy port area, Jamnagar, Tapkeshwari, Bhuj, Kutch, Gujarat, Jodhpur, Rajasthan and Betam cherala, Kurnool, Andhra Pradesh, India. The plant material was authenticated at BSI (Botanical Survey of India) Jodhpur, Rajasthan, India. Ref.no. BSI/AZC/I.12012/ Tech./ 2011-12 (PL.ID)-55. The shade dried roots were used for HPTLC analysis.

Chromatographic Conditions

Instrument

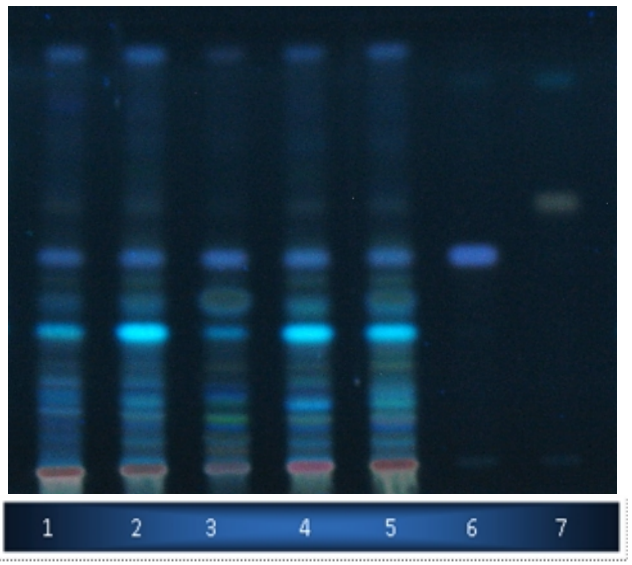
HPTLC plates: Merck, aluminium sheet precoated with silica gel 60 F254 procured from Anchrom laboratories, Mumbai, India. Applicator: CAMAG Linomat 5 sample spotter (Camag Muttenz, Switzerland) equipped with syringe (Hamilton). Developing chamber: CAMAG twin trough chamber, Densitometric scanner: Camag TLC Scanner 4 conjugated with win CATS software, Photo documentation: Camag Reprostar 3.

Reference standards and reagents

Reference compounds (standard) β-sitosterol (98 % purity, Figure 1) and lupeol (95 % purity, Figure 2) was procured from Sigma Aldrich, Steinheim, Germany. Solvents of analytical grade were procured from Merck specialties India Private Limited, India.

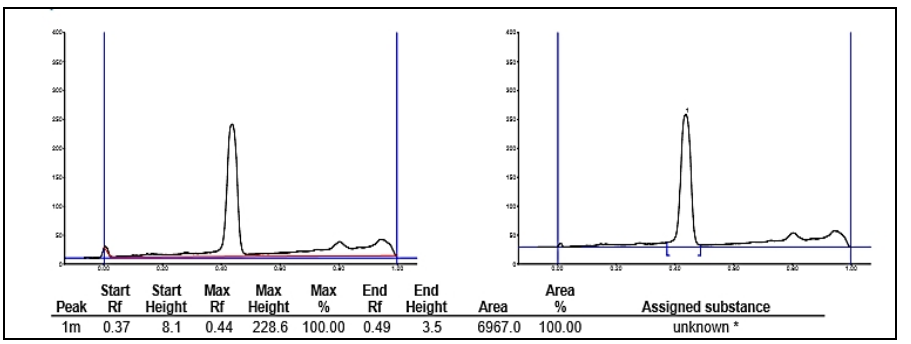
Extraction conditions

Each plant sample was accurately weighed (0.2 g) and extracted in methanol (5.0 mL). The mixture was vortexed for 1 minute and kept standing overnight. The mixture was filtered through Whatmann filter paper no. 41 and the filtrate obtained was diluted with methanol in equal proportion (1:1) and subjected to HPTLC analysis. (Regional, species, seasonal variation) The mobile phase constituted of Toluene: Methanol: 8: 1 (v/v). The plates were developed up to a distance of 85 mm in a Camag twin trough chamber previously equilibrated with mobile phase for 30 minutes. Their respective chromatograms are shown in the Figure.



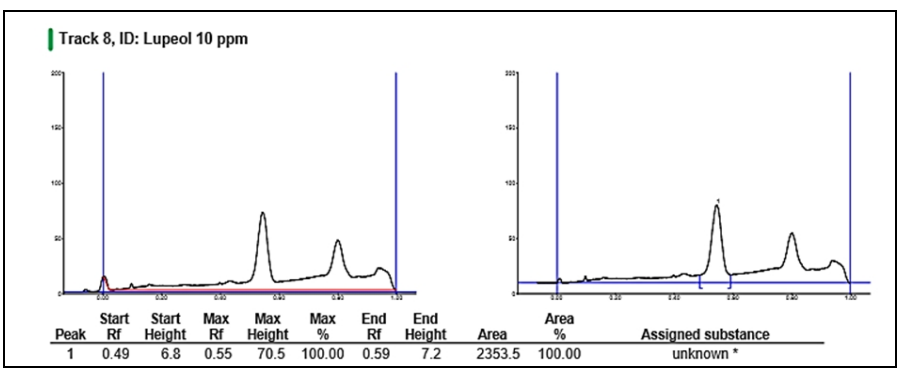
Track 1 Kutch, Track 2 Jamnagar, Track 3 Andhra Pradesh , Track 4 Jodhpur, Track 5 Rajkot, Track 6 Beta-sitosterol, Track 7 Lupeol

HPTLC plate photo representing regional variation of roots of *T. cuneifolia* with standards β-sitosterol and Lupeol Plate Photo at 336 nm



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1m	0.37	8.1	0.44	228.6	100.00	0.49	3.5	6967.0	100.00	unknown *

Figure 1: Chromatogram of β-sitosterol



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.49	6.8	0.55	70.5	100.00	0.59	7.2	2353.5	100.00	unknown *

Figure 2: Chromatogram of lupeol

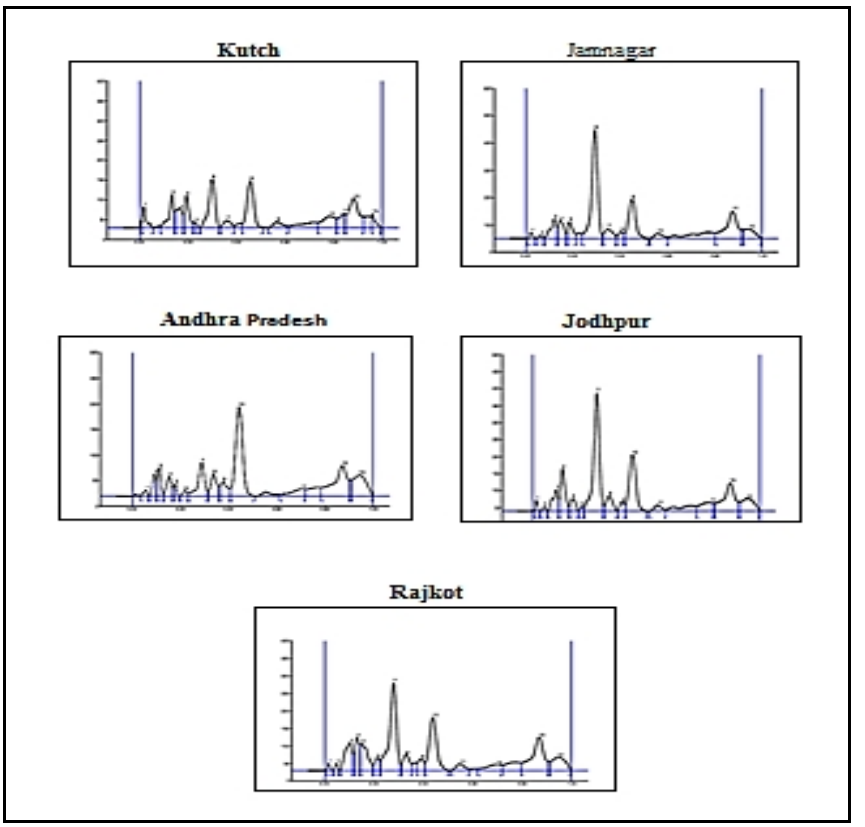
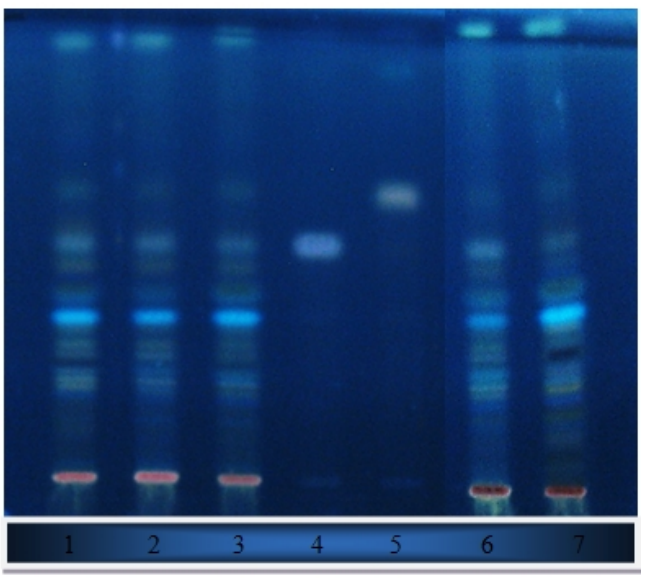


Figure 3: Chromatograms of *T. cuneifolia* roots from different geographical locations

HPTLC: Seasonal variation of *T. cuneifolia* and species variation of the roots of genus *Taverniera*

Plate Photo at 336nm



Track 1 *T. cuneifolia* summer (1:1), Track 2 *T. cuneifolia* monsoon (1:1), Track 3 *T. cuneifolia*winter (1:1), Track 4 betasitosterol 10 ppm, Track 5 lupeol 10 ppm, Track 6 Rajkot (*T. cuneifolia*) (1:1), Track 7 *T. abyssinica* (1:1)

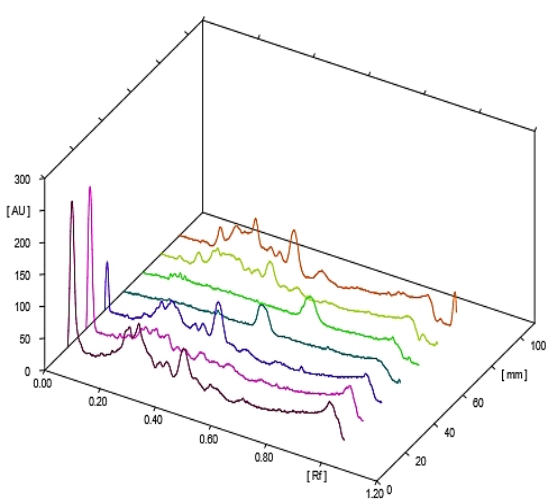


Figure 4: Spectrum of Seasonal variation

RESULTS AND DISCUSSION

Regional Variation

In this section the roots collected from different regions i.e. Kutch, Rajkot, Jamnagar, Jodhpur and Andhra Pradesh, India were compared with the standards of β-sitosterol and lupeol content. In Gujarat, only Kutch, India showed presence of lupeol both the other regions (Rajkot and Jamnagar) showed presence of both β-sitosterol and lupeol. Outside Gujarat, Jodhpur had presence of β-sitosterol and lupeol but Andhra Pradesh showed presence of only β-sitosterol. Andhra Pradesh has the highest amount of area percentage of β-sitosterol with 4.45 % and least is in Jodhpur with 1.90 %. Kutch has highest amount of lupeol (2.99 %) and lowest is in Jodhpur, India with 2.01 %.

Table 1: Regional variation of *T. cuneifolia* roots with β-sitosterol and lupeol

	Jamnagar	Rajkot	Kutch	AP	Jodhpur
β-sitosterol –	1.98 %	2.64 %	-	**4.45 %**	1.90 %
lupeol –	2.44 %	2.21 %	**2.99 %**	-	2.01 %.

Seasonal Variation

In this section of seasonal variation of roots, the study was done on summer, monsoon, and winter. All seasons showed presence of β-sitosterol but only summer season showed presence of lupeol. Monsoon season showed highest amount of β-sitosterol (7.08 %) and least was found in summer season with 2.64 %. Winter showed moderate amount.

Table 2: Seasonal variation of *T. cuneifolia* roots with β-sitosterol and lupeol

	Winter	Summer	Monsoon
β-sitosterol	3.89 %	2.64 %	**7.08 %**
Lupeol	-	1.42 %	-

Species Variation

Roots of two species of *Taverniera* i.e. *T. cuneifolia* and *T. abyssinica* were compared for their β-sitosterol and lupeol content. B-sitosterol concentration is higher in *T. cuneifolia* as compared to *T. abyssinica* while Lupeol was absent in both the species. Though lupeol is present in *T. cuneifolia* in summer, the content of lupeol is not present in the plant material collected during monsoon period.

Table 3: Species variation of *Taverniera* roots with β-sitosterol and lupeol

	T. abyssinica	*T. cuneifolia*
β-sitosterol -	6.89 %	**10.04 %**
Lupeol	-	-

CONCLUSION

Taverniera cuneifolia grows in all kinds of climatic variations and thus β-sitosterol is found to be present in all regions except Kutch. Lupeol is present in all regions except Andhra Pradesh. All seasons showed presence of β-sitosterol but only summer season showed presence of lupeol. B-sitosterol concentration is higher in *T. cuneifolia* as compared to *T. abyssinica* while Lupeol was absent in both the species. Thus, HPTLC analysis of dried roots of *Taverniera cuneifolia* can provide standard fingerprints and can be used as a reference for the identification and quality control of the drug. Such finger printing is useful in differentiating the species from the adulterant and act as biochemical markers for this medicinally important plant in herbal industries and plant systematic studies.

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