

Phytochemical characterisation of
***Taverniera cuneifolia* (Roth) Arn.**

Ph.D. synopsis submitted

To

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By

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Introduction

Phytochemical characterization is the branch of science that deals with the extraction, screening, identification and quantification of the medicinally active substances found in plants. The study of such compounds includes: their chemical structures, metabolism (biosynthesis and degradation), natural distribution, biological function, extraction and qualitative-quantitative evaluation. Some of the bioactive substances that can be derived from plants are flavonoids, alkaloids, carotenoids, tannin and other phenolic compounds. Plants are the foundation of life on Earth, and people have long relied on them for food and medicines. Majority of people in developing countries rely on traditional herbal medicine to address their basic healthcare requirements. The Indian subcontinent has a rich ethnobotanical legacy as a result of its great cultural diversity (Jain, 1991). Plant extracts have been widely used as traditional medicines, perfumes, culinary flavorings, and preservatives not only in India but all over the world. Pure chemicals or standardized extracts from medicinal plants provide an infinite supply of novel therapeutic leads due to their unparalleled chemical diversity (Cosa *et al.*, 2006).

Licorice (*Glycyrrhiza glabra*) is a perennial herb native to the Mediterranean region, central to southern Russia, and Asia Minor to Iran, now widely cultivated throughout Europe, the Middle East and Asia (Blumenthal *et al.*, 2000). They have been used medicinally since at least 500 BC and licorice has been described as 'the grandfather of herbs'. Licorice is used in more than 1250 herbal formulation in India; However, India's experience with licorice farming has not been particularly promising. The need for licorice involves importing it from nations like China, Afghanistan, Kazakhstan, Pakistan, Turkmenistan etc.(Rastogi and Mehrotra, 1989). Even though there are several substitutes for licorice in India, such as *Abrus precatorius*, *Alysicarpus longifolius*, and *Nardostachys* sp., *Taverniera cuneifolia* is one such promising alternative of Liquorice (Indian licorice) belonging to Fabaceae family. Description and ethnobotanical uses of the plant as an alternative of liquorice has been described in Vanaspati Shastra *Kathiyawadna Barda dungarni jadi buti teni pariksha ane upyog* (Thaker, 1908). It has been also mentioned in Nighantu sastra as an aquatic variety of liquorice, named as Klitanak. The text mentions 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.

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., 2002; Cinatl et al., 2003; Rastogi and Mehrotra, 1989). These properties have been mentioned for *T. cuneifolia* by Thaker (1910) wherein it has been mentioned to 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 reaffirmed by Nagar (2005). Khare (2007) has also mentioned the use of leaves in treating sloughing wounds.

In *Taverniera cuneifolia* roots, 25 phyto-components have been reported to be similar to that of *Glycyrrhiza glabra* (Mangalorkar 2012):

Class of compound	Functional group	Compounds
Lipids	Fatty acids	Hexadecanol
		Kumatakenin
		Cis- Linoleic acid
		1-Nonadecene
		n-Octadecanol
		Palmitic acid
		1-Pentadecene

Class of compound	Functional group	Compounds
Phenols	Flavone	Apigenin
		Dihydroxyflavone
		Kaempferol
		Naringenin
		Quercetin

Terpenoids	Triterpene glycoside	α -Amyrin
		β Amyrin
		Glycyrrhizin
		18- α -Glycyrrhetic acid
		18- β -Glycyrrhetic acid
phytosteroids	Phytosterols	β -sitosterol
		Stigmasterol

Apart from the above mentioned compounds, other chemical intermediates reported from the species are Benzaldehyde, Fasciolin, Furfural and 5-methyl Furfural.

The work done by earlier authors on *Taverniera cuneifolia* (Thaker, 1910; Zore *et al.*, 2008; Mangalorkar, 2013) were based on preliminary phytochemical studies, however, in order to prove that *T. cuneifolia* could be an alternative of *Glycyrrhiza glabra*, proper standardization and validation was the necessity to confirm their presence and quantity.

Standardization and validation of the phyto-chemicals entity of a drug's bulk form or pharmaceutical formulations contributes to the quality assurance process. In phytochemical analysis, the requirement for selectivity, speed, affordability, simplicity, sensitivity, precision, and accuracy led to the rapid adoption of novel techniques of analysis by pharmaceutical enterprises and chemical labs. The process of identifying and characterizing bioactive compounds is made more difficult by the fact that plant extracts typically occur as a combination of many different types of bioactive compounds or phytochemicals, each of which has a different degree of polarity (Sasidharan *et al.*, 2011). The bioactive molecules could be separated by various techniques such as thin-layer chromatography, column chromatography, flash chromatography, Sephadex chromatography, and high-performance liquid chromatography (HPLC).

Based on the above scientific review and scrutiny, an effort was made to standardize and validate the active phyto-constituents present in the roots of *Taverniera cuneifolia* in comparison with *Glycyrrhiza glabra*.

Hypothesis

Taverniera cuneifolia has been documented as an alternative of *Glycyrrhiza glabra*. However, in order to prove later statement, it is necessary to standardize and validate the presence of Glycyrrhizin, Naringenin, Apigenin, 18 alpha and 18 beta Glycyrrhetic Acids and other bioactive molecules present in both *T. cuneifolia* and *G. glabra*.

Objectives

- Selection and segregation of appropriate germplasm from different bio-geographical zones of Gujarat.
- Standardization and validation of sugars (sweeteners) and amino acids.
- Standardization and validation of active phyto-constituents of *T. cuneifolia*.
- Purification of the active fractions by Column chromatography.

Review of Literature

Taverniera cuneifolia belongs to the Fabaceae family are widely distributed in Africa, Middle East and south Asian countries but not much explored. This genus is known for its utilization in various folk and traditional medicines for the treatment of diseases as an anti- inflammatory, antitussive activity antiulcer, spasmolytic activity, gastroprotective activity, memory enhancing activity, blood purifier, spleen tumors, wound healing etc.

Taverniera DC. genus consists of 17 species distributed in African, Middle east and Asian countries, out of which two species occur in India (Mangalorkar, 2013). The species belonging to the genus *Taverneira* (Fabaceae) are branched herbs or shrubs, with few leaves and rosy or white coloured flowers (Cook, 1908). *Taverniera* genus belong to the family Fabaceae which has seventeen species: *Taverniera abyssinica* A. Rich., *Tavernier aeegyptica* Boiss., *Taverniera albida* Thulin, *Taverniera breviaolata* Thulin, *Taverniera cuneifolia* (Roth) Arn., *Taverniera diffusa* (Cambess.) Thulin, *Taverniera echinate* Mozaff., *Taverniera glabra* Boiss., *Taverniera glauca* Edgew., *Taverniera lappacea* (Forssk.) DC., *Taverniera longisetosa* Thulin, *Taverniera multinoda* Thulin, *Taverniera nummularia* DC., *Taverniera oligantha* (Franch.) Thulin, *Taverniera*

schimperi Jaub. & Spach, *Taverniera sericophylla* Balf.f. and *Taverniera spartea* DC. Geographically out of 17 species, 7 species are restricted to Africa (Djibouti, Egypt, Ethiopia, Somalia and Sudan) from which *T. abyssinica* and *T. schimperi* is endemic to Ethiopia, *T. longisetosa* is endemic to Somalia, *T. oligantha* is endemic to Djibouti and *T. sericophylla* is endemic to Socotra, as per IUCN criteria the species is extinct. Rest 11 species are found in middle east country (Bahrain, Iran, Iraq, Oman, Qatar, Saudi Arabia, South Yemen, United Arab Emirates and Yemen), of which *T. albida* and *T. glauca* are endemic to Yemen, *T. brevialata* is restricted to Oman, *T. echinata* is endemic to Iran. South Asian countries (India and Pakistan) have just five species: *T. cuneifolia* and *T. diffusa*, *T. glabra*, *T. lappacea*, and *T. spartea*, the first two of which are exclusively found in India.

All the species of *Taverniera* genera are perennial shrubs or shrublets. Leaves are unifoliate or digitate to ternate; leaflets are usually thick. Flowers are present in axillary racemes or sometimes reduced to 1–few-flowered clusters; Calyx teeth subequal or the 2 uppers more remote. Corolla much exerted; standard broadly obovate, narrowed at the base, scarcely clawed; wings small; keel about equal to the standard, obliquely truncate at the apex. Stamen Monadelphous; anther uniform. Pharmacognostic investigation on the roots of *T. cuneifolia* was carried out from the perspective of anatomy, powder and histochemical studies by Mangalorkar *et al.*, (2013); Gohil and Daniel (2014) and Prajapati *et al.* (2013; 2014). Pericyclic fibres, bast fibre, prismatic crystals, cork cells, and starch grains were discovered in the roots of *T. cuneifolia* in this study. So far, total 117 compounds were identified and isolated, which includes terpenoids, flavonoids, triterpenoid saponins, phenolic acids, saponins, organic acids, fatty acids, amino acids, sugars, vitamins, saikosaponins and sterols. Out of these, flavonoids and terpenoids were the most prominent. The study of literature on *Taverniera cuneifolia* has revealed the presence of some leading phyto-molecules such as Saponins, Phenols, Terpenes and Glycosides. The pharmacological activities documented by various *Taverniera* species are Anti-carcinogenic, Anti-HIV, Anti-inflammatory, Antimicrobial, Analgesic and Antipyretic, Antioxidant, Anti-tussive, Gastro-protective, Memory enhancer, Nematicidal, Anti-dementia, Spasmolytic, Wound healing properties (Khalighi *et al.*, 2014; Alexiades and Laird,

2002; Zore *et al.*, 2008; Keymanesh *et al.*, 2009; Dagne *et al.*, 1990; Jamdhade *et al.*, 2015; 2013; Prajapati and Patel, 2015; Noamesi *et al.*, 1990; Mangalorkar, 2013).

The Cytotoxic studies suggested that *T. cuneifolia* extract showed protection of 75% cells against EMS toxicity at 6mg/plate (Zore *et al.*, in 2008). Later, Mangalorkar (2013) has reported that 5gm/kg aqueous extract of *T. cuneifolia* did not show any toxic effect on adult female Charles foster rats and even the higher dose of short exposure was not able to cause marked alteration in important physiological processes. Similarly, the extract of *T. cuneifolia* showed good anti-microbial activity by inhibiting the germ tube formation in *C. albicans* (Zore *et al.*, 2008). The *T. cuneifolia* has also showed antioxidant activity. The methanolic extract of *T. cuneifolia* showed the highest free radical scavenging activity at the dose of 20µg/ml by DPPH assay whereas nitric oxide showed no significant radical scavenging activity (Jamdhade *et al.*, 2015).

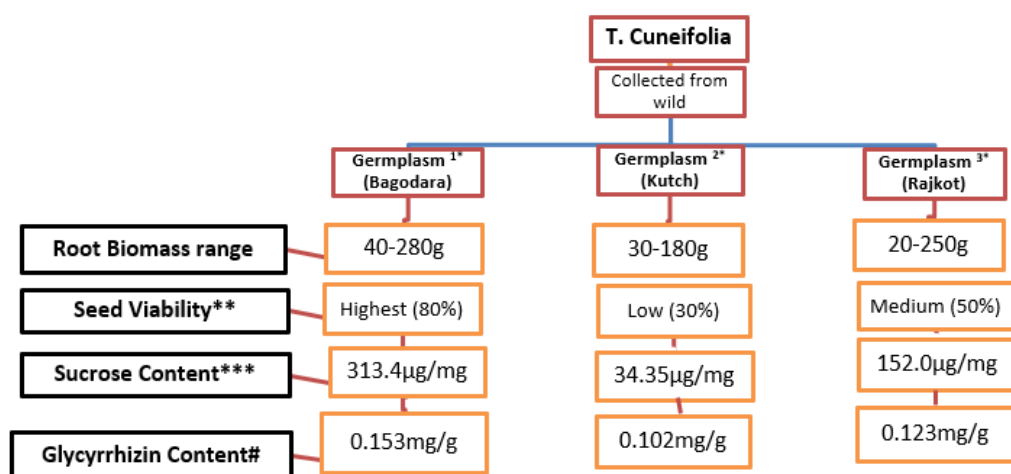
Methodology

Plant samples of *T. cuneifolia* were collected from three biogeographical zones, i.e., Munjaka gam (Rajkot), Bagodara (Ahemdabad) and Bhuj (Kutch). On the basis of various factors such as sugar content, root length and diameter, the best plant sample was selected for further analysis. Qualitative and quantitative analysis are done to determine the presence of different amino acids and sugars present in the plant samples, which play a major role in the growth of biological activity. Different solvents such as methanol, water, acetonitrile, and chloroform, the identification, quantification, and validation of various phytochemicals was done by using HPLC and LCMS instruments. The aforementioned studies demonstrated the possible phyto-constituents present in the samples. For the purification of the main components, the column was set up for the isolation of the compounds, and subsequently LCMS and GCMS done to validate the phyto-compounds. The process of Column chromatography was done to isolate the pure chemical compound from a mixture crude sample. It separates substances on the basis of differential adsorption of compounds to the adsorbent as the compounds pass along the column at various speeds, enabling fractional separation. The used silica ranges in size from 60 to 200 mesh.

Result and Discussion

1. Selection and segregation of appropriate germplasm from different biogeographical zones of Gujarat.

The raw material was collected in different season in order to understand the seasonal parameters. Raw material in different season was collected in order to study the appropriate time of harvesting. The parameters taken into consideration while collection were organoleptic properties, average root length and thickness.



*Raw material was collected in month of June – July

** Tetrazolium test/Petriplate

*** HPTLC Analysis

#HPLC Analysis

2. Standardization and validation of sugars (sweeteners) and amino acids.

Taverniera cuneifolia was analysed for 14 different types of sugars, of which five were located in the HPLC and HPTLC analysis. The sugars found in the analysis are as follows:

Sr. No.	Type of sugar	Name of the sugar component
1	Monosaccharide	Arabinose
2		Fructose
3		Glucose
4		Rhamnose
5	Disaccharide	Sucrose

Glucose, fructose, and sucrose were identified as the predominant sugars found in the roots of *T. cuneifolia* whereas Rhamnose and Arabinose were found in very less amount.

The amino acid analysis indicated that fifteen amino acids were found in the roots of *T. cuneifolia*. Essential amino acids included Arginine, Methionine, Phenylalanine, lysine, Histidine, Isoleucine, Leucine, Valine, and Threonine, while non-essential amino acids included Alanine, Asparagine, Glutamic acid, and Tyrosine.

Essential amino acids	Non essential amino acids
Arginine	Alanine
Methionine	Asparagine
Phenylalanine	Glutamic acid
Lysine	L-Glutamic acid
Histidine	Tyrosine
Isoleucine	
Leucine	
Threonine	
Valine	

3. Standardization and validation of active phyto-constituents of *T.cuneifolia*.

Standardization and validation of eleven active and economically important phyto-components were done which includes Naringenin, Glycyrrhizin, Apigenin, Liquiritigenin, Glabridin, Genistein, Kaempferol, 18 alpha Glycyrrhetic acid, 18 beta Glycyrrhetic acid, Stigmasterol and Beta Sitosterol. The presence of rest fifteen phyto-compounds were reconfirmed by HPLC and GCMS analysis. In addition, several other phytoconstituents with greater concentrations were discovered that were not previously reported by the LCMS-QTOF analysis.

4. Purification of the active fractions by Column chromatography.

The fractions obtained were composed of 100% hexane, 50% hexane+ 50% ethyl acetate, 100% ethyl acetate, 50% ethyl acetate+ 50% methanol, and 100% methanol. Column chromatography resulted in the collection of several fractions, few fractions of 50% ethyl acetate+ 50% methanol

were isolated in salt form or crystal form. LCMS and GCMS analyses were used to the acquired non-polar and polar fractions to identify the compounds.

Summary

- Collection of raw material was done from three different bio-geographical regions.
- Standardization and validation of sugars has been done
- Standardization and validation of active phyto-components were done.
- Column chromatography is done to purify the key components.

Conclusion

Based on the various phytochemical studies, it is justified that *T. cuneifolia* could be an potential alternative of *Glycyrrhiza glabra*.

OUTCOME OF THE RESEARCH

With proper scientific validation, it is possible to substitute of *G. glabra* with *T. cuneifolia*, which is grown domestically for various commercial usages. In majority of the cough syrups in India and outside India Licorice extract is widely used. Major ingredient used in such syrups is licorice in varying proportion, i.e. 40-50%, for example, Dabur Honeytus, Himalaya Koflet, Yogi Kanthika, Divya Mulethi kwath, etc., Many cosmetic creams also use *G. glabra*. These formulations and many more can be substituted by *T. cuneifolia*. A very good market for licorice exists in the European and American market. Similar kind of market can also be created for *T. cuneifolia* in Europe and in the other parts of the world. First and foremost validation of *Taverniera cuneifolia* for the presence of GLABRIDIN and GLYCYRRHIZIN will boost the herbal economy to the extent never thought off.

HERBAL ACCELERATION

- Cultivation of *T. cuneifolia* will make India self dependent in Licorice raw material.
- *T. cuneifolia* will improve the economy of the farmer staying in coastal and arid region of India.

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