Results
B. Bioprospecting

a. *Alternanthera sessilis* Linn. (DC) - Flower flavonoids and Phenolic acids

Alternanthera sessilis is a well known medicinal plant occurring as a weed everywhere. The herb is used as a galactagogue, cholagogue, abortifacient and febrifuge. It is also used for indigestion. It is claimed to be a good fodder which increases milk in cattle. In some parts of Bihar, the plant is used for hazy vision, night blindness, diarrhea, dysentery and post-natal complaints. Then, the roots are used externally, for inflamed wounds. The poultice of the herb is reported to be used for boils (Anon, 1985). The plant is reported to contain hydrocarbons and steroids and absolutely no data are available on the phenolics or flavonoids which are now considered as the best antioxidants. These compounds are also having a number of medicinal properties and that is, why *A.sessilis* is selected for a study on their flavonoids.

The flowers which form a major portion of the drug were analysed using standard procedures described in chapter 2.

The flowers are found to be rich in flavonoids. The flavonoids located include both flavones and flavonols. The flavones identified were two; luteolin (λ-max 255, 267' and 350) and 4'-OMe luteolin (λ-max 255, 267' and 347). The flavonol located were 3' -OMe quercetin (λ-max 255, 267' and 355). The identity of these compounds was confirmed by studying the spectral shifts and co-chromatography with authentic samples. The phenolics acids located were four in number and they were *cis* and *trans*- ferulic acid, vanillic acid and syringic acid. In addition, Ecdysterone also was located in flowers. The identity was confirmed by co-chromatography with authentic ecdysterone.

All the compounds identified from the flowers of this plant possessed distinct pharmacological properties. The flavonoids are known to have a positive role in strengthening the capillary walls, thinning blood by reducing the agglutination of RBCs and even preventing cancer. The anti-inflammatory properties of flavonoids also are well known. **Luteolin** exerts a number of pharmacological actions, such as antiallergic, antibacterial, anti-inflammatory, antipolio and antiherpetic. It is found to have profound

effect against tumours in the colon, kidney, lung, ovary, skin, stomach, pancreas, thyroid and brain. **4'-OMe Luteolin** is also similar to luteolin in its pharmacological properties. **3'-OMe Quercetin** is a derivative of quercetin which is a well known flavonol exhibiting cardiovascular protection, anticancer and anti-ulcer effects, anti-allergic activity and cataract prevention as well as antiviral and anti-inflammatory effects. It is antiarthritic, antiasthmatic, antibacterial, anticataract, antidiabetic, antiherpetic, antiviral, hepatoprotective and antimalarial in nature. It is known to act against tumours in the bladder, colon, lung, ovary and skin (Duke's database).

The phenolic acids obtained from *Alternanthera sessilis* also exert a number of medicinal properties. **Ferulic acid** is analgesic, antiallergic, anti-inflammatory, hepatoprotective and antihepatotoxic. It is antiviral, immunostimulant, antiallergic and acts as an arteriodilator. It is also found to acts against cancer in the colon, forestomach, liver and skin. **Vanillic acid,** one of the most commonly found phenolic acids in plants, is known to be anthelmintic, anti-fatigue, anti-inflammatory, antileukemic, antiseptic and anti-sickling. **Syringic acid** is known to be allelopathic, antioxidant, anti-peroxidant and anti-radicular.

b. *Alternanthera bettzickiana* – Flavonoids and Phenolic acids

This is a plant migrated from Northern India and has replaced almost all other weeds everywhere. It has got a gregarious growth and the plant is very sturd. Since, it is a migrant, no local uses are available. Since all other species of *Alternanthera* like *Alternanthera sessilis* and *Alternanthera pungens* to assume that this plant also may have similar uses and that is the reason why this species is taken up for bioprospecting.

The ethyl acetate fraction obtained after the hydrolysis of water soluble fraction of the methanolic extract yielded three flavonoids when they were chromatographed on paper, using 30% acetic acid. All these three compounds fluorescent yellow when viewed in UV-365nm. The areas of these three compounds in chromatograms are marked and portions were cut out and eluted in spectroscopic methanol. The first compound having Rf value of 0.4 gave the λ maxima of 255nm and 270' then 300', and 370'. The second compound having a slightly higher Rf value i.e., 0.45 gave a λ-maxima of 255, 267', 300', and 368'. The third compound having an Rf value of 0.6 showed a λ-maxima of

261, 290', 320', and 366'. These compounds were tentatively identified as Quercetin, IsoRhamnetin, and Kaempherol. The identity of these compounds was confirmed by co-chromatography with authentic sample.

The same ethyl acetate fraction (containing flavonoids) was analyzed for steroids by TLC. Two major spots (having Rf value 0.62 and 0.71 both gave a violet colour with sulphuric acid) were observed when the Thin layer chormatograms were sprayed with 25% sulphuric acid and warmed. On co-chromatography with standard samples, these steroids were identified as Ecdysterones and β-sitosterols.

The discovery of Ecdysterone in *Alternanthera bettzickiana* is highly significant. Ecdsteroids are now considered as anabolic steroids having stimulatory effect, hypocholesterolamic effects, hypoglycaemic effect, and having specific effect of muscle (increase muscle mass) by regeneration. It restore normal glomerular filtration rate in kindney, improves skin quality, and acts as a brain tonic (inducing neuro-transmitter synthesis and protecting neurons). Beta-sitosterol the other steroid located in this plant also exhibits androgenic, antibacterial, anticancer, antiestrogenic, antigonadotrophic, antiinflammatory, antileukemic, antioxidant, febrifuge, hypocholesterolemic properties.

The significance of locating flavonoids in this plant is of great importance. The pharmacological importance of quercetin is already explained above. **Isorhamnetin** is found to have antibacterial, antihistaminic, anti-inflammatory, antioxidant, antispasmodic, cancer-preventive, hepatoprotective, pesticide, vasodilator properties. **Kaempferol** induces growth inhibition and apoptosis in A549 lung cancer cells. It shows antihistaminic and antioxidant properties and is a reliable inhibitor of topoisomerase I. Kaempferol is known to be antiinflammmatory, antiherpetic, antileukemic, antiseptic, antiviral, hepatoprotective and acts as a vasodilator.

c. *Aloe barbadensis* Mil. Quinones from flowers

Aloe barbadensis is an extremely well known medicinal plant useful in many ailments and in cosmetics preparations. Normally, the plant never flowers in cultivation. But, they flower and produce seeds abundantly in certain parts of Saurashtra. Since the flowers, bracts, or seeds are almost unknown to commercial world, no data are available on their

constituents. Since, *Aloe* leaves are rich sources of quinones, the flowers and bracts were analyzed for this quinine content.

Quinones of Aloe flowers were extracted by methanol in a Soxhlet's apparatus for 24 hours. The methanol extract is then concentrated. The residue is dissolved in water. To this aqueous extract petroleum ether is added in a sparating funnel. Petroleum solubles are then discarded, and the water soluble are concentrated, this extract is spotted in Silica gel G TLC plates, and the solvent system used was, ethyl acetate: methanol: water (100: 13.5: 10); the developed chromatogram are viewed under UV 365 nm, where, the quinones appeared as fluorescent yellow spots. The chromatograms are then sprayed with 10% ethanolic KOH reagent. There were 4-5 flouresccnt spots in TLC chromatograms. On co-chromatography with Aloin and Emodin, isolated from Aloe leaves, it was found that two-spots corresponded to Aloin and Emodin. Aloin appeared at an Rf value of 0.5 and Aloe Emodin appeared near the solvent front (Rf-0.95). Flavonoids were found to be absent in the flowers but it contained the phenolic acids such as p-hydroxy benzoic, p-coumaric (both *cis* and *trans*), vanillic and syringic acids.

The discovery of aloin and emodin from the flowers of this plant is very significant, because these flowers are never utilized for any purpose and are going waste. Therefore, the flowers may be used as raw material for the extraction of these two pharmacologically important chemicals. Aloin is found to exhibit allergenic, antifertility, antiherpetic, antispermatogenic, antiviral, cholagogue, hypothermic, laxative, peristaltic, pesticide, purgative and uricosuric properties. Similarly, emodin also exhibit antibacterial, anticytomegalovirus, antiinflammatory, antileukemic, antimutagenic, antineoplastic, antiplaque, antiulcer, antiviral, cathartic, hypolipidemic, immunostimulant properties.

The phenolic acids identified also possess a number of medicinal properties. *p*-Hydroxy benzoic acid is antibacterial, anti-sickling, immunosuppressant, cancer preventive and fungistat. *p*-Coumaric acid is antibacterial, antiseptic and antitumour. The compound is also antiseptic, antioxidant and fungicidal. It is proved to inhibit the oxidation of L-tyrosine, which is an important precursor for the biosynthesis of a number of compounds. The properties of vanillic and syringic acids are explained above.

d. *Stemodia viscosa* – volatile oil.

Stemodia viscosa is an aromatic weed very common in the fields in and around Baroda. Though it is having a pleasant smell, there are no scientific data on its volatile oil. Therefore, this plant is taken up for bioprospecting of its volatile oil.

The volatile oil of *Stemodia viscosa* was light yellow in colour with a very pleasant clove like smell. On analysis using TLC, five major compounds were located, of which only two compounds *i.e.* eugenol and aceteugenol could be identified with the help of standards isolated from clove oil. The eugenol had an Rf value 0.6 and yellowish brown colour while aceteugenol was pinkish yellow at a low Rf value of 0.252. The other three monoterpenes could not be identified.

The extraction and analysis of the volatile oil from this species is significant in that one more additional source of eugenol could be identified. This plant, being abundant as a weed in cultivated fields, can be profitably used as sources of volatile oil which could be employed in perfumery or in medicines. The properties of eugenol includes acaricide, analgesic, anticonvulsant, antiedemic, antiestrogenic, antifeedant, antimitotic, antimutagenic, antipyretic, antiseptic, antispasmodic, antiviral, carminative, hepatoprotective, insecticide, vasodilator and vermifuge (Dukes' data). Aceteugenol also is found to have antispasmodic and sedative properties (Sung et al., 1998)

e. *Stemodia serrata* – Volatile oil

This is an endemic plant restricted in distribution to the Timbi region of Baroda. There were only few plants available and absolutely no work is conducted on any of the other chemical components. Since it is found sweet smelling, it is taken up for the analysis of volatile oil.

The volatile oil of *Stemodia serrata* was similar to the oil extracted from *Stemodia viscosa* in that it was light greenish yellow in colour with a pleasant clove like smell. On TLC analysis it gave four monoterpenes, of which two were identified as Eugenol and Aceteugenol. The other two compounds which were not identified were similar to those obtained from *Stemodia viscosa.*

In addition to *S. viscosa,* this plant also can be considered as a commercial source of eugenol. But the rarity of this plant in the study area does not permit its harvesting and

utilization, since it is an important commercial source, it has to be propagated in large scales so that commercial exploitation can be achieved.

f. *Bombax ceiba* Linn. - Flower calyces; Mucilages and Flavonoids

Bombax ceiba is a well known plant yielding gums and fibres. The flowerbuds and fleshy calyces are eaten; the immature calyx known as semargulla, is consumed as vegetable. The flowers are made into a conserve by boiling with the seeds of poppy and sugar goat's milk. The dried and powdered flowers are made into bread with or without corn (Anon. 1988). The bark exudes a gum known as Semul gum from natural wounds, or caused by insect or fungal attack. This gum is similar to gum Tragacanth. Since this gum is obtained from bark, it is not commercially available. But, during our survey, we have found that the fleshy calyx-cup of the flowers contained gums. This gum is never isolated or studied. Since, the flower fall of after or before fertilization it will form a good raw material which can be commercially exploited. This is the reason why the fleshy calyx of *Bombax ceiba* is selected for a detailed study on its mucilage and flavonoids.

The calyx of *Bombax ceiba* flowers is found to contain 20% dry weight mucilage. On hydrolysis, this gum yielded two sugars galacturonic acid and galactose. Therefore, it is a polymer of these two sugars. The calyces are found to be a rich source of phenolics, especially flavonols and phenolics acid. The flavonols located are quercetin, isorhamnetin and rhamnetin. The phenolic acids identified were vanillic acid and syringic acid.

The isolation of gums from the fleshy calyces of *Bombax ceiba* is of great economic importance because these compounds are extracted from a waste product that is the calyces get rotten when the flowers fall down. They possess a number of medicinal properties and are often used as emollients or demulcent, and thus act as remedies for wounds, to soothe itching, irritation and pain and also for drying and binding a tissue that is damaged. The demulcent action is continued in the lining of the digestive tract and this explains the remedies that mucilage bring for ulcers, lesions, inflammations in the gastrointestinal tract and for reducing the excessive acid secretions. The mucilage causes the irritation due to diarrhea, flatulence; colic and abdominal pain to get soothed and die down that the symptoms may be reduced. The mucilaginous remedies are also used in the

urinary and the respiratory system. The mucilaginous remedies have properties that are soothing for the urinary tracts, the bronchial tissues, and acts as a comfort where there is vigorous coughing syndrome and they increase the bronchial secretions if they go dry and also in an asthmatic condition they help to reduce the spasms.

All the three flavonoids isolated from the calyces show a number of pharmacological properties. The pharmacological properties of quercetin are explained above in *Alternanthera sessilis* and that of isorhamnetin in *Alternanthera bettzikiana*. The properties of rhamnetin includes antiaflatoxin, antibacterial, anticancer, antimutagenic, antioxidant, antitumor and hepatoprotective. The medicinal properties of the phenolic acids are explained earlier. Therefore, the consumption of the calyces as vegetable impart all the above mentioned health benefits to the consumer.

g. *Ageratum conyzoides* Linn. (Goat weed, White weed)

This is an aromatic, annual herb, 1 m in height, native to tropical America, naturalized as a weed throughout India, upto an altitude of 1,800 m. The plant is used internally as a stimulant tonic. The juice is reported to be a good remedy in *prolapsus ani*. Boiled with oil, it is applied in rheumatism. A decoction or infusion of the herb is given in stomach ailments such as diarrhoea, dysentery, and intestinal colic with flatulence. The data on the volatile oil of this plant is available. A large number of compounds such as flavonoids, chromones, and coumarins are reported from this plant. During the present study, the leaves are found to contain two flavones, which are derivatives of 6-hydroxy flavones. They are 6-hydroxy 4'-methoxy apigenin, and 6-hydroxy, 7, 4'-dimethoxy apigenin. The other flavonoids reported such as quercetin, kaempferol, scutallerin were found absent in the leaves. The phenolic acid located was vanillic acid. Apigenin and its derivatives are known to reduce DNA oxidative damage; inhibit the growth of human leukemia cells and induced these cells to differentiate; inhibit cancer cell signal transduction and induce apoptosis; act as an anti-inflammatory; and as an anti-spasmodic or spasmolytic. Therefore, the compounds isolated known from this plant also may exceed these properties. The pharmacological effects of vanillic acid are already explained above.

h. *Ageratum houstanianum* -volatile oil.

Ageratum houstanianum is an ornamental herb native to tropical America. This plant does not find much use even though its relative *Ageratum conyzoides* (Ajagandha) is a well known medicinal plant yielding a volatile oil i.e. used against leprosy and other cutaneous diseases. Since, *A. houstanianum* also possess aromatic leaves, this plant is taken up for a detailed study on its volatile oil and constituents.

The volatile oil from fresh shoots was extracted by steam distillation, and the amount of the oil obtained was 0.2%. This oil was analyzed by GC-MS from SICART, Vallabh vidyanagar, Gujarat. The GC-MS analysis was done by electron impact ionization method on auto system XL gas chromatography (Perkin Elmer Instrument, Germany) coupled to a Turbo Mass Spectrophotometer. The column was fused silica capillary column, 30x 0.25 mm ID; coated with D-I, 0.25 μm film thickness. The temperature of column was programmed at 70 to 250 °C at the rate of 10 °C/min. increase, injection port temperature at 250 C. Helium was used as carrier gas at constant pressure of 100 kpa and flow rate of 20 ml/min. Samples, dissolved in methanol, were run fully at range of 60-550 amu and the result were compared by using NIST 107 Spectral library search programme. Gas chromatogram resolved seven compounds of which three were having very low concentration. The compounds present in good concentration are precocene-II (74.25%); demethoxy ageratochromene (19.65%); caryophyllene (5.11%) and alpha-cubebene (0.39%). This oil is similar to the volatile oil of *Ageratum conyzoides* and can be used likewise for flavouring tobacco. Precocene II is found to be pesticidal in nature and therefore, this oil can be used as a biopesticide.

i. *Balanities aegiptiaca* – Seed oil.

This is a medicinally important plant of Gujarat and used to cure many diseases. The seeds are other plant parts which are known to contain steroidal sapogenins such as Diosgenin. The seed also yield a fixed oil. The amount of which varies from 30 to 40%. But, there is no data on the yield of oil from Gujarat plants. From the initial studies it is found that the seeds of plants growing in Sindh road, near Baroda contained 42 % dry weight of oil. This work is not completed because of publication of a patent on this oil

and its conversion to biodiesel. The potential of this plant as a source of biodiesel is very great and that is why it is selected for a detailed study on seed oil.

j. *Riedleia corchorifolia* - Flavonoids and Phenolic acids

The **Chocolate Weed**, or *Riedleia corchorifolia* DC., is a tropical weed that is typically seen in the wastelands and in open areas, such as highways. This plant was common in most areas of Maharastra state but now, it has now migrated to many places of Gujarat. Though this is not considered as a useful plant, it has been utilized as a homeopathic remedy. The leaves of *Riedleia corchorifolia* are eaten as a potherb in West Africa and southern Africa. The cooked leaves provide a popular, slimy side-dish in Malawi. Similar use of the leaves is reported from Indo-China and India. *Riedleia corchorifolia* is eagerly browsed, either green or dry, by cattle in Sudan but less so in Senegal. Fibres extracted from the bark are fine and strong and are used like those of *Triumfetta*, *Urena* and *Hibiscus*. The stems are used for tying bundles and are used in the construction of conical roofs for local houses. Leaves are used for unspecified stomach disorders in coastal East Africa. In Benin the seed is used to treat stomachache. In Malaysia and India the leaves and roots are used to treat a wide range of medical problems: urinary disorders, abdominal swelling, dysentery, snakebites and sores. An aqueous solution of leaves has insecticidal properties. Pulses stored in gunny bags treated with the solution have shown a reduction in the number of eggs laid and in damage done by the storage pest *Callosobruchus*. A number of compounds have been identified from this plant. They are Phytochemical analysis of leaves of *Riedleia corchorifolia* has revealed the presence of triterpenes (friedelin, friedelinol and β-amyrin), flavonol glycosides (hibifolin, triflin and melocorin), aliphatic compounds, flavonoids (vitexin and robunin), β-D-sitosterol and its stearate, β-D-glucoside and alkaloids. A pyridine alkaloid, 6-methoxy-3-propenyl-2-pyridine carboxylic acid, may be important as related pyridine derivatives are physiologically active.

During the present work a number of flavonoids which were not reported earlier were located. From the leaf quercetin, kaempferol and acacetin and a glycoflavone, ie., 6-C glucosyl acacetin were identified. The phenolic acids were vanillic and syringic acids.

The stem yielded quercetin, vanillic acid and syringic acid. The roots did not contained any flavonoid but being red in colour it yielded an anthocyanidin, cyanidin.

The presence of bioactive flavonoids such as quercetin, kaempferol and acacetin and a glycoflavone are very important because they form one of the best antioxidants available from the plants (Grubben, G.J.H. & Denton, O.A). So, this plant can be used as a commercial source for the extraction of these compounds. The presence of cyanidin in roots also is important because this compound also possesses a number of pharmacological properties besides being an effective antioxidant.

k. *Hygrophila schulli*(Ham.) M. R. & S.M. Almeida - Flavonoids

Hygrophila schulli (Ham.) M. R. & S.M. Almeida is a very well known medicinal plant of India known as **Kokilaksah**. It is concerned as a reputed remedy for arthritis and also possesses aphrodisiac, and demulcent properties. During field collections it was found that in majority of the places, these plants had bluish-white flowers. But, the plants growing in certain areas of Rajpipla possessed white flowers. Both these populations were subjected to a chemical study to find out taxonomic relevance of these variations in flower colour. The blue flowers contain anthocyanins like cyanidin while; the white flowers were devoid of them. The leaves of both these 'varieties' were then analyzed. The leaves of blue flower plant yielded luteolin, 4'-OMe luteolin and 3',4'-diOMe luteolin, while that of the white flowered plant contained luteolin and apigenin. The stems of these two plants also differed in their flavonoids. The stem of blue flower plant contained apigenin, and acacetin, whereas, the stem of the later plant, possessed luteolin, 4'-OMe luteolin and 3', 4'-diOMe luteolin. The phenolic acids of both the plant were the same.

Pharmacological properties of all these flavonoids are explained earlier. Apart from that the data obtained here are taxonomically important in that these two 'varieties' (blue and white flower) are chemically different, in their flavonoid composition in the vegetative parts (leaves and stem). The fact that, the flowers are chemically different in their flowers, *i. e.*, the blue flower contains anthocyanins, while, the white flowers are devoid of them. The fact adds to the phytochemical differences between these two 'varieties'.

More data are to be accrued before arriving at any conclusion on the taxonomic identities of these plants.