3. REVIEW OF LITERATURE

3.1. Medicinal plants

World literature on patents related to Medicinal and Aromatic Plants claimed the period 1980-1992 have been analysed. The study was based on literature survey from various patent disseminating services and from the Medicinal and Aromatic Plants publications. The study reveals that nearly 750 plants have been patented for Medicinal and Aromatic activities for various processes by twelve countries. Japan is the major contributor of plant derived patents, followed by USA, USSR (now Commonwealth of Independent States, CIS), UK, France, Germany, Canada and Romania. India has less than twenty five patents. Plants related patent information for facilitating better professional search has been briefly discussed. PID, New Delhi (Doreswamy and Jain, 1993).

*Aegle marmelos* (L.) Correa commonly known as Bael or Bilva belonging to the family Rutaceae has been widely used in indigenous systems of Indian medicine due to its various medicinal properties. Although this plant is native to northern India it is also widely found throughout the Indian peninsula and in Ceylon, Burma, Thailand and Indo-China. The decoction of the root and root bark is useful in intermittent fever, hypo-chondriasis, melancholia, and palpitation of the heart. The leaves and bark have been used in medicated enema. The leaves are also used in diabetes mellitus. The greatest medicinal value, however, has been attributed to its fruit and the unripe fruit is said to be an excellent remedy for diarrhoea and is especially useful in chronic diarrhoenas.
Biological activities of *Limonia crenulata* (Roxb.) (Nadkarni, 1954). The root is an important ingredient of the 'Dasmula' (ten roots) recipe (Chopra, 1982).

There are more than 650 species of medicinal plants available in the forests of the Kerala state. Fire, grazing, and collection of produce by the village folk poses serious threat to the medicinal and aromatic plant wealth of the state. Ways and means of protection, propagation and production of sufficient quantity of this plant wealth have been described. A thorough inventory of the medicinal and aromatic plant is an urgent necessity (Basha and Nair, 1995).

Members of Anacardiaceae, Burseraceae, Cneoraceae, Meliaceae, Ptaeroxylaceae, Rutaceae, and Simaroubaceae were analysed cladistically to evaluate the familial and subfamilial circumscriptions of Rutaceae. Taxa representing all subfamilies and tribes were sampled. The analysis shows that Rutaceae are paraphyletic, with *Spathelia* and *Dictyoloma* (Rutaceae), *Harrisonia* (Simaroubaceae), *Cneorum* (Cneoraceae), and *Ptaeroxylon* (Ptaeroxylaceae) forming a sister clade to all other Rutaceae. Circumscription of Rutaceae to include all of these taxa is recommended. This analysis indicates that Simaroubaceae and Meliaceae are the out groups closest to Rutaceae. Correlation of the molecular phylogenies with biochemical data indicates that chemotaxonomic information is more reliable than fruit type as an indicator of familial and subfamilial circumscriptions. The subfamilial classification needs revision none of the subfamilies of more than one genus is monophyletic (Mark et al., 1999).
**Limonia acidissima** L., syn. *Feronia limonia* is a moderate sized deciduous tree grown throughout India. The fruits are woody, rough and used as a substitute for bael in diarrhoea and dysentery. The fruits are used for tumors, asthma, wounds, cardiac debility and hepatitis. About 75 medicinal plants including *Limonia acidissima* L. were collected, identified botanically, arranged alphabetically along with their family names, local names for the purposes of Ethnobotanical Survey in Rural and Tribal people of Indo Nepal border. It describes the method of application of their part of plant and medicinal importance (Rajan, 2000).

India has a rich tradition in the use of medicinal plant to develop drugs from plants. Nowadays herbal drugs are prescribed widely even when their biologically active compounds are unknown because of their effectiveness, minimal side effects in clinical experience and relatively low cost (Valiathan, 1998). Last decade witnessed an increase in the investigations on plants as a sources of human disease management as well as various phytochemical constituents (Pegnyemb *et al.*, 2001; Baser *et al.*, 2002; Bezerra *et al.*, 2002; Chalchat *et al.*, 2002, Ciccio and Segrini, 2002; Ghosh and Bhattacharya, 2002 and Mounnissamy *et al.*, 2002).

The leaves of *Aegle marmelos* (L.) were used in indigenous system of medicine as astringent, laxative, expectorant, in the treatment of various gastro-intestinal affections (dysentery and piles), ophthalmia, deafness, inflammations, cataract, diabetes, diarrhoea, dysentery, heart palpitation, and asthmatic complications (Ghose, 1980; Kirtikar and Basu, 1993) The leaves are also reported in the treatment of abscess, backache, diabetes,
eye disorders, fever, heat in abdomen, jaundice, vomiting, wounds and cuts (Jain, 1991), while contraceptive property of the drug was also been claimed (Bhattacharya, 1982). Fresh aqueous and alcoholic leaf extracts were reported to have cardiotonic effects in mammals (Haravey, 1968; Nadkarni, 2000) said to possess hypoglycemic and antihyperglycemic activity (Paulose et al., 1993; Karunanayake et al., 1984; Gireesh et al., 2008) anti spermatogenic activity (Sur et al., 1999), antioxidant activity (Sabu and Ramadasan, 2004) and anticancer effect (Jagetia et al., 2005).

Extensive field studies were undertaken in order to study the utilization of wild medicinal plants which has resulted in the collection of 50 species belonging to 31 families. The plant name, family, vernacular name and ethno-botanical uses have been enumerated (Rajith and Ramachandran, 2010).

3.2. Qualitative phytochemical analysis

Ghosh et al. (1982) found that Limonia acidissima L. fruits contain flavonoids, glycosides, saponins and tannins. Krishnaveni et al. (2004) reported that phytochemical screening of various extracts of dried leaves of Cassia fistula showed the presence of phytosterols, flavonoids, glycosides, triterpenoids, alkaloids, saponins, tannins and steroids.

Berberine is an isoquinoline alkaloid found in the roots of Coptis japonica and cortex of Phellondendron amurense. This antibacterial alkaloid has been identified from a number of cell cultures, notably those of Coptis japonica (Sato and Yamada, 1984).
Phytochemical screening of fifty-one medicinal plants, which are used in indigenous systems of medicine as well as by local inhabitants either as single drugs or in combinations, for the cure of various ailments. The study carried out so far, revealed the presence of alkaloids in thirty-one plants of flavonoids in twenty eight glycosides in thirty four, saponins in thirty four, sterols in thirty seven and terpenoids in thirty three plants (Agarwal et al., 1989).

Phytochemicals exert their antimicrobial activity through different mechanisms, for example tannins act by iron deprivation, hydrogen bounding or non-specific interactions with vital proteins such as enzymes (Scalbert, 1991).

Khan et al. (1993) explained the antibacterial activity exhibited by ethanol extract of leaves and stems of Withania coagulans has been attributed to the presence of polar components viz., salts, alkaloids, glycosides, saponins, polyols, resins and amino acids.

Nishibe (1994) investigated the bioactive phenolic compounds in traditional medicines. Arctigenin, matairesinol, trachelogenin and notrachelogenin from Caulis trachelospermi, mauritianin from Herba catharanthi, acteoside and Plantaginin from Plantago herb, Suspensaside, Forsythiaside and (+) Pinoresino glucoside from Forsythia fruit and (+) Syringa resinol α-D glucoside from Eleutherococcus were isolated. The compounds isolated from these showed anti-tumerous activity, anti-inflammatory effects, anti-nephretic and immunosuppressive effects.
Advances in the area of cell cultures for the production of medicinal compounds has made possible the production of wide variety of pharmaceuticals like alkaloids, terpenoids, steroids, saponins, phenolics, flavonoids and amino acids. Taxol, a complex diterpene alkaloid found in the bark of the *Taxus* tree, is one of the most promising anticancer agents known due to its unique mode of action on the microtubular cell system (Jordan and Wilson, 1995).

The secondary metabolites flavonoid, phenol and phenolic glycosides unsaturated lactones, sulphur compounds saponins, cyanogenic glycosides and glyconsinolates produced by plant have known antifungal activity (Osbournce, 1996).

Sener *et al.* (1998) studied the biological activities of some Turkish medicinal plants as a resource of new chemistry for public health and plant protection. A systematical approach to the discovery of drugs from these plants had resulted in the identification of active compounds representing a wide range of structures, including alkaloids, terpenoids and phenolic compounds. Fifty five organosoluble extracts prepared from Turkish medicinal plants were investigated for their biological activities against insects, nematodes, plant pathogens and brine shrimp in addition to their biological activities such as antimalarial, anticholinergic, analgesic and antiplatelet activities. Assays for antimicrobial activity yielded 13 extracts with antibacterial activity and 4 with fungicidal activity.
Ahmad and Beg (2001) studied the effect of ethanolic extracts of 45 Indian medicinal plants for their antimicrobial activity against certain drug resistant bacteria and yeast. Of these, 40 plant extracts showed varied levels of antimicrobial activity against more bacteria and overall, broad spectrum antimicrobial activity was observed in 12 plants. Qualitative phytochemical tests, TLC and TLC bioautography of certain active extracts demonstrated the presence of common phytocompounds in the plant extracts including phenols, tannins and flavonoids as active constituents.

Mojab et al. (2003) studied a phytochemical screening of fifty five Iranian plants belonging to 21 families were carried out. A qualitative phytochemical analysis was performed for the presence of alkaloids, tannins, saponins and flavonoids. The medicinal uses of these plants are also reported.

Krasteva et al. (2004) studied that the phytochemical analysis of ethyl acetate extract from Astragalus corniculatus (Bieb.) and brain antihypoxic activity. Dry ethyl acetate extract containing flavonoids was obtained from above ground parts of Astragalus corniculatus (Bieb.). Seven flavonoids were isolated and identified as rutin, hyperoside, iso quercitrin, narcissin, quercetin, kaempferol and isorhamnetin for the first time. The extract was investigated for antihypoxic activity. Antihypoxic activity was especially pronounced in the model of circulatory hypoxia. This effect was attributed to the presence of flavonoids in the extract. The ethyl acetate extract was chromatographed on a cellulose column, using a 0.95% ethanol linear gradient. Seventy fractions were collected and analysed by TLC on silica gel. Identical fractions were put together and
rechromatographed and further purified by column chromatography. Five flavonol glycosides and three flavonol glycones were isolated.

Nalawade et al. (2006) observed the antimicrobial activity of the Spinach leaf extracts were investigated for presence of various chemical groups and antimicrobial activity. Qualitative chemical investigations showed presence of tannis, phenols, sugars, flavonoids, coumarins and sterols. Amongst all the extracts tested methanolic extract exhibited maximum inhibitory activity against all the bacteria culture used, compared with other extracts.

The bioactive chemical constituents to evaluate the antimicrobial activity of the ethanolic extract of traditionally used right medicinal plants of Nepal. A qualitative phytochemical analysis was performed for the detection of alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins and reducing sugar. The highest yield of ethanolic extract was found in Azadiracta indica (29.08%). Ocimum sanctum contained all the chemicals except flavonoids and reducing sugar hormone the Colquhounia coccina locked alkaloids and reducing sugar. The antimicrobial activities of these plants extract were also observed. The extract of Rhododendron setosum and the essential oil of Eucalyptus globules were most effective against Escherichia coli and Staphylococcus aureus respectively. But the extracts of Azadiracta indica and Elshotlzia feucticosa were found to be most effective against Klebsiella species (Chhetri et al., 2008).
From ancient days to recent civilization, human beings depend on nature for running their life smoothly from day to day. Plants remain a vital source of drugs and nowadays much emphasis have been given to nutraceuticals. Various parts of the plant have astringent, constipating, tonic for liver and lung, diuretic, carminative, and cardiotonic traditional uses. Various important phytoconstituents like alkaloids, phenolic compounds, triterpenoids, coumarins, tannins, steroids etc. have been isolated from Kavith (*Feronia limonia*). But only few pharmacological activities like antimicrobial, antiviral, antitumour and antifungal activity have been scientifically reported. From enormous traditional uses documented in various traditional system of medicine and presence of vital phytoconstituents make Kavith (*Feronia limonia*) an important plant to be studied scientifically to prove various traditional uses. In present review we explore Kvitha’s description, traditional medicinal uses, and phytoconstituents and investigated pharmacological activities in various parts of the plant to show potential ethnopharmacological importance of the plant (Qureshi Absar *et al.*, 2010).

Venkata *et al.* (2010) reported that 84 methanolic extracts prepared from the 54 Indian plants belonging to 33 different families collected from the forest located in Eastern Ghats of India. A qualitative preliminary phytochemical screening was performed on aforesaid extracts for the presence of alkaloids, flavonoids, steroids and terpenoids. Each analysis was carried out in triplicate, which resulted a total of 22, 19, 37 and 30 plant species were found to give positive results for alkaloids (41%), flavonoids (35%), steroids (69%) and terpenoids (56%), respectively.
Achras sapota (Linn.) belonging of Sapindaceae family and can be widely found in the world. The phytochemical study was carried by Monalisha et al., (2010) on the methanolic extraction of the modified stem of Achras sapota (Linn.) by standard method. The principal constituents of Achras sapota (Linn.) include alkaloid, steroid, flavonoid, saponin, reducing sugar, tannin, amino acid, protein, anthraquinone glycoside, deoxy sugar, phenolic compound. The main biological activity was found as an anti-oxidant.

3.3. Quantitative phytochemical analysis

Amount of Vitamin C in dry powder of Limonia acidissima L. was determined. Earlier workers observed that fresh juice of the plant contains more vitamin C content than that of dry powder (Anonymous, 1950).

The abundance of vitamin C (Ascorbic acid) in Limonia acidissima L serves to protect H⁺ carrier system and thus helps in tissue oxidation. Antioxidant along with hyaluronic acid maintains capillary tone by keeping the endothelium intact. Along with proline, ascorbic acid enhances collagen synthesis. Ascorbic acid increases Fe²⁺ absorption, which in turn increases H⁶ formation. Blood loss due to ulceration will be compensated by H⁶formation. Vitamin C also initiates the maturation of red and white blood cells (Rathan, 1986; Rama Rao, 1989).

3.4. Physico – chemical characters
Mammen et al. (2010) analysed the various parameters such as ash analysis, extractive values and moisture content for three plants *Aerva lanata, Hedyotis corymbosa* and *Leptadenia reticulata*. Each plant was collected during summer, monsoon and winter to study the effect of change of season on the proximate analysis values. Similarly the analysis was carried out for samples collected from Gujarat, Maharashtra and Kerala, to study the effect of geographical variation in the plants. Interestingly, the values were found to be change with season and region of collection of these plants. The results indicates that the importance of best time and place of collection for the plant.

Mathur et al., (2010) studied macroscopic, microscopic and preliminary phytochemical investigation of leaves of *Amaranthus spinosus* which includes leaf constants, physiochemical parameters like ash values, extractive values and moisture content. The total ash, acid insoluble ash, water soluble ash values and sulfated ash were observed to be 6.33%, 3.60%, 2.44% and 0.80% w/w respectively. Alcohol soluble and water soluble extractive values of the leaves were observed to be 6.40%, 3.30%, respectively.

*Naringi crenulata* stem wood is a traditional cosmetics in Southeast Asia. Physico – chemical analysis of *Naringi crenulata* showed moisture content and loss on drying were 6.125% ± 0.653 and 7.564% ± 1.146, respectively. Total and acid insoluble ash contents were 1.198% ± 0.515 and 0.035% ± 0.077, respectively. Extractive values of 95% ethyl alcohol, ethyl acetate and H$_2$O were 0.165% ± 0.058, 0.036% ± 0.008 and 0.533% ± 0.117, respectively (Kanlayavattanakul et al., 2009).
Pectic polysaccharides have been isolated from the fruits of *Naringi crenulata* by extraction with water. The water extract contains large amount of protein. The polymers present in the water extract are fractionated by graded precipitation with ethanol, anion exchange chromatography, and size exclusion chromatography. Characterization of the sub fractions obtained from various chemical and physico-chemical methods of analysis reveals that the water extract contain pectic polymers substituted to various degrees with side chains comprising mainly of terminal, 1,4-, 1,6-, 1,3,6-linked galactose, together with lesser amounts of 1,2,4- and 1,3-linked galactose residues. Arabinose residues are terminal, 1,5-, 1,3,5-linked. These polymers contain acetyl groups and give viscous solution in water (Mondal Saroj *et al*., 2003).

### 3.5. Elemental analysis

Analysis of twenty medicinal plants i.e. *Aganosma dichotoma*, *Ferula foetida*, *Citrullus colocynthis*, *Desmotrichum firmbriatum*, *Tinospora cordifolia*, *Tylophora indica*, *Vetiveria zizanoides*, *Clerodendron serratum*, *Mallotus philippinensis*, *Eclipta alba*, *Celastrus paniculata*, *Chloroxylon swietenia*, *Commiphora mukul*, *Viola odorata*, *Santaloides minus*, *Onosma bracteatum*, *Plumbago indica*, *Madhuca longifolia*, *Tephrosia purpurea* and *Gloriosa superba* for their mineral elements i.e. Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, Ag, Cd, Sr and Ce was carried out, using Atomic Absorption Spectrophotometer, Inductively Coupled Plasma and Flame Photometer. Mineral elements Na, Mg, K, Ca, Cr, Mn, Fe, Co and Zn were found to be common in all the medicinal plants analysed (Saily, 1994).
Rajurkar and Damame (1997) reported that the elemental analysis of some herbal plants used in the Ayurveda for curing of cardiovascular diseases has been performed using the techniques of neutron activation analysis and Atomic Absorption Spectroscopy (AAS). The concentration of elements Mn, Na, K and Cl has been estimated by NAA using a $^{252}\text{Cf}$ neutron source and a high purity germanium detector coupled to a multichannel analyser, while the elements Ca, Cr, Co, Cu, Fe, Pb, Zn, Ni, Cd and Hg were analysed by AAS using a Perkin Elmer 3100 instrument. The elements such as Na, Mg, K, Ca, Mn, N and Cl were detected in tested plants. *Solanum trilobatum* (Linn.) (Solanaceae-herbs) is an important medicinal plant. It contain rich amount of calcium, iron, phosphorus, carbohydrates, fat, crude fibre and minerals in the leaves (Jawahar *et al.*, 2004).

3.6. Microbial analysis

Idu *et al.* (2010) evaluated the microbial load on 17 randomly selected plant samples from 60 ethnobotanically collected medicinal plants from five local markets in Abeokuta, Ogun State, Nigeria. The pour plate method was used to cultivate serially diluted portions of the medicinal plant samples investigated. Enumeration of bacteria was carried out on nutrient agar while that of fungi was effected on Sabouraud agar. The identified microbial isolates include 12 bacterial and 6 fungal genera. The mean heterotrophic bacteria counts of the different herbal samples ranged from $1.3 \times 10^5$ cfu/g (*Cnestis ferruginea*) to $6.7 \times 10^6$ cfu/g (*Daniellia oliveri*), while total fungal propagule counts ranged from $0.0 \times 10^1$ cfu/g (*Terminalia superba, Cola gigantea, Rauwolfia*).
Biological activities of Limonia crenulata (Roxb.) vomitoria, Zingiber officinale and Argemone mexicana) to $7.1 \times 10^6$ cfu/g (Nesogordonia papaverifera). The synopsis and frequency (prevalence rate) of microbial species isolation showed that *Bacillus* spp. (82.4 %) and *Mucor* sp. (47.1 %) had the highest prevalence rates among bacteria and fungi, respectively. The results emphasized the need for constant quality assessment of herbal drugs on sale in order to ensure the production of therapeutic products suitable for human consumption.

Minea et al. (2004) studied fresh *Salvia officinalis* had $1 \times 10^4$ microorganisms/g, and these microorganisms were identified as bacteria and moulds (*Rhizopus, Mucor* and *Penicillium*). After irradiation with 0.2 kGy, the bacteria were decreased at $1 \times 10^3$/g and moulds were destroyed. No microorganisms were survived after irradiation with 0.5 kGy. Microbial load for *Salvia officinalis* and *Calendula Officinalis* have a high microbial load ($1 \times 10^5$ microorganisms/g). 1 kGy irradiation reduced the diameter of colonies and the number of microorganisms at $1 \times 10^3$/g. Microbiological contamination of medicinal herbs was a serious problem in the production of therapeutical preparations.

### 3.7. High Power Thin Layer Chromatography (HPTLC) analysis

A method for the estimation of quinine (Qn), cinchonine (Cn), and cinchonidine (Cnd) and a new method based on fluorescence enhancement and detection and quantification of quinidine (Qnd) from *Cinchona* stem bark and its formulations, using HPTLC has been reported. Standard solutions of Qn, Qnd, Cn and Cnd were applied on precoated HPTLC plates and developed with chloroform/diethylamine (9.6:104 v/v). The plates were scanned and quantified at 226 nm for Qn, Cn, Cnd and for Qnd at 366 nm in
fluorescents and reflectance mode (< K400 filter). The stem bark of *Cinchona officinalis* and some herbal were allowed for homeopathic formulations which was evaluated for their individual alkaloid content applying the developed method (Ravishankara *et al.*, 2001).

A simple sensitive and precise HPTLC method of analysis of trans - resveratrol in *Polygonum cuspidatum* (Polygonaceae) root extracts and in dosage forms was developed and validated. The separation was carried out on a TLC aluminium plates precoated with silica gel 60F – 254 as the stationary phase, eluted with chloroform, ethyl acetate and formic acid (2.5:1:0.1) as mobile phase. Densitometric analysis of trans-resveratrol was carried out in the absorbance mode at 313 nm. This system was found to give compact spot for trans - resveratrol (Rf value of 0.40 plus or minus 0.03). A good linear regression relationship was made between peak areas and the concentrations coefficient 0.9989. The limit of detection and quantification was found to be 9 and 27 ng/ spot. The method was validated for precision and recovery. The spike recoveries were within 99.85 to 100.70 percent. The method can be applied for identification and quantitative determination of trans-resveratrol in herbal extracts and dosage forms (Babu *et al.*, 2005).

Spectrophotometric study of *Aegle marmelos* (L.) showed presence of a compound with overlapping spectrum with the marker standard Umbelliferone - a coumarin glycoside which was further confirmed by the TLC and HPTLC studies of the ethanolic extract (Joshi *et al.*, 2009).
An HPTLC method was developed for the quantitative estimation of gallic acid, rutin and quercetin from aqueous and ethanolic extract of *Eruca sativa*, precoated HPTLC silica gel 60 F254 as stationary phase and mobile phase for gallic acid, toluene: ethyl acetate: formic acid (7:5:1) and mobile phase for quercetin and rutin, ethyl acetate: glacial acetic acid: formic acid: water (100:11:11:25). Detection and quantification were performed densitometrically at λ 280nm for gallic acid, 280 nm quercetin and 366nm for rutin. The standard Rf values of gallic acid, quercetin and rutin are 0.35±0.01, 0.98±0.01 and 0.34±0.02 respectively. The total peak areas of the standards gallic acid, quercetin and rutin and the corresponding peak areas of extracts were compared and the gallic acid, quercetin and rutin content were estimated to be 356.1, 4591.0 and 1277.1 (Sajeeth *et al.*, 2010).

3.8. Gas Chromatography-Mass Spectrometry (GC – MS) analysis

A new indole alkaloid, crenulatine, was isolated from the stems of *Limonia crenulata* (Roxb.). The below structure was identified by spectral means (Niu *et al.*, 2001).
Nayar et al. (1971) reported that the weak base 4-methoxy-1-methyl-2-quinolone(I). Sitosterol, 4-methoxy-1-methy-2-quinolone and four coumarins, one of which is a 1,2-epoxide, were isolated from the petrol extract of the root bark of *Hesperethusa crenulata* (Nayar and Bhan, 1972).

Das and Thakur, (1989) reported that the leaf cutin of *Limonia acidissima* L. was found to comprise *n*-alkanoic (*C*<sub>12</sub>), α,ω-alkanedioic (*C*<sub>3</sub>---*C*<sub>16</sub>), hydroxyalkanoic (*C*<sub>3</sub>-*C*<sub>16</sub>), dihydroxy alkanoic (*C*<sub>4</sub>---*C*<sub>20</sub>), hydroxy α,ω-alkanedioic (*C*<sub>14</sub>---*C*<sub>16</sub>) and aromatic acids, together with *p*-hydroxy benzaldehyde and heptadecane diol. The main constituents were 9, 16- and 10, 16-dihydroxyhexadecanoic acids (*ca* 30%), 10, 20-dihydroxyicosanoic acid (*ca* 10%) and 7-hydroxyhexadecane-1, 16-dioic acid (*ca*15%).

Lin and Hsu (1999) extracted tannin and related compounds from *Terminalia catappa* and *Terminalia parviflora* and isolated one novel complex type tannin, catappin A, together with two phenolcarboxylic acids, two phenol glucoside gallates, seven tannins, one other hydrolysable tannin, four flavon-3-ols and two complex type tannins from the bark of *T. catappa*. Their structures were elucidated on the basis of chemical and spectroscopic evidence and tested for antibacterial and antifungal activity and found to have very strong activity.

Three simple coumarins scoparone, limettin and psoralen have been isolated as major components from the leaves of *Euodia borbonica* var. *borbonica* (Rutaceae) together with xanthoxylin, a common phenolic compound in Rutaceae family. Their
structures were elucidated through GC - MS and NMR studies. A minor furocoumarin, bergapten, was also detected in the extracts (Valenciennes et al., 1999).

Acetone extract of *Limonia acidissima* L. dried leaves afforded a potent mosquito larvicide’s identified as n-hexadecanoic acid and found to be effective against fourth instars larvae of *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti* with LC_{50} 129.24, 79.58 and 57.23 ppm respectively (Abdul Rahuman et al., 2000).

The chemical constituents of the essential oil from *Plectranthus japonicas* have been identified by using GC, GC-MS and spectral analysis. The oil was screened for antifungal activity against *Aspergillus niger*, *Alternaria alternate*, *Penicillium citrinum*, *Rhizopus nigricans* and *Trichoderma viride* (Mathpal et al., 2002).

The taxonomic interest in the *Neoraputia* stimulated an investigation of *N. paraensis* searching for alkaloids. Fractions were monitored by Hydrogen Nuclear Magnetic Resonance (^{1}H NMR) and Electrospray Ionization Mass Spectrometry (ESI-MS) and only those which showed features of anthranilate alkaloids and flavonoids absent in the previous investigations were examined. Stems afforded the alkaloids flindersine, skimmianine, 8-methoxyflindersine and dictamnine; leaves yielded 3’,4’,7,8-tetramethoxy-5,6-(2”,2”-dimethylpyrano)-flavone, 3’, 4’, 5, 7, 8-penta-methoxyflavone, 5-hydroxy-3’, 4’, 6, 7-tetramethoxy flavone, 3’,4’-methylenedioxy- 5, 6, 7-trimethoxyflavone & 5-hydroxy-3’, 4’-methylenedioxy -6,7-dimethoxyflavone. A number of flavonoids isolated from *N. paraensis*, *N. magnifica*, *Murraya paniculata*, *Citrus
Biological activities of *Limonia crenulata* (Roxb.) *sinensis* graft (Rutaceae) and *Lonchocarpus montanus* (Leguminosae) were evaluated for their ability to inhibit the enzymatic activity of the protein glyceraldehyde-3-phosphate dehydrogenase from *Trypanosoma cruzi*. Highly oxygenated flavones and isoflavone were the most actives (Valéria *et al.*, 2003).

Skrzypek and Wysokinska (2003) studied the sterols and triterpenes in cell culture of *Hyssopus officinalis* L. Cell suspension cultures from hypocotyls derived callus of *Hyssopus officinalis* were found to produce two sterols, i.e., sitosterol and stigmasterol as well as several known pentacyclic triterpenes with an oleanene. The triterpenes were identified as oleanolic acid, ursolic acid, 2, 3 dihydroxyolean 12-en-28-oic acid, 2, 3-dihydroxyurs-12-en-28-oic acid, 2, 3, 24-trihydroxyolean-12-en-28-oic acid and 2, 3, 24-trihydroxyurs-12-en-oic acid.

Major constituents of fruit pulp of *Tamarindus indicus* (Pino *et al.*, 2004) were found to be Hexadecanoic acid; 27.4 % of this acid in the roots of *Salvia hypolecuca* (Bigdeli *et al.*, 2005). 31.9% in essential oil of *Astragalus microcephaus* (Rezaee *et al.*, 2006), palmitic acid 82.5% in *Carissa opaca* flower (Rai *et al.*, 2006) was estimated by GC-MS combination of haxadecanoic acid, oleic and linoleic acid was observed in *Coix lacryma – jobi* L.(Numata *et al.*, 1994) *Salavadora persica* (Hosamani and Pattanashettar., 2002) and *Malvastrum coromandelianun* (Hosamani *et al.*, 2004).

The secondary metabolism in the leaves of *Piper cernuum* produces cinnamic and dihydrocinnamic acid derivatives and the lignin cubebin. In the case of *P. crassinervium*
flavonoids and prenylated hydroquinones were characterized as major compounds. The cell cultures showed the production of the phenethylamines, dopamine and tyramine in *P. cernuum*, while in *P. crassinervium* four alkamides were isolated as major compounds, including the new 2,3,4-trimethoxy-N-methyl-aristolactam and 3-hydroxy-2-methoxy-N-methyl aristolactam (Danelutte *et al.*, 2005).

Simonsen *et al.* (2006) reported that 2-Methoxyjuglone was isolated from the leaves of *Lomatia hirsuta* and found to be active against the pathogenic fungus *Candida albicans*. Cinnamic acid and Vanillic acid were identified as major constituents in the tea by GC-MS. The tea was found not to be toxic against *Artemia salina*. The presence of phenolic acids with antimicrobial properties supports the traditional use.

Central Council for Research in Ayurveda and Siddha reported that petrol extracts of leaf in *Limonia crenulata* (Roxb.) contains the phytoconstituents such as xanthotoxin and sitosterol, which is used for analysis of crude drugs or herbal formulations (Patra *et al.*, 2010).

3.9. High-Performance Liquid Chromatography (HPLC) analysis

The biogenesis, structural diversity and distribution of simple, furano and pyranocoumarins in the Rutaceae is reviewed. The potential value of these compounds as taxonomic markers and their possible functions are discussed. The distribution of simple
cinnamic acid precursors of coumarins in the family is also reviewed (Alexander and Waterman, 1978).

Kajita et al. (1997) reported the structural characterization of modified Lignin in Transgenic Tobacco plants in which the activity of 4-coumarate: Coenzyme A Ligase is depressed. Transgenic tobacco plants in which the activity of 4-coumarate: coenzyme A ligase is very low, contain a novel lignin in their xylem. Details of changes in hydroxycinnamic acids bound to cell walls and in the structure of novel lignin were identified by base hydrolysis, alkaline nitrobenzene oxidation, pyrolysis-gas-chromatography, and $^{13}$C-nuclear magnetic resonance analysis. In the brownish tissue of the transgenic plants, the levels of three hydroxycinnamic acids, p-coumaric, ferulic and sinapic, which were bound to the cell walls, were apparently increased as a result of down regulation of the expression of gene for 4-coumarate: coenzyme A ligase. Their data indicated that the behaviour of some of the incorporated hydroxyl cinnamic acids resembles lignin monomers in the brownish tissue and their accumulation results in dramatic changes in the biosynthesis of lignin in transgenic plants.

Two coumarins, auraptene and marmin were isolated from roots of Aegle marmelos (Rutaceae). The isolation process involved extraction with various solvents and separation using chromatography techniques. Antimicrobial activity and cytotoxic tests of the crude extracts of the roots and the isolated compounds against T-cell lymphoblastic leukemia cells were carried out and found to be very weak effect (Riyanto et al., 2002).
Studies on certain chemical constituents in the leaves of *Ficus elastica* Roxb. and their biological activities were reported by Abdalla et al., (2002). The phytochemical screening of the leaves indicated the presence of four compounds emodin, sucrose, morin and rutin. The bioactivity screening showed that the crude extract and pure isolated compounds possessed antibacterial activity on *Bacillus cereus* and *Pseudomonas aeruginosa*.

Olszewska and Wolbis (2002) isolated two new flavonol glycosides, quercetin 3-o-(2-o-D-glucopyranosyl)-α-L-arabinofuranoside and kaempferol 3-o-(2-o-E-p-coumaroyl)-α-L-arabinofuranoside-7-o-α-L-rhamnopyranoside from the leaves of *Prunus spinosa* using HPLC. The known compounds, kaempferol, quercetin and their 3-arabinofuranosides, kaempferol 7-rhamnopyranoside, kaempferol 3,7-dirhamnopyranoside and kaempferol 3-arabinofuranoside 7-rhamnopyranoside were also identified.

From the flowers of *Ficaria verna* Huds. (Ranunculaceae), two flavonol triglycosides were isolated and their structures were elucidated by microscopic analysis (HPLC, UV, NMR, MS) as 3-o-(L-rhamnopyranosyl-(1→6)--D-glucopyranosyl)-7-o-(D-glucopyranosyl)-quercetin and 3-o-(L-rhamnopyranosyl- (1) D-glucopyranosyl) -7-o \((D-glucopyranosyl) -Kaempferol. In addition the structures were determined by using homo- and heteronuclear 2D NMR techniques (Tomczyk and Gudej, 2002).
The phytochemical studies on *Terminalia catappa* bark and leaves demonstrate the presence of tannins and flavonoid glycosides. Among them, gallic acid, corilagin, ellagic acid and rutin showed *in vitro* antibacterial activity (Thiem and Goslinska, 2004).

Six flavonoids, viz., quercetin, 3-O-methyl kaempferol, quercetin, kaempferol - 3-O-alpha-L-arabinofuranoside, rutin and neobudofficide and four sterols, namely, campesterol, stigmasterol, beta-sitosterol and stigmastanol were isolated by Neretina et al., (2004) from the aerial parts of *Hedysarum setigerum*.

Pharmacognostic specification of *Naringi crenulata* stem wood, traditional cosmetics in Southeast Asia was done by studying on twelve wood samples from different sources. The powdered stem wood had a sweet natural fragrance but tasteless. Stem wood fibers were predominately found with large amount of longitudinal cells in addition to high lignin content in cell wall. Wood parenchyma contained starch granules and calcium oxalate crystals with oil globules thoroughly distributed. Alkaloids and coumarin tests were positive. HPLC chromatograms of twelve wood samples were similar in patterns but diverse in quantity. Arbutin content was 0.750% ± 0.414 of the crude extract weight (Kanlayavattanakul et al., 2009).

3.10. Fourier Transform - Infra Red (FT-IR) spectroscopy analysis

Maoela et al. (2009) reported the FT-IR absorption spectra of catechin and ethyl acetate extracts of *Coprinus mellei* and *C. quadrifidus* confirming the presence of catechin in the plant extracts. The spectra show the characteristic absorption regions for
O-H group (3400 – 3100 cm⁻¹), C = C group around 1600 cm⁻¹, as well as C – O group (1150 – 1010 cm⁻¹).

Komal Kumar and Devi Prasad (2010) analysed the Fourier Transform Infrared technique to understand the composition, chemical structure and discrimination of biomolecules in medicinal plants of *Tephrosia tinctoria* and *Atylosia albicans*. IR spectrum in mid infrared region (4000–400 cm⁻¹) was used for discriminating and indentifying various functional groups present in two different species of medicinal plants belonging to the family Leguminosae. Presence of C=O, C–H, C=C and C–O, C–C, C–O were identified. These bonding structures were responsible for the presence of alkyl groups, methyl groups, alcohols, ethers, esters, carboxylic acid, anhydrides and deoxyribose. The results showed that *Tephrosia tinctoria* and *Atylosia albicans* are rich in phenolic compounds.

The FT-IR analysis of ethanol extract in *G.kollimalayanum* was confirmed the presence of the carboxylic acid and Alkenes-CH₂; CH₃ Aromatic stretching which shows major peaks at 1019.87 and 2922.33 cm⁻¹ (Ramachandran and Viswan, 2011).

Sebnem et al. (2006) isolated the secondary metabolites from *Phlomis syrica* and found out their antioxidant activities. An iridoid glucoside, lamiide, 4-phenylethanoid glycosides, acteoside, -OH acteoside, leucosceptoside A and S amioside, a caffeic acid ester, chlorogenic acid, 2 flavone glucosides, leuteolin-7-o-glucopyranoside and chrysoeriol-7-o-glucopyranoside and a flavanone aglycone, naringenin were isolated.
from the aerial parts of *Phlomis syriaca*. The structures of the isolated compounds were elucidated by means of spectroscopic (UV, IR, 1D and 2D-NMR and Fast Atom Bombardment Mass Spectrometry (FAB-MS) methods.

### 3.11. Ultra Violet (UV) - Visible spectroscopy analysis

The UV-Visible spectroscopy analysis of ethanol extract in *G.kollimalayanum* was evaluated. The peak value were 413.77, 469.15, 664.43 and its electron transition due to OH group (Ramachandran and Viswan, 2011).

Maoela *et al.* (2009) reported the the UV-vis absorption spectra of *Coprinus mellei* and *C. quadrifidus* overlap with that of catechin, which confirms the presences of the catechin. The UV-vis absorption spectra of *C. mellei* and *C. quadrifidus* show two absorption bands, a strong one at 218 nm (Band II) and a weak one at 282 nm (Band I). In general terms the band II absorption may be considered as having originated from the A ring benzoyl system and band I from the B ring cinnamoyl system.

Mishra *et al.* (2010) reported that a new anthraquinone, 1-methyl-2-(3’-methyl-but-2’-enyloxy)-anthraquinone (1) has been isolated from seeds of *Aegle marmelos* (L.) and was characterized on the basis of spectral analysis (UV, IR, $^1$H NMR, $^{13}$C NMR, 2D NMR and mass spectroscopy).

### 3.12. Antimicrobial acitivity
Padmaja and Thangasamy (1993) observed a pharmacological screening, substantial antibacterial, antifungal and antihelminthic activities of some medicinal plants. The hexane and ethyl acetate extracts of the root of _Uveria hanun_ wall and _Uvaria hookerii_ (Kins.) showed maximum activity. Chromatographic fascination of these extracts led to the isolation of the triterpene, glutinole, taraxerol, β-sitosterol.

Saxena _et al._ (1994) studied the antimicrobial activity of the methanol extract and isolated constituents of _Rhus glahra_ (Anacardiaceae), a species used in folk medicine by North American native people was evaluated against 11 microorganisms, including gram-positive and gram-negative bacteria. The extract was subsequently fractionated and monitored by bioassays leading to the isolation of three antibacterial compounds, the methyl ester of 3, 4, 5 trihydroxy benzoic acid (methyl gallate), 4-methoxy and 3, 5 dihydroxybenzoic acid and gallic acid.

Hessa _et al._ (1995) studied antimicrobial activity of some species of medicinal plants. _Vochysia divergens_ Pohl (Vochysiaceae) is a tree commonly found in wet soils of Pantanal of Mato Grosso, Brazil and used in folk medicine against infections and asthma. From the extracts of the stem bark β-sitosterol betulinic acid and sericic acid were isolated.

One hundred crude extracts obtained from various plant parts 59 species representing mostly the plant families _Scrophudariaceae_ and _Acanthaceae_ have been investigated for their antimicrobial activity. Plants were selected using ethanobotanical
Biological activities of *Limonia crenulata* (Roxb.) and chemotaxonomic information. Growth inhibition using agar diffusion assays was determined against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. Growth inhibitory activity against one or more of the microbial species was detected in over 40% of the samples (Meurer-Grimes *et al*., 1996).

From the Indian traditional medicines, 78 plants were selected on the basis of their use in the treatment of infectious diseases. Different concentrations of 80% ethanolic extracts were tested, using the agar dilution method, against four bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* using the agar well diffusion method and against two fungi *Candida albicans* and *Aspergillus niger*. In the lowest tested concentration of 1.6 mg/ml, 10% of the plant extracts were active; 44% in a concentration of 6.25 mg/ml and 90% of the plant extracts were active against at least two bacteria in a concentration of 25 mg/ml. Only 13% of the plant extracts were active against at least one fungus in a concentration of 50 mg/ml (Valsaraj *et al*., 1997).

Ethanol extract of 109 plants reported to be used in the traditional medicine of Baja California sur Mexicol were tested for antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus faecalis*, *Escherichia coli* and *Candida albicans* of these, and 64 were active against one or more test organisms (Dimayaga *et al*., 1998).

A comparative study on the antimicrobial properties of extracts from medicinal plants obtained by two different methods was carried out. The screening of the
antimicrobial activity of extracts from six plants was conducted by a disc diffusion test against gram-positive and gram-negative and fungal organisms. The most active extracts were assayed for the minimum inhibitory concentration and submitted to phytochemical screening by thin-layer chromatography and bioautography. The results obtained indicated that the diethyl ether extracts were the most efficient antimicrobial compounds. Bioautography showed that the antimicrobial activity was probably due to flavonoids and terpenes (Nostro, 2000).

Agarwal and Sudhir Singh (2000) isolated rhein, physeion, aloe-emodin and chrosophanol from *Rheun emodi* rhizomes and exhibited antifungal activities against *Candida albicans, Cryptococcus neoformans* and *Aspergillus fumigatus*.

Iswar Singh and Ved Pal Singh (2000) studied the antimicrobial activity of aqueous and organic solution extracts of 50 plants belonging to 27 families of seed plants which were screened for antifungal activity against *Aspergillus flavus* and *Aspergillus niger* using the agar well diffusion method. The results showed most of the plants have antimicrobial activity.

Perumalsamy and Ignacimuthu (2000) studied the antibacterial properties of medicinal plants. A series of 30 Indian folklore medicinal plants used by tribal to treat infections were screened for antibacterial properties at 10 mg/ml concentration by using disc diffusion method against *Bacillus subtilis, E. coli* and *Klebsiella aerogens*. 
Iwalokun et al. (2001) studied on three Nigerian medicinal plants and investigated their activities against multidrug-resistant Shigella species isolated from patients with bacillary dysentery in Lagos. Decoctions of Ocimum gratissimum and concoction of O. gratissimum and Terminalia avicennoides at crude concentration of 3000 µg/ml markedly inhibited the growth of all isolates tested. Minimum inhibitory concentration and maximum bactericidal concentration revealed at higher Shigelloidal property of Momordica balsamina. The results suggested that aqueous extracts of O. gratissimum and T. avicennoides as decoctions and concoctions could be useful in the treatment of Shigellosis and should be clinically evaluated specially in Nigerian region.

The aqueous extract of Limonia acidissima L. has antimicrobial activity against tested bacterial strain. Volatile oil in Limonia acidissima had also been found to have antimicrobial activity and anti helmintic activity (Garg, 2001).

Gnanamani et al. (2003) investigated antibacterial activity of crude alcoholic extract of Datura alba and Celosia argentica leaves were studied against pathogens isolated from infected burn patients. The disc diffusion method showed significant zone of lysis against all the pathogens studied and the results were comparable to the conventional antibiotic cream namely silver sulphadiazine (SSD). On comparing the efficiency of the two extracts of Datura alba exhibited more than 50% increase in antibacterial activity compared to Celosia argentica.
*Alangium saliifolium* is an ethnomedicinal plant used in folklore as a medicine. The leaves were extracted with water, ethanol and chloroform and each extract was evaluated for antibacterial activity against pathogenic strains of *Escherichia coli, Proteus vulgaris, Bacillus subtilis, Enterobacter faecalis, Serratia marcescens* and *Klebsiella pneumoniae* by using disc diffusion method. The zones of inhibition were recorded and compared with standard drugs i.e. chloramphenicol. Ethanolic extract showed the high degree of inhibition when compared with chloroform and aqueous extracts (Natarajan et al., 2003).

Alam et al. (2003) synthesized and studied the antibacterial activity of *Pongaglabol* was tested for antibacterial effects against *Shigella dysenteriae, Salmonella typhi, Streptococcus B-haemotyticus* and *Staphylococcus aureus*.

Somchit et al. (2003) screened the crude ethanol and water extract of leaves and barks from *Cassia alata* and they were tested *in vitro* against fungi, yeast and bacteria. Results showed that the water extracts exhibited higher antibacterial activity than the ethanol extract from leaves (inhibition zones of 11-14 and 9-11 mm respectively). *Escheretia coli* showed resistance to all types of extracts.

The methanol extracts of 306 plants of 52 families obtained from northeast of Iran (Khorasan province) were tested for antimicrobial activity (*in vitro*) using the cylinder plate assay method. Activity against *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Pseudomonas aeruginosa, Morganella morgani, Bacillus subtilis,*
Staphylococcus aureus and Candida albicans was showed significant results (Frazly et al., 2003).

Kartal et al. (2003) studied the antimicrobial activity of two propolis samples from Kazan and Marmaris regions in Turkey. They were tested with four different ethanolic extracts (30, 50, 70 and 96% ethanol) of each sample against seven gram-positive, four gram-negative bacteria and one fungus culture. The activity was found to be mainly due to caffeic acid and its esters.

Matu and Staden (2003) investigated antimicrobial activities of plant species in Kenya. Aqueous, hexane and methanol extracts of 12 plant species traditionally used in Kenya for the treatment of ailments of infections and inflammatory nature were screened for in vitro antibacterial activities.

Rani and Khullar (2004) screened some important plants in Ayurvedic system of traditional medicines in India to treat enteric diseases. Fifty four plant extract (methanol and aqueous) were assayed for their activity against multi-drug resistant Salmonella typhi. Strong antibacterial activity was shown by the methanol extracts of Aegle marmelos, Punica granatum, Myristica fragrans, Terminalia chebula, Acacia catechu, Solanum nigrum, Carum copticum, Apium graveolens, Ocimum sanctum and Butea monosperma etc.
Yogamoorthi and Srikala (2004) studied that the anti pathogenic bacterial properties of skin secretion (Mucus) of Narcine timelei. The fresh mucus extract was tested by the disc method against common pathogenic bacterial species namely Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus faecalis, and Vibrio cholerae.

Triterpenes of a hispidissima were investigated and found to be active against selected bacteria and fungi. Beta amycin demonstrated the maximum activity against Escherichia coli. Four new diterpenes were isolated from the leaves of Myrosernum frutescens as minor constituents. 6-Beta-1, 8-diaceotomycassan-13, 15 dinent, chargreslactone, chasresnone derivative products were obtained to test their activities against Chags’s disease. The hexane extract of the rhizome of Curcuma longa exhibited significant activity against gram-positive and insignificant activity against gram-negative bacteria assay as compared to standard antibiotics (Torres et al., 2004).

Kavitha et al. (2004) reported that alkaloids from the ethanolic extract of Holarrhena antidyserntica seeds were evaluated for their antibacterial activity against clinical isolated enteropathogenic Escheritia coli and their antidiarrhoeal activity on castor oil induced diarrhoea in rats, in vivo. The plasmid DNA, whole cell lysate and outer membrane protein profile of a clinical isolate of Enteropathogenic Escherichia coli (EPEC) was determined in presence of alkaloids of H. antidysenterica. The disc diffusion and agar well diffusion methods were used to evaluate the antibacterial efficacy. The loss of plasmid DNA and suppression of high molecular weight proteins were
observed on alkaloids treatment. The results suggest the usefulness of alkaloids of *H. antidysenterica* seeds as antibacterial and antidiarrhoeal agents.

Adamu *et al.* (2005) investigated the preliminary antimicrobial activity of the aqueous extracts of the 84 medicinal plants. Among the 84 plants, 75 exhibited antimicrobial activity against several tested organisms at the concentrations of 200 mg/ml. The extracts were found to show strong activity against *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *E. coli*.

De Boer *et al.* (2005) reported that 29 plants used for medicinal purposes and 41 plants used for non-medicinal purposes in Tanzania. Six medicinally used plants were selected for bioactivity analysis. Extracts of *Candida albicans*, *Aspergillus fumigatus*, *Fusarium culmorum*, *Staphylococcus aureus*, *Pseudomonas syringae* and *Erwinia amylovora*. All plants showed activity against several test organisms.

Kloucex *et al.* (2005) investigated the antimicrobial activity of 7 ethanol extracts of *Brunfelsia grandiflora*, *Caesalpinia spinosa*, *Dracontium loretense*, *Equisetum giganteum*, *Terminalia catapa*, *Phyllanthus amarus* and *Piper aduncum*. Among the plants tested, *Phyllanthus amarus* and *Terminalia catappa* showed the most promising antibacterial properties with minimum inhibitory concentration ranging from 0.25 to 16 mg/ml. The extract isolated from the aerial part of *Piper aduncum* was more active against gram-positive than gram-negative bacteria.
Phongpaichit et al. (2005) obtained 36 extracts from 10 plant species. They were screened for their antifungal activity against Candida albicans, Cryptococcus neoformans and Microsporum gypseum. The chloroform extracts of Alpinia galanga and Boesbergia pandurata showed strong antifungal activity against C. neoformans and M. gypseum, but weak activity against Candida albicans, Alpinia galanga and Boesbergia pandurata are excellent candidates for the development of a remedy from opportunistic fungal infections in AIDS patients.

Twenty five selected plants belonging to 19 families were collected from different localities of the island Soqutra dried, and extracted with the solvents chloroform, methanol and hot water to yield 80 extracts. The extracts were tested for their antimicrobial activity against several gram - positive and gram - negative bacteria and against one yeast species using agar diffusion method. Antibacterial activity was demonstrated especially against gram - positive bacteria including multi resistant Staphylococcus strains. The greatest activity was exhibited by the methanolic extracts of Boswellia elongata, B. ameero, Buxus hidebranchi, Commiphora parnifolia, Jatropha unicosstata (Ramzi et al., 2005).

Meryem (2005) reported that the methanol extract of Verbascum georgicum Bentham was investigated for its in vitro antimicrobial properties. A total 143 microorganisms belonging to 56 bacteria and four fungi and a yeast species were studied using the disk diffusion method and microdilution assays. The results indicated that the methanol extract of V. georgicum had an inhibitory effect on the growth of all Candida
albicans isolates and 17 strains in 10 different species of bacteria. Thus the results suggested that V. georgicum extract possesses compounds with antimicrobial properties that might be utilized for developing new drugs.

Santhi and Alagesabooopathi (2005) explained the antibacterial activity of the aqueous, ethanol and chloroform extracts from the leaves of Andrographis lineate Nees were determined by using the agar disc diffusion method against Staphylococcus aureus, Shigella dysentriae, Salmonella typhi and Vibrio cholerae. Ethanolic leaf extract showed high antibacterial effect against V. cholerae, and S. dysentriae. The antibacterial activity of ethanol extract was found to be higher than that of distilled water extract.

Akgul and Saglikogul (2005) investigated the antibacterial activity of the methanolic extract and its fractions of aerial parts of Anthemis tinctoria (Asteraceae) against gram positive Staphylococcus aureus (ATCC 25923) and Enterococcus faecalis (ATCC 29212) and gram negative Escherichia coli (ATCC 25922) and Pseudomonas aeruginosa (ATCC 27853). The activity was concentrated mainly in the dichloromethane (DCM) and hexane fractions of crude methanolic extracts. The 5mg of DCM extracts per disk produced 15-16 mm of inhibition zone against E. faecalis and P. aeruginosa, however, no activity was found against E. faecalis and E. coli. The hexane fraction showed activity against S. aureus, P. aeruginosa and E. faecalis.

Manikandan et al. (2006) studied antibacterial activity of Aristolochia bracteata Retz (Aristolochiaceae), a common annul herb, widely distributed in India and widely
used in indigenous system of medicine. The leaves were extracted with petroleum ether, chloroform and alcohol. The concentrated crude leaf extracts were tested against *Bacillus subtilis*, *Lactobacillus plantarum*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus faecalis* and *Pseudomonas aeruginosa*. Alcoholic extract showed significant antibacterial activity when compared to other extracts.

The ethanolic extracts of the leaves and flowers of *Cleome viscosa* and roots of *Gmelina asiatica* were tested for antimicrobial activity. The two plants exhibited a broad spectrum of antimicrobial activity, particularly significative against *E. coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*. The leaf extract of *C. viscosa* showed moderate activity against pathogenic fungi (Sudhakar et al., 2006).

Pereira (2006) reported the antimicrobial activity of *Indigofera suffruticosa*. Various organic and aqueous extracts of leaves of *I. suffruticosa* Mill (Fabaceae) obtained by infusion and maceration were screened for their antibacterial and antifungal activities. The extracts were tested against five different species of human pathogenic bacteria and 17 fungal strains by the agar solid diffusion method. Most of the extracts were devoid of antifungal and antibacterial activities, except the aqueous extracts of the leaves by infusion, showed inhibitory activity against the gram-positive bacteria *Staphylococcus aureus* with a minimal inhibitory concentration (MIC) of 5000 g ml$^{-1}$.

The ethanolic extract of *Teclea afzelii* together with three alkaloids identified as Kokusaginine (1), Maculine (2), Kolbisine (4) and a common terpenoid, Lupeol (3),
isolated from the stem bark of *Teclea afzelii* were tested for their antimicrobial activity against Gram-positive and negative bacteria, fungi and *Mycobacterium smegmatis*. Agar diffusion assay was used for the determination of the sensitivity of test organisms to the samples. The micro-dilution method was used to determine the Minimal Inhibition Concentration (MIC) and the Minimal Microbicidal Concentration (MMC). The results of the diffusion test showed that only compound 1 was active on all the tested microorganisms, whilst the inhibition effect of the crude extract and that of compounds 2 and 4 was observed on 87.5% of the tested microbial species. The lowest MIC value (19.53 µg/ml) for the crude extract was obtained on *Escherichia coli*, *Bacillus subtilis* and *Microsporum audorium*. The corresponding value for the tested compounds (2.44 µg/ml) was recorded with compound 2 on *B. subtilis*. The crude extract, compounds 2 and 3 showed moderate activity against *M. smegmatis*. The overall results provide promising basis for the use of the crude extract as well as the isolated alkaloids in the treatment of specific microbial infections (Kuete *et al.*, 2008).

The antimicrobial activity of methanolic extracts of some medicinal plants against *Escherichia coli*, *Salmonella typhimurum*, *Staphylococcus aureus* and *Enterococcus* sp. The methanolic extract of *Caryophyllus aromaticus* presented the highest anti *S. aureus* activity and was effective against all bacterial strains tested (Ushimalu *et al.*, 2007).

The *in vitro* evaluation of antibacterial and antifungal activity was carried out by the well agar diffusion method on a panel of gram - positive and gram - negative bacteria such as *Pseudomonas. aeruginosa, Escherichia coli, Streptococcus faecalis,*
Salmonella cholera, Proteus mirabilis, Morganella morganii and two group of fungi (filamentous, yeast). The results showed that 12 of 17 extracts demonstrated antibacterial activity against the pathogenic bacteria tested. The growth inhibition holes were ranged from 8.00 to 32.33. Among them extracts of Solanum aculeasteum (Solanaceae) and Syngicum guinensis (Myctaceae) showed and higher antibacterial activity. For the antifungal activity growth inhibition holes varied from 8.00 to 17.55 mostly against Geotrichum candidum and Penicillium species. The extracts Solanum acculeasteum demonstrated antibacterial and antifungal activity (Pieme et al., 2008).

Antimicrobial activity of the leaf and root extracts of Indigofera tinctoria (Linn.), Wrightia tinctoria (Br.) and Rungia repens against human pathogenic bacteria such as Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis and pathogenic fungi such as Aspergillus niger, Aspergillus flavus and Aspergillus fumigatus. Ethanolic leaf and root extracts were prepared and based on the susceptibility of the test organisms were determined. It was found that ethanolic leaf and root extracts were prepared and based on the susceptibility of the test organisms were determined. It was found that ethanolic extracts showed high inhibition zone those control experiments (Madhavan and Saritha, 2008).

The extracts of Acacia arabica, Nymphaea lotus, Sphaeranthus hirtus, Emblica officinalis, Ginchorium intybus and Cardus marianum were tested in vitro against bacterial species and fungal species by well diffusion method and micro dilution methods. The patterns of inhibition varied with the plant extracts, the solvent used for
Biological activities of *Limonia crenulata* (Roxb.) extraction and the organisms tested. *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* were the most inhibited microorganisms. The extract of *Sphaeranthus hirtus* was the most active against multi drug resistant *Pseudomonas aeruginosa* and Enterohemoerrhagic *E. coli* 0157. The ethanolic extract of *S. hirtus* exhibited a higher effect than the hot water extract. These plants extracts were analysed for elemental composition (Ammara Hassan *et al.*, 2009).

Patil *et al.* (2009) was reported that *Aegle marmelos* (L.) (Rutaceae) possess a number of medicinal properties including antidiarrhoeal, antimicrobial, antifungal and activities. The antiaflatoxigenic effects of ethanolic extract of the leaves of *Aegle marmelos* (L.) were studied on common aflatoxigenic fungal species. *Aegle marmelos* (L.) exhibited antifungal and antiaflatoxigenic activity at a concentration range of 0.5 to 2 mg/ml. The shake flask method was used to evaluate the antifungal and antiaflatoxigenic activity. The extract showed varied levels of antifungal and antiaflatoxigenic activity against the test fungi. Preliminary phytochemical tests of ethanolic extracts demonstrated the presence of major phytochemicals like phenols, tannins, flavonoids and alkaloids as major constituents.

Sumathi and Parvathi (2010) analysed the antimicrobial activity of the extracts of *Andrographis paniculata* Nees; *Phyllanthus niruri* Linn; *Terminalia bellerica* Roxb.; *Terminalia chebula* Retz.; and *Vitex negundo* Linn., against four gram - negative and one gram - positive bacteria. The results showed that the minimum inhibitory concentration (MIC) of *P. niruri* leaf extract was 50 µg/ml against *Salmonella typhi* and
*Staphylococcus aureus*, where as, the MICs of *T. bellerica* fruit extract against *Escherichia coli* and *S. aureus* were 50 and 200 µg/ml respectively. However, the leaf extracts of the *Andrographis paniculata*, *T. chebula* and *V. negundo* have not shown any antimicrobial activity in the tested concentrations.

Venkatesan and Karrunakaran (2010) reported *Aegle marmelos, Solanum nigrum* and *Cassia fistula* were extracted by soxhlet extraction method. Three plant materials were subjected to preliminary phytochemical screening activity against gram - negative organism of *Escherichia coli* and gram - positive organism of *Staphylococcus aureus* and they were compared with control drug Penicillin at different concentrations at 0.5, 1.0, 1.5, 2.0, and 2.5 mg/ml by disc diffusion method. At higher concentration of 2.5mg/ml *Cassia fistula* exhibits maximum zone of inhibition of about 30.9 mm against *Staphylococcus aureus*, and was considered as susceptible. The zone of inhibition was not found in *Aegle marmelos* and *Solanum nigrum* and considered as resistant. In case of *Escherichia coli*, *Solanum nigrum* exhibits maximum zone of inhibition of about 30.1mm such zones were not found in *Aegle marmelos* and *Cassia fistula* and were considered as resistant and control drug penicillin shows less activity compared to the plant extract *Aegle marmelos, Solanum nigrum* and *Cassia fistula*.

### 3.13. Antioxidant activity

*Naringi crenulata* (Roxb.) Nicolson belongs to the family of Rutaceae. Its stem had been claimed to cure prickly rash on the skin and Burmese people used as a whitening agent. Previous studies of *N. crenulata* found to exhibit antityrosinase, anti-inflammatory and antioxidative activities and active substances are umbelliferone and scopoletin. The aim of this special project is to develop *N. crenulata* to be a
sunscreen lotion from standardized alcoholic extract. The experiment was carried on the percentage of alcohol for the suitable extraction and the result showed that 70% ethanol was the suitable by evaluating the percentage of active compound (Thin Layer Chromatography) and antioxidative activity (2, 2-Diphenyl-1picrylhydrazyl - DPPH method). The lotion preparation was prepared from 1% alcoholic extract. The lotion contained 0.28 milligram% and 0.51 milligram% of umbelliferone and scopoletin, respectively. Its antioxidative activity was showed at the concentration of 23.32 microgram/milligram and sunscreen effective activity (Sun Protection Factor - SPF) was 1.25 (Pensri and Kaewprakan, 2005).

The antioxidant activities of methanol and ethyl ether extracts obtained from the leaves of Thymus zygis, collected during the flowering or non-flowering period, were evaluated and compared by Soares et al. (1997). The results showed that the methanolic extracts are more potent as scavengers of peroxyl and superoxide radicals than the ethyl ether extracts. Apparently, there is a relationship between antioxidant potency and the total phenolic groups content in each extract.

A novel flavonoid diglycoside, 5, 6, 7, 4' - tetrahydroxyflavone 3 - O - rutinoside, and a previously known compound, kaempferol 3 - O - neohesperidoside were isolated from an ethyl acetate extract of Daphyniphyllum calycinum leaves showed significant antioxidant activity was reported by Gamez et al., (1998).
The *in vitro* antioxidant and free radical scavenging properties of bark extracts of *Anadenanthera macrocarpa* (Benth.) (Fabaceae), *Astronium uruncleuva* (Engl.) (Anacardiaceae), *Mimosa verrucosa* (Benth.) (Fabaceae) and *Sideroxylon obtusifolium* T.D. (Penn.) were determined by monitoring the intensity of luminal-enhance Chemiluminescence (CL), using 2, 2’azo bis (2-amidinopropane) as a peroxyl radical source (Desmarchelier et al., 1999).

Aquino et al. (2001) examined the methanolic extract of the leaves of *Anthurium versicolor* and isolated two main fractions. Both the extract and the fractions were assayed for their radical - scavenging activity by means of an *in vitro* test (bleaching of the stable 1, 1'-diphenyl 1 - 2 - picrylhydrazyl 1-2 radical) and reported to have a significant radical - scavenging effect.

Methanol extracts, prepared separately from the roots, stems and leaves of four traditional Zulu medicinal plants (*Rhoicissus digitata*, *R. rhomboidea*, *R. tomentosa* and *R. tridentata*), were tested for their antioxidant activity. The extracts of *R.rhomboidea* and *R. tridentata* inhibiter the activities of the 1, 1'-diphenyl 1 - 2 picrylhydrazyl free radical, zanthine oxidase and also prevented the production of thiobarbituric acid reactive substances and free radical mediated Deoxyribonucleic acid (DNA) sugar damage. The extracts have a strong chelating effect on Fe"" ions. *R. digitata* and *R. tomentosa* extracts, however, possessed some prooxidative properties at high concentrations (Opoku et al., 2002).
The antioxidant properties of six medicinal herbs used in the traditional Paraguayan medicine were studied using free radical generating systems by Velazquez et al., (2003). The methanol extracts from Asristolochia giberti, Cecropia pachystachya, Eugenia uniflora, Piper fulvescens, Schinus weinmannifolia and Schinus terebinthifolia protect against enzymatic and non-enzymatic lipid peroxidation in microsomal membranes of rat. C. pachystachys, E. uniflora and S. terebinthifolia showed the highest scavenging activity on the superoxide and 2, 2 diphenyl-1-picrylhydazyl (DPPH) radicals.

The antioxidant activity of the different extracts and fractions of a aerial parts of Otostegia persia (Burm) Boiss were evaluated using beta-carotene bleaching and lipid peroxidation method. The inhibitory activity of the plant extracts on the peroxidation of linoleic acid was measured by ferric thiocyanate method in comparison to methanolic extracts of green tea, Ginkgo biloba, Vitamin E and BHA as positive control. Methanolic extract of the plant exhibited strong antioxidant activity (Shrififar et al., 2003).

Kim et al. (2003) suggested that the n-hexane and butanol fractions of Artemisia apiacea have significant free radicals scavenging effect. This was confirmed through DPPH free radical scavenging activity.

Bajpai et al. (2005) indentified promising sources of antioxidants, from the leaves, bark and fruits of Terminalia arjuna, Terminalia bellerica, T. chebula and Terminalia muelleri. The leaves and fruits of Phyllanthus emblica and the seeds of
Syzygium cumini were also found to have high total phenolic contents (72.0 - 167.2 mg/g) and high antioxidant activity (69.6 - 90.6%).

The phenolic extracts of the dried 11 Algerian medicinal plants have been performed using 70% ethanol. The antioxidant activity measurement, expressed as Trolox Equivalent Antioxidant Capacity (TEAC), ranged from 9.40 to 33.06mM Trolox equivalent (Djeridane et al., 2006).

Antioxidant activity of methanol extract and ethyl acetate extract of root bark of firing tree (Chionanthus virginicus L.) were evaluated. Total antioxidant activity was measured according to ferric thiocynate method methanol extract and ethyl acetate extract showed 69.4, 79.3, 72.3 and 83.7% inhibition on lipid peroxidation of linoleic acid emulsion, respectively at the 10 and 20g/ml concentrations (Gulcin et al., 2007).

Umanaheswari et al. (2007) evaluated the antiulcer and antioxidant activities of 70% ethanolic extract of leaves of Jasminum grandiflorum L. They suggested that Jasminum grandiflorum possess potential antiulcer activity, which may be attributed to its antioxidant activity. The in vitro antioxidant activities of the methanol extract of Paullinia pinnata leaves were evaluated using different testing systems by Jimoh et al., (2007). The results showed that P. pinnata possessed strong scavenging activity and moderate reducing power. The total phenol, flavonoid and proanthocyanidin contents of the extracts were very close to those reported for most medicinal plants and showed good correlation with its antioxidant activities.
Four extracts of *Enicostemma axillare* were examined for *in vitro* antioxidant activity using nine different methods. In the 2,2'-azino-bis 3-ethylbenzthiazoline-6-sulphonic acid (ABTS) method. All the four extracts of *E. axillare* showed potent antioxidant activity with half maximal inhibitory concentration (IC$_{50}$) values, ranging from 13.26 to 24.36 g/ml. All extracts showed moderate antioxidant capacity using the phosphomolibdenenum method (Jaishree *et al*., 2008).

Adeolu *et al.* (2008) were studied the antibacterial antioxidant activities and phenolic contents of methanol extracts of the leaves and stems of *Calpurnia aurea* using *in vitro* standard method. The antioxidant activities were determined by DPPH and Ferrous Reducing Antioxidant Pathway (FRAP) method which showed significant result.

Nickavar *et al.* (2008) evaluated the antioxidant and free radical scavenging properties and determine the phenolic content of the ethanol extract from five *Mentha* species. *M. piperita* exhibited the strong activity as a DPPH scavenger. All the extracts were active in ABTS$^+$ assay and no significant difference was observed in this assay. The total phenolic content of extract was determined by Folin-ciocalteu method and *M. piperita* showed the highest Total Phenolic Content (TPC).

Elango and Chithra (2009) studied that effect of *Limonia acidissima* L. (Rutaceae) on blood glucose levels and antioxidant enzyme levels in Alloxan induced diabetic rats. Alloxan (120 mg/kg, i.p) induced diabetic rats were treated with *Limonia*
*Limonia crenulata* (Roxb.) *acidissima* L. methanolic extract at a dose levels of 200 and 400 mg/kg for 21 days. Glucose level was measured in blood serum and antioxidant enzyme levels viz. Superoxide Dismutase (SOD), Catalase (CAT) and Lipid Peroxidation (LPO) were measured in pancreatic homogenate, methanolic extract of fruit pulp of *Limonia acidissima* L. significantly (P<0.01) lowered the Alloxan induced hyperglycemia. It also produced a significant (P<0.01) decrease in peroxidation products viz. Malonyl Dialdehyde (MDA) in blood serum. The activity of antioxidant enzymes such as SOD, CAT was found to be higher in the blood serum of diabetic animals treated with the *Limonia acidissima* L. extract. This confirms the antihyperglycemic and antioxidant activities of *Limonia acidissima* L. in Alloxan induced diabetic rats.

Dhruti *et al.* (2009) studied that the antioxidant activity of the methanolic and aqueous extracts of *Martynia annua* (Linn.) leaves were evaluated by several *in vitro* systems of assay namely such as reducing power assay, DPPH radical-scavenging activity, nitric oxide scavenging activity, H$_2$O$_2$ radical scavenging activity, superoxide radical scavenging assay, hydroxyl radical-scavenging activity, and total antioxidant capacity. Total phenolic content was measured by Folin–Ciocalteau reagent. The antioxidant property depends upon concentration and increased with increasing amount of the extract. The free radical scavenging and antioxidant activities may be attributed to the presence of phenolic and flavonoid compounds present in the extract. The results showed that the methanolic extract exhibited higher antioxidant activity than the aqueous extract. Chlorogenic acid is reported in this plant and a TLC densitometric method was developed for the quantification of chlorogenic acid.
Mahesh Kumar (2010) studied that in vitro antioxidant activity of methanolic extract of aerial parts of Salvia splendens was determined by DPPH free radical scavenging, hydrogen peroxide scavenging and superoxide anion scavenging assays. Ascorbic acid and butylated hydroxyl anisole were used as standard antioxidants for the analysis. All the analysis was made with the use of UV–Visible spectrophotometer (Schimadzu uv-vis 1700). The methanolic extract of aerial parts of Salvia splendens had shown very significant DPPH (1, 1, Diphenyl-2-picryl-hydrazyl) radical scavenging, hydrogen peroxide scavenging, and superoxide anion scavenging activity compared to standard antioxidants. The IC$_{50}$ values of methanolic extract in DPPH radical scavenging, hydrogen peroxide scavenging and superoxide anion scavenging assays are found to be 460 µg/ml, 358 µg/ml and 527 µg/ml respectively.

The fruits of Terminalia chebula, Terminalia bellerica and Emblica officinalis are important herbal raw materials containing polyphenols. They form the major constituents of widely used Ayurvedic formulations like Triphala churna. The extracts of these materials were standardized with respect to their total polyphenol contents as determined by Prussian blue method using gum acacia and phosphoric acid as stabilizers. The antioxidant activities were determined by DPPH (1,1-diphenyl-2-picryl-hydrazyl) method and inhibition of lipid peroxide formation induced by Fe$^{2+}$-ascorbate system. They were found to strongly correlate with total polyphenol contents. The half maximal effective concentration (EC$_{50}$) value (µg/ml) for free radical scavenging activity by DPPH method and IC$_{50}$ value (µg/ml) for lipid peroxidation inhibitory activity along with the total
polyphenol contents can be used as quality control parameters for standardization of herbal raw materials containing polyphenols as major phytoconstituents (Hazra et al., 2010).


Gossypin is a bioflavonoid obtained from *Hibiscus vitifolius* flower. The anti-inflammatory activity of gossypin has been studied in comparison with the standard non-steroid anti-inflammatory agent phenyl butazone against various experimental models of inflammation and increased vascular permeability. The migration of leucocytes and the formation of pleural exudates were also significantly reduced after pretreatment with gossypin in carageenin and turpentine induced rats (Palmer and Ghos, 1978).

Alcoholic extract of Teeburb has been found to produce significant anti-inflammatory activity at 2\textsuperscript{nd} and 3\textsuperscript{rd} hours of administration on carrageenin induced rat paw oedema. Phenylbutazone has been found to be having significantly higher anti-inflammatory activity than Teeburb suggesting similar mechanism of action of these drugs (Sharma and Srivastava, 1991).

Petroleum ether extract of *Ricinus communis* exhibited significant anti-inflammatory activity against induced rats paw arthritis. Drug was safe upto a dose of mg/kg per oral (p.o). and at a dose 150 mg/kg p.o. exhibited no significant analgesic activity in rats (Banerjee et al., 1991).
Triterpenoids are one of the most abundant class of compounds in plants. It has frequently been suggested that triterpenoids play an defensive role against pathogens and herbivores. They also have several interesting pharmacological activities that include anti-inflammatory (Recio et al., 1995), antibacterial (Cantrell et al., 2001), antiviral and cytotoxic properties.

Methanolic extract of dried leaves of *Alstonia macrophylla* and its fractions were investigated for its anti-inflammatory activity. The extract was concentrations of 200 and 50 mg kg$^{-1}$, p.o. showed the dextran - induced granuloma (Chronic model) in rats. Anti-inflammatory activity of the tested extract and its fractions was comparable with that of the standard drug indomethacin (10 mg kg$^{-1}$) (Arunachalam et al., 2002).

Petrovic et al. (2003) reported that oral administration of the chloroform extract from *Tanacetum larvatum* (Griseb. ex aptn) Kanitz caused a dose dependent anti-inflammatory effect in the carrageenan - induced rat paw oedema test and the results showed statistical significance at a dose of 50 mg/kg.

Patil et al. (2003) investigated the ethanolic crude extract of *Anacardium occidentale*. leaves and its five different crude fractions for anti-inflammatory activity in albino rats (300 mg/kg). Ethanol extract and butanone fraction exhibited significant anti-inflammatory activity when compared with control and standard drug diclofenac sodium (100 mg/kg).
Crude ethanolic extract of _Pergularia extensa_ leaves was fractioned with petroleum ether, solvent ether, ethyl acetate, butanol and butanone. The ethanolic extract and various fractions were investigated for anti-inflammatory activity in rats at a dose of 100 mg/kg intraperitoneally. Ethanol extract and its butanol fraction exhibited significant anti-inflammatory activity when compared with respective controls and were comparable with that of standard drug aspirin (Hukkeri _et al._, 2003).

Vishnoi _et al._ (2003) reported that the methanol and petroleum ether extracts of _Abies webbiana_ leaves exhibited significant anti-inflammatory activity against carrageenan induced rat hind paw oedema, the percentage protection was found to be effective as comparable to that standard drug diclofenac sodium.

Steroids, steroidal saponins, flavonoids, triterpenoidal and carbohydrates were detected in different extracts of the stem of _Neptunea oleracea_. The alcoholic extract was found to possess significant anti-inflammatory activity (Lakshmayya Joshi _et al._, 2003).

The anti-inflammatory effect of the methanol extract of the leaves of _Bambusa arundinacea_ against carrageenan induced as well as immunologically induced paw edema and also its antiulcer activity in albino rats have been studied and found to be significant when compared to the standard drugs. The combination of methanol extract and phenylbutayone (Non - Steroidal Anti-inflammatory Agent, NSAIA) has also been studied and found to have the most potent anti-inflammatory activity experimentally with least toxic (no ulcerogenic) activity (Muniappan and Sundararaj, 2003).
Various extracts of *Indigofera aspalathoides* were tested for anti-inflammatory activity using carrageenan induced paw edema assay, to isolate bioactive compounds from active extracts and to screen the isolated compounds for *in vitro* COX-1/COX-2 inhibitory activity. The flavonoid rich fraction was obtained from ethyl acetate extract. Repeated chromatography followed by crystallization of flavonoid rich fraction afforded the isolation of four compound viz., 4,2,4 – trihydroxyflavone and lutedin. The compounds, butein and 7,3,4 – trihydroxy flavone were tested *in vitro* for COX 1/COX 2 inhibitory activity. Both the compounds were COX-1 selective and the butein (28.4 micro M) was found to be potent than 7,3,4-trihydroxy flavone (35.7 micro M). Both the compounds showed moderate inhibitory activity towards COX-2. The observed percentage inhibition value at 100 micro g/ml where 37.2 and 30.3 percent for butein and 7,3,4-trihydroxy flavone, respectively (Brahmbhatt *et al.*, 2004).

The intra peritoneal administration of the methanol extract of *Oenothera rose* at a dose of 400 mg/kg produced a high reduction of hind - paw edema was reported by Meckes *et al* (2004). They also demonstrated a moderate inhibition of edema formation with the methanol extracts of *Astianthus viminalis*, *Brickellia paniculate*, *Chamaedorea tepejilote* and *Justicia spicigera*.

The antiociceptive, anti-inflammatory effect, and acute toxicity of the aqueous extract from leaves of *Pimenta racemosa* have been investigated by Garcia *et al.* (2004). The aqueous extract (125 and 250 mg/kg) significantly and in a dose - dependant manner
Biological activities of *Limonia crenulata* (Roxb.) reduced the nociception induced by the acetic acid intraperitoneal injection (P less than 0.001) and also reduced the carrageenan-induced paw in rat at 1, 3 and 5hrs (P< 0.001).

*Hibiscus* species have been used as folk remedy for the treatment of skin diseases, as an anti-inflammatory agent, antiseptic and carminative some compounds isolated from the sepsis, such as flavonoids, phenolic acids, are considered responsible for these activities (Vasudeva and Sharma, 2008).

Gupta1, *et al.*, (2010) investigated the various extracts of leaves of *Bryophyllum pinnatum* in chemically induced inflammation rodents model. The extracts/ inhibited formaldehyde induced paw edema in rats. These inhibitions were statistically significant (p<0.05-0.01, 0.001) as compared to control. Methanolic extract showed highest activity.

Modi *et al.*, (2010) analysed the anti-inflammatory activity of the water extract of *Argyreia nervosa*. Inflammatory diseases including different types of rheumatic diseases are very common throughout the world. *Argyreia nervosa* is used as a folk medicine for the treatment of inflammation in India. The plant *Argyreia nervosa* possesses a significant anti-inflammatory activity as evidenced in carrageenan induced paw edema method, which supports the folkloric claim of the anti-inflammatory activity of the plant.

### 3.15. Antiulcer activity

Peptic ulcer is a lesion of gastric or duodenal mucosa occurring at a site. The mucosal epithelium were exposed to aggressive factors (gastric acid & pepsin) and
mucosal defensive factors (blood flow, mucus, bicarbonate - HCO₃ secretion, etc) (Sun, 1974).

Ulcer is a benign lesion of the gastric or duodenal mucosa, which occurs at a site where the mucosal epithelium is exposed to acid and pepsin stress, smoking, nutritional deficiencies and ingestion of nonsteroidal anti-inflammatory drugs can all increase the incidence of gastric ulcer (Belaiche et al., 2002). The mechanism of formation of peptic ulcer (Baron et al., 1980; Piper and Stiel, 1986), its treatment (Sung et al., 1995; Soll, 1996) and the action of the antiulcer and aspects of their adverse reactions and the recurrence of the ulcer have been reviewed (Ariyoshi et al., 1986).

Okwari et al., (2000) studied the effect of an aqueous extract of the leaves of Dombeya buettneri on gastric acid secretion and ethanol-induced gastric mucosal damage in rats. Pretreatment with the extract also reduced the extent of gastric mucosal damage induced by oral ethanol (75%), but had no effect on mucus secretion. It is suggested that the consumption of an extract of the leaves of D. buettneri may be beneficial in the prevention and treatment of peptic ulcer disease.

The antiulcerogenic effects of ethanol extract of the bark from Vocanga atricana were studied by Jannet et al. (2000) using albino rats. The effects of the extract on the volume of gastric juice, gastric pH, acid output, mucus production and peptic activity were recorded. Oral administration of the extract (500 - 750 mg.kg) inhibited the formation of gastric lesions induced by hydrochloric - HCl or ethanol (40-63%
inhibition) was also studied. The extract significantly reduced gastric lesion formation in pylorus ligated rats.

The 10% ethanol extract of the aerial of *Calligonum comosam* at different concentrations (100, 200 and 400 mg/kg) produced a significant and dose dependent inhibition to the acute gastric ulcers induced by phenylbutazone indomethacin 0.2 N sodium hydroxide (NaOH) and 80% ethanol (Liu *et al*., 2002).

The effect of alcoholic extract of *Nigella sativa* in rats was investigated by Rajkapoor *et al*., (2002) to evaluate the antiulcer activity by using two models, i.e, pyloric ligation and aspirin induced gastric ulcer. The results indicated that the alcoholic extract significantly (P<0.001) decreased the volume of gastric acid secretion, free acidity, total acidity and ulcer index with respect to control.

*Eclipta alba* caused a significant reduction in ulcer index in all animal models and a significant inhibition was also observed in aspirin - induced gastric ulceration and secretion in pylorous ligated rats (Venkatesan *et al*., 2002).

The ulcer protective potential of methanolic extract of *Embla officinalis* was assessed in different acute gastric ulcer models in rats induced by aspirin, ethanol, cold restraint stress and Pylorus ligation healing effect in chronic gastric ulcers induced by acetic acid in rats. *Embla officinalis* extract, 10-50 mg/kg administered orally, twice daily for 5 days showed dose-dependent ulcer protective effects in all the above acute
models and significant ulcer healing effect in dose of 20mg/kg after 5 and 10 days treatment. The gastric mucosal factors showed that it significantly decreased the offensive factors like acid and pepsin and increased the defensive factors like mucin secretion, cellular mucus and life span of mucosal cells. Emblica officinalis extract showed significant antioxidant effects in stressed animals and did not have any effect on cell proliferation in terms of DNA micro g/mg protein or glandular weight (Sairam et al., 2002).

Aqueous extract of the leaves of the Limonia acidissima L., was administered orally for seven to fifteen days of the carbon tetra chloride CC14 administered rats. The results showed the extracts have antiulcer activity when compare to normal group and standard drug Liv52 administered rats (Karmat et al., 2003).

Oral administration of butanol fraction of Gynostoma pentaphyllum (GPB) at 200 and 400 mg/kg weight significantly inhibited gastric ulcer induced by indomethacin, HCl/EtOH and water immersion restraint stress in rats. In ethanol induced ulcerated rats, gastric wall mucus and hexosamine content were markedly preserved by GPB pretreatment. The findings indicated that the butanol fraction of G. pentaphyllum possesses gastric protective potential related to the preservation of gastric mucus synthesis and secretion (Rujjanawate et al., 2004).

Ocimum sanctum (OS) is known to possess various therapeutic properties. Dharmani et al., (2004) evaluated its antiulcerogenic activity in aspirin, alcohol and pyloric ligation (PL) induced gastric ulcer models in Sprague Dailey rats, histamine
induced duodenal ulcer in guinea pigs and ulcer healing activity in acetic acid induce chronic ulcer model. The result indicated that *O. sanctum* not only decreased the incidence of ulcers but also enhanced the healing of ulcers.

The decoctions were collected from the aerial parts of *Malva niglecta* (Kallr.) (Malvaceae), leaves of *Potentilla reptans* L. (Rosaceae) fruits of *Runex patientia* L. (Polygonaceae), aerial parts of *Siderites caesarea* Daman (Lamiaceae) and flowers of *Verbasaem cheicanthi* Boits var. (Scrophulariaceae) showed significant gastric protection against the ethanol induced gastric ulcer model in rats. Furthermore, healing effects were also confirmed through histopathological examination (Gurbuz et al., 2005).

Peptic Ulcer Disease (PUD) is a serious gastrointestinal disorder that requires a well targeted therapeutic strategy. A number of drugs including proton pump inhibitors and $\text{H}_2$ receptor antagonists are available for the treatment of peptic ulcer, but clinical evaluation of these drugs has shown incidence of relapses, side effects, and drug interactions. This has been the rationale for the development of new antiulcer drugs and the search for novel molecules has been extended to herbal drugs that offer better protection and decreased relapse. Drugs of plant origin are gaining popularity and are being investigated for a number of disorders, including peptic ulcer. The antiulcerogenic and ulcer healing property of *Ocimum sanctum*, *Allophylus serratus*, *Desmodium gagenticum*, *Azadirachta indica*, *Hemidesmus racemosus*, *Asparagus racemosus* and *Musa sapientum* were evaluated. The above plants reported that they have ulcerhealing activity. Ayurvedic knowledge supported by modern science is necessary to isolate,
characterise, and standardise the active constituents from herbal sources for antiulcer activity (Dharmani and Palit, 2006).

The bark of *Anogeissus latifolia* (Combretaceae) has been reported to be used in the treatment of various disorders including stomach and slain disease. Govindarajan *et al.* (2006) studied the anti-ulcer potential and antimicrobial activity of the 50% aqueous alcoholic extract in order to validate ethnobotanical claims. *A. latifolia* extracts possessed gastro protective activity as evidenced by its significant inhibition in the formation of ulcers induced by physical and chemical agents.

Andrade *et al.* (2007) evaluated the antiulcerogenic property of the hydroalcoholic extract of aerial parts of *Maytenus robusta*. The effects of the extract on gastric content volume, pH and total acidity in the ethanol induced ulcer model showed that the extract significantly reduced the lesion index such as 75.1 ± 8.9 ± 7.4 and 75.5 ± 5.37 when treated with 50, 250 and 500 mg/kg of *M. robusta* respectively. They also observed significant inhibition in lesion index in the indomethacin induced ulcer model. Regarding the model of gastric secretion, a reduction in gastric juice and total acidity were observed, as well as an increase in gastric pH.

The *Plectranthus barbatus* (Lamiaceae) aqueous extract and isolated compounds were assayed *in vivo* in pylorus ligated mice, and *in vitro* on acid secretion measured as (14 C-aminopyrine (14C-AP) accumulation in rabbit gastric glands and gastric H+, K+, adenosine triphosphate - ATPase preparations injected into the duodenal lumen, the
aqueous extract of the plant leaves (0.5 and 1.0g/mg) decreased the volume (62 and 76 percent) and total acidity (23 and 50 percent) of gastric acid secretion in pylorus ligated mice showing antiulcer activity (Schultz et al., 2007).

Ashok Kumar et al., (2010) reported the antiulcer activity of aqueous extract of Physalis minima was investigated in experimental animal (rats). Gastric ulcers were induced by oral administration of 1ml ethanol 80%, the animals were divided in to six groups; in each group contain six animals. Administered aqueous extract of Physalis minima in two dose 100 mg & 200 mg/kg body weight by oral route before one hour to administration of ethanol. The extract at doses of 100 and 200 mg/kg significantly (P<0.05) showed an antiulcer effect characterized by reduction of acid volume (AV), free acidity (FA), total acidity (TA), total protein (TP), ulcer index (UI), Lipidperoxidation (LPO), and increasing rate of pH, Glutathione (GLU), and Catalase (CAT) when compared to the control group. The results suggest that the extract acts to produce significant ulcer protective property.

Bhalke et al., (2010) investigated the antiulcer effect of ethanolic extract of leaves of S. grandiflora using different models of gastric ulceration in rats. Acute gastric ulceration in rats was produced by oral administration of various noxious chemicals including aspirin or ethanol or indomethacin. Gastric total acid output was estimated in the pylorus ligated rats. Gastric tissue was also examined histologically. The ethanolic extract of leaves of S. grandiflora was administered in the dose of 400 mg/kg orally in all experiments. Omeprazole, ranitidine, misoprostol were used as a reference drug. The
ethanolic extract of leaves of *S. grandiflora* at the dose of 400 mg/kg produced a significant reduction in the ulcer index.

### 3.16. Antidiarrhoeal activity

The effectiveness of *Aegle marmelos* fruit in diarrhoea and dysentery has resulted in its entry into the British Pharmacopoeia. This plant was also called as a *Rasayana* (Pandeya *et al.*, 1983). The pathogenesis of infectious diarrhoea has been widely studied. Enteric pathogens have evolved a remarkable array of virulence traits that enable them to colonize the intestinal tract. These organisms colonize and disrupt intestinal function to cause mal-absorption or diarrhoea by mechanisms that involve microbial adherence and localized effacement of the epithelium, production of toxin and direct epithelial cell invasion (Guerrant *et al.*, 1999). The traditional use of *A. marmelos* unripe fruit has an antidiarrhoeal which was also reported. *A. marmelos* is effective in chronic cases of diarrhoea due to the presence of large quantities of mucilage, which act as a demulcent. Additionally, *A. marmelos* has been shown to be effective in experimental models of irritable bowel syndrome and physiological diarrhoea (Shoba and Thomas, 2001; Jagtap *et al.*, 2004; Dhuley, 2003).

Diarrhoea is one of the main causes of morbidity and mortality in children under age of 5 years. In view of this problem, the WHO has a Diarrhoea Disease Control Program, which includes studies of traditional medical practices together with the evaluation of health education and prevention approaches. A review of the last 7 years
about the studies of extracts of plants used to combat diarrhoea in different countries has been done (Gutierrez et al., 2007).

The antidiarrhoeal effect of ethanolic extract of the dried fruit pulp of *Aegle marmelos* (L.) was studied on various intestinal pathogens. It showed excellent activity against *Shigella boydii*, *S. sonnei* and *S. flexneri* whereas the activity was found to be moderate against *S. dysenteriae*. The minimum inhibitory concentration against the strains of *Shigella* was recorded between 250 to 500 μg/ml. Preliminary phytochemical tests of extract demonstrated the presence of common phytochemicals including phenols, tannins and flavonoids as major active constituents (Joshi et al., 2009).

*Aegle marmelos* (L.) Correa has been widely used in indigenous systems of Indian medicine due to its various medicinal properties. However, despite its traditional usage as an antidiarrhoeal there is limited information regarding its mode of action in infectious forms of diarrhoea. The hot aqueous extract (decoction) from dried unripe fruit pulps of *A. marmelos* and effect on various aspects of pathogenicity of infectious diarrhoea (Brijesh et al., 2009).

Meite et al. (2009) studied the ethyl acetate extract of *Morinda morindoides* (Baker) Milne-Redh (Rubiaceae) properties against experimental diarrhoea induced by castor oil in albino Wistar rats. The ethyl acetate extract of *Morinda morindoides* (250, 500, and 1000 mg/kg body weight) was administered orally to three groups of rats (five animals per group) in order to evaluate the activity of the extract against castor oil-induced diarrhoea model in rat. Two other groups received normal saline (5 mg/kg) and
loperamide (5 mg/kg) as positive control. The effect of the extract on intestinal transit and castor oil-induced intestinal fluid accumulation (enteropooling) was assessed. At oral doses of 250, 500, and 1000 mg/kg body weight, the plant extract showed pronounced and dose-dependent antidiarrhoeal activity. The protective role of the extract at 1000 mg/kg was comparable to that of the reference drug, loperamide (5 mg/kg). The extract (1000 mg/kg) produced a decrease in intestinal transit comparable to atropine (5 mg/kg), and significantly (p < 0.01) inhibited castor oil-induced enteropooling. No mortality and visible signs of general weakness were observed in the rats following the extract administration of up to a dose of 6000 mg/kg. The results showed that the extract of *M. morindoides* has a significant anti-diarrhoeal activity which supports its use in traditional herbal medicine practice.

Senthilkumar et al. (2010) reported that the antidiarrhoeal activity and gastrointestinal motility reducing activity of alcoholic and aqueous extract of bark of *Limonia acidissima* L., was evaluated at two dose levels. Both the extracts showed significant antidiarrhoeal activity and reduced the mean weight of faeces and reduced the gastrointestinal motility significantly.

Akter et al. (2010) was scientifically evaluated hydromethanol extract of *Curcuma alimatifolia* leaves for its antidiarrhoeal and antioxidant properties. Antidiarrhoeal property was studied using castor oil and MgSO₄ induced diarrhoeal models and charcoal induced gastrointestinal motility test in swiss albino mice. In all of these experimental models the extract, at higher dose (500 mg/kg body weight), exhibited
significant ($p < 0.05$) antidiarrhoeal property compared to the control. The extract was found to possess high amount of phenols and flavonoids, expressed as gallic acid and quercetin equivalents respectively, that indicate the usefulness of *C. alismatifolia* leaves in diarrhoeal disease and other disorders linked to free radical-mediated oxidative stress.

The bioassay guided fractionation of the n-hexane extract of the seeds of *Murraya koenigii* Spreng (Rutaceae) resulted in the isolation of three bioactive carbazole alkaloids, kurryam (I), koenimbine (II) and koenie (III). Of the three compounds (I) and (II) exhibited significant inhibitory activity against castor oil-induced diarrhoea and Prostaglandin E2 -induced enteropooling in rats. The compounds also produced a significant reduction in gastrointestinal motility in the charcoal meal test in Wistar rats (Mandal *et al.*, 2010).

3.17. Volatile oil

The effects of the time of year and the time of day of harvesting on essential oil concentrations, yield, and percentage of the principle component in *Lemon verbena* were reported by Vogel *et al.* (1999). Maximum essential oil concentration was found in young leaves in October with 0.95 ml/100 g dry weight, decreasing to values of 0.09 to 0.20 ml/100 g dry weight from December to April. Essential oil yield increased from October to March, reaching a maximum of 27.3 l/ha. Citral content was highest in November (up to 64%), than decreased to reach a minimum in December (34%). From February values maintained stable (52–45%). In November largest leaf areas were found with 9.2
cm$^2$/leaf. No differences in essential oil concentration between different times of day of harvesting could be found.

The amount and composition of the essential oil from leaves of *Hypericum androsaemum* (Linn.) cultivated in Arouca (Portugal) were determined in six samples harvested during 1 year at intervals of 2 months. The seasonally dependent essential oil content ranged from 0.7 mg/g biomass dry weight in September to 3.4 mg/g in February. The oil contained more than 80 compounds, 70 of which (constituting 88–93% of the total oil) were identified by GC and GC-MS. An approximation of the absolute quantification of each compound and compound class was performed using a GC method with an internal standard. The relative and the absolute content of each compound and compound class changed during the year. At the end of the winter and in the spring, the essential oil was dominated by sesquiterpene hydrocarbons and accumulated a high number of intermediate to long chain $n$-alkanes and 1-alkenes. In September, the essential oil contained the lowest levels of sesquiterpene hydrocarbons (43%) and the highest levels of 1-octene and 2-hexenal (38%). In February, the essential oil had the highest level of sesquiterpene hydrocarbons (73%) and the highest diversity of intermediate to long chain $n$-alkanes and 1-alkenes (Guedes *et al*., 2004). The total amount of essential oil obtained was 22 percent (w/w), which is higher than any species of the genus *Pistacia* (Delazar *et al*., 2004).

3.18. GC–MS analysis of volatile oil
Essential oils of nine cultivars of ginger (*Zingiber officinale*) were evaluated by gas chromatography. The selection of these cultivars is based on the yield of essential oils and gingerol content in oleoresin. The levels of 13 identified and 6 unidentified compounds of ginger oil are presented. The role of beta-sesquiphellandrene, zingiberene and curcumene in ginger aroma is described by Gopalam and Ratnambal (1989).

The essential oil of *Cedrus deodara* obtained in a yield 2.1 percent hydro distilling its saw dust, GC-MS analysis of its oil revealed the presence of 23 compounds out of which 7-compounds are uncharacterized sesquiterpenoids (Nigam *et al*., 1990).

The essential oil, obtained from the shade-dried leaves of *Buddleia asiatica* by hydodistillation in a yield of 0.3 percent, was analysed by chromatographic spectroscopic and chemical methods. Eighteen components including various monoterpenoids and sesquiterpenoids were characterized and measured by area normalization. The oil was found rich in beta-caryophyllene oxide (21.7 percent), citronellol (16.7 percent) remained uncharacterized. The oil has been reported to possess good *in vitro* antifungal, antibacterial and anthelmintic activity (Garg and Dengre, 1992).

The essential oil of *Mammillaria bombycina* was produced by hydro distillation from fresh leaves. The oil was analysed by GC - MS and 40 components were identified, which constituted 93.7 percent of the oil. The major components of the oil were decanal (12.5 Percent), 11-dodecenal (8.1 percent) and dodecanal (26.5 percent) (Choudhury and Leclercq, 1995).
The cultivation of *Eucalyptus globules* and *E. citriodora* at about 7000 feet height in the two hills has attained considerable economic importance, former as the source of 1,8-cineole rich essential oil and the latter for citronellal rich essential oil for perfumery and fragrance industry. The leaves are used for the production of essential oil about 80 percent of the country’s total production of about 500 tons of *E. globules* oil is from Palni and Nilgiri hills of Tamilnadu. *E. citriodora* was cultivated in a limited area in Nilgiris (Kakaraparthi and Sushil Kumar, 1999).

Qualitative and quantitative variations of the essential oil from leaves of *Limonia acidissima* L., collected from the different regions was observed by the use of GC-MS. The oils contains rich amount of methyl chavicol (68.3%), Linalool, caryophyllene, cis-anethole, p-methoxy phenyl-2-propanone, elemicine, 3,4-dimethoxy benzaldehyde, 3-dimethoxy cinnamic aldehyde and p-methoxy cinnamic alcohol are first reported in the oily (Garg, 2003).

The essential oil of the aerial parts of *Achillea albicaulis* was analysed by Capillary GC - MS. The major constituents were 1, 8 - cineole (10.1%), camphoir (9.2%), germacrene D (7.8%), piperitone (6.2%), alpha - pinene (5.9%) and artemisia ketone (5.7%) (Feizaakhsh et al., 2003). Bello et al., (2003) studied the chemical composition of the leaf oil of *Psidium salutare* by GC-MS. Thirty - four compounds were identified with caryophyllence oxide (39.8%) and turmerone (17.3%) as the major ones.
Agarwal and Rangari (2003) phytochemically investigated and evaluated the anti-inflammatory and anti-arthritic activities of essential oil of *Strobilanthes ixiocephala* (Benth.) Column chromatographic fractionation of essential oil obtained by hydrodistillation from the flowering tops of *S. ixiocephala* resulted in the isolation of β-caryophyllene, fenchyl acetate, T-cadinol and a new sesquiterpene alcohol for which a name ixiocephol has been proposed. The GC-MS analysis of the essential oil has also revealed the presence of various monoterpenoids and sesquiterpenoids. The β-caryophyllene and fenchyl acetate were identified by Co-TLC with authentic samples whereas T-cadinol and ixiocephol were structurally elucidated by UV, IR, $^1$H NMR, $^{13}$C NMR and mass spectral data.

Asadipour *et al.* (2003) investigated the volatile oil obtained from the aerial parts of *Cymbopogon oliveri* grown in Kerman Province, Iran by GC and GC-MS. Fifteen components were detected, representing 93.5% of the total oil, piperitone (61.10%), beta-caryophyllence (14.4%), delta-O-carene (6.5%) and beta-eudesomol (4.2%) were the major constituents of the oil.

The volatiles of the fruit of *Limonia acidissima* L. were analysed by GC and GC - MS. Character Impact odorants of the fruit were systematically characterized by Aroma Extract Dilution Analysis (AEDA) with GC–Olfactometry (GC-O). A total of 75 compounds were identified including 28 esters, 11 alcohols, one acetal, 10 ketones, four lactones, one heterocyclic, four aliphatic hydrocarbons, one of furan and five acids. However, only 44 volatiles were identified by GC-O. Among these, compounds, with the
most impact were ethyl butyrate (Fruit, Sweet, Banana like) and methyl valeric acid, 1-octen-3-01, Pentyl isobutyrate, 2-ethylhexanoic acid, ethyl octanoate, gamma-decalactone, 2,3-pentanedione, 3-octanone, 5-methyl-3-heptanone, 9-methyl-5-imdecene and (E)-2-hexenyl butyrate seem to contribute to Wood apple fruit flavor (Apriyantono and Kumara, 2004).

The volatile compounds, obtained by hydro distillation of the aerial parts of Rosmarinus officinalis, were analysed by GC - MS. Thirty compounds were characterized representing 98.2% of the essential oil with 1, 8 - cineole (29.5%), 2 - ethyl 1-4, 5 - dimethylphenol (12.0%) and camphor (11.5%) as the major components (Touafek et al., 2004).

The volatile components of the aerial parts of Ruta graveolens and Haplophyllum suaveolens, as well as leaves of Zanthoxylum limoncello, Z. panamense and Z. setulosum have been studied by GC - MS analysis. The biggest amount of aliphatic fatty acids was found in Z. panamense (17.2%), followed by R. graveolens (16.5%), Z. limoncello (14.7%), H. suaveolens (9.2%) and Z. setulosum (5.3%). Pentanoic, hexanoic, octanoic and nonanoic acids are common compounds in the volatiles of the five species (Ivanovaa et al., 2004).

The oleoresin of Pistacia atlantica var. mutica growing in different regions of Iran, is a popular, naturally occurring chewing gum and has been used traditionally in the treatment of peptic ulcer. The GC - MS analysis of the essential oil, obtained from steam
distillation of the oleoresin terpenoids, showed alpha pinene was the major constituent (70%) followed by limonene oxide (9%) and myrienol (5.31%) (Delazar et al., 2004).

*Cestrum diurnum* is a single or multistemmed shrub that is also known as Day Jasmine. The essential oil of the mature leaves of *C. diurnum* was analysed by Gas Liquid Chromatography (GLC) and Gas Liquid Chromatography – Mass Spectrometry (GLC-MS) and altogether 14 components were detected. The main constituents were palmitic acid, stearic acid and oleic acid (Bhattacharjee et al., 2005).

Nurettin et al. (2006) reported that the essential oil of air-dried *Minuartia meyeri* Bornm. (Caryophyllaceae) was analysed by GC-MS and fifty-two components were identified in the oil. The main components in the essential oil of *M. meyeri* were nonacosane (6.2%), 6,10,14-trimethyl-2-pentadecanone (5.1%), nonanal (4.6%), and α caryophyllene (2.9%).

Rai et al. (2006) studied the highly fragrant essential oil of the flowers of *Carissa opaca* (Apocynaceae). A total of 20 compounds accounting for 99.5 percent of the oil, were identified. The main component was palmitic acid (82.5 percent). Other major compounds were benzyl salicylate (6.0 percent), benzyl benzoate (4.6 percent) and (E,E)-alpha-farnesene (3.5 percent). Flowers on system distillation gave an essential oil (yield-0.02%) rich (-90%) in ionones in which β-ionone predominated (Sukhdev, 2006).

Jasim Unddin and Nemal (2007) was identified the essential oil of the fresh leaves of *Lantana camera* growing in Dehra Dun was by GC and GC - MS. The major
constituents identified in the oil included β-caryophyllene (23.3%), hemulene (11.5%) germacrène D (10.9%) davanone (7.3%) and γ-curcumene (6.3%).

Comparative analysis of essential oils from the leaves of *Aegle marmelos* and *Limonia acidissima* L. was carried out with the help of GC-MS. *Aegle marmelos* oil contains 16 compounds including alpha phellandrene (35.7%), dilimonene (29%), subinene (16.7%), and alpha-pinene (6.9%). The *Limonia acidissima* L., has been contain 26 compounds including methyl chavicol (74.6%) and anethole (20%) (Chowdhury and Yusuf, 2007).

3.19. Acute toxicity study

LD$_{50}$ of > 1250 mg/kg body weight, no change in the behaviour and physiological activity was recorded (at this dose) in the acute oral toxicity test in mice with the ethanolic extract of *A. marmelos* (L.) dried fruit pulp (Joshi et al., 2009).

Mondal *et al.* (2009) investigated the acute and sub-acute toxicity of the methanolic extract of *Cleome rutidosperma* (family: Capparidaceae) root, *Neolamarckia cadamba* (family: Rubiaceae) and *Spondias pinnata* (family: Anacardiaceae) bark. In the acute toxicity study, the extracts were administered orally at dose ranging from 100-3000 mg/kg p.o. and the animals were observed continuously for the first 4 hours for any behavioral changes and they were then kept under observation up to 14 days after drug administration to find out the mortality if any. However, there was no mortality in any of the above plants extract at 3000 mg/kg dose. In sub-acute toxicity study was tested at the
Biological activities of *Limonia crenulata* (Roxb.)

A single dose of 600 mg/kg p.o. once daily for 14 days. The results concluded that the methanol extract of *C. rutidosperma* root, *N. cadamba* and *S. pinnata* bark at doses of 600 mg/kg, p.o. is nontoxic since no marked changes in haematological and biochemical parameters were observed.

Baghel *et al.* (2011) evaluated the acute toxicity study of *Coccinia indica* roots. Rats were orally administrated single dose of 100, 500 and 1000mg/kg of aqueous extract of *Coccinia indica*. Mortality, signs of toxicity, body weight, food consumption and gross findings were observed for 07 days post treatment of *Coccinia indica* extract. In addition, no significant differences were noticed in the body and organ weights between the control and treated groups. These results state that aqueous extract of *Coccinia indica* is toxicologically safe by oral administration.

Sikarwar Mukesh *et al.* (2009) analysed the pharmacognostic, phytochemical parameters and acute toxicity of *Crateva nurvala* stem bark (family: Capparidaceae). The acute toxicity study of plant extract was also carried out in female albino rats (50mg to 5000 mg/kg body weight) as per OECD guidelines. In the acute toxicity study, oral administration of 5g/kg of *Crateva nurvala* stem bark extracts produced neither mortality nor changes in behaviour or any other physiological activities.

### 3.20. Herbal capsules
The pharmacognostical study of leaf of *Desmodium triflorum* was conducted with a view to help in correct botanical identification of the drug, including leaf in powdered form. Diagnostic features of leaf are described. NSL, New Delhi (Deokule, 1992).

Herbal drug technology is used for converting botanical materials into medicines, where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge is important. Herbal medicines are gaining more and more attention all over the world, due to their long historical clinical practice and less side effects. This paper reviewed the traditional methods in the quality control of herbal medicines, including, the traditional chromatographic methods and comprehensive methods, such as fingerprint and multi-component quantification are emphasized; hyphenated techniques, like HPLC, GC-MS. In a few word, the analysis and quality control of herbal medicines are moving towards an integrative and comprehensive direction, in order to better address the inherent holistic nature of herbal medicines (Patra et al., 2010).

Wangthong *et al.* (2010) studied that the stem bark powder of *Hesperethusa crenulata* or Thanaka has been used on the face by Myanmar women for more than a thousand years as a skin care regiment.

### 3.2.1. Herbal syrup

Contributions of plants are numerous in every sector of human life. They help to grow human body and also protect human beings from sickness due to their extensive use as medicine. A large number of plants are used as medicinal agents (Ghani, 1990). In
Bangladesh, about two hundred fifty plant species are used as medicinal agents. It has now been established that plants which naturally synthesize and accumulate some secondary metabolites like alkaloids, glycosides, tannins, volatile oils, minerals and vitamins possess medicinal properties (Ghani, 1998). Most important concept underlying antimicrobial therapy is selective toxicity, i.e., Selective inhibition of the growth of microorganism without damage to the host (Warren and Ernest, 1998). Natural products are known to play an important role in both drug discovery and chemical biology (Holetz et al., 2002).