6. DISCUSSION

6.1. Medicinal plants

Medicinal plants (MPs) continue to play a significant role in the people welfare as they have been for several millennia. Since the medieval time plants played an important role in the life of human as the major source of food, as well as maintenance and improvement of health by elimination of the disease causing microbes. The World Health Organization (WHO) estimated that about 80 percent of the world's population still relies on plant based medicines for their primary health care (Khalil et al., 2007). Various medicinal plants have been used for years in daily life to treat diseases all over the world. The relatively lower incidence of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost, is encouraging both the consuming public and national health care institutions to consider plant medicines as an alternatives to synthetic drugs (Nair et al., 2005). Recently various modern procedures and techniques were used for the determination of biological activity of plant extract and bioassay techniques. (Ahmad et al., 2002; Zafer et al., 2002). The plants have also been used as source of medicine. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times (Farombi, 2003). Over 50 percent of all modern clinical drugs are natural product origin and natural products play an important role in drug development programmes in pharmaceutical industry (Baker et al., 1995).
Many studies made tremendous contribution for the therapeutic usage of medicinal plants. Large number of plants belonging to different families has been studied for their therapeutic usages (Bowers, 1976; Coredell, 1981; Stuﬄiness and Crodell, 1987, Mukhtar et al., 2002).

Rutaceae is a large family comprising 160 genera and 1,650 species largely distributed in the tropical and subtropical parts of the world (Jones, 1995). Limonia acidissima L. syn. Feronia elephantum Correa is a multipurpose tree species belonging to Rutaceae family. Different parts of this tree, fruits, seeds, and leaves, have been reported to possess many medicinal properties and are widely used in folk medicine. The fruits and leaves are prescribed for vomiting, dysentery, indigestion and slight bowel affections in children (Kirtikar and Basu, 1935).

Limonia crenulata (Roxb.) is an Indian medicinal plant. All parts of the tree are medicinally useful. Literature in Indian traditional medical systems like Ayurveda, Siddha and Unani were prescribed this as an Indian folk medicine which has much potential information on its therapeutic uses. In Ayurveda Limonia crenulata (Roxb.) is used as a folk medicine for renitent fever, puerperal fever, lightening of skin, diarrhoea, ulcer, inflammation, skin irritation, dyspepsia, diabetes and many other diseases. With this background, the present study plant Limonia crenulata (Roxb.) was undertaken to analyse phytochemical, pharmacological, pharmacognostical and pharmaceutics studies.
Morphological and anatomical observations

Microscopic and macroscopic characters are distinct and showed various morphological and anatomical characters. As the species is an untouched specimen most of the data’s are newly identified and checked with the similar genus.

6.2. Qualitative phytochemical analysis

Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but such information may be the value in disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances, etc. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Farnsworth, 1966).

Recently Attarde et al., (2011) reported that preliminary phytochemical analysis of methanolic leaf extracts of Limonia acidissima L. contains good amount of polyphenolic compounds. Khyade and Vaikos (2009) exhibited that the phytochemical screening of Wrightia tinctoria is considered to be very effective because of phytochemicals such as lipid, saponins, alkaloids, phenol, steroid, flavonoid and some other chemical constituents.

In the present investigation revealed that the qualitative phytochemical analysis of hydroalcoholic extract of Limonia crenulata (Roxb.) has preliminary identification of
bioactive compounds such as alkaloids, carbohydrate, protein & aminoacids, glycosides, flavonoids, fixed oil and fats were present except phytosterol and saponins.

Similarly the phytochemical studies were investigated in leaf of *Limonia acidissima* L revealed that the presences of wide range of phytoconstituents like sterols, terpenoids, glycoside, flavonoids, polyphenols, coumarin, and carbohydrates (Parial *et al.*, 2010).

6.3. Quantitative phytochemical analysis

Secondary metabolites are produced by plants which play certain biological and ecological roles towards combating other plants, animals, insects and man (Mann, 1987).

Among the secondary metabolites flavonoids are the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants (Jeremy, 2008). The widespread distribution of flavonoids, their variety and their relatively low toxicity compared to other active plant compounds (for instance alkaloids) mean that many animals, including humans, ingest significant quantities in their diet. Preliminary research indicates that flavonoids may modify allergens, viruses, carcinogens, and so may be biological "response modifiers". *In vitro* studies of flavonoids have displayed antiallergic, anti-inflammatory, antimicrobial (Cushnie and Lamb, 2005) and anticancer activities (De Sousa *et al.*, 2007). Flavonoids (both flavones and flavonols) are most commonly known for their antioxidant activity. Additionally, at high experimental
concentrations that would not exist in vivo, the antioxidant abilities of flavonoids in vitro are stronger than those of vitamin C and E (Manashi et al., 1999). Hence many of secondary metabolites have been shown to present interesting biological and pharmacological activities and are used as chemotherapeutic agents for centuries to treat a variety of diseases or serve as the starting point in the development of modern medicines (Verpoorte, 1998).

All human beings require a number of complex organic or inorganic compounds in diet to meet the need for their activities. Evidently, crude tannins were isolated from leaves of Naringi crenulata exhibited antihelmintic activity. It contains many phytochemical constituents (Ramalingam et al., 2010). When it compared with the present study the total amount of medicinal principles present in Limonia crenulata (Roxb.), the active constituents are carbohydrates (7.80±2.3 mg/100gm), tannins (2.52 ± 0.3), phenolic compounds (13.20±0.1mg/100gm), proteins (32.0 ± 1.5 µg/100gm), vitamin C (0.33 ± 0.2 µg/100gm) and vitamin E (0.45 ± 0.2 µg/100gm). Natural antioxidants such as vitamin C and vitamin E directly influence the biological activity. Similarly methyl extract of Limonia acidissima L. contains rich amount of total polyphenolics (1.2 mg/gm), total flavonoids (0.7 mg/gm) and total flavonols (0.85mg/gm) (Attarde et al., 2011). The important constituents of diet are carbohydrates, fats, proteins, vitamins, minerals and water (Indrayan et al., 2005).
The total amount of compounds present in *Limonia crenulata* (Roxb.) were glycosides (47.77%), flavonoids (43.18%) and tannins (41.81%). Thus from the data it is evident that the study plant could make useful in treating different ailments and having potential for providing useful drug for human use. This is because of the pharmacological activity of this plant is used to trace the particular compound. The strong presence of glycosides, flavonoids and tannins are responsible for the biological activities of this plant. They are known to show medicinal potential and physiological activities (Sofowara, 1993).

### 6.4. Physico - chemical characters

However the present analytical studies of *Limonia crenulata* (Roxb.) were carried out with a view to evolve standard and to validate its ethnobotanical uses. The ash of any material is composed of their non-volatile inorganic components. Inorganic elements play an important role in physiological process involved in human health. Controlled incineration of crude drugs results in the ash residue consisting of an inorganic material. This value varies within fairly wide limits and is therefore an important parameter for the purpose of evaluation of crude drugs. In certain drugs, the percentage variation of the weight of ash from sample is very small and any marked difference indicates a change in quantity. More direct contamination, by sand of earth, is immediately detected by the ash value. According to Mukherijee, (2002) the ash values can be determined by three different methods to measure the total ash, the acid insoluble ash and the water soluble ash. Crude fibre consists of the material other than ash which cannot be dissolved in
water and cannot be digested by boiling with sulphuric acid or with sodium hydroxide which represents the more important resistant part of the plant cells as well as some less resistant cell wall component like cellulose and pectin. This work was established that total ash value, water soluble ash, acid insoluble ash and crude fibre content of *Limonia crenulata* (Roxb.) were found to be 4.42%, 2.50%, 1.57% and 2.87% respectively. The earlier studies of Vermani *et al.*, (2010) that the physico-chemical analysis of ash of some medicinal plants growing in Uttarakhand results were shown as the percentage ash value was highest in acid followed by alcohol and water. Recently in the same parameters were evaluated in *Gymnema sylvestre*. The total ash for aerial parts was found to be 8.22% of which, acid insoluble ash was 1.08% and water soluble ash was 3.39% (Kalidass and Mohan, 2010).

However the ash analysis method determines the amount of active constituents in a given amount of medicinal plant material when extracted with solvents. It is employed to that material for which no chemical or biological assay method exists. As mentioned in different official books the determination of water soluble and alcohol soluble extractives, is used as a means of evaluating crude drugs which are not readily estimated by other means.

Hence the extraction of any plant with a particular solvent yields a solution containing different phytoconstituents. The composition of these phytoconstituents in that particular solvent depends upon the nature of the plant and solvent used. The use of
a single solvent can be the means of providing preliminary information on the quality of a particulars plant sample. For example, in a plant where the extraction procedure for the constituents commences with water as the solvent any subsequent aqueous extraction on the re-dried residue will give a very low yield a soluble matter. Thus the present study revealed that water and ethanol soluble extract of Limonia crenulata (Roxb.) were found to be 6.83% and 13.15% respectively. Similarly Kalidass and Mohan (2010) reported that the Gymnema sylvestre extraction values were found to be 18.21% and 20.19% for water and alcohol respectively.

6.5. Elemental analysis

This analysis was carried out to understand the importance of elements. Plants are the rich source of all the elements essential for human beings. There is a relationship between the element content of the plant and its nutritional status. Some elements are essential for growth, structure formation, reproduction or as components of biologically active molecules while others have some other beneficial effects (Newwall et al., 1996).

Qualitative or quantitative determination of mineral elements present in plants is important because the concentration and type of minerals present must often be stipulated on the label of a food. The quality of many foods depends on the concentration and type of minerals which they contains, also play a very significant role against a variety of degenerative diseases and processes, they may also prevent and reduce injury from environmental pollutants and enhance the ability to work and learn, some minerals are
essential to a healthy diet (e.g. calcium, phosphorus, potassium and sodium) where as some can be toxic (e.g. lead, mercury, cadmium and aluminium). It is clear that mineral nutrition is important to maintain good health and because of that determination of As, Ca, Fe, Mg, Na, K, Zn, Ni, Co etc. have been added to Ayurvedic Pharmacopoeia of India (Anonymous, 1999). The study plant also consist of essential mineral nutrients.

From ancient times, Swarna bhasma (gold ash) has been used in several clinical manifestations including loss of memory, defective eyesight, infertility, overall body weakness and incidence of early aging. It was supported with Mitra et al., (2002) who stated the qualitative analyses indicated that Swarna bhasma contains not only gold but also several microelements Fe, Al, Cu, Zn, Co, Mg, Ca, As, Pb etc.

Potassium is important as diuretic and it takes part in ionic balance of the human body and maintains tissue excitability. Potassium is the principal intracellular cation and also consider as a very important constituent of the extracellular fluids. Potassium ions are concerned with the transmission of electrical impulse in the nerve cells and in maintaining the fluid balance of the body.

Analysis were made to confirm the activity of elements with this background Venkataraman and Gopal Krishnan (2002) reported maximum concentration of Ca, Fe and K in nine plants traditionally used for jaundice and concluded that high concentration of K in the medicinal plants could be related to the diuretic action of drugs
prepared from medicinal plants. Calcium imparts strength and rigidity to bones and teeth. Calcium ions are also needed in neuromuscular transmission, in excitability of nerves for normal excitability of heart, in clotting of blood and promoting muscular contraction. It also acts as an activator of the enzymes phospholipase, arginine kinase, adenosine triphosphatase and adenyl kinase.

Manganese is essential for haemoglobin formation but excess is harmful. Zinc is an essential component of a number of enzymes present in animal tissue including alcohol dehydrogenase, alkaline phosphatase, carbonic anhydrase and procarboxypeptidase, is also essential for the normal growth and reproduction and helps in the process of tissue repair and wound healing. Zinc deficiency causes growth retardation and skin lesions (Chatterjee and Shinde, 1995).

Some elements are essential for all the biological activities especially few elements are having high beneficial effects. Which was encompassed by Kar et al. (1999) that the inorganic parts (containing K, Zn, Ca traces of Cr etc) of Tinospora cordifolia (stem) showed more pronounced action of glucose-tolerance factor than their corresponding organic parts.

Nickel aids the synthesis of haemoglobin in the bone marrow. Perhaps Iron is the most well known in biological system. It performs a wide range of biological functions. Many of these functions are connected with oxidation-reduction and processes by which
energy is conserved in the body. However it forms an integral part of cytochromes, haemoglobin, myoglobin, etalloflavo proteins and certain enzymes such as catalase and peroxidases. Thus, iron is absolutely essential for transport of oxygen to the tissue and for operation of oxidation systems within the tissue cells, without which life would cease within a few seconds. Iron deficiency causes anemia.

The present study revealed that the amount of calcium (78.05 mg/l) and potassium (140.30 mg/l) were higher in *Limonia crenulata* (Roxb.). The content of iron was 1.98 mg/l, manganese (0.20 mg/l), nickel (0.11 mg/l) and molybdenum (0.22 mg/l) were found to be less in *Limonia crenulata* (Roxb.). Our study accordance with the Vermani *et al.*, (2010) that the analysis of eight mineral elements of plant’s ash by AAS of *Cassia fistula, Tinospora cordifolia, Quercus infectoria* and *Cedrela toona* showed maximum concentration of potassium (K) in comparison to other elements. *Butea monosperma* showed maximum results with magnesium. Calcium is present in all plants in good quantity it was highest in *B. monosperma* and lowest in *C. toona*. Iron concentration was found maximum in *Q. infectoria* and minimum in *C. toona*. Phosphorus concentration was found to be highest in *T. cordifolia* and lowest in *C. toona*.

6.6. Microbial analysis
Microbial contamination usually medicinal plants containing bacteria and molds are coming from soil and atmosphere. Analysis of the limits of \textit{Escherichia coli} and molds clearly throws light towards the harvesting and production practices. The substance known as aflatoxin will produce serious side effects if consumed along with the crude drugs. The microbial data will relate to the product safety and quality inputs of microbiology are essential in maintaining the integrity of products whilst protecting consumers safety.

The microbial analysis of \textit{Limonia crenulata} (Roxb.) revealed the presence of pathogenic microorganisms within WHO limit. The total heterotrophic bacterial count was 112 X 10^4 (Cells in sample/g). \textit{Salmonella} sp., \textit{Shigella} sp., and \textit{Enterobacter} sp. were absent. Indeed the similar work was carried out by Shrivastava and Leelavathi (2010) in \textit{Catunaregum spinosa} Thunb. Reports were stated that the total bacterial count (9 X 10^3), total fungal count (8 X 10^4), \textit{Escherichia coli} (7 X 10^3) and \textit{Salmonella} was absent.

\textbf{6.7. High Power Thin Layer Chromatography (HPTLC) analysis}

HPTLC is a reliable method for quantification of a nanogram level even when present in complexes formulation. HPTLC finger print analysis is used for rapid identity check, for monitoring purity of drugs, for detection of adulterants, for determining whether a material is derived from defined botanical species, also to know whether the constituents are clearly characterized (Sethi, 1996).
The recent study revealed that *Feronia limonia* (Linn.) (Rutaceae) have gained traditional therapeutic importance owing to their high essential oil and coumarins content. A simple, sensitive and accurate High Performance Thin Layer Chromatographic (HPTLC) method has been developed for the estimation of marmesin in the methanolic extract of stem bark of *Feronia limonia* (Linn.). The calibration curve was linear in the concentration range of 20 – 100 ng spot⁻¹. This method was validated for precision, repeatability and accuracy. This technique has been applied, for the first time, for the estimation of marmesin. Therefore it holds potential for detection, monitoring and quantification of marmesin in *Feronia Limonia* (Linn.) and its related formulation Jain *et al.*, (2010).

Based on the results obtained from *Limonia crenulata* (Roxb.) in the HPTLC fingerprint, it has been shown that the alcoholic extract of contains eight compounds displayed at 5 µl, 10 µl and 10 compounds displayed at 15 µl.

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

Gas Chromatography - Mass Spectroscopy (GC-MS) plays a key role in the analysis of unknown components of plant origin. Generally, the plant materials are highly complexes, which make GC-MS well suited for their analysis because of its high sensitivity and selectivity. GC - MS ionizes compounds and measures their mass numbers. It provides additional information on structure of these profiles.
The overall evaluation of the compounds present in the plant extract were analysed by using GC – MS. Alcohol was used as a solvent for the separation of bioactive compounds present in the plant leaves. Totally 13 compounds were identified in *Limonia crenulata* (Roxb.). The results indicated the presence of benzene acetic acid, benzaldehyde, 4-hydroxy-, benzene, 1, 2 – dimethoxy – 4 - (2 - propenyl) -, n - hexadecanoic acid, estragole, 7H – Furo (3,2-g) (1) benzopyran-7-one, 4-methoxy-, phytol and 4H - pyran --4-one, 2,3 dihydro-3,5 dihydroxy -6methyl -. These compounds contain the following properties such as antibacterial, antifungal, antioxidant, antiulcer, antiviral, anticancer, anti-inflammatory, fungicide, pesticide, anti acne, larvicide, gastro protective, flavour, hemolytic, preservative and analgesic (Dr. Duke's Photochemical and Ethobotanical database). Among the identified phytochemicals, n- Hexadecanoic acid have the property of antioxidant antimicrobial and larvicidal activities (Bodoprost and Rosemeyer, 2007; Falodun, et al., 2009). The phenolic constituents of the extracts of *Mentha spicata* namely phytol was reported for its antimicrobial and antiviral activities, strong antioxidant and antitumor action (Mckay and Blumberg, 2006).

6.9. High-Performance Liquid Chromatography (HPLC) analysis

HPLC, is a chromatographic technique that can separate a mixture of compounds and used to identify, quantify and purify the individual components of the mixture. It has been developed and validated for stability, linearity, accuracy and precision.
Previous works were supported that the presence of bioactive compounds such as gallic acid (GA), catechin (CA), rutin (RU), ellagic acid (EA) and quercetin (QU) are phenolic compounds. Structurally they have phenolic groups which serve as a source of readily available hydrogen atoms such that the subsequent radicals produced can be delocalized over the phenolic structure (Robards et al., 1999; Nikolic, 2006). The interest in these compounds is due to their pharmacological activity as radical scavengers (Azzi et al., 2004; Baydar et al., 2007). They have been proved to have potential preventive and therapeutic effects in many diseases, where the oxidative stress has been implicated, including cardiovascular diseases, cancer, neurodegenerative disorders and in aging (Wu et al., 2006; Duthie et al., 2000; Myhrstad et al., 2002, Sun and Chen, 1998). Interestingly many authors were reported the phenolics are in food, cosmetic and pharmaceutical industries, as substitutes for synthetic antioxidants. These five phenolics widely distribute in the plant kingdom (Justesen and Knuthsen, 2001; Soong and Barlow 2004; Rizzo et al., 2006.).

Samee and Vorarat (2007) studied that gallic acid (GA), catechin (CA), rutin (RU), ellagic acid (EA) and quercetin (QU) were analysed simultaneously by HPLC with UV detection at 280 nm. Calibration curves were found to be linear with ranges of 2.62 - 21.00, 10.85 - 86.80, 10.00 - 80.00, 10.05 - 80.40 and 10.05 - 80.40 mg.ml$^{-1}$ for GA, CA, RU, EA and QU respectively. The contents of GA, CA, RU, EA and QU in the flower extracts of *Michelia alba, Caesalpinia pulcherrima* and *Nelumbo nucifera* were successfully determined at 5.0, 6.8, 9.2, 9.8 and 11.3 mins respectively with satisfactory reproducibility and recovery.
HPLC procedure provided excellent identification and quantification of four phenolic compounds presented in *Limonia crenulata* (Roxb.). This method was used to isolate the compounds such as Gallic acid (0.145 µg/ml), Caffeic acid (0.399 µg/ml), Rutin (3.776 µg/ml) and Quercetin (0.416 µg/ml). The experimental results indicated that *Limonia crenulata* (Roxb.) extracts were contained an especially high concentration of rutin (3.776µg/ml). Since the phenolic compounds have been of interest of health benefits, the present analytical study could be a potential application to identify and quantify the phenolic compounds in plant extracts.

Similarly, a new indole alkaloid, crenulatine (1), along with twenty known compounds such as four alkaloids, four coumarins, two flavonones, three tetranortriterpenoids, one triterpenoid, three steroids, two lignans and two aromatic compounds were isolated from *Limonia crenulata* (Roxb.) (Niu et al., 2001).

The observations of Chatterjee et al. (1980) insisted a new coumarin, 7 – phenylacetoxycoumarin, has been isolated from *Limonia crenulata* (Roxb.) along with luvangetin, xanthotoxin, umbelliferone and limonin. This is the first report a coumarin phenol ester from a natural source. Michael (2003) reported that the isolation, structure determination synthesis and biological activity of quinoline, quinazoline and acridone alkaloids from plant, microbial and animal sources. The integriquinolone compound isolated from *Limonia crenulata* (Roxb.).
6.10. Fourier Transform - Infra Red spectroscopy (FT- IR) analysis

IR Spectroscopy is a valuable tool in the determination of the structure of organic molecules and in establishing the identity of the sample. IR is used for the identification of functional groups like OH, NH CO and C=C, CH and NO\textsubscript{2} in the molecules. The absence of such characteristic bands is definite proof of absence of such groups.

The FT –IR and UV spectrum were used to identify the functional group of the active components based on the Peak value in the region of infrared radiation. The ethanol extract of *Gymnema kollimalayanum* was passed into the FT - IR, the functional groups of the components were separated based on its peak ratio and the same was passed into UV spectroscopy for the electron transition of compounds. The results of FT - IR analysis was confirmed the presence of the carboxylic acid and Alkenes-CH\textsubscript{2}; CH\textsubscript{3} Aromatic stretching which shows major peaks at 1019.87 and 2922.33cm (Natarajan *et al.*, 2011).

IR spectrum analysis of gallic acid, caffeic acid, rutin and quercetin isolated from *Limonia crenulata* (Roxb.) were recorded using Perkin-Elmer Paragon 580 B FT-IR spectrophotometer. The IR results indicated the following functional groups were present in gallic acid, such as polymeric O-H stretch, C=O stretch, phenol or tertiary alcohol, O-H bend, C-O stretch and CC-C C-C stretch. The functional group of caffeic acid were polymeric O-H stretch, C=O stretch, phenol or tertiary alcohol, O-H bend, C-O...
stretch and C-C stretch. In rutin organic molecules such as polymeric O-H stretch, C=O stretch, phenol or tertiary alcohol, O-H bend, C-O stretch and CC-C-C stretch. The spectrum showed polymeric O-H stretch, C-H group in aromatic ring, C=O stretch, phenol or tertiary alcohol, O-H bend, C=C stretch and C-C stretch were present in quercetin.

6.11. Ultra Violet (UV) – Visible spectroscopy analysis

The technique of UV spectroscopy is one of the most frequently method employed in plant drug analysis. It involves the measurement of the amount of ultraviolet (190-380 nm) radiation absorbed by a substance in solution. Ultraviolet - visible absorption spectra provide a useful source of supporting evidence in the elucidation of structures of organic compounds. Moreover, selective absorption also serves as an identifying fingerprint for a particular structure in many cases.

The value of UV - visible spectra in identifying unknown constituents is indicative of the compounds. The absorbance of the extract of Limonia crenulata (Roxb.) recorded using lambda 35 model UV-Visible spectrophotometer. The UV results were supported that the functional group of isolated compounds such as gallic acid, caffeic acid, rutin and quercetin.

6.12. Antimicrobial activity
There has been an increasing incidence of multiple resistance in human pathogenic microorganisms in recent years, largely due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of diseases. The number of resistant strains of microbial pathogens is growing, ever since penicillin resistance and multi resistance Pneumococci were reported (Meurer-Grimes et al., 1996; Elloff, 1998). This situation, coupled with the under desirable side effects of certain antibiotics and the emergence of previously uncommon infections are a serious problem (Marchese and Shito, 2001; Poole, 2001). This has forced scientists to search for new antimicrobial substance from various sources like the medicinal plants.

The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Meurer-Grimes et al., 1996; Rabe and Van Staden, 1997; Afolayan, 2003). The presence of antibacterial and antifungal substances in the higher plants is well established (Fridous et al., 1990; Didry et al., 1998; Javed and Ali, 2002; Belboukhari and Cheriti, 2005).

The present investigation involving Limonia crenulata (Roxb.) also lends credence to the above observations. The leaf extracts showed significant antimicrobial activity against gram - positive bacteria as well as fungi. The different plant extracts differ significantly in their activity against tested microorganisms. These differences may be attributed the fact that the occurrence of different antimicrobial compounds with different solvents. The benzene leaf extract of Limonia crenulata (Roxb.) exhibited
maximum antimicrobial activity against *Salmonella typhi* (22 mm) and least activity against *Sheigella shigae* (14 mm) at 250 µg/ml. *Sheigella shigae* (25 mm) and *Candida albicans* (25 mm) were strongly inhibited by chloroform leaf extract and minimum activity was recorded in *Pseudomonas aeruginosa* (17 mm). The significant antimicrobial activity was observed against *Pseudomonas aeruginosa* (26 mm) and less activity was showed against *Aspergillus niger* (16 mm) in ethanol leaf extract. In the acetone leaf extract showed very promising antimicrobial activity against *Pseudomonas aeruginosa* (28 mm) and no activity was observed against *Aspergillus niger*. In aqueous extract showed minimum and maximum antimicrobial activity against *Aspergillus niger* (12 mm) and *Klebsiella aerogenes* (20 mm) respectively. So that the present study concluded that *Limonia crenulata* (Roxb.) had effective antimicrobial activity against pathogenic bacteria and fungi species. GC-MS analyses revealed the occurrence of more number of antimicrobial compounds in the leaf extracts.

Suresh *et al.*, (2009) evaluated that methanolic extracts of *Aegle marmelos* (L.) have great potential as antimicrobial agent against both gram - positive and gram - negative organisms such as *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Salmonella typhi* and *Staphylococcus aureus*.

### 6.13. Antioxidant activity
Antioxidant means “Against oxidation” antioxidants work to protect lipids from peroxidation by free radicals. Antioxidants are effective because, they are willing to give up their own electrons to free radicals.

When a free radical gains the electron from an antioxidant it no longer needs to attack the cell and the chain reaction of oxidation is broken. After donating an electron an antioxidant becomes a free radical by definition. Antioxidants in this state are not harmful because they have the ability to accommodate the change in electrons without becoming reactive. The human body has an elaborate antioxidant defense system. Antioxidants are manufactured within the body and can also be extracted from the food humans eat such as fruits, vegetables, seeds, nuts, meats, and oil. There are two lines of antioxidant defense within the cell. The first line, found in the fat soluble cellular membrane consists of vitamin E, β – carotene, and Co – enzyme Q of these, vitamin E is considered the most potent chain breaking antioxidant within the membrane of the cell. Inside the cell water soluble antioxidant scavengers are present (Kaczmarski et al., 1999).

The antioxidants may be enzymatic or non – enzymatic, super oxide dismutase, glutathione peroxidases, catalase and peroxidases are some examples which come under enzymatically potential antioxidants. In the non-enzymatic category some of the known and documented antioxidants are vitamin C, vitamin E, vitamin A, carotenoids, uricacid, ubiquinone and synthetic compounds like melatonin, Dihydro eplandrosterone (DHEA)
The ethyl acetate extract of *Pereskia grandifolia* (Haw.) (Cactaceae), played a considerable role in antioxidant activity by DPPH method. This is the first report on the antioxidant activities on leaves of *P. grandifolia* (Sim *et al*., 2010).

However, hydro ethanolic extracts of *Agle marmelos* and *Trigonella foenum* was found to be more active in DPPH scavenging ability in comparison to the methanol and aqueous extract which contains higher levels of phenols. (Vijaya *et al*., 2002). Recently Attarde *et al*., (2011) reported that petroleum ether, chloroform and methanolic extract of leaves of *Limonia acidissima* L. have potential antioxidant ability using DPPH method.

The present study to evaluate the *in-vitro* antioxidant activity of ethanol, methanol and aqueous leaf extracts of *Limonia crenulata* (Roxb.) by DPPH method. The DPPH radical scavenging activity of ethanol extracts showed a challenging result (83.69%), methanol and aqueous extracts were found to be 77.89% and 70.59% respectively.

Similar work was done by Sathishkumar *et al*., (2010) that the ethanol and acetone extracts of *Polyalthia longifolia* leaves showed significant DPPH radical scavenging activity were found to be 87.92% and 92.84% respectively.

There is an increasing interest in the study of anti-inflammation. Anti-inflammation is the response of living tissues to injury. It involves a complex array of enzyme activation, mediator release, and extravasations of fluid, cell immigration, tissue breakdown and repair (Vane and Bolting, 1995). It is also known that anti-inflammatory effects can be elicited by a variety of chemical agents and that there is little correlation between their pharmacological activity and chemical structure (Sertie et al., 1990).

Inflammation is a complex in the pathogenesis of inflammatory diseases (Conner and Grisham, 1996). Also, it is well known that inflammation sites present a high concentration of free radicals and oxidants, which play an important role in different inflammation process. Therefore antioxidant compounds can be helpful to avoid this process (Salvemini et al., 1996).

The previous investigation suggested that carrageenan induced paw edema is the most widely used primary test for the screening of new anti-inflammatory agents (Di Rosa et al., 1971; Winter et al., 1962). The mechanism of action of carrageenan induced paw oedema is described as biphasic. The development of edema in the paw of the rat after the injection of carageenan is due to release of histamine, serotonin and prostaglandin like substances. Hence the initial phase seen at the 1st hour is attributed to the release of histamine and serotonin. The second phase is related to the release of prostaglandins like substance in 3rd hour. (Brooks and Day, 1991; Vinegar et al., 1969).
Winter et al., (1992) reported that the second phase of edema is sensitive to drugs like hydrocortisone, phenylbutazone and indomethacin. Perhaps this associated with the complexity of the inflammatory process, makes the use of different experimental models essential when conducting pharmacological trials.

In Indian system of medicine, certain medicinal plants are claimed to provide relief of pain and inflammation. The claimed therapeutic reputation has to be verified in a scientific manner. Plants which belong to Rutaceae family are rich in flavonoids and bioflavonoids are known for their anti-inflammatory and antioxidant activities. Hence, in this study anti-inflammatory properties of Limonia crenulata (Roxb.) leaf extracts were tested using a number of experimental rat models representing different phases of inflammation. The carrageenan paw inflammation has been accepted as a useful diagnostic tool for investigation of systemic anti-inflammatory activity of drugs. The percentage inhibition of paw volume of alcoholic extract of Limonia crenulata (Roxb.) was found to be significant (50.95%) as that of the standard drug Indomethacin treated group which was 63.80%. The chloroform, aqueous, benzene and acetone extracts of Limonia crenulata (Roxb.) were found to be 40.44%, 27.33%, 29.05%, and 37.25% of paw volume inhibition respectively. The results of exhibited that the study plant has reasonable anti-inflammatory activity.

Presence of terpenes, glycosides and sterols in plants has been found to exert active anti-inflammatory effects (Chawla et al., 1987). The more pronounced activity of
these plants may be due to the presence of certain polar constituents such as flavonoids and glycosides (Katith et al., 1996). The results of phytochemical analysis of *Limonia crenulata* (Roxb.) revealed the presence of flavonoids, alkaloids, glycoside and sterols. Thus it has been taken into consideration which has made tremendous contribution of anti-inflammatory activity.

Similar work was carried out by Rao, (2003) insisted the significant anti-inflammatory activity at a dose level of 200 mg/kg body weight by ethanolic extract of *A. marmelos* fruits. It contains several biological activities such as anti-inflammatory, analgesic and antibacterial effect (Ponnapalli, 2005). *Trichodesma indicum*, belonging to Boraginaceae showed anti-inflammatory activity with different models (Periyanayagam et al., 2006).

The therapeutic use of *Cassia fistula* bark was treated for inflammatory conditions by practitioners of Ayurvedic system of medicine. The bark extracts of *Cassia fistula* possess significant anti-inflammatory effect in the acute and chronic anti-inflammatory model of inflammation in rats. The presence of alkaloid and flavonoids in *Cassia fistula* may be responsible for the anti-inflammatory and antioxidant effects (Yadava and Verma, 2003; Gupta et al., 1989).

### 6.15. Antiulcer activity
Gastric ulceration has been attributed to various causes such as stress, hormones, drugs, alcohols, smoking and ingestion of certain foods (McGuigan, 1991). Plant extracts are some of the most attractive sources of new drugs and have shown promising results for the treatment of gastric ulcer. Plant extracts are more of the most attractive sources of new drugs and have shown promising results for the treatment of gastric ulcers. Several medicinal plants and herbs have been used to treat gastric intestinal disorders, including gastric ulcers (Adami et al., 1964; Best et al., 1984; Ageel et al., 1987; Goel et al., 1990; Disi et al., 1998; Alkofahi and Atta, 1999).

A significant reduction in gastric ulcer in experimental animals was reported by many workers with ethanolic extracts of different plants such as *Hemidesmus indicus* (Anoop and Jagadeesan, 2003), *Triumfetta rhomboidea* (Pradhan et al., 2003), *Mimusops elengi* (Shah et al., 2003), *Carallum arabica* (Zakaria et al., 2003), *Solanum variable* (Antonio et al., 2004), *Allophylus serratus* (Dharmani et al., 2005), *Terminalia pallida* (Gupta et al., 2005), *Commiphora opobalsamum* (Howiriny et al., 2005) and *Kaempferia parviflora* (Rujjanawate et al., 2005).

The present investigation was carried out to evaluate the antiulcer activity of different extracts of *Limonia crenulata* (Roxb.) against *Acacia* mucilage induced ulcer models of rats. The effect of the alcoholic, chloroform, aqueous, acetone and benzene extracts of *Limonia crenulata* (Roxb.) (200 mg/kg) and Famotidine (20 mg/kg) on gastric secretary volume, pH, total acidity, free acidity and gastric ulcers were evaluated.
The result of present investigation revealed that the antiulcer activity of the leaf extracts of *Limonia crenulata* (Roxb.) has moderate effect when compared with the control. The plant extracts reduced gastric secretory volume, acidity and ulceration in pylorus legated rats. It can be used to discover bioactive natural products that may be serve as feeds for the development new pharmaceutical products.

Many investigations have suggested that the antiulcer effect is by the decrease in the aggressive factors like pepsin and protein and an increase in the resistance actor like pH, hexose hexoseamine, fucose and acid protection against experimental ulcers may be due to the effect of protogandins in the parietal cells (Takeuchi and Nobubara, 1985; Lauritsen and Rask Madsen, 1986; Sumangala *et al*., 1998), as prostaglandins enhance the mucosal resistance, perhaps by increase the secretion of mucous and bicarbonates (Hogan *et al*., 1994), strengthening the mucosal barrier, decreasing the gastric motility (Szabo, 1984), increasing the release of endogenous mediators (Olsen *et al*., 1984) scavenging the free radicals (Szabo, 1984) decreasing the release of endogenous amines (Whittle *et al*., 1985), stimulation of cellular growth and repair (Goal *et al*., 1985; Eugene and Jacobson, 1990). The antiulcer activity of plant extracts was not only related to local neutralization of gastric content, but also that it was effective after absorption of extracts indicating a systemic effect. This effect is also indicative of antihistaminic activity (Andrade *et al*., 2007).
Ahmed et al. (2010) observed the significant antiulcer activity of *Cassia auriculata* leaf extract against pylorous ligation induced gastric ulcer. The methanolic leaf extract of *Cassia auriculata* at dose of 300 mg/kg p.o. markedly decrease the incidence of ulcers in pyloric ligated rats. In pyloric ligated rats, there was an increase in the gastric volume, free acidity, total acidity and ulcerative index as compared to the control group. The methanolic leaf extract of *Cassia auriculata* at dose of, 300 mg/kg showed significant reduction in the above parameters which was comparable to the standard drug famotidine (10 mg/kg). *Cassia auriculata* extract showed protection index 79.4%, whereas standard drug famotidine showed protection index 90.7%.

Pasquale et al. (1995) have reported that plant drugs containing saponin, terpenoids or amino acid have antiulcer activity. Presence of beta sitosterol in the samples may also enhance the antiulcer activity (Malini and Vanithakumari, 1989). It is well known fact that many flavonoids display antisecretary and cytoprotective properties in different experimental models of gastric ulcer (Zayachkivska et al., 2005.) Indeed flavonoids and tannins are one of the most important phytochemical compounds with antiulcer and gastroprotective activities (Alarcon de la Lastra et al., 1994; Borrelli and Izzo, 2000; Gonzalez et al., 2000). In the present study plant also contains flavonoids and tannins.

6.16. Antidiarrhoeal activity
Diarrhoea is considered as a consequence of altered motility and fluid accumulation. It has long been recognized as one of the most important health problems in the developing countries (Snyder and Merson, 1982). Diarrhoea is the second leading cause of death among children under five globally. Nearly one in five child deaths about 1.5 million each year is due to diarrhoea. The objective of WHO/UNICEF report was to focus attention on the prevention and management of diarrhoeal diseases as central to improving child survival. Secretory diarrhoea is the most dangerous symptoms of gastrointestinal problems and is associated with excessive defecation and stool outputs, the stools being of abnormally loose consistency (Park, 2000).

Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract accompanied by hurry resulting in an excess loss of fluid in the faeces. In some diarrhoea the secretory component predominates while other diarrhoea is characterized by hypermotility (Chitme et al., 2004). The magnesium sulphate (MgSO₄) induced diarrhoea is acknowledged to be by osmotic properties and cholecystokinin production. It has been reported to induce diarrhoea by increasing the volume of intestinal content through prevention of reabsorption of water. It has also been reported that it promotes the liberation of cholecystokinin from the duodenal mucosa, which increases the secretion and motility of small intestine and thereby prevents the reabsorption of sodium chloride and water. Numerous reports have been available regarding magnesium sulphate (MgSO₄) induced diarrhoea (Mujumdar et al., 2000 and 2001; Bajod, 2001; Galvez et al., 1993; Zavala et al., 1998).
The antidiarrhoeal activity of the extract may also be due to denature proteins forming protein tannates which make intestinal mucosa more resistant and reduce secretion. In addition to other previous mechanisms to explain the diarrhoeal effect, recently nitric oxide has been claimed to contribute to the diarrhoea (Mascolo et al., 1994). Antidiarrhoeal effect may be attributed, at least in part, to nitric oxide scavenging activity of the extract.

The aim of the therapy in diarrhoea is to treat the patient promptly to reduce the loss of electrolytes and water. There are several potent antidiarrhoeal drugs in the modern system of medicine however on prolonged use they do have some adverse effect (Galvez, 1993). For this reason uses of herbal medicines have increased as they are devoid of any adverse side effects. A range of medicinal plants with antidiarrhoeal properties is widely used by traditional healers. However, the effectiveness of many of these antidiarrhoeal traditional medicines has not been scientifically evaluated.

An integrated approach was made to prepare medication after the thorough observation of antidiarrhoeal activity in epsom salt (MgSO₄) induced diarrhoea. The antidiarrhoeal activity of alcohol, chloroform, aqueous, acetone and benzene extracts of *Limonia crenulata* (Roxb.) were found to be 89.88%, 89.00%, 51.85%, 86.99% and 85.06% respectively. From the above results it can be concluded that the alcoholic leaf extracts of *Limonia crenulata* (Roxb.) showed the challenging result (89.88%) when compared with the standard drug Diphenoxylate (93.80%). The different parameters
enables in drawing out important conclusions regarding the antidiarrhoeal activity. The use of *Limonia crenulata* (Roxb.) leaf extracts as antidiarrhoeal drug as justified in folk medicine indicates that the people in rural area, where they are more aware of decoction procedures of plants than the electrolyte therapy or conventional antidiarrhoeal drug can very well use it. Similar results have been obtained in extract of *Aegle marmelos* which cause dose dependent decrease in the numbers of faecal matter in caster oil induced diarrhoea (Amresh *et al*., 2003).

It is indeed worthy that the flavonoids have been reported to inhibit intestinal motility and secretion (Discarlo *et al*., 1993), they may presumably exert antidiarrhoeal action (Rao *et al*., 1997). Earlier reports suggested that antidiarrhoeal properties of medicinal plants might be attributed to tannins, alkaloids, saponins, flavonoids, sterols and reducing sugars (Longanga *et al*., 2000). In the study plant also contains alkaloids, flavonoids, tannins, and vitamin C. These constituents may mediate the antidiarrhoeal property of the extract. Flavonoids, present in the plant extract were reported to inhibit release of autacoids and prostaglandins, thereby may inhibit motility and secretion (Veiga *et al*., 2001).

6.17. Volatile oil

Many investigations were suggested that essential oils are natural mixtures of terpenes or terpenoids, most of which are obtained from aromatic medicinal plants. The chemical composition of essential oil differs in each species or subspecies and is
characteristic for the species in question (Tunalier et al., 2002; Sefidkon et al., 2004; Adams, 1995).

Syamasundar et al. (2010) found that the Limonia acidissima L. leaf collected from Karanthai malai from Tamil Nadu State gave the highest yield of essential oil (0.5%). All the other four samples from the central part of the Western Ghats and nearby plains, which are in the Karnataka State region, gave from 0.3 to 0.2% as the oil yield. In the present investigation revealed that the volume of volatile oil isolated from leaves of Limonia crenulata (Roxb.) was 1.00 ml/ kg.

6.18. GC–MS analysis of volatile oil

One of the most popular methods of studying essential oil composition is Gas Chromatography - Mass Spectrometry (GC-MS), which allows the identification of the specific natural compounds found in an essential oil by comparing their relative retention times indicates and their mass spectra (Yayli et al., 2005).

The composition of essential oil has been investigated by GC - MS analysis from different parts of various plants such as aerial parts of Sinapis alba (Sefidkon et al., 2002b), Thymus persicus (Sefidkon et al., 2002a), Lantana xenica (Juliani et al., 2003), leaves of Zanthoxylum procerum (Vila et al., 2002), Agastache scrophulariaefolia (Lognay et al., 2002), roots of Acorus calamus (Ozcan et al., 2002), rhizome of Rhodiola rosea (Rohloff, 2002) and seeds of Myristica fragrans (Simpson and Jackson, 2002).
Nor Azah et al. (2010) reported that the leaf oil of *Zanthoxyllum acanthopodium* was characterized by the presence of 1, 8-cineole (34.6%) and limonene (30.8%) and the other main constituents were -terpineol (6.2%), α - pinene (5.3%) and citronellal (3.9%). Limonene (58.5%) was the most abundant compound in the leaf oil of *Tetractomia tetranda* and other chemical components such as α - pinene (5.8%), caryophyllene (3.9%), terpinolene (3.3%) and safrole (2.5%).

The present study revealed the major chemical constituents of volatile oil of *Limonia crenulata* (Roxb.) were anisole, p – allyl- (34.82%), methanimidamide, N,N – dimethyl – N’ phenyl (28.29%) and eugenol methyl ether (12.45%) . The other important minor constituents are α-pinene, caryophyllene oxide and caryophyllene.

The similar work was evaluated by Syamasundar et al. (2010) that the *Limonnia acidissima* L. leaf collected from Karanthai malai from Tamil Nadu State showed that methyl chavicol is the major constituent (91.2%) and the other important minor constituents were α-pinene, myrcene, limonene, linalool, anisaldehyde, and p-methoxycinnamaldehyde. Interestingly, the leaves collected from Bangalore and Mysore showed a low percentage of oil, and β -pinene as the major compound. All the other four samples from the central part of the Western Ghats and nearby plains, which are in the Karnataka State region β –pinene as the major compound, ranging from 63.9–76.9%. The other major compounds that contribute to these samples are β –pinene (4.7–6.1%).
sabinene (4.4–6.0%), and limonene (4.5–6.2%). The other important minor constituents are camphene, myrcene, E- ocimene, linalool, -terpineol, methyl chavicol, -caryophyllene, -cadinene, and glubulol.

Similarly there are numerous reports emphasis that the presence of active phytoconstituents such as methyl chavicol (72.7%) and anethole (26.2%) (Bhati and Deshpande, 1949) trans-anethole (4.7%), methyl eugenol (3.6%), and anisaldehyde (4.4%), methyl chavicol (68.3%) (Garg, 2003), trans-anethole (10.9%), methyl chavicol (27.2%), and thymol (24.4%) (Ahmad et al., 1989) in volatile oil of Limonia acidissima L.

6.19. Acute toxicity studies

The definition "toxic" is ultimately a matter of viewpoint. Traditionally, herbs and herbal products have been considered to be non toxic and have been used by the general public and traditional medicine practitioner’s world wide to treat range of ailments. The active ingredients of plant extracts are chemicals that are similar to those in purified medications and they have the same potential to cause serious side effects. While the literature documents severe toxicity of herbs and herbal products it has not been recognized. Two kinds of side effects have been reported for herbal medicines. The first considered to be intrinsic to herbal drugs themselves, is mainly related to predictable
toxicity due to toxic constituents of the herbal ingredients and over dosage and second is allergy.

For several reasons it would be difficult to establish absolute safety standards for herbal preparations based solely on epidemiological studies. First, the types of studies would be costly, second, there is little published data in countries where the major use of medicinal plant occurs and thus general standards based on limited numbers of reports would have limited meaning. Third, the exact identification of the products implicated in side effects claimed for medicinal plants is usually lacking. In spite of these inadequacies there are a number of general comments that can be made with regard to avoiding potential serious side effects from herbal medicines.

Perhaps the major problem with regard to the safety of herbal medicines is related to the manufacturing practice, including contamination, substitution, incorrect preparation and dosage, intentional addition of unnatural toxic substances, interaction involving synthetic prescriptions, drugs and herbal medicines either intentional or unintentional mislabeling and presence mislabeling and presence of natural toxic contaminants. Many ordinary foods contain constituents that could be regarded as poisonous. Alpha gladin produced by gluten in wheat, oats and rye, the cyanogenic glycosides in many fruit skin and seeds, thiocyanates of the Brassica vegetables and lections of many pulses including soya and red kidney bean are such examples. Cyanogenic glycosides present in the kernel of many fruits can undergo gastric hydrolysis, resulting in the release of hydrogen.
cyanide, viscotoxin which are constituents of mistletoe are both cytotoxic and cardiotoxic.

**In these context herbs can be broadly classified in to three major categories**

The food herbs: Medicines such as pepper, mint, ginger, garlic, lemon, betel leaves etc., are gentle in action have low toxicity and are unlikely to cause any adverse response. They can be consumed in substantial quantities over long period of time without any acute or chronic toxicity. However they may bring about allergic reactions in certain individuals.

The Medicinal herbs: These are not daily "tonics" and need to be used with greater knowledge for specific conditions (with a medical diagnosis) and usually for a limited period. They have a greater potential for adverse reaction and in some cases drug interaction. They include comfrey, ephedrine, gingko, ginseng, kava kava, senna etc.

The poisonous herbs: They have a strong potential for either acute or chronic toxicity and should only be prescribed by trained clinicians who understand their toxicology and appropriate use. *Aconite, Belladonna, Datura, Digitalis*, and Male fern are some examples (Ahmad and Aquil, 2006).

Before going for the preparation of any medication obviously the standardization is very important. To check the toxicity level of any plant the ED₅₀ and LD₅₀ values are
important for knowing the safety of a drug. The ratio between LD$_{50}$ and ED$_{50}$ represents the therapeutic index which is an index of safety of the drug. Greater the therapeutic index, safer is the drug.

The acute oral toxicity was carried out to determine the acute toxicity of single oral administration of the extract, in a dose of 2000 mg/kg. as per OECD, revised draft guidelines 423, received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India (OECD, 2001).

In the present study investigation was made to check weather the plant has any strong potential for either acute or chronic toxicity. With that motive *Limonia crenulata* (Roxb.) underwent with the following strategies such as death, convulsion, tremor (shiver), straub tail, sedation, excitation, jumping, abnormal gait (step), motor in coordination, altered muscle tone, akincsia, catalepsy, loss of traction (grip), loss of balance, writhing, fore paw treading, stereotypy, head movement, scratching, altered respiration, aggression (violent behaviour), altered fear, altered reactivity to touch, loss of righting reflex, loss of corneal reflex, analgesia, defecation/diarrhoea, salivation, lacrimation, body temperature and myosis / mydriasis were analysed. After administration of the extract, the animals were observed individually for 4 hrs and thereafter 14 days to check mortality and their behavioral pattern. Result shows that there is no significant respond for toxicity. The extracts were found to be safe at the tested
dose level of 2500 mg/kg body weight indicating the high margin of safety of these extracts. Thus we conclude the extract of *Limonia crenulata* (Roxb.) leaves have a high margin of drug safety.

6.20. Herbal capsule

According to United Nations Development project report in 1994 the annual value of medicinal plants derived from developing countries is about 32 billion. Theoretically there is the possibility of discovering 328 new modern drugs lying hidden in nearly 3,25,000 species found in tropical rainforest. There are 47 major modern plant based drugs on the world market and predicted 328 more potential drugs have an estimated value 147 billion. India is ranked among the major exporters of medicinal plants and vegetative sap extracts (Atal and Kapur, 1982).

Musthaba *et al.* (2010) evaluated greater global interest in non synthetic, natural medicines derived from plant sources due to better tolerance and minimum adverse drug reactions as compared to synthetic medicines. Herbal products are also commonly used by the patients with certain chronic medical conditions, including breast cancer, liver disease, human immunodeficiency, asthma and rheumatological disorders. WHO estimates that about three quarters of the world's population currently uses herbs and other forms of traditional medicines for the treatment of various diseases. The herbs are formulated in different modern dosage forms, such as Tablets, Capsules, Topical cream, Gel, Ointment and even some novel drug delivery forms, like extended release, sustained
release, and micro encapsules dosage forms. Patenting of herbal formulations has increased over the past few years and scientific evidence of therapeutic activity has been reported by performing various in vitro and in vivo experiments.

The only drawback in the promotion of these herbal medicines is the lack of standardization. Also in some areas confusion in nomenclature and controversial botanical identification exist. Hence development of quality control and standardization of plant drugs with adoption of good manufacturing practices and validation of claims of therapeutic efficacy are the challenges in the years ahead if the revival of global interest obvious today in these drugs is to be strengthened in future.

Due to the continuous increase of medical costs many countries provide support to encourage scientific research alternative therapies. Furthermore, the putative efficacy of medicinal herbs relies on empirical or anecdotal data and tradition of use, which frequently cannot satisfy the requirements of evidence based medicine. Thus, the steps back by the analysis of traditional herbal medicine and new drug development from herbs are started recently with high effort.

Few study had been made on Aegle marmelos was considered as one of the effective medicine for the treatment of non-specific diarrhoea. Therefore, its efficacy for the treatment of dysentery was tested in 25 patients, all the patients were treated with powdered unripe fruits of A. marmelos 5gms thrice daily for 21 days (Singh et al., 2000).
Drug from *A. marmelos* plays a vital role in diabetes in pregnant women and nursing mother (Bombarde and Bombarde, 1995). *A. marmelos* used as insertion of foreign body in vagina for reduction of vault is common practice in rural India. It also helps in stopping foul smell discharge from vagina (Karea *et al*., 1998).

In view of the diverse medicinal applications of *Limonia crenulata* (Roxb.) the present investigation dealt with the preparation and evaluation of capsule. Thus *Limonia crenulata* (Roxb.) have great potential use as phytomedicine in terms of pharmacognostical, phytochemical and pharmacological aspects. The present study plant having strong biological activities and biochemical compounds which may be responsible for arresting the diarrhoea. The pharmacological studies showed outstanding results when compared with the standard drug. There is a need to discover bioactive natural product that serves as a drug for the development of new pharmaceuticals. Development of phytomedicine is relatively inexpensive and less time consuming moreover it is suitable to our economic conditions. Thus the present prepared capsule would be highly beneficial for all range of people without any side effect.

### 6.21. Herbal syrup

India can emerge as the major country and play the lead role in production of standardized, therapeutically effective ayurvedic formulation. India needs to explore the medicinally important plants. This can be achieved only if the herbal products are
evaluated and analysed using sophisticated modern techniques of standardization such as UV-visible, TLC, HPLC, HPTLC, GC-MS, spectrofluorimetric and other methods.

For the purpose of research work on standardization of herbal formulations and neutraceuticals a profound knowledge of the important herbs found in India and widely used in Ayurvedic formulation is of utmost importance.

The subject of herbal drug standardization is massively wide and deep. There is so much to know and so much seemingly contradictory theories on the subject of herbal medicines and its relationship with human physiology and mental function. The herbal syrup of *Limonia crenulata* (Roxb.) has great potential to heal the diarrhoea. Our investigation is optimistic for the development of safe drug which has great therapeutic potential to control diarrhoea.