5. DISCUSSION

5.1 Physicochemical Standardization of Leaves

The macroscopical examination revealed that leaves are green in colour, attenuate, trifoliate and also very rarely five foliate. The shape of the leaves is lanceolate. The size is varying, ranging from 7 cm to 13 cm in length and 3 to 6 cm in width. The margin is crenate with acuminately apex and symmetric base with petiole around 2.3 to 6.5 cm long. Surface of leaves is glabrous and shiny showing parallel venation with brittle texture. Leaves bear aromatic odour and characteristic taste.

The lamina portion of the transverse section of *A. marmelos* leaf showed the presence of upper and lower epidermis both comprised of round to oval shaped cells. The Epidermis is single layered occasionally interrupted with sunken stomata on both surfaces and over-lined by a thick layer of cuticle. Both upper and lower epidermal layers bear stomata. Each stoma has two guard cells and two subsidiary cells and they correspond to rubiaceous type. Numbers of stomata are high in upper epidermis compared to lower epidermis and both epidermis have shown presence of covering trichomes. Interior to the epidermis is a many layered palisade tissue, which consists of closely packed oval cell without much intercellular space.

The palisade layer is continued midrib in upper portion while it is discontinued in lower portion. Interior to the palisade, spongy parenchyma layer is present which makes the bulk of lamina. Spongy parenchyma also shows presence of cluster crystals of calcium oxalate. The chloroplasts are more abundant in the palisade cells and less in the spongy tissue. Midrib portion shows continuation of the epidermal layer of lamina over midrib. Below the upper epidermis and above the lower epidermis are seen strips of the collenchymas (3-4 layered). Midrib shows the presence of xylem and phloem arranged in an arc. The microscopic photograph of transverse section had shown the characters
The microscopic examination of leaf material was on dried powder. Character observed was covering trichomes and stomata were present in the sample. The covering trichomes were multicellular, uniseriate and the stomata were paracytic type. It was found that the powdered leaf showed groups of fibres with calcium oxalate crystals. Calcium oxalate crystals were numerous and mainly of cluster crystal type. Some xylem vessels (pitted vessels) were also visible which were lignified and cells of palisade and spongy parenchyma were also observed.

Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with solvent. The extractions of any crude drug with a particular solvent yield a solution containing different phytoconstituents. The compositions of these phytoconstituents depend upon the nature of the drug and solvent used. The use of a single solvent can be the means of providing preliminary information on the quality of particular drug. Extractive value also gives the information regarding the quality of the drug. The extractive values of dried leaf powder in petroleum ether, chloroform, ethanol, hydroalcohol and water are calculated in terms of air dried sample. Ethanol followed by hydroalcohol proved to be highly effective for cold and hot extractive values.

The air dried plant materials were subjected to fluorescence analysis using different chemicals and lights and results are depicted in table 1. The fluorescence analysis of powdered leaf material was subjected to analyze under Daylight and Long Ultra Violet light (254 nm & 366 nm) after treatment with various chemical and organic reagents like sodium hydroxide, nitric acid, sulphuric acid, iodine, concentrated hydrochloric acid, ammonia, ferric chloride, Glacial acetic acid, Picric acid, Petroleum ether, chloroform and water. Table 1 shows a detailed fluorescence behavior of crude drug powder. The evaluation of a crude drug is an integral part of establishing its correct identity. Before any crude drug can be included in herbal pharmacopoeia, pharmacognostical parameters and standards must be established (Sayyed et al., 2012). Therefore some diagnostic features like fluorescence have been
evolved to identify and to differentiate the *Aegle marmelos* leaf from the other crude drugs and adulterants.

The physicochemical parameters like ash value, extractive value and loss on drying were determined for the powdered drug values and the findings are depicted in Table 2. Phytochemical investigation was performed on the extract developed by the successive extraction technique and the presence of chemicals in individual extract was reported. The percentage of loss on drying, total ash values, water soluble ash, acid insoluble ash and resin content were determined. The results noticed were; loss on drying (0.79%), total ash (5.79%), water soluble ash (1.28%) and acid insoluble ash (2.23%) respectively. Ash values are indicating the purity of drug, extractive values are representing the presence of polar or nonpolar compounds and loss on drying value indicates whether the drug is safe regarding any contamination of growth of bacteria, fungi and yeast.

The ash value of any organic material is composed of their non volatile inorganic components. Control and incineration of crude drugs result in ash residue consisting of an inorganic material (metallic salt and silica). This value varies within fairly wide limits and is therefore an important parameter for the purpose of evaluation of crude drugs. In certain drug, the percentage variation of ash from sample to sample is very small and any marked difference indicates the change in quality. Unwanted adulterants of drug, some time possess a character that raises the ash value. Ashing involves an oxidation of the components of the product. A high value is indicative of contamination, substitution, adulterations or carelessness in preparing the crude drug for marketing. The total ash value, acid insoluble ash value, water-soluble ash values were determined and the results are presented in Table 2.

5.2 Qualitative Phytochemical Analysis

The result of phytochemical screening of the aqueous and alcoholic extracts of *Aegle marmelos* revealed the presence of alkaloids, flavanoids, phytosterols, tannins
and phenols. The plant extract of *Aegle marmelos* used for the present work was chosen on the basis of their medicinal values. The natural plant parts are having a wide range of medicinal properties like antiulcer, hepatoprotective, antimicrobial, diuretic, emollient, febrifuge, narcotic, purgative and sedative. Previous study in the naturally the extracts of *Aegle marmelos* were subjected for phytochemical analysis. Phytochemical screening of the crude extract revealed the presence of alkaloids, cardiac glycosides, terpenoids, saponins, tannin, flavonoids, and steriods, but reducing sugars, carbonyl (aldehyde) and Phlobatanin show negative results (Venkatesan *et al*., 2009).

In the present investigation alcoholic and aqueous extract of the plant showed the presence of alkaloids, flavanoids, phytosterols, tannins and phenols. The production of secondary metabolites by the plant cells growing in culture has been studied by several scientists (Ramawat, 1999). He observed the production of indole alkaloids, ajmalicine in cell suspension culture of *Catharanthus roseus*.

These plants growing under natural conditions contain the spectrum of secondary metabolites such as phenols, flavanoids, quinones, coumarins, tannins and their glycosides, alkaloids, essential oils etc., the importance of these substance as microbial agents against the pathogen has been emphasized by several workers (Sofowara, 1993). In the present study, it was clearly understood that the alcohol extracted maximum amount of the different type of metabolites present in the *Aegle marmelos*. The phytochemical analysis of the *H. indicum* and *C. procumbens* extracts showed the presence of tannins, alkaloids, flavonoids and phenolic compounds. Tannins have been found to form irreversible complexes with proline-rich proteins (Boominathan and Ramamurthy, 2009).

Qualitative phytochemical analyses for alkaloids, carbohydrates, tannins, phenols, gums and mucilage, fixed oils and fats, saponins, proteins, volatile oils, flavonoids and steroids were screened in water and ethanolic extracts of the selected
medicinal plants of *Aegle marmelos*. The screening of the extracts indicated the presence of alkaloids, tannins and saponin in the ethanolic extracts of leaves (Table 3). Plants constitute an important source of active natural products which differ widely in terms of structures, biological properties and mechanisms of actions. It is not surprising that there are differences in the antimicrobial effects of plant species, due to the phytochemical properties and differences among species. It is quite possible that some of the plants that were ineffective in this study do not possess antibiotic properties, or the plant extracts may have contained antibacterial constituents, just not in sufficient concentrations so as to be effective. It is also possible that the active chemical constituents were not soluble in methanol or water. The drying process may have caused conformational changes to occur in some of the chemical constituents found in these plants (Parekh and Chandra, 2007).

Preliminary qualitative phytochemical analysis of all the extracts was carried out by employing standard conventional protocols. The results of qualitative phytochemicals are presented in the table 3. Alkaloid test was positive for all the extracts. Qualitative phytochemical studies of carbohydrate showed a good characteristic colour and precipitate in ethanolic extract. Strong presence of flavonoids in the ethanolic extract was confirmed by yellow colouration test and slight presence in all the other extracts was observed. Phenolic compounds, flavanoid and tannin were abundantly present in the water and ethanolic extract. The cardiac glycoside was copiously present in water and ethanolic extract. Our finding of the previous phytochemical screening in the naturally growing *Heliotropium indicum* revealed the presence of secondary metabolites (Boominathan and Ramamurthy, 2009), steroidal alkaloids, (Beb Oliver, 1986) saponin and tannin, (Nadkarni, 1976). The leaves, stems and roots are used externally as a poultice, and in the treatment of cancerous sores, boils, leucoderma and wounds (Duke and Ayensu, 1985; Moerman, 1998).

The present study carried out on the plant samples revealed the presence of medicinally active constituents. The phytochemical characters of the *Aegle marmelos*
results were summarized. The extract of plants showed higher proportion of carbohydrate, proteins, amino acids and saponins content. Moderate amount of alkaloids, phytosterols, tannins and phenols were also recorded. Secondary metabolites were found in good proportion in ethanolic and aqueous extracts when compared with petroleum ether and chloroform extracts. These secondary metabolites may be responsible for various pharmacological effects of ethanolic extract of Aegle marmelos leaves. Different chemical compound such as carbohydrates, alkaloids, flavonoids, tannins, terpenoids, saponins, quinones, glycosides, coumarins are detected more in the ethanolic extract of Aegle marmelos leaves than the other extracts, which could make the plant useful in treating different ailments and having potential for providing useful drug for human use. The phytochemical screening and quantitative estimation of the percentage crude yields of chemical constituents of the plants studied showed that the leaves and stems were rich in alkaloids, flavonoids, tannins and saponins. They were known to show medicinal activity as well as exhibiting physiological activity (Sofowara, 1993). Also, the presence of saponin in S. anthelmia contradicts the observation of Taylor-smith (1966) who reported that saponin was absent in this taxon.

Steroids and tannins were found to be present in all the plants. It has been found that some of these investigated plants contained steroidal compounds. It should be noted that steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones (Okwu, 2001). This may be the reason the leaves of C. rutidosperma are used as vegetable for expectant mothers or breast feeding mothers to ensure their hormonal balance, since steroidal structure could serve as potent starting material in synthesis of these hormones (Okwu, 2001).

The powder of Aegle marmelos leaves was taken in an apparatus and refluxed serially using distilled water and ethanol solvent system depending upon the polarity. The extracts of ethanol solvent system were transferred separately in previously weighed beaker; the weight of the sample was calculated. Weight and character of the
sample was found to be more in ethanol leaching out of the compounds. Other researchers have also reported the presence of terpenoids in *H. indicum*, and this plant is widely used in herbal medicine (Hayashi *et al.*, 1993). *E. heterophylla* contains tannins and alkaloids and this confirms the report of Gill (1992). The later also observed that some of the *Euphorbia* species including *E. heterophylla* are used as a purgative. They are also used in the treatment of cough, asthma and hay fever (Burkill, 1994 and Gill, 1992).

Alkaloid test was positive for all the extracts, except petroleum ether. Qualitative phytochemical studies of carbohydrate showed a good characteristic colour and precipitate in ethanolic extract. Strong presence of flavonoids in the ethanolic extract was confirmed by yellow colouration test and slight presence in all the other extracts was observed. Phenolic compounds, flavanoid and tannin were abundantly present in the ethanolic extract. The cardiac glycoside was copiously present in ethanolic extract. Various phytochemical components, especially polyphenols (such as flavonoids, phenyl propanoids, phenolic acids, tannins etc.,) are known to be responsible for the free radical scavenging and antioxidant activities of plants. Flavonoids and terpenoids are polar substances effective in acute inflammation whereas glycosides and steroids are non-polar substances effective in chronic inflammation (Singh and Pandey, 1997).

Polyphenols possess many biological effects. These effects are mainly attributed to their antioxidant activities in scavenging free radicals, inhibition of peroxidation and chelating transition metals. In general all polyphenols share the same chemical patterns, i.e., one or more phenolic groups for which they react as hydrogen donors and in that way neutralize free radicals (Heinonen *et al.*, 1998). Crude extracts from multiple parts of the bael plant are used to treat various disorders in different Indian traditional systems (Jain, 1991). Fresh leaves are used as an astringent, laxative, digestive and febrifuge. They are also useful in opthalmia, hearing loss and inflammation (Kirthikat and Basu, 1935). Secondary metabolites were found in good
proportion in ethanolic and aqueous extracts when compared with petroleum ether and chloroform extracts. These secondary metabolites may be responsible for various pharmacological effects of ethanolic extract of *Aegle marmelos* leaves. Different chemical compound such as carbohydrates, alkaloids, flavonoids, tannins, terpenoids, saponins, quinones, glycosides, coumarins are detected more in the ethanolic extract of *Aegle marmelos* leaves than the other extracts, which could make the plant useful in treating different ailments and having potential for providing useful drug for human use.

5.3 Quantitative Phytochemicals analysis

Phytochemicals are naturally occurring biochemicals that plants developed in order to protect themselves from oxidation, insects, disease organisms and other hazards in their environment. These chemicals give plants their characteristic colour, flavour, smell and texture. Epidemiological studies indicate that populations consuming high levels of plant derived foods have low incidence rates of various cancers.

From the table 4 the total phenolic content in the *Aegle marmelos* aqueous and alcoholic leaf extract was found to be 52.5 ± 0.24 and 60.2 ± 0.31mg/g of extract respectively. Phenolic acids are plant metabolites widely spread throughout the plant kingdom. Phenolic compounds are essential for the growth and reproduction of plants, and are produced as a response for defending injured plants against pathogens. The importance of antioxidant activities of phenolic compounds and their possible usage in processed foods as a natural antioxidant have reached a new height in recent years. Plant phenolics, originally hypothesized to inhibit carcinogenesis by virtue of antioxidant or electrophile trapping mechanisms, can also act as potent modulators of arachidonic metabolism cascade pathways. Certain plant phenols can be effective inhibitors of chemical mutagens, in vitro, and/or carcinogenesis in vivo. Phenolics can enhance the body's immune system to recognize and destroy cancer cells as well as inhibiting the development of new blood vessels (angiogenesis) that is necessary for
tumour growth. They also attenuate adhesiveness and invasiveness of cancer cells thereby reducing their metastatic potential. Augmentation of the efficacy of standard chemo and radiotherapeutic treatment regimes and the prevention of resistance to these agents is another important effect of plant phenolics that warrants further research. Plant phenolics appear to have preventative and treatment potential in combating cancer and warrant further in-depth research (Wahle et al., 2010).

Analysis of total flavonoid content in *Aegle marmelos* aqueous and alcoholic leaf extract has been done using colourimetric method and from the table 4, the flavonoid content of *Aegle marmelos* aqueous and alcoholic leaf extract was 78.12 ± 0.21 and 82.24 ± 0.04 mg/g of extract. Flavonoids are polyphenolic compounds that are ubiquitous in nature and are categorized, according to chemical structure, into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones. Numerous in vitro and animal model data suggest that flavonoids modulate important cellular and molecular mechanisms related to carcinogenesis, a multistep process involving the transformation, survival, proliferation, invasion, angiogenesis, and metastasis of the tumor cells (Clere et al., 2011). Flavonoids act as protein kinase inhibitors for cancer chemoprevention. Recent studies show that flavonoids can bind directly to some protein kinases and then alter their phosphorylation state to regulate multiple cell signaling pathways in carcinogenesis processes (Hou and Kumamoto, 2010). Flavonoids have also been shown to increase mucous secretion, prostaglandin synthesis and blood flow (Singh et al., 1998).

The total tannin content was also observed from the table 4 as 38.9 ± 0.30 and 43.26 ± 0.22 mg/g of *Aegle marmelos* aqueous and alcoholic leaf extract. Tannins are a group of natural products which are recognized as health protecting antioxidants. Tannins are plant polyphenolic compounds that are contained in large quantities in food and beverages (tea, red wine, nuts, etc.) consumed by humans daily. It has been shown that various tannins exert broad cancer chemoprotective activity in a number of animal models. An increasing body of evidence demonstrates that tannins act as both
anti-initiating and antipromoting agents. In view of the fact that tannins may be of valid medicinal efficacy in human clinical trials, attempts are made to integrate results from animal studies, and consider their possible application in humans (Nepka et al., 1999). In the present investigation alcoholic extract of the plant leaves and root showed the presence of alkaloids, flavanoids, phytosterols, tannins and phenols. The production of secondary metabolites by the plant cells growing in culture have been reported by Ramamurthy et al. (2009). These plants growing under natural conditions contain the spectrum of secondary metabolites such as phenols, flavanoids, quinones, coumarins, tannins and their glycoside, alkaloids, essential oils etc., the importance of these substance as microbial agents against the pathogen has been emphasized by several workers (Mahadevan, 1979).

Analysis of alkaloid content in aqueous and alcoholic leaf extract of *Aegle marmelos* has been done using colourimetric method and from the table 4, the alkaloid content of *Ocimum sanctum* and *Annona squamosa* methanolic leaf extract was 4.12 ± 1.05 and 4.51 ± 0.03 g/100g of extract. Quantitative phytochemical analysis of the leaf powders of two extract showed that total alkaloid contents were almost similar. Previous phytochemical screening in the naturally growing *Heliotropium indicum* revealed the presence of secondary metabolites (Boominathan and Ramamurthy, 2009) steroidal alkaloids (Beb Oliver, 1986), Saponin and tannin (Nadkarni, 1976).

Analysis of crude protein content in *Aegle marmelos* aqueous and alcoholic leaf extract has been done using colourimetric method and from the table 4 the crude protein content of *Aegle marmelos* aqueous and alcoholic leaf extract was 28.5 ± 0.12 and 34.9 ± 0.14 g/100g of extract. According to Gupta et al. (2006) 1.8% proteins, 2.9% fibre with 137 Kcal calorific values are presented. Suvimol and Pranee (2008) reported that fruit pulps had crude protein, total, soluble, and insoluble dietary fiber contents of plants respectively.
The glycosidal content was also observed from the table 4, the plant aqueous and alcoholic leaf extract of *Aegle marmelos* was 1.79 ± 1.50 and 1.96 ± 0.25 g/100g of extract. Tannins are a group of natural products which are recognized as health protecting antioxidants. Uttara Singh *et al.* (2012) reported that the *Aegle marmelos* leaf, pulp and seed powder had glycosidal content of phytic acid respectively. According to Agroforestry Database (2009) the bael pulp contains 9% tannin and glycosidal content.

From the table 4, the total terpenoid content of the aqueous and alcoholic leaf extract of *Aegle marmelos* was found to be 3.52 ± 0.02 and 3.85 ± 0.08 g/100g of extract respectively. The terpenoids are plant metabolites widely spread throughout the plant kingdom. In the present investigation alcoholic extract of the plant showed the presence of alkaloids, flavanoids, total terpenoid and phenols. The production of secondary metabolites by the plant cells growing in culture has been reported by several scientists (Ramawat, 1999). He observed the production of indole alkaloids, total terpenoid in cell suspension culture of *Catharanthus roseus*.

Ascorbic acid (Vitamin C) content of leaf powders of aqueous and alcoholic extract of *Aegle marmelos* were 0.89 ± 0.16 and 0.76 ± 0.13 g/100g. Ascorbic acid content of the both samples does not vary much and the estimated value was respectively for aqueous and alcoholic extract of *Aegle marmelos*. Analysis of β-Sistosterol content in *Aegle marmelos* aqueous and alcoholic leaf extract has been done using colourimetric method and from the table 4, the β-Sistosterol content of *Aegle marmelos* aqueous and alcoholic leaf extract was 20.78 ± 0.07 and 0.82 ± 0.05 g/100g of extract.

**5.4. Trace elements of *Aegle marmelos***

The trace elements are useful in human physiological activities. It is therefore concluded that the plants under study which are rich in elements may also help in biodiversity function etc. A total four elements have been determined in medicinal
plants; these are commonly used in curing various ailments. The results indicated that there are variations in elemental composition and concentration between the species and different extracts. The reflecting difference in physiological functioning of the specific plants depends upon the elemental interaction within it. Levels of the trace elements iron, phosphorus, sodium, potassium, calcium, zinc, copper and manganese (ppm, dry weight) in plant samples collected from different extracts of *Aegle marmelos* are presented in table 5. The plants used as biomonitors were sampled with two different extracts. Atomic absorption spectrometry was used in order to determine the concentrations of elements. The following mean concentrations were determined in the sample.

The sources for accumulation of some trace elements have been explained by various researchers. For example, anthropogenic activities are the main sources of copper and Zn according to Alfani *et al.* (2000), Blok (2005) and Oliva and Rautio (2005). The burning of coal and oil, production of Cu, Ni and Pb, mining operations, steel works and cement industry are cited as major anthropogenic sources of Ni (Nriagu and Pacyna, 1988). Although airborne Mn mainly comes from soil (Bargagli *et al.*, 2003; Oliva and Rautio, 2005), and Fe originates from both anthropogenic and natural sources (Oliva and Rautio, 2005), it was reported that the contamination of soil by Fe and Mn highly affects plants in the Mediterranean climate zone (Loppi *et al.*, 1999).

Selection of the plants used for this study was based on their extensive use in traditional medicinal system of India. The medicinal uses of these plants in Ayurveda cures a number of ailments including hypertension, neurological disorders, asthma, immuno-stimulants, antibacterial, menstrual disorders, rheumatism and urinary tract infection etc. Since the major elements are either direct or indirect involvement in biological activity, analysis of four major elements namely Fe, Zn, Mn and Cu was performed in a plant samples collected.
The average iron content of aqueous and alcoholic extract of *Aegle marmelos* was 178 ± 1.22 and 199 ± 1.05 ppm/100g (Table 5). The highest amount of Fe is recorded in the alcoholic extract. The variation due to the solvents variability and presence of mining activities are reported. It is an important hemoglobin component responsible for oxygen transport in human body (Martin et al., 1985). The normal tolerable range of iron is 15-120 mg/day. Iron is undoubtedly the most important nutrient and its deficiency causes several disorders (Chen et al., 1993). The concentration of Fe in tulsi *Ocimum sanctum* and neem *Azadirachta indica* were 129 and 355 mg/g-1 respectively Samudralwar and Garg (1996); Sheded et al. (2006) reported that the highest Fe is estimated in *Pergulariato mentosa* 292 mg/g-1 among the estimated seven medicinal plants.

The average zinc content of aqueous and alcoholic extract of *Aegle marmelos* was 78.2 ± 2.65 and 91.3 ± 3.79 ppm/100g (Table 5). The highest amount of zinc is recorded in the alcoholic extract. It is clear from the above results that there is an accumulation of high amount of Zn content and rich amount of minerals in the soil due to mining area and it is a second abundant element of medicinal plants. The physiological activities of the plant influence the Zn absorption and the interaction with many elements like Fe, Mn and Cu, affects Zn intake (Kandala et al., 1974). Zn is the component of more than 270 enzymes and its deficiency causes many physiological disorders. Besides, it is responsible for stimulating growth of epidermal and epithelial cells (Keplan et al., 2003). The normal per day intake of Zn level is 12-15 mg/day. The similar kind of report in medicinal plants has been reported by Morabad et al. (2013).

The Manganese content of aqueous and alcoholic extract of *Aegle marmelos* was 36.2 ± 5.02 and 41.5 ± 3.25ppm/100g (Table 5). The highest amount of zinc is recorded in the alcoholic extract. Mn is an important electrolyte also responsible for proper bones and liver function. It also works as co-factor in more than 300 metabolic reactions (Berdanier, 1994). Normal daily intake of Mn is 2-8 mg/day. Shededet al.,
(2006) estimated the manganese from seven medicinal plants; in *Acacia ehrenbergiana* 339 mg/kg the highest amount was detected. According to Reddy and Reddy (1977) most of the plants examined are safe.

The copper content of aqueous and alcoholic extract of *Aegle marmelos* was 43.1 ± 1.16 and 49.6 ± 1.22 ppm/100g (Table 5). The highest amount of copper is recorded in the alcoholic extract. Cu is the main constituent of the bone, connective tissue, brain, heart and many other body organs (Ekinci and Rekinci, 2004). Normal daily intake of copper is 2-5 mg/day. The Cu is macronutrients, which is essential for human health and nutrition by Reddy and Reddy *et al.* (1997). Sheded *et al.* (2006) reported the range of Cu contents in 50 medicinally important leafy materials growing in India.

Minerals are known to play an important metabolic and physiologic roles in the living system (Morabad *et al.*, 2013). Micronutrients present in 100 gm of *Aegle marmelos* leaf extract are shown in the table 5. Copper is the third most abundant trace mineral in the body, but it is often deficient in a person's diet because food sources high in this mineral are not always eaten frequently. Copper deficiency is rare and is most frequently seen in cases of severe anorexia, starvation, or rare kidney problems. The human body contains approximately 100-500 mg of copper but it's role is important. Copper works together with iron to form red blood cells and it is the major component of the outer coating of nerve fibers and collagen. Copper is involved in maintenance of immunity, fertility, formation of melanin, and the promotion of consistent pigmentation. It is believed to play a role in preventing high blood pressure, heart arrhythmia, oxidation of the cells, and keeping cholesterol low. Copper is used by the body to synthesize numerous enzymes, many of which work as antioxidants.

Johnson and colleagues (Johnson *et al.*, 1982) have reported that the solid tumor rats when treated with the copper complexes (such as copper salicylate) have decreased the tumor growth and increased survival rates. Numerous copper complexes
with SOD activity prevented or retarded the spontaneous development of cancers in mice and possessed anticancer, anti-carcinogenic, and anti-mutagenic effects both in vitro and in vivo (Dollwet HH and Sorenson, 1985). Copper complexes did not kill cancer cells but often caused them to revert to the growth patterns of normal (differentiated) cells. The low copper also decreased the expression of various protein kinase C isozymes, a series of proteins involved in the signal transduction pathway within the cell, thus upsetting normal cell regulation (Klevay, 1998).

Phosphorus was observed in 100 mg of *Aegle marmelos*. Phosphorus is the second most abundant mineral in the body and 85% is found in the bones. The rest of the body's phosphorus is found in the blood, the fluid around and in cells, and in various organs like the heart, kidneys, brain, and muscles, where it is involved in many critical functions. Its main purpose is for building strong bones and teeth, but this mineral is used by every cell in the body. Phosphorus is involved in virtually all physiological chemical reactions in the body, and calcium & Vitamin D are essential for proper functioning of the phosphorus.

This element protects and strengthens cell membranes, assists other nutrients, hormones, and chemicals in their processes, and is necessary for normal bone and tooth structure. Phosphorus is needed for healthy nerve impulses, normal kidney functioning, and the utilization of carbohydrates, fats, and proteins for growth, maintenance, and repair of cells and for energy production. Phosphorus is a component of DNA and RNA and serves in the preparation of glucose for energy formation. Estramustine phosphate is a chemotherapy drug that is known as an alkylating agent and it used in the treatment of hormone-refractory prostate cancer and prostate cancer with bone metastasis. Estramustine phosphate works by binding the two strands of DNA that make up the double helix. The DNA cannot replicate,

Zinc and magnesium found in traces was below detectable level in 100 mg of *Aegle marmelos*. Zinc is an important mineral that the body uses in a variety of
processes. Zinc plays an important role in cell division, growth, and repair. It helps in wound healing and maintaining a normal sense of taste and smell. Zinc works as an immune booster and can be instrumental in fighting colds, flu, and other infections. Zinc is a component of more than 200 enzymes, most of them involved in protein and DNA synthesis. Zinc has beneficial effects on sex and thyroid hormones. The male prostate gland has heavy concentrations of zinc and this gland synthesizes prostatic fluid in which sperm cells are mixed to make semen, and zinc helps to regulate the metabolism of testosterone in the prostate. Zinc is especially important in the prostate and may protect it from early damage that could lead to cancer, although studies with prostate cancer cells in cell culture labs indicate that zinc supplementation may be less useful in treating prostate cancer (Barbara and Minton, 2009).

Magnesium is an important mineral to promote health because it enhances the activity of 300 enzyme-related processes. This mineral facilitates effective nerve and muscle functioning and it is involved in bone and tooth formation. Magnesium (along with calcium and potassium) regulates heart rhythm, clots blood, and assists the body in producing and using insulin. Magnesium's role as a cofactor in calcium utilization is very important, for without it the enzyme that facilitates the passage of calcium across cell membranes fails to act, resulting in both calcium and magnesium deficiencies (Morabad et al., 2013).

5.5 PROFILING OF SECONDARY METABOLITES

5.5.1 Thin Layer Chromatography of Aegle marmelos leaf extract

TLC plates were prewashed with methanol. Activation of plates was done in an oven at the rate of 500 for 5 min. The chromatographic conditions maintained were precoated silica gel 60F254 aluminum sheets (10×10 cm) as stationary phase, chloroform: methanol: glacial acetic acid (6:2: 0.1 v/v/v) as mobile phase, chamber and plate saturation time of 30 min, migration distance 13.5 mm, wavelength scanning at 260 nm keeping the slit dimensions at 5×0.45 mm. A deuterium lamp provided the source of radiation.
TLC plate was dried, developed and analyzed photometrically as described earlier. The standard calibration curve was generated using regression analysis with Microsoft excel. Sample solutions of the marketed formulation were spotted on the same plate followed by development scanning. The analysis was repeated in triplicate. The content of the drug calculated from the peak areas was recorded. The developed method was validated in terms of linearity, accuracy, limit of detection, limit of quantification and repeatability.

For the pharmacological as well as pathological discovery of novel drugs, the essential information’s regarding the chemical constituents are generally provided by the qualitative phytochemical screening of plant extracts. TLC profiling of *Aegle marmelos* leaf extract gives an impressive result, directing towards the presence of number of phytochemicals. Various phytochemicals gives different Rf values in different solvent system. This variation in Rf values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by Column Chromatography. Compounds showing high Rf value in less polar solvent system have low polarity and with less Rf value have high polarity. Mixture of solvents with variable polarity in different ratio can be used for separation of pure compound from plant extract. The selection of appropriate solvent system for a particular plant extracts can only be achieved by analyzing the Rf values of compounds in different solvent system (Das Talukdar *et al.*, 2010). In the present state of affairs, TLC profiling of *Aegle marmelos* ethanolic leaf extract indicated three major bands in long UV and iodine sprayed plates. Rf was calculated as distance travelled by solute/ Distance travelled by solvent. Quinones and steroids at Rf 0.72, Glycoside and polysaccharides at Rf 0.70 and Amino acids and peptides at Rf 0.58 are clearly seen from the figure II. Different Rf values of the compound also reflects an idea about their polarity. This information helps in selection of appropriate solvent system for further separation of compound from the plant extract.
5.5.2 HPLC Analysis of *Aegle marmelos* leaf extract

*Aegle marmelos* leaf extract was subjected to HPLC and the obtained records were super imposed on the retention time values of the extract. Rutin, Quercetin, Kaempherol, Farmarixetin, Isorhamnetin, Marmesin, Ursolic acid were used as standards. Table 7 shows the chromatogram of *Aegle marmelos* leaf extract, which was compared with the standards and found to contain Rutin, Quercetin, Marmesin and Ursolic acid.

Rutin is a bioflavonoid (flavonol glycoside) comprised of the quercetin and the disaccharide rutinoside. Rutin is effective in reducing hemorrhoids, by strengthening and improving the permeability of blood vessels and capillaries. Rutin has also been found to be useful and effective against poor blood circulation, high blood pressure, varicose veins, capillary fragility and other conditions where the blood vessels are weak. Rutin, a flavonoid, obtained from the plant, *Sophora japonica*, markedly reduced the infarct size and prevented the loss of the ‘R’ wave in anaesthetized rats subjected to coronary artery ligation. It, however, had no effect on heart rate and systolic blood pressure. It also reduced the ligation-induced increase in serum malonyldialdehyde levels and prevented the loss of glutathione peroxidase activity. Rutin inhibited, invitro, luminol-induced chemiluminescence of rat PMN’s, thus indicating that its beneficial effect is probably due to its ability to impair the generation of reactive oxygen species (Chopra and Singh, 1994).

Quercetin is the most abundant of the flavonoid molecules and it is found in many medicinal plants. It has been reported to prevent gastric mucosal lesions induced by ethanol (Alarcon de la Lastra *et al.*, 1993). Quercetin increases the amount of neutral glycoproteins in the gastric mucosa and thus participates in the recovery of the mucosal defensive capacity against aggregation from absolute ethanol. Other possible mechanisms include inhibition of lipid peroxidation, inhibition of the gastric proton pump (Di Carlo *et al.*, 1999) and scavenging of free radicals associated with a significant enhancement in the glutathione peroxidase activity. Rutin along with
Quercetin can significantly inhibit the oxidation of HDL induced by Ca^{2+}. Marmesin, a linear dihydrofuranocoumarin, isolated from the bark of *Aegle marmelos*, has been reported to be used as a remedy in melancholia, intermittent fevers and palpitation of heart (Chopra *et al*., 1956). Marmesin has also been isolated from *Ammi majus*, a plant used for the treatment of leucoderma (Abumustafa *et al*., 1958).

Ursolic acid is a pentacyclic triterpene acid, used in cosmetics, also capable of inhibiting various types of cancer cells by inhibiting the STAT 3 activation pathway (Shishodia *et al*., 2003) and human fibrosarcoma cells by reducing the expression of matrix metalloproteinase-9 by acting through the glucocorticoid receptor. It may also decrease proliferation of cancer cells and induce apoptosis (Wang *et al*., 2011). Ursolic acid can serve as a starting material for synthesis of more potent bioactive derivatives, such as anti-tumor agents (Ma *et al*., 2005). It has been found to reduce muscle atrophy and to stimulate muscle growth in mice (Kunkel *et al*., 2011). Ursolic acid has potential use as a cardioprotective compound (Liobikas *et al*., 2011).

### 5.5.3 HPTLC Analysis of *Aegle marmelos* Leaf Extract

HPTLC is an invaluable quality assessment tool for the evaluation of plant compound. It allows the analysis of a broad number of compounds both efficiently and cost effectively. HPTLC studies have shown that it is more versatile than ordinary TLC methods, as the spots are well resolved. Repeatability of sample application was assessed by spotting of drug solution 10 times on a TLC plate followed by development of plate and recording the peak area for 10 spots and major active compounds presented in 5. The % for peak area values of *Aegle marmelos* was found to be 3.60 to 20.1 respectively.

In this study the HPTLC fingerprinting of *Aegle marmelos* leaf extract revealed 10 spots with Rf values 0.04, 0.1, 0.16, 0.25, 0.28, 0.41, 0.62, 0.69, 0.79, 0.88 and purity of the sample was confirmed by comparing the absorption spectra at start, middle and end position of the band. Figure 12 shows the HPTLC chromatogram of
*Aegle marmelos* leaf extract at 520nm, showing different peaks of phytoconstituents. These studies were carried out at three levels i.e. Retention factor, % area and % Yield recovery studies. Sample stock solutions from compounds identification of *Aegle marmelos* were prepared. Dilutions were made and recovery studies were performed. Percentage of yield was found to be within the limits as listed in Table 8. The developed HPTLC technique is simple, precise, specific and accurate and the statistical analysis proved that method is reproducible and selective for the analysis of *Aegle marmelos* in bulk drug formulations.

The composition of the mobile phase for TLC was optimized by testing different solvent mixtures of varying polarity. The best results were obtained using chloroform: methanol: glacial acetic acid (6:2: 0.1 v/v/v) as mobile phase. The selected mobile phase showed good resolution. The compound with Rf value was identified and the reported HPTLC method was recorded. Modi *et al.* (2008) reported that the gradient elution technique was used and mobile phase was consisted of water, methanol, phosphoric acid and methanol and the pH was adjusted to 2.0. Thus as compared to the reported method, our proposed method requires simple solvent system to separate the L-dopa from the extract. The peak purity was assessed by comparison of overlay spectra of standard L-dopa and *Mucuna pruriens* seed extract at peak apex and peak base which confirmed the method, selective.

The HPTLC method was validated in terms of precision, accuracy and repeatability. The method was found to be specific as it well resolved active compound with Rf value of 0.87, in the presence of other components in the sample. Linearity was evaluated by determining various standard working solutions containing 10 to 100 mg/ml of active compounds. Peak area and concentrations were subjected to least square linear regression analysis to calculate the calibration equation and correlation co-efficient. The linearity of calibration graph and adherence of the system to Beer’s law was validated by high value correlation co-efficient. In the reported HPTLC method by Siddharaju and Becker (2001) linearity was in the range of 2 to
100 mg/ml while in our method the linearity range was 10 to 120 mg/ml. Hence it is comparable with the reported method. Repeatability of sample application was assessed by spotting of drug solution 10 times on a TLC plate followed by development of plate and recording the peak area for 10 spots and major active compounds presented in 5. The % for peak area values of Aegle marmelos was found to be 10.68 to 19.10 respectively.

Accuracy parameter was checked by recovery studies. For Aegle marmelos 0.69% recovery was observed. Four micro litres of sample solutions of the marketed formulation were spotted on to the same TLC plate and developed. The analysis was repeated in triplicate. The content of the drug calculated from the peak areas was recorded. Joshi et al., (2009) reported the recovery studies of the drugs carried out for the accuracy parameter. These studies were carried out at three levels, i.e. multiple level recovery studies. Sample stock solution from tablet formulation of 100µg/ml was prepared. Dilutions were made and recovery studies were performed.

The decoction contained carbohydrates, glycosides, amino acids, proteins, tannins, flavanoids, and phytosterols. The results were similar to earlier reports (Brijesh et al., 2009). The chromatogram of the HPTLC fingerprinting analysis of the ethanol soluble fraction of the decoction scanned at 254 nm has been analyzed.

Detection of adulteration and quantitative determination of marker substances are other widely used applications. The advancement of instrumentation and methodological concepts has created a basis for reliable qualitative and quantitative results in HPTLC. Integration of biological detection systems as well as hyphenation to mass spectrometry has widened the applicability of planar chromatography as an analytical technique that is both orthogonal and complementary to HPLC (Reich and Widmer, 2009)
In this study the HPTLC fingerprinting of *Aegle marmelos* leaf extract revealed 10 spots with Rf values 0.04, 0.1, 0.16, 0.25, 0.28, 0.41, 0.62, 0.69, 0.79, 0.88 and purity of the sample was confirmed by comparing the absorption spectra at start, middle and end position of the band. Sobhani *et al.* (2002) reported that the Harmane (Rf 0.70) was not detected in the extract. The plots of the ratio of peak area of the alkaloids to quinidine sulfate (Rf 0.40), which was used as an internal standard, versus concentration, were straight lines with slopes of 0.0274 for harmine and 0.0211 for harmaline. In order to correlate topoisomerase I inhibitory effect of *Peganum harmala* extract with its α-carboline content, HPTLC method was used. The results of HPTLC analysis show that harmine and harmaline constitute 13.5% of dry weight of the extract. Harmane content of the extract, if present, was below the detection limit of the HPTLC analysis that was used. Our *in vitro* findings demonstrate that *Aegle marmelos* root extract do inhibit topoisomerase and based on the results of HPTLC analysis, it appears that the biological activity of the extract can be explained by its α-carboline content. The developed HPTLC technique is simple, precise, specific and accurate and the statistical analysis proved that method is reproducible and selective for the analysis of *Aegle marmelos* in bulk drug formulations.

### 5.5.4 GC-MS Analysis of *Aegle marmelos* Leaf Extract

Use of GC/MS enabled identification of the most components in leaves samples of *Aegle marmelos* was analyzed by phyto-constituents. The compounds identified are listed in Table 9. In these, natural compounds have been a source of medicinal agents for antimicrobial, anti-inflammatory compounds and some essential fatty acids are analyzed in this plant. The components of the infusion differ from those found in tincture except organic acids derivatives. The concentrations (in %MS) of these derivatives in infusion and tincture are a in great deal close to each other; may be that is why in traditional medicine are used both types of extracts are used with success. Chandra Mohan *et al.* (2012) reported the preliminary phytochemical, antibacterial and GCMS analysis of ethanol extract of the plant. Phytochemical screening of leaves extract revealed the presence of alkaloids, tannins, steroids,
saponins, flavonoids, glycosides and phenolic compounds. Six compounds were identified by GCMS analysis for *Aegle marmelos*.

Thirty three compounds were identified in alcoholic fraction of *Aegle marmelos* leaf extract by GC-MS analysis. The chromatogram is obtained by alcoholic fraction of *Aegle marmelos* leaf. The active principle, area of the peak, Concentration (%), Retention Time (RT), Molecular formula and Molecular weight were presented in Table 9. The prevailing compounds were 3,4-Dimethoxybenzoic anhydride (21.01%), Cinnamic acid (10.9%), Palmitic acid (4%), 1-Phenylpyrrole (7.73%), Cinnamamide (3.6%), 4-Methoxybenzaldehyde (1.7%), Gamma-Sitosterol (1.27%), Caryophyllene oxide (1.27%), Alpha-amyrin (1.31%) and Loliolide (0.59%).

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained in Table 4. The extract of *Aegle marmelos* was subjected to GC-MS study for identification of medicinal properties. According to the results, the Phytocomponents are screened, and most of the medicinal properties are 1H-Pyrrole-2,5-dione, 1-ethenyl, 3,8-Nanodiene-2-one, (E)-, Proline, 3,4- didehydro-, 4-Amino-3-methoxypyrazolo [3,4- d] pyrimidine, Propanenitrile, 3-(5-diethylamino-1-methoxy-3-pentyloxy)- compounds.

The differences between the compounds that we have found in the roots, stems and leaves of *Aristolochia clematitis* were studied by GC-FID. This study was performed on the alcoholic extracts of the three parts of the plant. From this study we have concluded that the compounds found in the root and stem are very similar. The aristolochic acid derivatives are present in both extracts, but in the leaves these derivatives are in very low concentration (Podea *et al.*, 2001).
Ramamurthy (2010) reported the difference between the compounds that we have found in the roots and leaves of *H. indicum*, studied by GC-MS. This study was performed on the alcoholic extracts of the two parts of the plant. From this study it is concluded that the compounds found in the root and leaves are very dissimilar. The organic acid derivatives are present in both extracts, but in the leaves these derivatives are in very high concentration. On the other side the study shows that their concentration is higher in the roots and stems. In the leaf extracts organic acid derivatives and vitamin F (polyunsaturated fatty acids) are present in very higher amount. In conclusion terpenic compounds, fatty acids, phytol, alkaloids and especially organic acid derivatives are responsible for the therapeutic activity of this plant. In the present study the compounds that they have found in the *Aegle marmelos* were studied by GC-MS. This study was performed on the alcoholic extracts of the plant. From this study it is concluded that the compounds found in the root are very dissimilar. The organic acid derivative presents in ethanolic extracts of leaves are in very high concentration.

The analytical methods GC/MS is suitable for medicinal herbs organic compounds determination. The sample preparation method is rapid and precise. There is a difference between the compounds extracted from herbs by infusion and tincture, but the important thing is that the organic acid and fatty acids derivatives are present in both of them. In the leaf extracts organic acid derivatives and vitamin (polyunsaturated fatty acids) are present in very higher amount. In conclusion terpenic compounds, fatty acids, phytol, alkaloids and especially organic acid derivatives are responsible for the therapeutic activity of this plant.

5.6 Antimicrobial activity of *Aegle marmelos*

*Aegle marmelos* possessed very high levels of alkaloids and flavonoids, and are employed in medicinal uses. The plants studied here can be seen as a potential source of useful drugs. Further studies are going on these plants in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds. The
antimicrobial activities of these plants for the treatments of the diseases as claimed by traditional healers are also being investigated.

In this study, medicinal herbs, i.e., *Aegle marmelos* was extracted with water and 95% ethanol. The extracts were used to study antifungal and antibacterial effects by the agar diffusion technique. The bacterial strains such as *Streptococcus pyogenes*, *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumonia*, *Helicobacter pylori*, *Pseudomonas auroginosa* and fungal strains such as *Aspergillus niger*, *Candida albicans* and *Trichoderma viride* were used. Until the late eighties, it was widely believed that superficial infection was no longer a threat to public health. Microbial infection seems especially controllable due to good hygienic condition, but development of microbial resistance to antimicrobial drugs is almost an inevitable consequence of their application. Microorganism that acquired resistance to a particular antimicrobial agent becomes clinical important, particularly when the use of individual drug is wide spread. Mechanism of drug resistance by microorganisms to antimicrobial agents can be categorized into the enzymatic modification in activation of the antibodies or receptor modification as well as limiting access of the drug to its susceptible host of pathogen (Ramamurthy *et al.* 2013).

The crude alcoholic extracts of *Aegle marmelos* and the control drug were subjected to antimicrobial activity. In the case of *Streptococcus pyogenes*, *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumonia*, *Helicobacter pylori*, *Pseudomonas auruginosa* activity is high when compared with the plant aqueous extract of *Aegle marmelos*. Venkatesan *et al.* (2009) reported that in gram positive bacteria, *Aegle marmelos* and the control drug penicillin, the organisms exhibit a similar zone of inhibition and hence they are considered as resistant. In this study, the results of the investigation show that the plant extracts from *Aegle marmelos* have good antimicrobial activity against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumonia*, *Helicobacter pylori* and *Pseudomonas*
*A. auroginosa* due to the presence of alkaloids, cardiac glycosides, terpenoids, saponins, tannin, flavonoids, and steriods.

Suressh *et al.* (2009) have concluded that the leaf and flower methanol extracts were found to be more effective against all microorganisms. The flower extracts was found to induce maximum inhibitory effect against all these microorganisms. In the leaf methanol extracts of *Aegle marmelos* antibacterial activity was maximum in *Salmonella typhi* followed by others. In the flower methanol extract *Aegle marmelos* antibacterial activity was higher in *Staphylococcus aureus* followed by others. This is due to astringent antipyretice and also contains tannins. The results support earlier result as antibacterial activity of Amry card power (formulation) consists of *Aegel marmelos* against *E. coli*, *Staphylococcus* and *Streptococcus*.

The result in this study revealed that the alcohol and aqueous extracts of the plant have antimicrobial activity against the test organisms. The inhibitory action of the crude extracts was recorded even at very low dose, which is a clear indication that the crude extract contains active commencements that have antifungal properties. Similarly, the methanol crude extract of *C. procera* have antifungal activity against *Microsporum canis* and *Trichophyton rubrum* at 5.0 mg/ml concentration. Ramamurthy *et al.* (2013) had reported that patients of dematophytoses in Nigerian are faced with the challenges of re-occurrence of ringworm infections. Inspite of the fact that the crude extract inhibited the growth of the test organisms and the use of the extract for therapeutic purpose should be encouraged, the crude extracts had less inhibitory effect in some cases as revealed in the results (Tables 10 & 11). The ethanol leaf extracts produced significant increase in the zone of inhibition compared to aqueous that was found to be ineffective.

The *Aegle marmelos* was screened against six pathogenic bacterial strains and three pathogenic fungal strains for antimicrobial activities. The inhibition zones of extracts against the specific test organisms were measured. The extracts showed the
inhibitory effect on the all test organisms. The extract restricted the growth of pathogen on the media around the well. The maximum inhibition zone (22 mm) was observed in the extract of alcohol extract of *Aegle marmelos* against the *Helicobacter pylori* and the minimum inhibition zone (2.6 mm) was observed with the water extract of *Aegle marmelos*. All the extracts showed the inhibitory effect on the test organisms. Maximum inhibition was noticed in *Helicobacter pylori* when compared to other microbes. Similarly extracts from water and ethanol leaves extract of *Aegle marmelos* showed maximum zone of inhibition in all the concentrations tested over the control. Significantly, the ethanol extracts of *Aegle marmelos* showed the maximum zone of inhibition when compared to aqueous extract of *Aegle marmelos*. Our study is the first of its kind that demonstrated the antimicrobial activity of the extracts of *Aegle marmelos*. Such a property could be related to the presence of enzymes and stable cysteine proteases in the latex (Dhanaraj et al., 2011).

Further, the antimicrobial activity of latex suggests that it might be effective against other fungal strains as well. Boominathan and Ramamurthy (2009) reported that the ethanolic extracts were tested against bacteria and fungi. Among the extracts, the leaf extract of *Heliotropium indicum* were effective against bacteria and fungi. The other three extracts have less inhibitory effect which has been noted in bacteria and fungi. In the present study it was interesting that the traditional method of treating a microbial infection was by administering a decoction of the plant, whereas according to our results an alcoholic extract was better; hence this may be more beneficial. Amongst the six bacterial and three fungal strains investigated *Helicobacter pylori* is the most resistant and *Trichoderma viride* is less resistant.

In the present study ethanol extract was tested for its antimicrobial activity against some human bacterial pathogen such as *Streptococcus pyogenes*, *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumonia*, *Helicobacter pylori* and *Pseudomonas auroginosa*. These results are consistent with previous reports on related plants regarding Gram-positive bacteria (Cowan, 1999). The resistance of
Gram-negative bacteria (*Staphylococcus aureus*) to plant extracts was not unexpected as in general, this class of bacteria is more resistant than Gram-positive bacteria. Such resistance could be due to the permeability barrier provided by the cell wall or the membrane accumulation mechanism (Adwan and Abu-Hasan, 1998).

The methanol extracts of *Castanopsis acuminatissima* leaves, stem and root barks were partitioned (petrol, dichloromethane, ethyl acetate). Though all of the crude methanol extracts and obtained fractions from them, showed a broad spectrum of antibacterial activity, in most cases the activity was decreased on fractionation. None was active against tested moulds (Khan *et al.*, 2001). In this study alcohol and aqueous extract of *Aegle marmelos* showed a broad spectrum of antibacterial activity.

The extract restricted the growth of pathogen on the media around the well. The maximum inhibition zone (22 mm) was observed in the extract of alcohol extract of *Aegle marmelos* against the *Helicobacter pylori* and the minimum inhibition zone (2.6 mm) was observed with the water extract of *Aegle marmelos*. All the extracts showed the inhibitory effect on the test organisms. Maximum inhibition was noticed in *Helicobacter pylori* when compared to other microbes. Similarly extracts from water and ethanol leaves extract of *Aegle marmelos* showed maximum zone of inhibition in all the concentrations tested over the control. The antibacterial activity of *Aegle marmelos* extracts on *S. aureus* was detected ethanol. Extract from leaves. *B. subtilis* showed resistance to all types of extracts. Based on the current findings, it can be concluded that this plant has antimicrobial activity, which is as potent as standard antimicrobial drugs against certain microorganisms (Somchit *et al.*, 2003).

According to Boominathan and Ramamurthy (2009) medicinal plants and microorganisms are the proper candidates and should receive continuous research attention. The use of higher plants to treat infections is an age-old practice in a large part of the world population. Furthermore, because of the side effects and the resistance that pathogenic microorganisms build against the common antibiotics, much
recent attention has been paid to extracts and biologically active compounds isolated from plants used in herbal medicine. They found plant derivatives of gamma-linolenic acid and arachidonic acid act bactericidally on a significant number of multi-resistant *P. aeruginosa* isolates, but did not mention the plant names. In many parts of Iran there is a rich tradition in the use of herbal medicine for treatment of various infectious diseases and since Iran possesses vast number (Senda *et al.*, 1999; Jones *et al.*, 2001; McCallum *et al.*, 2001; Cos *et al.*, 2002; Essawi, 2000; Kokoska *et al.*, 2002 and Shahidi *et al.*, 2002) of medicinal plants, their antimicrobial and (Ghahraman and Attar, 1998) phytochemical studies would provide valuable information to the media of the world knowledge. The present survey forms the basis for investigation on antimicrobial and phytochemical determination of the most promising components for *in vitro* evaluation of these plants in human pathogenic studies. In the present study ethanol extracts were tested for their antimicrobial activity against some human bacterial pathogen such as *Streptococcus pyogenes*, *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumonia*, *Helicobacter pylori* and *Pseudomonas aeruginosa*.

In the present study the ethanol extracts were found to inhibit the growth of six organisms tested. Similarly, *Aegle marmelos* has been reported to contain anthraquinone, the principal laxative constituent of many plants used as purgatives (Ogunti *et al.*, 1993). Thus, from literature search, there is no evidence that flavonoid glycoside; a main constituent of the leaf extract of *Aegle marmelos* was responsible for antifungal activity. Meanwhile, the ethanol extracts of leaves, flowers, stem and root of *Aegle marmelos* had been shown to have a broad spectrum of antimicrobial activity after fractionating with petroleum spirit, dichloromethane and ethyl acetate. The dichloromethane fraction of the flower extract was found to be very effective (Khan *et al.*, 2001). In a recent study, the methanolic fraction of the leaves has been shown to be active against *Candida albicans* at a concentration of 50mg/ml but has no activity against *Trichophyton mentagrophytes* (Villasenor *et al.*, 2002). Much earlier, the
antimicrobial activity of *Cassia alata* leaf extract has been reported (Palanichamy et al., 1990).

The Ayurvedic system of traditional medicine is used in India to treat enteric diseases. Fifty-four plant extracts (Chloroform, ethanol and methanol) were assayed for their activity against multi-drug resistant *S. mutans*, *B. subtilis* and *S. aureus*. Strong antimicrobial activity was shown by the ethanol extracts of *Aegle marmelos*, *S. trilobatum*, *Salmalia malabarica*, *Calotropis procera*, *Punica granatum*, *Myristica fragrans*, *Holarrhena antidysenterica*, *Terminalia arjuna* and *Triphal*. Moderate antimicrobial activity was shown by *Picorhiza kurroa*, *Acacia catechu*, *Acacia nilotica*, *Cichorium intybus*, *Embelia ribes*, *Solanum nigrum*, *Carum copticum*, *Apium graveolens*, *Ocimum sanctum*, *Peucedanum graveolens* and *Butea monosperma*.

In the present study alcoholic extracts were tested for their antimicrobial activity against several Gram-positive and Gram-negative bacteria as well as yeast species using agar diffusion method. Antibacterial activity was demonstrated especially against bacteria including *Helicobacter pylori* strains. The greatest activity was exhibited by the ethanol extracts when compared to aqueous extracts. In similar reports the greatest activity was exhibited by the ethanolic extracts of *Boswellia elongata*, *Boswellia ameero*, *Buxus hildebrandtii*, *Commiphora parvifolia*, *Jatropha unicostata*, *Kalanchoe farinacea*, *Pulicaria stephanocarpa*, *Punica protopunica*, *Withania adunensis* and *Withania riebeckii*. Only the methanolic extract of *Buxus hildebrandtii* displayed significant antifungal activity (Ramesh et al., 2001).

The better antibacterial activity of the European herbal drugs is probably based on their relatively high amount of tanning agents. On the other hand, all tested plant preparations inhibited not at all or only insufficient growth of the Gram-negative bacteria is tested and that of *Candida albicans* (Simin et al., 2001). In this study growth of the Gram-negative bacteria tested against the *Aegle marmelos*. Accordingly, medicinal plants and microorganisms were the proper candidates and should receive
continuous research attention. The use of higher plants to treat infections was an age-old practice in a large part of the world population. Furthermore, because of the side effects and the resistance that pathogenic microorganisms build against the common antibiotics, much recent attention has been paid to extracts and biologically active compounds isolated from plants used in herbal medicine.

The methanolic extract of the roots of \(C.\ majus\) revealed a high resistance to \(Fusarium\). Several flavonoids and phenolic acids were isolated from the aerial parts, which exhibit interesting antiviral and antimicrobial properties both \textit{in vitro} and \textit{in vivo}. A glycoprotein isolated from \(C.\ majus\) exhibits good antibacterial activity against methicillin resistant staphylococci and multiresistant enterococci. Among the study, aqueous and ethanol extracts of \(Aegle\ marmelos\) leaves were observed to have the good resistance to test microorganisms. Kugelman \textit{et al.} (1976) isolated the N-oxide of the alkaloid indicate from \(C.\ alata\) and observed significant antimicrobial activity of the compound in flavones. On the basis of these results the compound was selected for human clinical trials. Studies related to the uses in Mali have not been performed.

Wound healing activity has been reported by Reddy \textit{et al.} (2002). They showed that topical application of 10% w/v of \(C.\ alata\) increased the percentage of wound contraction and completed wound healing by 14\textsuperscript{th} day indicating rapid epithelization and collagenization. The control used, healed a similar wound in 23 days. An increase of the tensile strength indicated the increase in collagen facilitating wound healing. In the present study wound healing microorganism effects were killed in \(Aegle\ marmelos\) plants.

A study reported that \(Urera\ baccifera\) from Urticaceae showed no antimicrobial activity against the test microorganisms; \textit{Streptococcus pyogenes}, \textit{Staphylococcus aureus}, \textit{Salmonella typhi}, \textit{Klebsiella pneumonia}, \textit{Helicobacter pylori}, \textit{Pseudomonas auroginosa} and fungal strains such as \textit{Aspergillus niger}, \textit{Candida albicans} and \textit{Trichoderma viride}. The extracts of \(Urtica dioica\) showed no
antimicrobial effects against the tested microorganisms in this study. Also, some studies confirm our findings (Keles et al., 2001). The extracts obtained from *Aegle marmelos* inhibit considerably the growth of all tested microorganisms such as *Streptococcus pyogenes, Psudomonas auroginosa* and *Staphylococcus aureus*, having an inhibition of zone.

*Aegle marmelos* was used for the treatment of inflammation, wound healing, antitumor and antianelgesic and hence different formulations could be prepared for clinical trials. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin. This research work states that the presence of alkaloids, cardiac glycosides, terpenoids, saponins, tannin, flavonoids and steroids in the ethanol extract of *Aegle marmelos* was responsible for its antimicrobial activity. These compounds exhibit a maximum zone of inhibition against *Helicobacter pylori, Streptococcus pyogenes, Salmonella typhi, Klebsiella pneumonia, Staphylococcus aureus* and *Pseudomonas aeruginosa* when compared to the control fungal pathogen. Such a zone of inhibition was not found in the case of *Helicobacter pylori*, which is considered a resistant. Hence, the present study suggests that pathogenic microorganisms may become resistant to existing drugs. Moreover, this study shows that some plants show much promise in the development of phytomedicines having antimicrobial properties. In this endeavour, traditional herbal medicines must perforce be granted the benefits of modern science and technology to serve further global needs. The drugs derived from herbs may have the possibility of use in medicine because of their antibacterial activity.

5.7 Antiulcer activity of *Aegle marmelos*

Most of the studies demonstrate the importance of natural products in drug discovery. In these studies antiulcer activity of aqueous and alcoholic extract of *Aegle marmelos* has been studied. The antiulcer study was evaluated using aspirin induced in rats. Most of the studies demonstrate the importance of natural products in drug
discovery. The use of phytoconstituents as drug therapy to treat major ailments has proved to be clinically effective and less relatively toxic than the existing drugs. The acute oral toxicity study result showed that the plant leaf is safe.

There is a balance between the aggressive and the mucosal protective factors in stomach. Thus drug therapy of peptic ulcer has been commonly targeted at either counteracting the aggressive factor or stimulating defensive ones. Despite the progress in conventional chemistry and pharmacology in producing highly effective drugs, some of them are expensive and have different adverse effects (Mahendran et al., 2002), however screening plants for active drugs is still important and might provide a useful source of new antiulcer compounds for developing pharamaceutical drugs or alternatively as simple dietary adjuncts to existing therapies.

The present study was carried out to evaluate the antiulcer activity of *Aegle marmelos* against Aspirin induced toxicity in albino rats. The effectiveness of this medicinal plant was screened by assessing biochemical changes of different groups of experimental animals. The results were expressed as mean ± standard deviation. The phytochemical constituents of the same plant were also analyzed and the results are given here.

The anti-ulcer activity of the plant of *Aegle marmelos* was evaluated by employing aspirin, alcohol and pylorus ligation ulcer models. These models represent some of the most common causes of gastric ulcer in humans. Many factors and mechanisms are implicated in the ulcerogenesis and gastric mucosal damage induced by different models employed in the present study involving, depletion of gastric wall, mucin mucosal damage induced by non-steroidal anti-inflammatory drugs and free radical production. NSAIDs like aspirin causes gastric mucosal damage by decreasing prostaglandin levels through inhibition of PG synthesis (Vinothapooshan and Sundar, 2010). The present study was carried out to evaluate the antiulcer activity of *Aegle marmelos* against Aspirin induced toxicity in albino rats. The effectiveness of this
medicinal plant was screened by assessing biochemical changes of different groups of experimental animals. The results were expressed as mean ± standard deviation.

Aspirin induced gastric injury is associated with significant production of oxygen free radicals leading to increased lipid peroxidation, which causes damage to cell and cell membrane. The extracts of the *Mimosa pudica* has significantly protected the gastric mucosa against ethanol challenge as shown by reduced values of lesion index as compared to control group suggesting its potent cytoprotective effect. It has been proposed that in pyloric ligation, the digestive effect of accumulated gastric juice and interference of gastric blood circulation are responsible for induction of ulceration (Gordan, 1990). The antiulcer activity of *Aegle marmelos* extracts in pylorus ligation model is evident from its significant reduction in gastric volume, total acidity, free acidity, ulcer index and increase in pH of gastric juice. Because of animals treated with *Aegle marmelos* extracts significantly inhibited the formation of pylorus ulcer in the stomach and also decreased both acid concentration, gastric volume and increased the pH values, it is suggested that *Aegle marmelos* extracts can suppress gastric damage induced by aggressive factors.

The present study was carried out to evaluate the antiulcer activity of *Aegle marmelos* against Aspirin induced toxicity in albino rats. The effectiveness of this medicinal plant was screened by assessing biochemical changes of different groups of experimental animals. The results were expressed as mean ± standard deviation. The phytochemical constituents of the same plant were also analyzed and the results are given here. Ramamurthy and Selvarani (2011) carried out studies to evaluate the antiulcer activity of *Holostema reedi* against Aspirin induced toxicity in albino rats. The effectiveness of this medicinal plant was screened by assessing biochemical changes of different groups of experimental animals. There is balance between the aggressive and the mucosal protective factors in stomach. Thus drug therapy of peptic ulcer has been commonly targeted at either counteracting the aggressive factor or stimulating defensive ones. Despite the progress in conventional chemistry and
pharmacology in producing highly effective drugs, some of them are expensive and have different adverse effects (Mahendran et al., 2002), however screening plants for active drugs is still important and might provide a useful source of new antiulcer compounds for developing pharmaceuticaal drugs or alternatively as simple dietary adjuncts to existing therapies.

The ulcer index and pH for the different groups and the % inhibition of ulceration are reported below in Table 12. The results showed that the control did not show any ulceration, whereas the group treated with acid showed maximum ulcer index of 2.80 and high protein 495 while the acid challenged administered with Ranitidine showed minimum protein and ulcer index. Similarly the % inhibition of ulceration was minimum in Aegle marmelos treated groups. The effectiveness of Aegle marmelos actually attributed to different active constituents which exist in the aqueous and alcoholic extract. Ramamurthy and Umamaheswari (2012) reported the effect of ethanolic extract of Nigella sativa seeds on pylorus ligated induced ulcer model. The ulcer index and number of lesions present on the gastric mucosa is an index of the severity of the ulcer. It was observed that there is an increase the ulcer index and number of ulcer lesion in ulcer control rats. Significant reductions in ulcer index and number of ulcer lesion were observed in N. sativa (100mg/ body weight). Therefore, the decreases in the ulcer index in the N. sativa extracts treated groups are an ulcer indication of the ulcer curative nature of extracts. Our results are concordant with earlier result reported by Rajkapoor et al. (2003).

The pH of the supernatant for all the samples was estimated using pH meter and was shown in the Table 12. The pH of gastric juice was found to be decreased in the ulcer induced rats. After herbal extract of Aegle marmelos administration, the level was gradually returned to normal. In aspirin plus pylorus ligation model, ulcer index parameter was used for the evaluation of anti-ulcer activity, since ulcer formation is directly related to factors such as gastric volume, free and total acidity. In vehicle control animal, aspirin plus pylorus ligation increased the acid secretion, which in turn
caused increase in gastric volume, low pH, increased free and total acidity resulting in higher ulcer index. Although in most of the cases, the aetiology of the ulcers is unknown, it is generally accepted that they result from an imbalance between aggressive factors and the maintenance of mucosal integrity through endogenous defense mechanisms (Bettarello, 1985; Piper and Stiel, 1986; Venkatesh et al., 2009; Ramamurthy and Selvarani, 2011).

The increase in volume in the ulcer control rats is undoubtedly due to increased production of hydrochloric acid as is evident from the total acidity and decreased pH value of gastric juice. Takagi et al. (1964) have reported that inhibition of acid secretion accelerated ulcer healing. The decrease in volume of the gastric juice and concomitant decrease in the acidity and increase in pH prove the anti-ulcer activity of seeds of N. sativa.

It is generally accepted that gastric ulcers result from an imbalance between aggressive factors and the maintenance of the mucosal integrity through endogenous defence mechanisms. The excess gastric acid formation by prostaglandin (PG) includes both increase in mucosal resistance as well as a decrease in aggressive factors, mainly acid and pepsin. Inhibitions of PG synthesis by aspirin coincide with the earlier stages of damage to the cell membrane of mucosal, parietal and endothelial cells (Cheesman, 1993). Although in most of the cases the aetiology of the ulcers is unknown, it is generally accepted that they result from an imbalance between aggressive factors and the maintenance of mucosal integrity through endogenous defense mechanisms (Bettarello, 1985; Piper and Stiel, 1986).

To regain the balance, different therapeutic agents including plant extracts are used. Aegle marmelos extract is one such herbal drug undertaken for the present study primarily to evaluate its antiulcerogenic potential. The number of lesions in the untreated ulcer group was quite high and among the treated groups, the group pretreated for study periods had a dramatic decrease in the number of lesions. The
numbers of lesions present on the gastric mucosa are indicative of the ulcer severity (West, 1982). A significant reduction in the number of lesions in the pretreated *Garcinia cambogia* groups may be due to the appetite suppressant effect of the drug, thereby inhibiting gastric acid secretion, an important factor in ulcer formation (Clouatre and Rosenbaum, 1994).

In aspirin plus pylorus ligation induced gastric ulcer model the ethanol extracts of *Aegle marmelos* reduced the gastric volume, free acidity, total acidity and ulcer index thus showing the anti-secretory mechanism involved in the extracts for their anti-ulcerogenic activity. Ulcer index parameter was used for the evaluation of anti-ulcer activity since ulcer formation is directly related to factors such as gastric volume, free and total acidity. In case of vehicle control, aspirin plus pylorus ligation increased the acid secretion, which in turn caused increase in gastric volume, low pH, increased free and total acidity resulting in increase in ulcer index (Goel and Bhattacharya, 1991; Malairajan *et al.*, 2008).

The etiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanisms (Piper and Stiel, 1986). To regain the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanisms by increasing mucosal production, stabilizing the surface epithelial cells or interfering with the prostaglandin synthesis. The causes of gastric ulcer pyloric ligation are believed to be due to stress induced increase in gastric hydrochloric acid secretion and/or stasis of acid and the volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating acid (Ramamurthy and Selvarani, 2011).

Aspirin induced gastric ulcer was employed to study the cytoprotective effect of the extracts. Aspirin induced gastric lesion formation may be due to stasis in gastric
blood flow which contributes to the development of the haemorrhage and necrotic aspects of tissue injury. Alcohol rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage leading to increased intra cellular membrane permeability to sodium and water. The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium (Soll, 1990 and Surendra, 1999). The extract shows protection against characteristic lesions produced by Aspirin administration and thus antiulcer effect of *Aegle marmelos* may be due to both reductions in gastric acid secretion and gastric cytoprotection.

Gastric acid is an important factor for the genesis of ulceration of pylorus ligation ulcer in rats. Table 4 shows that the data of mean and standard deviation of gastric secretion, free acidity and total acidity activity in aspirin treated rats. The volume and total acidity were significantly increased in the untreated ulcer group relative to the normal group. The extract of *Aegle marmelos* reduced the gastric volume, free acidity, total acidity and hence ulcer index showing the anti-secretary mechanism (Goel and Bhattacharya, 1991). HCl-Ethanol induced gastric damage is ranging from endothelial microvascular damage to development of macroscopic gastric mucosal lesions, which is attributed mainly to the inhibition of biosynthesis of cytoprotective PG resulting in overproduction of leukotrienes and other products of the 5-lipoxygenase pathway (Nasuti *et al*., 2006). These agents break the mucosal barrier, provoke an increase in gastric mucosal permeability to $\text{H}^+$ and $\text{Na}^+$ ions reducing the transmucosal potential difference and induce formation of erosions and ulcers. In this model *Naravelia zeylanica* extract was able to produce a significant reduction of the gastric mucosal damage, indicating a probable local increase in PG synthesis (Chandaka Madhu *et al*., 2012).

Muthusamy *et al*. (2009) reported that the acid secretary parameters such as pH, gastric volume, free acidity and total acidity were increased significantly in the aspirin administered group. Administration of ethanolic extracts of *Azima tetracantha*
exhibited a significant reduction in all the parameters and the results were comparable with the standard drug Ranitidine 50 mg/kg. Determination of the concentrations of various muco-proteins such as total protein, total hexoses, hexosamine, fucose and sialic acid revealed a decrease in ulcer induced group. Ramamurthy and Selvarani (2011) reported that the Holostema reedi showed significant dose-dependent ulcer protective effect against aspirin plus pylorus ligation induced gastric ulcers. H. reedi used in the study have been found to be effective against aspirin pylorus ligation model. It is evident from the results that these drugs produce reduction in the intensity of gastric ulceration as observed from reduced ulcer index in the drug treated groups. However, inconsistent results were obtained as regards to other parameters such as volume of gastric acid secretion, free and total acidity and pepsin activity. The extract at 400 mg/kg increased the level of the muco-proteins significantly and comparably with the standard drug. The ulcer scores were obtained in ulcer induced group of Aspirin plus pylorus ligation and Cold restraint stress induced ulcers exhibited an increased score. Administration of the extract exhibited a moderate decrease in the ulcer scores in a dose dependent manner in Cold restraint stress induced ulcers models and a significant decrease in Aspirin plus pylorus ligation models.

* Aegle marmelos showed significant dose-dependent ulcer protective effect against aspirin plus pylorus ligation induced gastric ulcers. The anomaly in ulcer protective effect may be due to variability of factors affecting ulcerogenesis in different models. Ulcers are caused due to imbalances between offensive and defensive mucosal factors (Goel and Bhattacharya, 1991) and hence the effects of ethanolic extracts of *Azima tetracantha* can be explained based on these factors. Mucin is a viscous glycoprotein with physiochemical properties producing relatively resistant acid barrier (Flemstrong and Garner, 1982). It makes up the major part of the mucus, an important pre-epithelial factor that acts as a first line of defense against ulcerogens (Zalewsky and Moody, 1979). Increase in mucin can be due to increased levels of individual mucopolysaccharide like sialic acid and total hexoses. The increase in mucosal defense may also be due to decrease in cell exfoliation
(Mukhopadhyay, 1987). Hence, the protection afforded by *Azima tetracantha* in APL induced ulcers may be predominantly due to strengthening mucosal defense (Goel RK and Bhattacharya, 1991). The ability of *Azima tetracantha* to protect stomach against ulcerogens by neutralizing intra gastric acidity can as well lead it to classify as a cytoprotective agent (Robert, 1979).

*Aegle marmelos* used in the study have been found to be effective against aspirin pylorus ligation model. It is evident from the results that these drugs produce reduction in the intensity of gastric ulceration as observed from reduced ulcer index in the drug treated groups. However, inconsistent results were obtained as regards to other parameters such as volume of gastric acid secretion, free and total acidity and pepsin activity.

*Aegle marmelos* and Ranitidine showed reduction in volume of gastric secretion without affecting pepsin activity of gastric juice. These findings go parallel with the findings reported by Brage *et al.* (1986) who suggested that calcium is not involved in the physiological secretion of pepsin. It appears that calcium may be involved only in acid secretory activity of GI tract but not in pepsin activity.

Based upon above discussion, the protective effect of these drugs may be attributed to some reason other than their antisecretory activity. Along with the gastric acid secretion, reflex or neurogenic effects have also been proposed to play some role in the formation of gastric ulcers in PL model (Anichkov *et al.*, 1971). It is a well known fact that gastric secretion is under vagal controls (Brodie, 1996). A possible central effect of verapamil interfering with any ulcerogenic action in triggering vagal outflow cannot be ruled out, since vagal overactivity appears to contribute substantially to any stress ulcer formation (Ogle *et al.*, 1985). It has been postulated that histamine plays a mediating role in the gastric acid secretion stimulated by gastrin, vagal excitation and cholinergic agents (Parmar and Ghosh, 1981).
Total Carbohydrate such as hexoses, hexosamine, fucose and sialic acid were determined in experimental rats. Group Aspirin treated shows decrease level of total carbohydrates (165.1 (µg/ml)) when compared to normal control, alcoholic and aqueous extracts of *Aegle marmelos* leaves treated groups. Aspirin induced ulcer study were not able to show a uniform rise in individual carbohydrate content but, total carbohydrate content of gastric juice was significantly increased in the presence of alcoholic and aqueous extracts of *Aegle marmelos* leaves and Ranitidine treated groups. Our study has established various causes responsible for the development of ulcers due to ulcer induced model such as increased metabolism of carbohydrates, increased synthesis of nucleic acids and also exhaustion of carbohydrates and other compensatory mechanisms.

Ramamurthy and Selvarani (2011) reported the level of total carbohydrate such as hexoses, hexosamine, fucose and sialic acid in experimental rats. Group Aspirin treated group shows decrease level of total carbohydrates when compared to normal control and treated groups. Aspirin induced ulcer study were not able to show a uniform rise in individual carbohydrate content, but total carbohydrate content of gastric juice was significantly increased in the presence of *Holostema reedi* and Ranitidine treated groups. Our study has established various causes responsible for the development of ulcers due to ulcer induced model such as increased metabolism of carbohydrates, increased synthesis of nucleic acids and also exhaustion of carbohydrates and other compensatory mechanisms.

Relative to the normal levels the hexose, hexosamine and sialic acid content of the gastric juice decreased considerably in the ulcer group while the protein level increased. The increase in protein content of the gastric juice indicates damage to the gastric mucosa as a result of which plasma protein leaks into the gastric juice (Goel *et al.*, 1985). The decrease in the glycoprotein moieties in the gastric juice may be attributed to the decreased activity of defense mechanisms as a result of damage to the gastric mucosa. In other words disintegration and degradation of glycoprotein moieties
into their simpler components in the process of Aspirin induced injury might have resulted in minimal quantities of glycoprotein in the gastric juice. The levels of protein, hexose and hexosamine were maintained at near normal levels in the group pretreated with *Aegle marmelos*. *Aegle marmelos* inhibits vagus nerve stimulation, thereby reducing the HCl output and acidity. Being an effective appetite suppressor (Clouatre and Rosenbaum, 1994), it protects the quantity and quality of mucus secretion against the offensive assault of acid. *Aegle marmelos* appears to regulate both acid output and mucus secretion.

It has been proposed that aspirin induces gastric ulceration by reducing the gastric mucus which protects the gastric mucosa from acid and pepsin and by weakening the gastric mucosal barrier. Ramamurthy (2012) determined the hexosamine, one of the major components of mucus, in the tissues of the corpus and antrum. Although the hexosamine level fell to a low level abruptly after aspirin administration, ulceration did not develop until several hours after the treatment. This observation suggests that the low hexosamine level is a cause of ulceration and not a result, that the visual ulcer is exerted by acid and/or pepsin after the gastric mucus has been reduced by aspirin. The reduced hexosamine level in the tissues of both the corpus and antrum was restored to the intact level by pretreatment with clotiazepam.

*Lasianthera africana* leaves though used as a vegetable has been reported by Okokon *et al.* (2007) to be used traditionally in the treatment of ulcer. For this reason, the antiulcer activity of the leaf extract of *Aegle marmelos* was evaluated using aspirin induced ulcer models. Aspirin plus, a known ulcerogen especially in an empty stomach (Bhargava *et al.*, 1973) causes ulcer mostly on the glandular (mucosal) part of the stomach (Evbuonwa and Bolarinwa, 1990; Nwafor *et al.*, 1996) by inhibiting prostaglandin synthetase through the cyclooxygenase pathway (Rainsford, 1987). Suppression of prostaglandins synthesis by aspirin results in increased susceptibility of stomach to mucosal injury and gastroduodenal ulceration. The extract was observed to
significantly reduce mucosal damage in the Aspirin plus–induced ulcer model, suggesting the possible extract mobilization and involvement of prostaglandin in the anti ulcer effect of the extract.

Okokon et al. (2007) reported that the leaf extract contains mainly flavonoids, terpenes, saponins, alkaloids and cardiac glycosides among others. Flavonoid such as quercetin has been reported to prevent gastric mucosal lesions in various experimental models (Di carlo et al., 1999; Zayachkivska, 2005) by increasing the amount of neutral glycoproteins (Di carlo et al., 1999). Flavonoids have been reported to protect the gastric mucosa from damage by increasing the mucosal prostaglandin content and by inhibiting histamine secretion from most cells by inhibition of histidine decarboxylase. Free radical scavenging ability of flavonoids has been reported to protect the gastrointestinal tract from ulcerative and erosion lesion (Borrelli and Izzo, 2000).

Mozsik et al. (1969) have established various causes responsible for the development of ulcers due to PL model such as increased metabolism of carbohydrates, increased synthesis of nucleic acids and also exhaustion of carbohydrates and other compensatory mechanisms. Moreover, the status of mucus secretion is important to determine the status of mucosal barrier (Blum, 1985). As evident from the study, both soluble (dissolved) as well as insoluble mucosubstances of gastric juice are increased in the presence of all three Aegle marmelos treated groups which indicates rise in glycoprotein content of the gastric mucosa.

It is also observed from the study that there is a significant fall in protein content of gastric juice by all CCBs, suggesting decrease of leakage of plasma protein into gastric juice (Grossman, 1978). Thus TC: PR ratio is increased by CCBs at higher dose levels, in this study; the gastric wall mucus content is increased with all the three treated groups of drug which goes parallel with the findings of Ogle et al. (1985).
They also reported that antiulcer action of verapamil in stress induced ulcer model is associated with prevention of stomach wall mucus loss.

The presence of dissolved mucosubstances in the gastric juice seems to be a reliable index of an effective mucosal barrier (Sanyal et al., 1983). Present study confirms that *Aegle marmelos* not only increase mucosal cellular mucus but also secrete more dissolved mucus in gastric juice. Thus the gastroprotective effect of these compounds may be attributed to above findings i.e., *Aegle marmelos* may act through prevention of gastric mucosal damage by strengthening the mucosal barrier.

### 5.8 Antipyretic activity of *Aegle marmelos*

The subcutaneous injection of a yeast suspension elevated the rectal temperature markedly after 24 h of administration. Treatment with the aqueous extract of *Aegle marmelos* and Ethanolic extract of *Aegle marmelos* leaves at doses of 50 mg/kg body wt. and 100 mg/kg body wt. decreased the rectal temperature of the rats in a dose-dependent manner. The antipyretic effect started as early as 2h, and the effect was maintained for 4 h, after its administration. The standard drug paracetamol (100 mg/kg body wt.) reduced the yeast-provoked elevation of body temperature significantly. The present results show that the *Aegle marmelos* possesses a significant antipyretic effect in yeast-provoked elevation of body temperature in rats, in which Ethanolic extract of *Aegle marmelos* shows more significant followed by aqueous extract of *Aegle marmelos* and its effect is comparable to that of paracetamol (standard drug).

Amber Vyas *et al.* (2011) aimed our study for screening antipyretic activity of different extract of *Aegle marmelos* (L.) Correa leaves and observed that it possesses a significant antipyretic effect in yeast-provoked elevation of body temperature in rats in a dose-dependent manner up to 4 h after its administration. Fever was induced as described by Abena *et al.* (2003). Fever may be a result of infection or one of the sequelae of tissue damage, inflammation, graft rejection, or other disease states.
Antipyretic are drugs, which reduce the elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained (Goodman and Gilman, 1996).

Results of effect on hyperexia induced in rats by brewer’s yeast are given in table 16. It revealed that ethanolic extracts as well as aqueous extracts of *Aegle marmelos* (L.) leaves (50 mg/kg body wt. and 100 mg/kg body wt.) showed a significant antipyretic effect in yeast-provoked elevation of body temperature in rat. It has been observed that ethanolic extracts are more effective against elevated body temperature in rats in comparison to aqueous extract in dose dependent manner. In both the cases, the extracts caused a significant lowering of body temperature, with the effect being comparable to that of paracetamol. The present pharmacological study confirms the therapeutic value of *A. marmelos*. However, there are several area to be explored. Very less information is available regarding the chemical constituents of leaves of this plant. The standardization of the extracts, identification and isolation of active principles and pharmacological studies of isolated principle may be considered for detail studies.

### 5.9 Antioxidant activity of *Aegle marmelos*

The aqueous and alcoholic extract of *Aegle marmelos* leaves was studied for antioxidant activity by nitric oxide method & GSH method. In these two methods all the doses like 50 mg and 100mg/kg possessed significant antioxidant activity. Both extract of aqueous and alcohol showed dose-dependent anti-oxidant activity. More and significant activity was exhibited by alcohol extracts. The maximum antioxidant activity was identified at the dose of 100mg/kg in aqueous and alcoholic extracts of *Aegle marmelos* leaves. A growing body of experimental and clinical evidence have shown that gastric mucosal damage by stress (Das *et al.*, 1997), ethanol (Mizui *et al.*, 1986), NSAIDs (Vaanannen *et al.*, 1992 and Yoshikawa *et al.*, 1993) and *H. pylori* is mediated through the involvement of reactive oxygen species. It has been shown that
OH- radical plays a critical role in gastric mucosal tissue damage and causes ulcer. The present investigation of herbal drug was carried out to study their antioxidant and free radical scavenging activities.

The present study revealed that oral administration of *Aegle marmelos* for experimental periods at a dose of 50 mg/kg and 100 mg/kg in rats significantly elevated the activity of the enzymes involved in scavenging reactive oxygen species such as superoxide dismutase and catalase. This indicated that *A. marmelos* improved the enzymatic antioxidant status in rat liver since it is known that a marked increase in SOD and CAT activity can offer first line protection against the damaging effects of superoxide radicals in the liver. However no significant change in the enzyme levels was observed in the groups treated with 50 mg/kg. Verma *et al.* (2012) studied the antioxidant property of 50% ethanolic extract of *Moringa oleifera* Lam; was found to be changed in SOD, CAT, and LPO levels in rat gastric mucosa. During the ulcer condition there is an increase in gastric mucosal SOD and LPO activities. This indicated that the generation of reactive oxygen species during stress might be the causative factor for the inactivation of gastric peroxidase. *Moringa oleifera* Lam. exerts its antioxidant defense mechanism probably by metabolising lipid peroxides and scavenging endogenous H$_2$O$_2$ (Bhattacharya *et al.*, 2000).

Antioxidant activity of aqueous and alcoholic extract of *Aegle marmelos* leaves was studied by LPO method and all doses 50mg and 100mg/kg produced significant antioxidant activity as given in Table 17. The maximum antioxidant activity was exhibited at dose 100mg/kg in alcoholic extract of *Aegle marmelos*. Among the two extracts, the alcoholic extracts exhibited more antioxidant activity. The activity was dose dependent. The superoxide anion (O$_2^-$), H$_2$O$_2$ and hydroxyl radical (OH') are the major reactive oxygen species (ROS) which induce cell degeneration by increasing LPO of cell membrane lipids.
The toxic end products of peroxidation induce damage of the structural and functional integrity of cell membrane, break DNA strands and denature cellular proteins. The natural cellular antioxidant enzyme includes SOD which scavenges superoxide radicals by speeding up their dismutation, CAT, a heam enzyme which removes H$_2$O$_2$. Detoxification of the superoxide anion is not a terminating step in free radical scavenging, since the enzyme catalysed dismutation results in the production of H$_2$O$_2$ which ultimately accumulates in the mitochondria and cytosol. Our result is thus similar with the finding that dimethylsulfoxide, an OH- scavenger, reduces cold restraint stress induced damage in the stomach (Cochran et al., 1983 and Verma et al., 2012).

Higher amounts of ROS have been shown to play a role in the development of diabetic complications as well as in a number of other disease states. As a safeguard against the accumulation of ROS, intracellular enzymatic antioxidant activities exist. ROS generated during metabolism can enter into reactions that, when uncontrolled, can affect certain processes leading to clinical manifestations (Halliwell, 1993; Winkler and Moser, 1996; Kesavulu et al., 2000). Oxidative stress induced by excessive production of superoxide and an imbalance in antioxidant enzymes has been linked to the development of diabetic complications. ROS are key participants in the damage caused by diabetic complications. Diabetes is one of the pathological processes known to be related to an unbalanced production of ROS, such as hydroxyl radicals (HO), superoxide anions (O2) and H$_2$O$_2$. Therefore, cells must be protected from this oxidative injury by antioxidant enzymes (Lyons, 1991 and Galloue et al. 1993). An overproduction of ROS especially in diabetes can not be properly balanced by antioxidant enzymes. Therefore, when oxidative stress arises as a consequence of a pathologic event, a defense system promotes the regulation and expression of this enzyme. Our results indicate the presence of some alteration in oxidant–antioxidant balance in the erythrocytes of diabetic rats. The increase in the erythrocyte antioxidant enzymes such as catalase is related to the oxidative damage of membrane protein and lipid by increased oxygen free radicals in the body.
The increased lipid peroxidation in the RBC’s is also due to an Inhibition or changing the activity of non enzymatic and enzymatic components of the oxidative system (reduced glutathione (GSH), Superoxide Dismutase (SOD) and Catalase (CAT) activities. The glutathione peroxidase system consists of several components, one of which is reduced glutathione (GSH) (Wu and Cederbaum, 2003). The enzymatic antioxidant defense system including Superoxide Dismutases (SODs) and Catalases (CATs) which can decompose superoxide and hydrogen peroxide in the cells are the main defense against oxidative injuries. SOD catalyses the rapid removal of superoxide radical. Because the SOD enzyme generates H$_2$O$_2$, it works in collaboration with H$_2$O$_2$ removing enzymes. Catalase present in the peroxisomes of nearly all aerobic cells, serves to protect the cell from the toxic effects of hydrogen peroxide by catalysing its decomposition into molecular oxygen and water without the production of free radicals.

Reaction of alloxan that produced toxic ions in vivo resulted in decreased activity of enzymatic antioxidant system accompanied by increased lipid peroxidation and there was a decreased activity of SOD and CAT in erythrocytes of alloxan induced diabetic rats. Decrease in antioxidant enzymes is an indication that there might be generation of an active radical a factor which is a key to alteration of these enzymes. The decrease in both enzyme activities could be the result of a reduced synthesis of these enzymes proteins as a result of higher accumulation of free radicals or there may be a drug-enzyme interaction resulting in the deactivation of these enzymes (Crouch et al., 1981).

5.10 Effects of the Aegle marmelos extract on the testicular follicle of the adult insect Gryllotalpa africana
5.10.1 Effect of plant extracts on insect biochemical constitutions

Data obtained from the biochemical effects of the treated leaf extracts of Aegle marmelos also confirmed different degrees of action on total glycogen content, total protein content and lipid content of Gryllotalpa africana. In the present investigation it
was shown that the glycogen content was changed due to antifeedant assay and significant decreases were noticed in plant extract treatments. The recorded effect was more pronounced with the effect of *Aegle marmelos*. These findings coincide with those of Abdo *et al.* (1995). Chitra and Reddy (2000) showed reduction in carbohydrate content of different instar larvae treated with *Ammi majus*, *Apium graveolens*, *Melia azedarach* and *Vinca rosea* extracts. As reported by another investigator (Hashem *et al.*, 1993) Amylase is the most sensitive enzyme to the action of plant extracts and inhibition of such enzyme activity in turn reduce the glucose level in both leaf extract treatments though decreasing the hydrolytic rate of glycogen. Etabari and Matindoost (2004) believed that starvation may reduce biochemical component such as glycogen in heamolymph of *Bombyx mori*.

Total glycogen content in the tissue of *Gryllotalpa africana* was greatly reduced when orally treated with leaf extracts mixed diet of plants. Reductions were 5.5, 5.0 and 4.7 mg/g for the treatment of 0.01, 0.05 and 0.1% of extracts of *Aegle marmelos* treatment while percent control group was 7.3 mg/g respectively (Table 18). Carbohydrates are the dominate carbon source of chitin, a participant in energy metabolism as well as substrate for lipid synthesis (Pant and Kumar, 1979). It was clear that the total carbohydrate was gradually increased with increasing of the pupal age's development in the control group. The tested compounds produce a highly significant decrease in the total carbohydrate content. The significant decrease was produced by seed extract of *E. cyclocarpum*.

The present investigations are comparable with the findings of Gordon and Burford (1984) who stated that exposure of early 4th instar larvae of *Aedes aegypti* to juvenile hormone analogue, methoprene significantly increased the concentration of carbohydrates in the haemolymph of the late larval instar. Ismail and Fouad (1985) showed that topical application of the juvenile hormone analogus (Isopropyl, 3, 7, 11-trimethyl, 2, 4 Dodencadienoate) to the pre-pupae of *S.litura* respectively increases the total carbohydrates contents during the pupal instar in the case of *S. litura* and
decrease in *Chrysomia albiceps*. Abou El-Ela *et al*. (1990) showed that the concentration of total carbohydrate content in one day old pupae of *Musca domestica*, previously treated as larvae with JHA ZR-515 was significantly lower than normal. In three days old pipae there was a marked significant increase and in five days old, the carbohydrate content was significantly higher than controls.

El-Sherif (1995) concluded that treatment of the first and the sixth instar nymphs of *S. gregaria* with JHA pyriproxifen increased the haemolymph carbohydrate level. Khalaf (1998) reported that treatment of the second instar larvae of *Muscina stabulans* with two plant oils of *Cymbopogon citratus* and *Rosmarinus officinalis* was induced a significant reduction in the carbohydrate content of the whole pupal period and Shoukry and Hussein (1998) have obtained the same findings with the larvae of *Galleria mellonella* when they were treated with volatile oils of *Lantana camara* and *Vitex aganus custus* plants.

Shoukry *et al*. (2003b) showed that the content of haemolymph carbohydrate significantly decreased in treated larvae of *Plodia interpunctella*. The fixed oil extracts of *Trigonella foenum grceum, Acacia nilotica, Rumex dentatus* were the most effective followed by the volatile oil extracts of *Piper cubebae* and *Salvia officinalis*. Sabry (2004) found that the content of haemogenate carbohydrates of *Chrysomyia albiceps* larvae significantly increased by treatment with plant oils. The fixed oil extracts were the most effective one followed by the volatile oil extracts.

*Aegle marmelos* leaves caused 19.2, 16.3 and 14.8 mg/g reductions into total protein content of insects tissues of *Gryllotalpa africana* at 0.01, 0.05 and 0.1 percent concentrations tested. Similar reductions were also noticed in the total protein contents of insect treated with leaf extract. The protein value of control group insect was 21.2 mg/g (Table 18). It is likely that the larvae degrade proteins to resultant amino acids in order to let them enter in to TCA cycle as a keto acid for compensation for the lower energy caused by stress (Nath *et al*., 1997). Exsensive work has been carried out in
order to determine how various toxic agents such as phytochemicals affect protein synthesis. Schmid et al. (1998) reported reduction in protein content in haemolymph of *S. littoralis* and *Agrotis ipsilon* when *Melia azedarach* fruit extract was treated. They also reported lower protein content in third instar larvae of *Xanthogaleruca luteola* treated with *Artemisia annua* leaf extract. The insect proteins are numerous; among them are storage protein, vitellogenin, lipophrin and a large amount of other major proteins with unknown fractions (Kyung and Kim, 1990).

Results which are comparable with that obtained by Abdel-Hafez et al. (1983) found that the IGR’s diflubenzuron and triflumuron application caused reduction in the levels of proteins of the treated *S. littoralis* larvae. Ismail and Fouad (1985) found that the topical application of juvenile hormone analogue (Isopropyl, 3, 7, 11-trimethyl, 2, 4, Dodencadenoate) on the pre-pupae of *Chrysosoma albiceps* induced an increase in the total protein content all over the pupal period. Schloter (1985) found that, treatment of the last larval instar of *Epilachna varivestis* with high doses of azadirachtin caused metabolic defects. He also added that storage of proteins in the fat bodies, which is necessary for pupation, did not occur.

El-Sheakh et al. (1990a) noticed an increase in total soluble protein in the 4th instar larvae of *S. litura* treated with *Soybean phytoalexins* (plant extract). Mostafa (1993) recorded a decrease in the total soluble protein in 4th and 6th instar larvae of *S. litura* treated with Margoson-O (neem extract). Abou El-Ela et al. (1995) reported a significant decrease in the total protein content in the house fly *M. demostica* larvae treated with plant extracts. Khalaf (1998) showed that the volatile oils of *C. citratus* and *R. officinalis* induced biochemical disturbance in the pupae of *M. stabulans*, which originated from treated larvae by decreasing the protein content. Shoukry and Hussein (1998) reported similar results on the greater wax moth *Galleria mellonella*. Shoukry et al. (2003b) showed that haemolymph proteins content of *Plodia interpunctella* larvae was significantly increased in all treatments by fixed and volatile oils. Sabry
(2004) reported significantly decreased total protein content of *Chrysomyia albiceps* larvae with treatment by fixed and volatile oils.

Fell *et al.* (1982); Rajender (1990) and Shakoori and Saleem (1991) attributed the greater protein synthesis with insecticidal treatment, to the synthesis of the proteinease needed for insecticide detoxification. This may be due to the conversion of carbohydrates and lipids to proteins as stated by Kinnear *et al.* (1971) who suggested that increase of protein level is due to increased synthesis of new proteins by fat body, haemolymph and other tissues of the larvae. Thus, the increase in the total protein may be a kind of detoxification mechanism. In this respect, Wilkinson (1976) stated that protein helps to synthesize microsomal detoxifying enzyme, which assists to detoxify the toxicants that entered into the body.

Total lipid content in the tissue of *Gryllotalpa africana* was greatly reduced when orally treated with leaf extracts mixed diet of plants. Reductions were 21.5, 17.8 and 14.2 mg/g for the treatment of 0.01, 0.05 and 0.1% of extracts of *Aegle marmelos* treatment while percent control group of lipid was 25.1 mg/g respectively (Table 18). Regarding the total lipid content a number of toxic agents have been caused to cause disturbances of fats in different body organs of vertebrates and invertebrates (Rawi *et al.* 2011). Lipids are an important source of energy and are reserved in fat body.

Insects preferentially store lipid in the fat body rather than glycogen as an energy source. These are obtained from the diet as well as synthesized by the insect. The storage of lipids has adaptive advantage especially for relatively small insects. This is because an isoelectric quantity of lipids occupies less storage places than the equivalent amount of glycogen, and in addition, the metabolism of lipid generates more water than carbohydrates (Chippendale, 1973).

The present results are comparable with the findings of Ismail (1980) who found that the JHA (Isopropyl, 3, 7, 11-trimethyl, 2, 4, Dodencadienoate), induced an
increase in the total lipids in the pupae of *S. litura*. Abou El-Ela *et al.* (1990) showed that the total lipid of one day old pupae of *Musca domestica* resulted from treated larvae with JHA ZR-515 was significantly increased; followed by a highly significant decrease in five day old pupae and. Abou El-Ela *et al.* (1995) found the same result on *Musca domestica* after treatment with water extracts of some plants.

Shoukry and Hussein (1998) showed that treatment of the third instar larvae *Galleria mellonella* with sublethal concentrations of *Lantana camara* and *Vitex agnus castus* reduced the total lipids in the last larval instar. Shoukry *et al.* (2003b) found that haemolymph lipids content of *Plodia interpunctella* was significantly increased in all treatments expect treatment with garden sage volatile oil and significantly decreased the haemolymph lipid content. Sabry (2004) found that the content of haemogenate lipids of *Chrysomyia albiceps* larvae significantly decreased in treatment with volatile oils while treatment with the fixed oils increased the lipid content.

### 5.10.2 Histopathological effects of the plant extract on insect *Gryllotalpa africana*

#### 5.10.2.1 Effect on apical cell

The present study shows the existence of marked changes in the apical cell of the testicular follicles of *G. africana* due to treatment with the extract of the plant *A. marmelos*. The cell shows signs of hypertrophy and the nucleus seems to have lost its characteristic shape and size. Insecticides, chemicals, X-irradiations, pesticides and plant extracts have been shown to produce somewhat similar changes in the apical cell. According to Drowpathi (1987), the apical cells have lost their characteristic shape and size and they appear scattered with considerable intercellular spaces and scanty amount of cytoplasm, when treated with the insecticide rogor in *Serinetha augur*. Another insecticide phosphamidon has caused hypertrophy in the apical cells of *Catacanthus incarnates* (Nirmala Devi, 1990). Apholate, an alkylating agent has caused pycnosis and subsequent degeneration of the apical cells and in some cases displacement of these cells are evidenced by their occurrence in the centre of the testicular follicle in *Locusta migratoria* (Vishwanth *et al*., 1978). It has been reported
for *Anthomonus gradis* that exposure to 1000 R results in the interruption of apical cell development for some time after irradiation, probably reflecting death among the spermatogonial cells (Riemann and Flint, 1967).

Vishwanth et al. (1978) has concluded that disturbance in the apical cells may lead to malnutrition of the developing germ cells ultimately resulting in the necrosis of germ tissue. The vijay neem treated *Odontopus varicornis* exhibited remarkable changes in testis such as disintegration in nutritive cells which affects nourishment to the germ cells and spermatids. Cell enlargement and pycnotic nuclei were also noticed (Ambika, 2012). The findings referred to above suggest that the *A. marmelos* plant extract alters the morphological structure of the apical cell of *G. africana* and the extract appears to act like an insecticide, chemicals, x-irradiation, phyto pesticides and plant extracts.

### 5.10.2.2 Effect on spermatogonia

The extract of *A. marmelos* at the dose of 0.01 ml of 0.05 per cent and 0.1 per cent has resulted in marked changes in the spermatogonial cells. The primary spermatogonia have increased in size with pycnotic nuclei while the secondary spermatogonia seems to be affected with nuclear degenerative symptoms. Similar studies by Gangrade and Paul (1970) have revealed the reduction of the spermatogonial cells in *Cadra cautella* when treated with chemosterilant. Pycnosis of spermatogonia was observed in *Locusta migratoria* when treated with apholate, an alkylating agent (Vishwanath et al., 1978).

Nuclear degenerative symptoms such as pycnosis karyorrhexis and hyperchromatosis have been reported for the secondary spermatogonia of *Dyssercus koenigii* after x-irradiation (Srivastava et al., 1985). Radiation has induced several changes in gonial cells of *Acarus siro* (Szlendak et al., 1992). Abnormally thickened chromosomes with pycnotic nuclei and reduced cytoplasmic contents were observed.
after the treatment of hexachlorocyclohexane in the primary spermatogonia of *Poecilocercus pictus* (Janak Ahi, 1988).

Reda *et al.* (2010) observed degeneration and necrosis of germcells, spermatogonia on *S. gregaria* treated with IGR Lufox and consult. Schultz and Schulte (1984) found the damage in the apical region, i.e in spermatogonia and spermatocytes in *Ephilachana varicestis* treated with azadirachtin. The testis follicle of the male insect *Melanoplus sanguinpus* showed destruction of cells and reduction in cell size in the apical part of testicular follicle, degeneration of spermatogonia after the azadirachtin treatment (Tayade, 2012). Mohamed *et al.* (2010) also found the architectural defects such as spermatogonial degeneration, displacement of testicular cysts and depopulation of germ cells on neem treated weevil *Rhynchophorus ferrugineus*. The same observation was also made by Ambika and Selvi Sabanayakam (2012) on *Odontopus varicornis* treated with vijay neem oil. The leaf extract of *O. sanctum* treated male albino mice testis has resulted in the clumping of spermatogonial cells (Kasinathan *et al.*, 1972). Similarly the plant extract of *Achyranthus aspera* has produced clumping of spermatogonia in the slug *Leavicaulis alte* (Rajeswari, 1988).

It has been reported that in the present study the 0.01 per cent concentration of plant extract has not produced significant effects in the spermatogonial cells in *G. africana*. Thus it may be inferred that a higher concentration of plant extract of *Aegle marmelos* appears to affect the spermatogonia. Further, the effect of the plant extract observed in *G. africana* seem to be similar to the effect caused by chemicals, chemosterilants, insecticides, X-irradiation, IGR, Phytopesticides and plant extracts.

5.10.2.3 Effect on spermatocyte

The present study also reveals that treatment with this extract at the concentration of 0.05 per cent and 0.1 per cent has resulted in shrinkage of spermatecyte with pycnotic nuclei and scanty amount of cytoplasm. The chromosomes
of the spermatocytes are deeply stained with haematoxylin showing chromosome clumping. Similar effects have been produced with other chemicals in spermatocyte. Amarjit Kaur et al. (1983) have reported histopathological changes such as disintegration of cells, nuclear pycnosis and chromatin clumping in spermatocyte of *Leptocoris coimbatorensis* after hydroprene treatment. Nakayama et al. (1979) have reported that the differentiation of spermatogonium into spermatocyte has been inhibited due to the treatment with metepa and hempa in *Mamestra brassicae*. Srivastava et al. (1985) have found out the breaking of cyst wall of spermatocyte in the red cotton bug *Dysdercus koenigii* after X-irradiation. Gourvishwavidhya (1988) has observed the pycnotic spermatocyte in the X-irradiated testes of *Poecilocercus pictus*. Janak Ahi (1988) has reported the vacuolated spermatocyte with reduced cytoplasmic contents, fragmentation of chromatin, thus leading to their disintegration in *Poecilocercus pictus* when treated with hexachlorcyclohexane.

The pycnosis of primary spermatocyte was accompanied by fragmentation of chromatin and subsequent karyolysis in some primary spermatocytes but hypertrophy of the nucleus was observed in the secondary spermatocyte of *Locusta migratoria* when treated with apholate (Vishwanath et al., 1978). Clumping of chromatin material in the primary spermatocyte and breaking of the cyst wall in secondary spermatocyte were observed in *Catacanthus incarnates* treated with the pesticide phosphamidon (Nirmala Devi, 1990). A similar effect has been reported for *Serinetha augur* under the influence of rogor (Drowpathi, 1987).

Vijayan (1987) has found out the crowding of spermatocytes and the occurrence of phagocytic cells in the rats after the *Andrographis paniculata* plant extract treatment. Blockade of spermatocyte was observed in the slug *Laevicaulis alte* after the treatment of *Achyranthus aspera* plant extract (Rajeswari, 1988). Structural damage to the spermatocyte cells of the testes *Ephilachana varivestis* treated with azadirchitin was observed by Schultz and Schluter (1984).
Reda et al. (2010) found degeneration and necrosis of germ cells, spermatocytes in *S. gregaria* treated with IGR Lufox and consult. The same finding such as spermatocyte degeneration was observed in azadirachtin treated *Schistocerca gregaria* by Nisbet et al., (1995). The prominent architectural defects such as germ cells degeneration and spermatocytes destruction was observed in neem treated weevil *Rhynchophorus ferrugineus* by Mohamed et al. (2010). Tayade (2012) observed that the testis follicle of the azadirchtin treated male insect *Melanoplus sanguinpus* showed destruction of germ cells and reduction in spermatocytes size in the apical part of testicular follicles. The disintegration of primary and secondary spermatocytes in the histology of Vijay neem treated testis of *Odontopus varicornis* was observed by Ambika (2012). The same observations were also made on *Odontopus varicornis* by Jayakumar et al. (1988) and Lousia (2010) and on *Spodotera rusticum* by Prasanna (1998) and Umamageswari (2006).

It has been shown that a lower concentration of 0.01 per cent extract has no effect on the spermatocyte of *G. africana*. Similarly, the male insect of *Poecilocercus pictus* injected with 0.01 per cent HCH has revealed not much of change after four days, but as the treatment is continued, the damage is further increased (Janak Ahi, 1988). The severity of effects has increased with the increase, in the number of doses (Verma et al., 1980) and the effects appear to be dose dependent (Rajeswari, 1988). From these observations it may be concluded that the higher concentration of *A.marmelos* plant extract affects the spermatocytes while the lower concentration has no effect on these cells. Further, the effect of the plant extract observed in *G. africana* seem to be similar to the effect caused by chemicals, chemosterilants, insecticides, X-radiation, IGR, Phytopesticides and plant extracts.

5.10.2.4 Effect on spermatid

It has been shown on *G. africana* that treatment with the concentration of 0.01 per cent, 0.05 per cent and 0.1 per cent of the plant extract has resulted in hypertrophy of spermatids and they are seen displaced from their original position. Clumping and
disorganization of spermatids are the two important changes observed in the present study. Such hypertrophy of spermatids and their disorganization have been observed in *Poecilocercus pictus* when treated with hexachlorocyclohexane (Janak Ahi, 1988). Exposure to 30 K rad of X-ray has produced abnormalities in the spermatids of *Dermestes frischii* (Hodges, 1983). In another experiment the spermatids exhibit degenerating condition with large cellular spaces affecting their compact arrangement in *Catacanthus incarnates* when treated with Phosphamidon (Nirmala Devi, 1990).

In *Serinetha augur* treatment with the pesticide rogor has caused intercellular spaces in spermatid region (Draowpathi, 1987). In the plant extract *Clerodenron infortunatum* treated male beetle *Oryctus rhinoceros*, the orderly arrangements of spermatids was lost and the number and the size were seen very much reduced. Reduction in the size of the testis and scattered and Shrunken spermatids are also observed (Sreelatha and Geetha, 2011). The degeneration and necrosis appeared in many spermatids after the treatment of IGR consult and Lufox on the male insect *S. gregaria* (Reda et al., 2010). The same result was also observed by many workers on same male insects treated with different plant extracts i.e., Azadirachtin treated on male insect *Mamestra brassicae* (Schmizu (1988) on *Ephilachana varivestis* (Schultz and Schluter, 1984) and on *Melanoplus sanguinpus* (Tayade, 2012) and neem treated testes of *Rhynchophorus ferrugineus* (Mohamed et al, 2010) and Vijay neem treated *Odontopus varicornis* (Ambika, 2012).

These findings suggest that both the lower and higher concentrations of *A. marmelos* plant extracts seems to affect the spermatids, as it has been reported for other insects making use of chemosterilants, insecticides, X-irradiation, IGR, Phytopesticides and plant extracts.

5.10.2.5 Effect on sperm and sperm bundles

It has been shown on *G. africana* that treatment with the concentrations of 0.01 per cent, 0.05 per cent and 0.1 per cent of the *A. marmelos* extract has resulted in
hypertrophy of the sperms intensely stained with haematoxylin and eosin. They appear to have lost their tails and are found displaced from their original position. Clumping of sperms and sperm bundles are also observed.

These observations are consistent with those of others in some species of insects. Amarjit Kaur et al. (1983) have observed the formation of defective spermatozoa in *Leptocoris coimbatorensis* due to treatment of hydroprene. The formation of sperms has been inhibited in *Dysdercus cingulatus* due to the treatment with tepa (Ahamed, 1980). Redfren et al. (1980) have reported that the sperm transfer from male to female in *Oncopeltus fasciatus* has been prevented due to treatment with penfluron.

Maheswari et al. (1981) have revealed in their study that maturities of sperms are inhibited after treatment with tepa in *Dysdercus koenigii*. Hypertrophied and dislocated spermatozoa were observed in *Poecilocercus pictus* when the insect was treated with hexachlorocyclohexane (Gourvishwavaidhaya, 1988). In another experiment, it has been shown that treatment with apholate in *Locusta migratoria* has produced hypertrophied sperms possessing flagella and acrosomes but they failed to form bundles (Vishwanath et al., 1978).

In *Catacanthus incarnates* the treatment with the pesticide phosphamidon has resulted in the reduction in the length of sperms (Nirmala Devi, 1990). The histopathological abnormalities on the testes of ten day old adult male insect *S.gregaria* treated with IGR consult and Lufox showed Degeneration and necrosis in spermatogenic stages and inhibition in the formation of sperm bundles (Reda et al., 2010). The spermatids and spermatozoan tails had dissolved, affecting the motility of the spermatozoa observed on the azadirachtin treated testes of *Ephilachana varivestis* (Shultz and Schluter, 1984).
Mohamed *et al.* (2010) observed decreased number of spermatozoa in neem treated weevil *Rhynchophorus ferrugineus*. The observed reduction in the spermatozoal number is in agreement with the study of Linton *et al.* (1997). Disintegration of sperm bundles and reduction in their number in terminal zone are also observed on *Melanoplus sanguinpus* treated with azadirachtin (Tayade, 2012).

Schutz and Schiter (1983) found that in *Epichana varivestis* the degeneration of sperm bundles without sperm formation has been reported after the treatment of azadirachtin. Ambika et al., (2012) observed clumping of sperm in the histology of Vijay neem treated testis of *Odontopus varicornis*. The observations were also made on *Odontopus varicornis* by Jayakumar *et al.* (1988) and Lousia (2010) and on *Spheroderma rusticum* by Prasanna (1988) and Umamageshwari (2006).

Sperms were found scattered all through the tubular lumen in the male mice after treatment with the extract of *O. sanctum* (Kasinathan *et al.*, 1972). Gossypol, a constituent of cotton seed oil is a substance suppressing spermatogenesis and damaging germ cells in male Hamsters (Waller, 1981). The crude chloroform *Carica papaya* seed extract treatment has induced abnormalities in sperms of the male albino rats (Lohiya and Ravibalagoyal, 1992). Apparently it is clear that three different concentrations of the extract of *A. marmelos* used in the present experiment appear to act in a way similar to chemical agents, X-rays, chemosterilants, insecticides, IGR, phytopesticides and other plant extracts in *G. africana*.

### 5.10.2.6 Antispermaticogenic and sterility effect of *A. marmelos* on *G. africana*

Thus, it is evident that the treatment with *A. marmelos* has caused structural and functional changes in the apical cell, spermatogonia, spermatocyte, spermatids and sperms leading to an arrest of spermatogenesis and perhaps it might result in sterility. This inference gains supports from the observations of other workers. Crude foliage extract of *Podocarpus gracilior* seems to sterilize the Egyptian cotton leaf worm *Spodoptera littoralis* (EL-Ibrashy, 1974). Sukumar and Osmani (1981) have reported
that the leaf and root alkalids of *Catharanthus roseus* held promise as good sterilants against *Dysdercus cingulatus*.

The *Catharanthus roseus* extract can successfully sterilize the male house fly *Musca domestica* (Kumuda Sukumar, 1987). Aqueous ethylmethane sulphonate induced more sterility as compared to acetone ethylmethane sulphonate in the fruit fly *Dacus dorsalis* (Thakur and Ashokkumar, 1988). Sterilization of cabbage looper moths *Trichoplusia sp* has been accomplished with the application of several chemicals (Howland *et al*., 1965). Terramycin, likuden and flavomycin have been reported to induce sterility in blackbean aphid (Lal, 1972).

Apholate has produced sterilization in the boll weevil (Lindquist *et al*., 1964). Thio aziridine chemosterilants uptake has induced sterility in pupae and adults of *Pipiens quinquefasciatus* (Seawright *et al*., 1971). Vishwanth *et al*. (1978) have stated that the hypertrophied isolated sperms reflect the disturbed physiological state of activity and this might lead to infertility in *Locusta migratoria*. A study into the invitro effect of 3 ppm azadirachtin affects spermatogenesis in diapausing male *mamestra brassicae* (Schimizu, 1988). Nisbet *et al*., (1996) concluded that azadirachtin concentrations above $10^{-5}$ m invitro caused a time dependent reduction in the motility of sperm bundles liberated from the accessory glands in *S. gregaria* and it also showed a major cytogenetic effect on spermatogenesis.

Reda *et al*. (2010) observed histopathological abnormalities in the ten day old adult males such as degeneration and necrosis of germ cells and necrosis in spermatogenic stages and inhibition in the formation of sperm on *S. gregaria* treated with IGR Consult and Lufox. These results also agree with that on *Heteracris littrolis* when treated with azadirachtin (Ghazawi *et al*., 2007). The plant extract of neem are capable of disrupting growth, development and reproduction in *Rhynchophorus ferrugineus*. It has effects on histological structure of gonads. In this way it disrupts the gamete productions (Mohamed *et al*., 2010). The flower extract of *Thevitia*
nerifolia has been shown to induce sterility in the male red cotton bug Dysdercus similis accompanied by anatomical defects, including incomplete testis follicles (Raju et al., 1990).

Neem products are known to have antifertility effects (Schmutter, 1990). Growth regulatory and sterilizing effects are observed in insects treated with azadirachtin (Klocke, 1990). In Male, azadirachtin induced over aged nymphs of Oncopeltus fasciatus showed reduction of reproductive potentials (Dorn et al., 1987). Continuous feeding of oleander Nerium indicum sap during the larval period significantly affects the testicular development, spermatogenesis and results in infertility in male Helicoverpa assulta. These male specific infertility effects are associated with profound abnormalities in spermatogenesis (Seon eun Jeong et al., 2001). These effects on male sexual development and particularly on spermatogenesis are similar to those induced by injecting α-difluoromethyl ornithine (DFMO) and α-difluromethylorgine (DFMA) into H. assulta (Jeong and Kown, 1996). The histological destruction and arrest in spermatogenesis are also detected in different insects with IGR and botanical extracts by Younes et al. (1994); Mohamed et al. (2000).

The anti spermatogenic and sterility effects are also studied on rats by many workers. The aqueous leaf extract of A. marmelos administration on male rat showed significant decrease in the weight of testis (Sathyaraj et al., 2010). The ethanolic extract of Bael leaves has shown anti spermatogenic activity in the form of significant decrease in the weight of primary as well as some accessory sex organs and reduced sperm count (Sur et al., 1999). Again the same workers, including Mohamed (2002) presented data of antimotility of rats sperms through invitro study. Similarly Sharma and Gupta (2009) studied the effect of ethanol extract leaves of A. marmelos for their invitro effect on sperm motility and suggested that the extracts had a considerable effect on the motility of sperm. It was also proposed that an increase in concentration
of the extracts decreased the motility of sperms. The aqueous leaf extract of *A. marmelos* has potent antitesticular effect at a specific dose on rats (Das *et al.*, 2006).

Nazia and Pandey (2009) observed that the oral administration of alcoholic extract of *Clerodendron sithonanthus* leaf extract at different doses have shown a significant decrease in the weight of testis and reduction in the size of rats. Administration of *Azadirachta indica* neem bark extract has inhibitory effect on spermatogenesis and the production of spermatocytes and spermatids have been significantly reduced in rats (Purohit and Dixit, 1940).

Similarly alcoholic flower extract of *Malvaviscus conztiiii* has resulted in complete arrest of spermatogenesis with mass atrophy of spermatogenic elements in the male albino mice (Verma *et al.*, 1980). Further the leaf extract of *O. sanctum* has been shown to cause impairment of spermatogenesis in male albino mice (Kasinathan *et al.*, 1972). In another case, the leaf powder of *Andrographis paniculata* has been shown to possess antifertility effect and inhibition of spermatogenesis in rats (Vijayan, 1987). In the same way the extract of *Vinca rosea* plant has been shown to have antispermatogenic effect on male swiss albino mice (Murugavel and Akbarsha, 1991).

The seed extract of *Carica papaya* has induced abnormalities in sperms of male albino mice (Lohiya and Ravi Balagoyal, 1992). The male rats fed with neem oil, exhibited remarkable loss of reproductive functions (Khare *et al.*, 1984). Chinoy *et al.* (1986) have reported that the treatment with the extract of *Carica papaya* seed has resulted in complete sterility along with the alterations in sperm motility and morphology in rodents. Isopropyl methane sulphonate and methyl methane sulphonate have induced complete sterility in mouse (Moutschen, 1969). In this sequence, it is obvious that the effect of *Aegle marmelos* on *Gryllotalpa africana* is in accordance with the inhibition in spermatogenesis and sterility caused by the various chemicals, chemosterilants, heat, IGR, phytopesticides and plant extracts.