5. DISCUSSION

Herbal drugs are playing an important role in health care programmes worldwide and there is resurgence of interest in herbal medicines for treatment of various ailments. The World Health Organization estimated that about 80 per cent of the world's population still relies on plant-based medicines for their primary health care (Khalil et al., 2007). Various medicinal plants have been used for years in daily life to treat disease all over the world. The relatively lower incidence of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost, is encouraging both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs (Nair et al., 2005). Recently various modern procedures and techniques have been developed for the determination of biological activity of plant extract and bioassay techniques (Ahmad et al., 2002; Zafar et al., 2002). The plants have also been used as source of medicine. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times (Farombi, 2003). Over 50 per cent of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programmes in pharmaceutical industry (Baker et al., 1995).

Large numbers of plants belonging to different families have been studied for their therapeutic properties (Bowers, 1976; Cordell, 1981; Stuffiness and Cordell, 1987; Mukhtar et al., 2002). However, plants such as T. chebula and C. sinensis belonging to Combretaceae and Theaceae, which have many many therapeutic properties, have not been studied for their photochemical constituents and pharmacological properties and hence the present study focused on those plants.

5.1. PHYTOCHEMICAL ANALYSIS

Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being Igbinosal et al., (2009). Now-a-day modern drugs have been isolated from natural sources; many of these isolations were based on the uses of the agents in traditional medicine Doughari et al., (2008), because of higher plants have the capacity to produce a large number of organic phytochemicals with complex structural diversity that is known as secondary metabolites. A knowledge of the
chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because such information may be of great value in disclosing new sources of economic phytochemicals for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies (Milne et al., 1993). Hence a thorough validation of the herbal drugs has emerged as a new branch of science emphasizing and prioritizing the standardization of the natural drugs and products because several of the phytochemicals have complementary and overlapping mechanism of action. Mass spectrometry, coupled with chromatographic separations such as Gas chromatography (GC/MS) is normally used for direct analysis of components existing in traditional medicines and medicinal plants. In recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non polar components and volatile essential oil, fatty acids, lipids (Jie et al., 1988) and alkaloids (Betz et al., 1997).

In the present study, the quantitative GC/MS Phytochemical investigations of the two different plants T. chebula and Camellia sinensis have been reported on presence of tannins, carbohydrates, glycosides, phenols, alkaloids, terpenoids and flavonoids. GC-MS analyses, totally 26 compounds identified from the methanol fractions of the T. chebula are presented in Table 3. The plant samples revealed the synthesis of 2-Cyclopenten-1-one, 2-hydroxy-; 2-Furan carboxaldehyde, 5-methyl-; 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one; Phenol; 1,2-Cyclohexanediolone; Cycloheptanone; 5H-1,4-Dioxepin, 2,3-dihydro-2,5-dimethyl-3-Methoxytetrazolo(1)pyridazine; 1-Piperidineacetoniitrile; Benzoic acid, hydrazide; 2,3-Dimethylfumaric acid; Levoglucosenone; Acetamide, 2,2,2-trifluoro-N-[2-(hexahydro-1(2H)-azocinyl)ethyl]-; Piperazine, 1-(aminocacetyl)-; Resorcinol; 2-Furan carboxaldehyde, 5-(hydroxymethyl)-; Ethanone, 1-(2-hydroxy-5-methylphenyl); N-(5-Amino-4-cyano-1-pyrazolyl)phthalimide; 2-Butenoic acid, 4,4-dimethoxy-, methyl ester; 2,2-Bis(2'-methoxyphenyl)propane; 1,2,3-Benzene triol; D-Allose; Phenol, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, (R)-; Phenethylamine, 3,4,5-trimethoxy-3-methyl-; Tridecanoic acid, methyl ester; Dodecanoic acid, 10-methyl-, methyl ester. All these compounds are of pharmacological importance as they possess the properties such as analgesic, anti-diabetic, antibacterial, and antifungal. Based on the results, we believe the plants used in this study have potential as sources for antibacterial drug, and we have experiments underway leading to the identification of the
active molecules present in these plants. This was supported by an earlier study on Ko et al., (1994) identified the Squalene from leaves of *Terminalia catappa* L through gas chromatography-mass spectrometry. However, the seed extracts only exhibited potent scavenging activity. Trees of the genus *Terminalia* have long been used in the traditional medicine of Kenya (East Africa). In an ethno pharmacological approach, extracts of the stem bark of *Terminalia spinosa* were investigated for antibacterial and antifungal activity due to the presence of poly phenol and terpenoids. (Zhang et al., 1997). Ghosh et al., 2008 and stated that *T. bellerica* showed the highest inhibition zones against *P. aeruginosa* and *K. aerogenes*. This higher antibacterial activity due to the alcoholic nature and presence of biological active components alkaloids, flavonoids, essential oil, terpenoids and tannins. Adigun et al., 2000 stated that Some members of the Combretaceae have high concentrations of flavonoids, terpenoids, tannins or polyphenolic compounds. These compounds are known have *in vitro* antimicrobial activity. 3,4,3'-Tri-O-methylflavellagic acid and its glucoside were isolated from Combretaceae species. These compounds were analysed by GC-MS. Antimicrobial effect of the Combretaceae species possesses anti bacterial and antifungal activity due to the presence of glucosides.

Besides the present investigation on GC-MS analysis, totally 20 compounds were identified from the methanol fractions of the *Camellia sinensis* is presented in Table 4. The plant samples revealed the synthesis of 1,2,5,6-Tetrahydropyridin-2-one, 5-methyl, 2, 3-Pentanedione, 4-methyl-3H-Pyrazol-3-one, 2, 4-dihydro-2, 4, 5-trimethyl, 1, 2, 5, 6-Tetrahydropyridin-2-one, 5-methyl, 3-Amino-2-oxazolidinone, 4H-Pyran-4-one, 2, 3-dihydro-2-[1-(benzyloxy)ethyl], 2-Methoxyresorcine, Eugenol, Naphthalene, 6-ethyl-1, 2, 3, 4-tetrahydro-1, 1, 4, 4-tetramethyl-7-(1-methylene), 1, 2, 3-Benzenetriol, Sucrose, D-Allose, Oxalic acid, 2-ethylhexyl hexyl ester, Bicyclo[3.1.1]heptan-3-ol, 2, 6, 6-trimethyl, 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol, 1HPurine-2, 6-dione, 3, 7-dihydro-1, 3, 7-trimethyl, 1H-Purine-2, 6-dione, 3, 7-dihydro-1, 3-dimethyl, Phytol, Myristoyl chloride and Squalene. All these compounds are of pharmacological importance as they possess the properties such as analgesic, anti-diabetic, antibacterial, and antifungal activity.

In early study, Kararti et al., (1995) reported that *Camellia olifera* contain phenylethanol (14.7%), linalool (7.9%), (E)-linalool oxide furanoid (3.5%), epoxy linalool (1.6%), geraniol
(2.3%) and hotrienol (1.5%), m-Xylene (2.6%), (E)-linalool oxide pyranoid (5.4%), p-myrcene (5.2%), alpha-cadinol (4.3%), methyl palmitate (2.9%), 3-hexenol (2.1%) (E)-4,8-dimethyl-1,3,7-nonatriene (20.9%) and linalool (35.1%) which was determined through GC-MS. Likewise, Nishikitanet al. (1996) was isolated a new glycosidic aroma precursor from green tea leaves (Camellia Yabukita) along with the known primeverosides of cis-linalool 3,6-oxide, linalool and geraniol. The chemical structure of the new unknown glycoside was confirmed as geranyl 6-O-alpha-L-arabinopyranosyl-beta-D-glucopyranoside (geranyl beta-vicianoside) by GC-MS study. Yao et al. (2005) analyzed the major volatile constituents present in group of special black teas in China. GC-MS analyses of special black teas shown that Forty-nine different compounds such as longifolene, longicyclene, guaiacol, 4-methylguaiacol, 4-ethylguaiacol, etc., Longifolene and alpha-terpineol were the most abundant compounds and its responsible for aroma.

Stapleton et al. (2004) identified the polyphenols compound from methanolic extracts of Japanese green tea (Camellia sinensis) through GC-MS analysis and stated that polyphenolic compound (-)-epigallocatechin gallate (EGCG) had a moderate capacity to modulate oxacillin activity against S. aureus, but ECG, as both (-)-epicatechin and (-)-epicatechin-3-cyclohexylcarboxylate were unable to reverse resistance to oxacillin. Octylgallate exhibited direct antibacterial activity against S. aureus. Neyestani et al. (2005) observed that Gallic acid in black tea extract and epigallocatechin and epigallocatechin gallate in green tea extract from GCMS analysis and this compound exhibit antibacterial activity against gram positive and gram negative bacteria. Ferrazzano et al. (2009) reported that polyphenols occurring in cocoa, coffee and tea can have a role in the prevention of cariogenic processes, due to their antibacterial action. Tea polyphenol pentamers significantly reduce biofilm formation and acid production. Osterburg et al. (2009) and Peng et al., (2010) identified that polyphenol, (-)-epigallocatechin-3-gallate (EGCG), from green tea (Camellia sinensis), had antimicrobial effects against multiresistant clinical isolates of A. baumannii, and methicillin-resistant Staphylococcus aureus (MRSA). Enzveiler, et al. (2012) also stated that the potential antimicrobial activity of aqueous extracts of white tea due to the catechin compound.
Besides, Sagesaka et al., (1996) isolated the tea-leaf saponin, from the leaves of white tea using GCMS analysis. Tea-leaf saponin inhibited rat paw edema induced by carragecin in a dose dependent manner. The results strongly recommends that tea-leaf saponin have both have high anti-inflammatory and antimicrobial activity. Sur et al. (2001) reported the two groups of saponins, from tea root extract through GCMS analysis and it contains anti-inflammatory and in vitro antioxidant activity.

The result of this study suggested that the presence of these phytochemical in, T. chebula and Camellia sinensis might be the reason for its antibacterial activity, anti-inflammatory and hepatoprotective activity. T. chebula contains more bioactive compound than Camellia sinensis. The result of this experiment indicates that these medicinal plants could be studied further in detail and its beneficial effects could be utilized to create a healthy environment.

5.2. HEPATOPROTECTIVE ACTIVITY

Liver is the most important organ concerned with the biochemical activity in the human body and it has great capacity to detoxicate toxic substances and synthesize useful metabolities (Meyer et al., 2001). Liver plays a central role in co-ordinating the various metabolic functions of the body. Chronic consumption of ethanol induces lipid peroxidation causing hepatotoxicity by increasing the free radical formation which in turn increases the level of lipid peroxide in hepatic tissue and causes cell injury (Lieber, 1991). Hepatic damage is associated with distortion of these metabolic functions (Wolf, 1999). Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects (Guntupalli et al., 2006). In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity. A single drug cannot be effective for all types of severe liver diseases (Shahani, 1999). Therefore an effective formulation has to be developed using indigenous medicinal plants, with proper pharmacological experimental study and clinical trials.
Carbon tetrachloride is the one of the most commonly used hepatotoxins in the experimental study of liver diseases (Johnson et al., 1998). It induces liver cell necrosis and apoptosis and can be used to induce hepatic fibrosis or cirrhosis by repetitive administration (Shi et al., 2005). The hepatotoxic effect of carbon tetrachloride is mainly due to its active metabolite, trichloromethyl radical. (Srivastava et al., 1990). This activated radical bind covalently to the macromolecules and induce lipid peroxidation and forms lipid peroxides which produce damage to the membrane. (Mujeeb et al., 2009). The increase in the levels of serum bilirubin reflected the depth of jaundice and the increase in transaminases and alkaline phosphatase which are cytoplasmic in location and released into circulation after cellular damages was the clear indication for the loss of functional integrity of the cell membrane. (Saraswat et al., 1993). Amino transferases are present in high concentration in liver, an important class of enzymes linking carbohydrate and amino acid metabolism. Alanine amino transferase and aspartate amino transferase are well known diagnostic indicators of liver disease. In cases of liver damage with hepatocellular lesions and parenchymal cell necrosis, these marker enzymes are released from the damaged tissues into the blood stream (Chaudhary et al., 2009). Estimating the activities of serum market enzymes like SGOT and SGPT can make assessment of liver function. When liver cell plasma membrane is damaged, a variety of enzymes, normally located in cytosol, are released into the blood stream. Their estimation in the serum is a useful quantitative market of the extent and type of hepatocellular damage (Reitman et al., 1998).

In the present study, CCL4 is used as inducer and it increased the levels of serum markers AST, ALT, ALP, γ-glutamyl transpeptidase (GGTP), total bilirubin, total protein and lipid peroxidation (LPO), indicating liver damage. Because liver is considered to be highly sensitive to toxic agent in all over part of the body. However, treatment of methanolic fruit extracts of T. chebula and methanolic leaves extract of C. sinensis CCl4-induced hepatotoxicity, which was compared to that of standard drug Silymarin. The enzymatic antioxidant defense system is the nature protector against lipid peroxidation for important scavengers of superoxide ion and hydrogen peroxide (Dash et al., 2007). The levels of superoxide dismutase (SOD), Catalase, glutathione peroxidase (GPx), and glutathione S transferase (GST) were decreased by induction of CCL4. Treatment with T. chebula and C. sinensis were recovering the decreased antioxidant levels when compared with CCL4 treated rats. Silymarin treated animals also showed a
significant increase in antioxidant enzymes levels compared to CCL₄ treated rats. Histopathological examination of the liver section of the rats treated with toxicant showed intense centrilobular necrosis and vacuolization. The rats treated with silymarin and extracts along with toxicant showed sign of protection against these toxicants to considerable extent as evident from formation of normal hepatic cards and absence of necrosis and vacuoles. In conclusion, *T. chebula* extract possess hepatoprotective activity against CCL₄ intoxication rats than *C. sinensis*.

5.3. ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY

Inflammation is a biological complex of vascular tissues in harmful stimulated by pathogens and irritants (Meena *et al.*, 2009) and has been major health problems in the world (*Li et al.*, 2003). The anti-inflammatory effects can be elicited by a variety of chemical agents and that there is little correlation between their pharmacological activity and chemical structure (*Sertic et al.*, 1990). Although, several agents are known to treat inflammatory disorders, their prolonged use often leads to gastric intolerance, bone marrow depression, water and salt retention (*Rajasekaran et al.*, 2003). Now-a-days herbal treatments are becoming increasing by popular as the herbal preparations have no or least side effects (*Sharma et al.*, 2009). World Health Organization (WHO) estimates that 80% of the population relies on plant based products for human health care (*Gurib-Fakim*, 2006). Hence, in the present study pharmacological properties of *T. chebula* and *C. sinensis* were tested using a number of experimental rat models, representing different phases of inflammation and their antioxidant properties.

Most living organisms possess enzymatic and non-enzymatic defense system against excess production of reactive oxygen species. However, different external factors such as smoke, diet, alcohol, drugs and aging could decreases the capability of such productive systems resulting in disturbances of the redox equilibrium that is established in healthy conditions that scavenge reactive oxygen species may be of great value in preventing the onset and/or propagation of oxidative diseases (*Willett, 1994*). Antioxidants are also compounds that inhibit or delay the oxidation of the molecules by inhibiting the initiated or propagation of oxidizing chain reaction. Herbal drugs are playing an important role in health care programmes worldwide and there is resurgence of interest in herbal medicines for treatment of various ailments.
In our present study, anti-inflammatory and antioxidant activities of the Methanolic extracts of *T. chebula* and *C. sinensis* Fruits and leaves were evaluated by carrageenan-induced rat paw oedema method and the result showed that Carrageenan-induced rat paw oedema has been used as an inflammation model in order to investigate the anti-inflammatory effect of drug. The extracts were tested at two different dose levels such as 100 and 200 mg/kg. The parameters studied were TBARS, SOD, CAT, GPX, GSH Vit-E and C. in Plasma, RBC and tissues. The results showed that higher dose of alcoholic extracts of *T. chebula* and *C. sinensis* (200 mg/kg) showed 48% and 47.05% of inhibition on carrageenan induced rat paw oedema at 180 min. This result indicated that alcoholic extracts with a dose of 100 mg/kg b.w showed a maximum anti-inflammatory activity is similar to the reference drug indomethacin, which showed 46.68% of inhibition. In general, oedema has an early stage of inflammation (10) is due to release of histamine and serotonin like substances (Maridass *et al.*, 2008). Higher dose (200 mg/kg) of *T. chebula* and *C. sinensis* extract reflects anti-inflammatory activity may be due to inhibition of the mediators of inflammation histamine, serotonin and prostaglandin at after 180 min. Such a phenomenon, a number of medicinal plants are used in various medical systems for pain and inflammation relief at after 180 min, and has already been observed in *Jatropha gossypifolia* (Panda *et al.*, 2009) *Bambusa vulgaris* (Carey *et al.*, 2009), *Tabernaemontana catharinensis* (Gomes *et al.*, 2009) and *Tagetes erecta* (Chatterjee *et al.*, 2009).

The study of lipid peroxidation is attracting much attention in recent years due to its role in disease process. Lipid peroxidation in the biological system involves all peroxidation of fatty acids with two or more double bonds are susceptible to oxidation than the saturated and monounsaturated fatty acids (Halliwell and Gutteridge, 1999). It is a highly destructive process and alters the structure and functions of the cell membrane (Kale and Sitarawad, 1990). It is now generally accepted that lipid peroxidation and its products play an important role in liver, kidney brain toxicity and anti-inflammatory activity.

The enzymatic antioxidant defense system is the nature protector against lipid peroxidation for important scavengers of superoxide ion and hydrogen peroxide (Dash *et al.*, 2007). In the present study, elevated level of LPO or TBARS in carrageenan treated rats is a clear indication of exclusive formation of free radicals and activation of lipid peroxidation system. The significant decline in the level of these constituents in animals treated with plant
extracts *T. chebula* and *C. sinensis* indicate anti-lipidperoxidative effect of these plants. Methanolic extract of *T. chebula* fruit showed greater anti-lipidperoxidative activity than *C. sinensis*.

Glutathione (GSH) is a ubiquitous thiol containing tripeptide, which plays a central role in cell biology. It is implicated in the cellular defense against xenobiotics and naturally occurring deleterious compounds, such as free radicals. It is a highly sensitive indicator of cell functionality (Meister, 1991). Glutathione is a major non-protein thiol in living organisms which coordinates the body’s antioxidant defense process. Perturbation of GSH status of a biological system can lead to serious consequences (Valenzuela et. al., 1985). Glutathione peroxidase is an antioxidant enzyme that reduces hydrogen peroxide and lipid peroxide (Knapen et. al., 2000). In plasma, GSH levels are usually less than 20 vtmol/L in human. GSH not only protects cell membrane from oxidizing damage, but also helps to maintain the sulphohydrolyl groups of many proteins. Irreversible cell damage supervenes when the cell is no longer able to maintain GSH content. Decreased level of GSH in the ethanol intoxicated animals in the present study revealed that the GSH was utilized for peroxide radicals. Decline in GSH content in serum of carrageenan treated rats and its subsequently elevated in ethanol with extracts of *T. chebula* and *C. sinensis* administered group also expose anti-lipidperoxidative effect of these plants. The effect is more significant in *T. chebula* than other extracts.

Glutathione Peroxidase (GPx, EC 1.11.1.9) family of enzymes play important roles in the protection of organisms from oxidative damage. GPx converts reduced glutathione (GSH) to oxidized glutathione (GSSG) while reducing lipid hydroperoxides to their corresponding alcohols or free hydrogen peroxide to water. Several isozymes have been found in different cellular locations and with different substrate specificity. Low levels of GPx have been correlated with free radical related disorders (Lai et. al., 2010). Decreased level in GPx content in serum of carrageenan treated rats and its subsequently elevated in ethanol with extracts of *T. chebula* and *C. sinensis* administered group also expose the effect of these plants. The GPx content in serum more significant in *T. chebula* than other extracts. Carrageenan treated rats and its high level in ethanol with extracts of *T. chebula* and *C. sinensis* administered.
Superoxide dismutase (SOD) is one of the most important antioxidative enzymes. It catalyzes the dismutation of the superoxide anion into hydrogen peroxide and molecular oxygen. In present study, low level of SOD content in serum of group which exhibited the effect of these plants. The SOD content in serum and plasma more significant in *T. chebula* than *C. sinensis*.

Similarly Catalase(CAT) (EC 1.11.1.6) is a ubiquitous antioxidant enzyme that is present in nearly all living organisms. It functions to catalyze the decomposition of hydrogen peroxide (H₂O₂) to water and oxygen. In the present analysis, CAT content is very low level in serum and plasma of carrageenan treated rats and its high level in ethanol with extracts of *T. chebula* and *C. sinensis* administered group which exhibited the effect of these plants. The CAT content in serum and plasma more significant in methanolic extract of *T. chebula* fruit.

Vitamin E and C has been shown to function as antioxidant in various settings. It is an important water soluble antioxidant and readily scavenges Reactive Oxygen Molecule (ROM), ozone, HNO₃, NO₂, NO⁻ and hypochlorous acid (Noroozi *et. al.*, 1998). Vitamin C rejuvenates vitamin E making it an indirect contributor to the fight against free radical damage in the lipids. These two nutrients can be effective partners in reducing the destructive process of lipid peroxidation (Karagezian and Gerorkian, 1989). In the present investigation, a significant reduction in vitamin E and C content was noticed in carrageenan induced rat that may be due to the reduced availability of glutathione which has been utilized in detoxification process. Contrary to this, carrageenan induced rat with *T. chebula* and *C. sinensis* fruits and leaves treated animals showed an increase in level of Vitamin E and C. Among the treatments, *T. chebula* exhibited significant increase over *C. sinensis* (Table 12).

In present study, the levels of TBARS was increased and superoxide dismutase (SOD), Catalase, glutathione peroxidase (GPx), and glutathione S transferase (GST) were decreased in carrageenan induced rat. Treatment with *T. chebula* and *C. sinensis* were increased antioxidant levels when compared with carrageenan induced rat. Moreover, Methanolic extracts of *T. chebula* produce a significant anti-inflammatory antioxidant activity than *C. sinensis*. 
The present study results supported by Sagesaka et al., (1996) isolated the tea-leaf saponin from the leaves of white tea. Tea-leaf saponin inhibited rat paw edema induced by carrageenin in a dose dependent manner. The results strongly recommends that tea-leaf saponin have both have high anti inflammatory and antimicrobial activity. Following this Akihisa et al.,(1997) identified the triterpene s from the seeds of Camellia japonica L. and this led to the isolation of twenty-seven triterpene alcohols, and the results suggest that triterpene from C japonica have the anti inflammatory activity.

Sur et al.,(2001) reported the two groups of saponins, from tea root extract (TRE) were tested for antiinflammatory and in vitro antioxidant activity. Both TS-1 and TS-2 inhibited carrageenan-induced paw oedema in rats. Like wise, Chattopadhyy et al.,(2001) stated that methanol-water (1:1) extract of dried tea root extract (TRE) possess anti-inflammatory, analgesic and antipyretic activities Wang et al.,(2010) investigated the anti-inflammatory and analgesic effects of theacrine (1, 3, 7, 9-tetramethyluric acid), a purine alkaloid which is abundantly present in Camellia kucha, Ellis et al.,(2011) reported that Epigallocatechin-3-gallate (EGCG), the major polyphenolic component of green tea, has been demonstrated to possess anti-inflammatory, antioxidant, anti-mutagenic and anti-carcinogenic properties.

Moreover, Punicalagin and punicalin isolated from the fruits of Terminalia catappa L. exhibited the high anti-inflammatory activity in carrageenan-induced hind paw oedema in rats (Lin et al.,1999) and Methanolic extracts of Terminalia catappa leaves showed anti-inflammatory activity due to the presence of triterpenic acids Fan et al.,(2004). Like wise, isolation of anolignan B from Terminalia sericea showed the antibacterial and anti-inflammatory activities (Eldeen 2006).

This study reports for the first time to our knowledge that T. chebula has anti-inflammatory activities than C. sinensis. Further studies may reveal the exact mechanisms of action responsible to treat for the analgesic and inflammatory activities. Though the study has highlighted the anti-inflammatory activity of C. sinensis and T. chebula could be a potential new natural source as well as scientific proof of its ethno-pharmacological use in inflammatory disorders.
5.4. ANTIBACTERIAL ACTIVITY

Bacterial infection is one of the most serious global health issues in the 21st century (Morris et al., 2002). The emergence of bacterial resistance to antibiotics is a major health problem and therefore, it is critical to develop new antibiotics with novel mechanisms of action to overcome these problems (Wang et al., 2003). Antimicrobial chemotherapy is severely troubled by the emergence and rapid spread of multiresistant bacteria (Cohen, 1992 and Neu, 1992). Although the use of antibiotics has been the major weapon in combating infectious diseases, the rapid development of antibiotic resistance has resulted in treatment failures and outbreaks of infections caused by antibiotic-resistant bacteria (Chiu et al., 2001, Ho et al., 1995, Wong et al., 1999, Wong et al., 2000 and Yuen et al., 1990). There has been an increasing incidence of multiple resistance in human pathogenic microorganisms in recent years, largely due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of diseases. The number of resistant strains of microbial pathogens is growing, ever since penicillin resistance and multiresistance pneumococci were reported (Maurer-Grimes et al., 1996; Ellof, 1998). This situation, coupled with the undesirable side effects of certain antibiotics and the emergence of previously uncommon infections are a serious problem (Marchese and Shito, 2001; Poole, 2001). This has forced scientists to search for new antimicrobial substances from various sources like the medicinal plants. The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Maurer-Grimes et al., 1996; Rabe and Van Staden, 1997; Afolayan, 2003). The presence of antibacterial and antifungal substances in the higher plants is well established (Fridous et al., 1990; Didry et al., 1998; Javed and Ali, 2002; Belboukhari and Cheriti, 2005).

Plants remain the most common source of antimicrobial agents. Their use as traditional health remedies is most popular in 80% of the world population in Asia, Latin America, and Africa, and is reported to have minimal side effects (Bibitha et al., 2002). Plant materials remain an important source to combat serious diseases in the world. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in developing countries. The medicinal value of these plants lies in some chemically active substances that produce a definite physiological action on the human body. The most important among the bioactive constituents of plants are alkaloids, tannins, flavanoids, and steroids.
(Edeoga et al., 2005). The demand on plant-based therapeutics is increasing in both developing and developed countries due to growing recognition that they are natural products, non-narcotic, easily biodegradable, pose minimum environmental hazards, have no adverse side-effects and are easily available at affordable prices. The present investigation involving T. chebula and C. sinensis also lends credence to the above observations.

T. chebula and C. sinensis is an important medicinal plant in Indian traditional medicine and it is most frequently used herb in Ayurveda. In the present study, Antimicrobial activity of extracts of fruits and leaves from T. Chebula and C. sinensis was tested against important human pathogens including Gram positive and Gram negative bacteria by agar disc diffusion method, using methanolic solvents. Antibacterial activity of both leaves and roots of both plants against different pathogens is shown in the Figures 12 and 13. Maximum antibacterial activity (inhibition zone in mm) was recorded against K. aerogenes (12 mm) followed by S. aureus (9.5 mm) B. subtilis (8.2 mm) and P. aeruginosa (7.6 mm) with the fruit extract of T. Chebula. On the other hand leaf extract of C. sinensis showed maximum inhibitory effect on K. aerogenes (12.5 mm) followed by B. subtilis (8.7 mm), P. aeruginosa (7.8 mm) and S. aureus (7.3 mm). Of the plants tested, activity was more with T. Chebula than C. sinensis. Among fruits and leaves, the leaf extract of T. Chebula showed more activity against all the bacteria tested than that of C. sinensis. Among the pathogens, K. aerogenes was more susceptible to leaf extracts of both plants followed by S. aureus, B. subtilis, and P. aeruginosa. The extract showed a broad spectrum of antibacterial activity against gram-positive and gram-negative bacteria.

This was supported by an earlier study on an alcoholic extract that exhibited greater activity than the aqueous and hexane extracts against bacteria, with no cellular toxicity (Ahmad et al., 1998). The broad spectrum of antibacterial activity was reported for T. arjuna (Singh et al., 2008). The ethanol extract at a concentration of 1 mg/disc showed maximum inhibition against S. epidermidis, followed by B. subtilis. Gupta et al., (2002) reported that a T. pallida fruit methanolic extract showed maximum activity against gram-negative bacteria, while that of T. bellerica showed the highest inhibition zones against P. aeruginosa and E. coli (Ghosh et al., 2008). Two possibilities that may account for the higher antibacterial activity of alcoholic extracts are the nature of biological active components (alkaloids, flavonoids, essential oil, tarenoids, tannins, etc.), which may be enhanced in the presence of ethanol; and the stronger
extraction capacity of ethanol that may have yielded a greater number of active constituents responsible for antibacterial activity (Ghosh et. al., 2008). Bag, A. et. al., 2009 and Chaudhari, and Mengi, 2005 performed the antimicrobial activity on *Terminalia* species on various microorganisms to obtain the result which was similar to that found in our showing *Terminalia* species to possess antimicrobial property which also supports data obtained.

Adigun et. al., 2000 stated that Some members of the Combretaceae have high concentrations of flavonoids, terpenoids, tannins or polyphenolic compounds. These compounds are known have *in vitro* antimicrobial activity. 3,4,3'-Tri-O-methylflavellagic acid and its glucoside were isolated from Combretaceae species. These compounds were analysed by GC-MS. Antimicrobial effect of the Combretaceae species possesses anti bacterial and antifungal activity due to the presence of glucosides.

Similarly, the result of methanolic extract of *C. sinensis* leaves shows that has an antibacterial activity against gram-positive and gram-negative bacteria. This finding is in agreement with the previous studies about Chinese and Japanese green tea (You et. al., 1993; Sakanaka et. al., 1989; Sasaki et. al., 2004). Tea is known to possess antibacterial activity against a number of bacteria due to presence of polyphenols especially catechins which play an important role in green tea's inhibition of bacterial growth. Several significant catechins include: epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), epicatechin (EC), and gallocatechin-3-gallate (GCG) (Tiwari et. al., 2005). Moreover, The daily consumption of green tea can kill Gram positive staphylococcus aureus including many other harmful bacteria. Tea constituents also possess antibacterial, antiviral action, anticarcinogenic and anti mutagenic properties. (Toda et. al., 1991).Antimicrobial activities of tea extracts are very selective. This difference in their activity depends upon the concentration and type of the extracts. These effects may also differ depending on the bacterial species so that they may be either growth inhibitory or stimulatory (Isogai et. al., 2001).Green tea possesses antimicrobial activity against a variety of pathogenic bacteria that cause cystitis, pyelonephritis, diarrhea, dental caries, pneumonia, and skin infection (You et. al., 1999).
The results noticed in the study showed that the extract obtained from C. sinensis and T. Chebula had shown strong antibacterial activity and can be serve as a very good source for the invention of new therapeutic agents to kill pathogenic bacteria isolated from oral samples.