2. REVIEW OF LITERATURE

2.1. PHYTOCHEMICAL ANALYSIS

India has a rich tradition in the use of medicinal plant to develop drugs from plants. Now-a-days herbal drugs are prescribed widely even when their biologically active compounds are unknown because of their effectiveness, minimal side effects in clinical experience and relatively low cost. Last decade witnessed an increase in the investigations on plants as a source of human disease management as well as various phytochemical constituents Camellia and Terminalia species have rich source of bioactive compounds which are effective against various diseases which are follows.

Guo et al., (1994) observed three glycosides, namely 6-O-beta-D-xylopyranosyl-beta-D-glucopyranosides (beta-primeverosides), linalool, 2-phenylethanol, and benzyl alcohol which were isolated from the tea leaves (oolong tea). The isolation was guided by acid or enzymatic hydrolysis, and subsequent GC and GC-MS analyses. The linalyl glycoside is the first example of naturally occurring (S)-linalyl beta-primeveroside.

Ko et al., (1994) identified the Squalene from freeze-dried abscisic leaves of Terminalia catappa L through gas chromatography-mass spectrometry and high-performance liquid chromatography (HPLC). When the freeze-dried abscisic, senescent, mature, and immature leaves and seeds were subjected to supercritical CO₂ extraction at 40 degrees C and 3000 psi and HPLC quantitation, squalene contents were 12.29, 2.42, 1.75, 0.9, and 0% in the extracts and corresponding to 1499, 451, 210, 65, and 0 microg/g in the freeze-dried sample, respectively. However, the seed extracts exhibited potent inhibition of CDHP formation and very low DPPH scavenging activity.

Kararli et al., (1995) reported that Camellia oleifera contain phenylethanol (14.7%), linalool (7.9%), (E)-linalool oxide, furanoid (3.5%), epoxy linalool (1.6%), geraniol (2.3%) and butenol (1.5%), m-Xylene (2.6%), (E)-linalool oxide pyranoid (5.4%), p-mycene (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 were determined through GCMS.
Nishikitane et al. (1996) isolated a new glycosidic aroma precursor from green tea leaves (*Camellia sinensis var. sinensis* cv. *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-vicinanside) by GC-MS and HPLC analyses.

The fatty oils in *Terminalia chebula* were extracted by supercritical-CO₂ fluid extraction, and their fatty acids and its relative contents were determined by GC-MS. Totally 12 fatty acids were identified, of which Palmitic acid, linoleic acid and oleic acid of them are main constituents. Compared with petroleum ether extraction method, the extraction rate from SFE-CO₂ is higher and extraction time is shorter (Zhang et al., 1997).

Qi-Mei Liu et al. (1999) analysed the biomedical Components of *Camellia oleifera* Leaf by GC/MS. There are 25 components identified in the leaf extractive, and the main constituents are butyraldehyde, semicarbazone (11.58%), hexatriacontane (8.04%), 1,6-anhydro-beta-D-glucopyranose, (7.54%), octadecane (7.53%), benzenecetic acid 4-hydroxy- (6.59%), 1,3-dioxolane 4-ethyl-5-hexyl-2,2-bis(trifluoro methyl)-1cis- (6.53%), etc. The extraction of kernel hull also contains abundant constituents identified as 2-flurancarboxaldehyde 2- (hydroxymethyl) - (32.37%), 1,3-propanediol, 2-ethyl-2- (hydroxymethyl) - (28.34%), 1,2,3- benzenetriol (15.10%), etc. As the first report here, the analytical result by GC/MS showed that the benzene/ethanol extractives of both leaf and kernel hull of *C. oleifera* contain abundant components of rare natural medicinal materials, and also contain many components which can be developed into two value-added materials of high-grade cosmetic, bioenergy, and industrial solvent.

Xiao-Hong et al. (1999) observed the aroma components from the fresh flowers of *Camellia renshania* by GC-MS analysis. A total of 50 components were detected, 19 of them were identified, which accounted for 71.72%. The result indicated that the main aroma components were aromatic compound, sesquiterpenoid, alkane, alkene, and fatty acid ester. The content of sesquiterpenoids is the highest, accounting for 46.31%, the next is alkane, accounting for 19.79%.

Yanagimoto et al. 2003 investigated the major volatile constituents of green tea and roasted green tea extracts, which exhibited significant antioxidative activities, were analyzed using gas chromatography-mass spectrometry. The major volatile chemicals with possible
antioxidative activity identified were alkyl compounds with double bond(s), such as 3,7-dimethyl-1,6-octadien-3-ol (8.04 mg/kg), in the extract from green tea and heterocyclic compounds, such as furfural (7.67 mg/kg), in the extract from roasted green tea. Benzyl alcohol, which was proved to be an antioxidant, was identified both in a green tea extract (4.67 mg/kg) and in a roasted tea extract (1.35 mg/kg).

Gupta et al., (2004) isolated and characterized the brassinosteroids from leaves of *C. sinensis* (L.) O. Kuntze. Brassinosteroids are of ubiquitous occurrence in plants and elicit a wide spectrum of physiological responses. In our study, brassinosteroids were isolated and identified in topmost dormant leaves of tea plants. Six brassinosteroids, i.e., 6-deoxoastaxasterone, 24-epibrassinolide, 3-dehydroasteasterone, typhasterol, 3-deoxo-typhasterol and 28-homodolicholide, were isolated and identified by GC–MS. It suggests that the most active brassinosteroid in tea plant is the brassinolide Zeng Hong-yan et al., (2005) extracted the fatty acids from tea-seed oil by different methods (supercritical CO₂, microwave, ultrasonic, and squeezing) by gc–ms analysis. Chemical composition of the seed oil extracted with the three methods was similar except squeezing. However, vitamin E and squalene were not found in the constituents extracted by squeezing. It is suggested that the extraction with supercritical CO₂ is an ideal method by which the oil is up to the first degree of national standard for gc–ms analysis.

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 which are responsible for the aroma.

Zhang et al., (2008) observed the bioactive components of benzene/ethanol extractive and pyrolyze from the kernel hull of *C. oleifera* seed. There are 15 components identified in the benzene/ethanol extractive, and the main constituents are 2- furancarboxaldehyde, 5-(hydroxymethyl)- (32.37%), 1,3-propanediol, 2-ethyl-2-(hydroxymethyl)- (28.34%), 1,2,3-benzenetriol (15.10%), 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (5.39%), 2-furancarboxaldehyde (3.68%), etc. However, 38 compounds were identified from kernel hull pyrolyze, and the main constituents are ethylene, fluoro– (21.95%), acetic acid (23.53%), butane (11.88%), acetate, dehydrohydroxy– (5.63%), 2-propanone, hydroxy– (5.59%), heptadecane, 9-octyl– (4.00%), etc, the analytical result by GC/MS showed that both the
benzene/ethanol extractive and pyrolyzate of kernel hull of C. oleifera seed contain abundant components of rare natural medicinal materials, and also contain components which can be developed into two value-added materials of high-grade cosmetic, bioenergy, and industrial chemical and solvent.

Qi-mei Liu et al., (2009) reported the biomedical components of Camellia oleifera leaf and kernel hull by ge/ms. There are 25 components identified in the leaf extract, and the main constituents are butyraldehyde, semicarbazone (11.58%), hexatriacontane (8.04%), 1,6-anhydro-beta-D-glucopyranose, (7.54%), octadecane (7.53%), benzenecetic acid, 4-hydroxy- (6.59%), 1,3-dioxolane, 4-ethyl-5-hexyl-2,2-bis(trifluoro methyl)-1cis- (6.53%), etc. The extractive of kernel hull also contains abundant constituents identified as: 2- furancarboxaldehyde, 5 - (hydroxymethyl) - (32.37%), 1,3-propanediol, 2-ethyl-2-(hydroxymethyl) - (28.34%), 1,2,3- benzenetriol (15.10%), etc. As the first report here, the analytical result by GC/MS showed that the benzene/ethanol extract of both leaf and kernel hull of C. oleifera contain abundant components of rare natural medicinal materials, and also contain many components which can be developed into two value-added materials of high-grade cosmetic, bioenergy, and industrial solvent.

Shi et al., (2010) observed the fatty acid composition Pu’er tea seed oil using gas chromatography. It was found that the quality fraction of oleic acid in Pu’er tea seed oil is higher than in peanut oil and rapeseed oil, and lower than in camellia seed oil and olive oil, and the quality fraction of erucic acid in Pu’er tea seed oil is lower than in peanut oil, rapeseed oil and camellia seed oil, while slightly higher than in olive oil. Therefore, Pu’er fruit tea is an ideal oil resource. At the same time 30 kinds of volatile compounds in Pu’er tea seed oil were identified by gas-chromatography-mass spectrometry. They are mostly alcohols, aldehydes, ketones and esters, and most of these compounds contain unsaturated bond.

The different cultivation methods affect tea quality by altering the basic metabolite profiles. In this study, the metabolome changes were investigated in green tea and shade cultured green tea (tencha) by liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) coupled with a multivariate data set. The principal component analysis (PCA) and orthogonal projection to latent structures discriminate analysis (OPLS-DA) of green tea clearly showed higher levels of galloylquinic acid, epigallocatechin, epicatechin, succinic acid, and fructose, together with lower levels of
gallo catechin, strictinin, apigenin-glucosylarabinoside, quercetin p-coumaroylglucosyl-
rhamnosylgalactoside, kaempferol p-coumaroylglucosylrhamnosylgalactoside, malic acid,
and pyrog glutamic acid than tencha. The effects of some seasonal variations were also
observed in the primary metabolite concentrations such as amino acids and organic acids. In
addition, green tea showed stronger antioxidant activity than tencha in both April and July.
The antioxidant activity of green tea samples were significantly correlated with their total
phenol and total flavonoid contents. This study delineates the possibility to get high umami
and less astringent green teas in shade culture. It highlights the metabolomic approaches to
find out the effect of cultivation methods on chemical composition in plants and the
relationship with antioxidant activity by et al., (2010).

Joshi et al. (2011) characterized the volatile components of tea flowers (Camellia
sinensis) growing in Kangra by GC/MS. Phenylethanol (14.7%), linalool (7.9%), (E)-linalool
oxide furanoid (3.5%), epoxy linalool (1.6%), geraniol (2.2%) and hotrienol (1.5%) were
major components. m-Xylene (2.6%), (E)-linalool oxide pyranoid (5.4%), p-mentha (5.2%),
alpha-eleinol (4.3%) and methyl palmitate (2.9%) were major compounds isolated from tea
flowers.

Rajendra Gyawali and Kyong-Su Kim (2012) isolated various compounds from the
volatile organic compounds of Terminalia chebula by simultaneous distillation-extraction
(SDE) technique and then analyzed by gas chromatography–mass spectrometry (GC-MS).
Totally 56 compounds were identified from which camphor, borneol, capric acid, furfural,
myrtenal, β-pinene, β-terpeniol, perillaldehyde, 2-carene, butyrophenones, furfural,
β-caryophyllene, 2-nitropropane were detected as a bioactive compounds with various
proportions among the studied plants.

LI Xin-Lei and Sun Zhen-Yuan (2012) investigated the volatile components of
Camellia azalea Wei by GC-MS. There were twenty components identified in sepal, twenty-
two in petal, twenty-first in stamen and thirteen in pistil. The main flower parts of
volatile releasing were petal, and stamen. of Alkanes were the highest in petal, sepal
and stamen, accounting for 49.86%, 51.59% and 44.66% of total volatile components
respectively. The content of aldehydes and ketones was the highest in pistil, accounting for
83.87%.
2.2. HEPATOPROTECTIVE ACTIVITY

Plants are the basic source of knowledge of modern medicine. 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 alternative to synthetic drugs. Now-a-days herbal drugs are prescribed widely even when their biologically active compounds are unknown because of their effectiveness and minimal side effect in clinical experience. Large number of plants belonging to different families has been studied for their therapeutic properties. However, plants such as *Terminalia Sp* and *Camellia sp* have enormous source of hepatoprotective activity and their review follows.

Lin et al.,(1997) studied antihepatotoxic activity the free radical scavenging and from *Terminalia catappa*. Treatment with *T. catappa* water extracts showed antihepatotoxic activity against CCl4-induced toxicity in the rat liver that was tested. The crude drug also exhibited anti-oxidant effects in FeCl2-Ascorbic acid induced lipid peroxidation in the rat liver homogenate. The results indicate that *T. catappa* possesses good antihepatotoxic activity and superoxide radical scavenger activity.

Anand et al.,(1997) isolated 3,4,5-trihydroxy benzoic acid (gallic acid) from fraction TB5 of *Terminalia belerica* and evaluated for hepatoprotective activity against carbon tetrachloride (CCl4)-induced physiological and biochemical alterations in the liver. The main parameters studied were hexobarbitone-induced sleep, zoxazolamine induced paralysis, serum levels of transaminases and bilirubin. The hepatic markers assessed were lipid peroxidation, drug metabolising enzymes, glucose-6-phosphatase and triglycerides. Administration of Compound I led to significant reversal of majority of the altered parameters. Our results confirm the presence of hepatoprotective activity in Compound.

Lin et al.,(1998) elucidated Punicalagin and punicalin from the leaves of *Terminalia catappa L.*, are used to treat dermatitis and hepatitis. Both compounds have strong antioxidative activity. The antihepatotoxic activity of punicalagin and punicalin on carbon tetrachloride (CCl4)-induced toxicity in the rat liver was evaluated. Levels of serum glutamate-oxalate-transaminase and glutamate-pyruvate-transaminase were increased by administration of CCl4 and reduced by drug treatment. Histological changes around the liver central vein and oxidation damage induced by CCl4 also benefited from drug treatment. The results show that both punicalagin and punicalin have anti-hepatotoxic activity but that the
larger dose of punicalin induced liver damage. Thus even if tannins have strong antioxidant activity at very small doses, treatment with a larger dose will induce cell damage.

Ulina et al. (2002) investigated the hepatoprotective properties of rooibos tea (Aspalathus linearis) in a rat model of liver injury induced by carbon tetrachloride (CCL₄). Rooibos tea, like N-acetyl-L-cysteine which was used for the comparison, showed histological regression of steatosis and cirrhosis in the liver tissue with a significant inhibition of the increase of liver tissue concentrations of malondialdehyde, triacylglycerols and cholesterol. Simultaneously, rooibos tea significantly suppressed mainly the increase in plasma activities of aminotransferases (ALT, AST), alkaline phosphatase and bilirubin concentrations, which are considered as markers of liver functional state. The antifibrotic effect in the experimental model of hepatic cirrhosis of rats suggests the use of rooibos tea as a plant hepatoprotector in the diet of patients with hepatopathies.

HP-1 a herbal formulation comprising of Phyllanthus niruri and extracts of Terminalia beherica, Terminalia chebula, Phyllanthus emblica and Tinospora cordifolia has been evaluated for hepatoprotective activity against carbon tetrachloride (CCL₄) induced toxicity. Results show that HP-1 reversed the leakage of lactate dehydrogenase (LDH) and glutamate pyruvate transaminase (GPT) and prevented the depletion of glutathione (GSH) levels in a primary monolayer culture of rat hepatocytes (in vitro). HP-1 attenuated the serum toxicity as manifested in elevated levels of transaminases (glutamate oxaloacetate transaminase (GOT), and GPT). The antioxidative enzymes in liver (catalase and superoxide dismutase (SOD) were restored to normal values after the oral administration of HP-1. HP-1 suppressed the formation of the superoxide anion radical and reduced CCL₄ mediated lipid peroxidation (LPO). Silymarin and antioxidants (ascorbic acid, beta-carotene and alphatocopherol) were used for comparison. The present study showed that HP-1 is a potential hepatoprotective formulation with an additional attribute of being anti-oxidative (Tasduq et al., 2003).

Tang et al., (2004) studied the hepatoprotective effects of the extract of Terminalia catappa L. leaves (TCE) against D-Galactosamine (D-GalN)-induced liver injury and the mechanisms underlying its protection. In acute hepatic injury test, it was found that serum ALT activity was remarkably increased (3.35-fold) after injection of D-GalN in mice. But with oral pretreatment of TCE (20, 50 and 100 mg/kg/d) for 7 days, change in serum ALT was notably reversed. In primary cultured hepatocytes from fet.al., mice, it was
found that cell viability was decreased by 45.0% after addition of D-GalN, while incubation with TCE (0.1, 0.5 and 1.0 mg/ml) for 36 hours could prevent the decrease in a dose-dependent manner. Meanwhile, D-GalN-induced both the increase of AST level (1.9-fold) and the decrease of SOD activity (48.0%) in supematant of primary cultured hepatocytes could also be inhibited by pretreatment with TCE. It was concluded that TCE has hepatoprotective activity and the mechanisms underlying its protective effects may be related to the direct mitochondrion protection.

Gao et al. (2004) evaluated the effect of the chloroform extract of *Terminalia catappa* L. leaves (TCCE) on carbon tetrachloride (CCL4)-induced acute liver damage and D-galactosamine (D-GalN)-induced hepatocyte injury. Moreover, the effects of ursolic acid and asiatic acid, two isolated components of TCCE, on mitochondria and free radicals were investigated to determine the mechanism underlying the action of TCCE on hepatotoxicity. In the acute hepatic damage test, remarkable rises in the activity of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (5.7- and 2.0-fold) induced by CCl4(4) were reversed and significant morphological changes were lessened with pre-treatment with 50 and 100 mg kg(-1) TCCE. In the hepatocyte injury experiment, the increases in ALT and AST levels (1.9- and 2.1-fold) in the medium of primary cultured hepatocytes induced by D-GalN were blocked by pre-treatment with 0.05, 0.1, 0.5 g L(-1) TCCE. In addition, Ca(2+)-induced mitochondrial swelling was dose-dependently inhibited by 50-500 microM ursolic acid and asiatic acid. Both ursolic acid and asiatic acid, at concentrations ranging from 50 to 500 microM, showed dose-dependent superoxide anion and hydroxyl radical scavenging activity. From this study it is concluded that TCCE has hepatoprotective activity and the mechanism is related to protection of liver mitochondria and the scavenging action on free radicals.

Oyejide and Olushola (2005) studied the hepatoprotective and antioxidant effects of water extracts of black tea (*Camellia sinensis*) in sodium oxalate-treated rats. Lipid peroxidation was induced in rats by administration of 100 mg/kg body weight sodium oxalate. The protective effect of black tea was assessed by monitoring the serum and tissue levels of malondialdehyde, catalase activity, aspartate transaminase (AST) and alanine transaminase (ALT) as well as serum vitamin C content in the normal, control and experimental rats after 10 and 20 days of tea administration. It was observed that tea administration lowers significantly (p<0.05) the serum and tissue levels of malondialdehyde, as well as AST and
ALT activities in a dose dependent manner. The serum level of vitamin C and activity of catalase in the serum and tissues were however shown to be significantly elevated (p<0.05). After 10 days of administration of 200 mg/kg body weight of tea extract, serum level of malondialdehyde was reduced from 47.855±1.050 to 21.86±0.882nmol, AST activity from 5912.95 to 31±1.40 IU and ALT activity from 39±2.51 to 25±1.25 IU. Moreover, administration of 200 mg/Kg body weight of tea for 10 days caused an increase in serum catalase activity from 7 to 10% and serum vitamin C level was increased from 45.39±9.75 to 79.11±5.13 mg/100ml. In the tissues, the same trend was observed. The result also indicated that prolonged tea administration (for 20 days) significantly increased serum vitamin C level and the activity of catalase in both the serum, liver and the kidney (p<0.05). Also, the serum and tissue levels of malondialdehyde and transaminase activities (AST and ALT) were significantly reduced (p<0.05).

Tang et al., (2006) analysed the protective effects of chloroform extracts of Terminalia catappa L. leaves (TCCE) on carbon tetrachloride (CCL4)-induced liver damage and the possible mechanisms involved in the protection were investigated in mice. We found that increases in the activity of serum aspartate aminotransferase and alanine aminotransferase and the level of liver lipid peroxidation (2.0-fold, 5.7-fold and 2.8-fold) induced by CCL4 were significantly inhibited by oral pretreatment with 20, 50 or 100 mg/kg of TCCE. Morphological observation further confirmed the hepatoprotective effects of TCCE. In addition, the disruption of mitochondrial membrane potential (14.8%), intramitochondrial Ca2+ overload (2.1-fold) and suppression of mitochondrial Ca2+/ATPase activity (42.0%) in the liver of CCL4-insulted mice were effectively prevented by pretreatment with TCCE. It can be concluded that TCCE have protective activities against liver mitochondrial damage.

Asiatic acid (AA) is one of the triterpenoid components of Terminalia catappa L., which has antioxidative, anti-inflammatory and hepatoprotective activity. This research focused on the mitochondrial protection of AA against acute liver injury induced by lipopolysaccharide (LPS) and D-galactosamine (D-GalN) in mice. It was found that pretreatment with 25, 50 or 100 mg kg(-1) AA significantly blocked the LPS + D-GalN-induced increase in both serum aspartate aminotransferase (sAST) and serum alanine aminotransferase (sALT) levels, which was confirmed by ultrastructural observation under an electron microscope, showing improved nuclear condensation, ameliorated mitochondrial
proliferation and less lipid deposition. Collectively, the above data suggest that AA could protect liver from damage and the mechanism might be related to up-regulating process of mitochondria (Gao et al., 2006).

Tasduq et al., (2006) stated that Terminalia chebula Gertm. (Combretaceae) is an important herbal drug in Ayurvedic pharmacopea. In the present study, a 95% ethanolic extract of T. chebula (fruit) (TC extract), which was chemically characterized on the basis of chebuloside II as a marker, was investigated for hepatoprotective activity against antituberculosis (anti-TB) drug-induced toxicity. TC extract was found to prevent the hepatotoxicity caused by the administration of rifampicin (RIF), isoniazid (INH) and pyrazinamide (PZA) (in combination) in a sub-chronic mode (12 weeks). The hepatoprotective effect of TC extract could be attributed to its prominent anti-oxidative and membrane stabilizing activities. The changes in biochemical observations were supported by histological profile.

El-Beshbishy et al. (2007) studied the hepatoprotective effect of green tea (Camellia sinensis) extract against tamoxifen-induced liver injury in rats. Tamoxifen citrate (TAM), is widely used for treatment of breast cancer. It showed a degree of hepatic carcinogenesis. So, the liver injury in female rats was done by intraperitoneal injection of TAM in a dose of 45mg Kg(-1) day(-1), i.p. for 7 successive days. GTE in the concentration of 1.5 %, was orally administered 4 days prior and 14 days after TAM-intoxication as a sole source of drinking water. The antioxidant flavonoid; epicatechin (a component of green tea) was not detectable in liver and blood of rats in either normal control or TAM-intoxicated group, however, TAM intoxication resulted in a significant decrease of its level in liver homogenate of tamoxifen intoxicated rats. The model of TAM-intoxication elicited significant declines in the antioxidant enzymes (glutathione-S-transferase, glutathione peroxidase, superoxide dismutase and catalase) and reduced glutathione concomitant with significant elevations in TBARS (thiobarbituric acid reactive substance) and liver transaminases; sGPT (serum glutamate pyruvate transaminase) and sGOT (serum glutamate oxaloacetate transaminase) levels. The oral administration of 1.5 % GTE to TAM-intoxicated rats, produced significant increments in the antioxidant enzymes and reduced glutathione concomitant with significant decrements in TBARS and liver transaminases levels. The data obtained from this study speculated that 1.5 % GTE has the capacity to scavenge free radical and can protect against
oxidative stress induced by TAM intoxication. Supplementation of GTE could be useful in alleviating tamoxifen-induced liver injury in rats.

Lee et al., (2007) investigated the hepatoprotection of tea seed oil (Camellia oleifera Abel) against CCl₄-induced oxidative damage in rats. Male SD rats (200±10 g) were pre-treated with tea seed oil (50, 100, and 150 g/kg diet) for six weeks before treatment with a single dose of CCl₄ (50% CCl₄, 2 mL/kg of bw, intraperitoneally). The livers were excised for evaluating peroxidation products and antioxidant substances, as well as the activities of antioxidant enzymes. Pathological histology was also performed. The results showed that a tea seed oil diet significantly (p<0.05) lowered the serum levels of hepatic enzyme markers (alanine amino transferase, aspartate aminotransferase, and lactate dehydrogenase), and elevated the content of GSH. Pre-treatment of animals with tea seed oil (150 g/kg diet) could increase the activities of glutathione peroxidase, glutathione reductase and glutathione S transferase in liver when compared with CCl₄-treated group (p<0.05). Therefore, the results of this study show that a tea seed oil diet can be proposed to protect the liver against CCl₄-induced oxidative damage in rats, and the hepatoprotective effect might be correlated with its antioxidant and free radical scavenger effects.

Sengottuvelu et. al., (2008) appraised the hepatoprotective activity of aqueous extract of Camellia sinensis leaves and its possible mechanism of action. Liver damage was induced by intraperitoneal administration of carbon tetrachloride/olive oil (50% v/v, 0.5 mL/kg) in male Wistar rats (150-220g) once daily for 7 days and the extent of damage was studied by assessing biochemical parameters such as alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), total protein and albumin in serum and concentrations of lipid peroxides (LPO), glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) in liver. The aqueous extract of Camellia sinensis (100 mg and 200 mg/Kg) were administered orally to the animals with hepatotoxicity induced by carbon tetrachloride and its effects on biochemical parameters were compared with those in animals treated with vitamin E (100 mg/Kg). Histopathological studies were also done. Camellia sinensis 100 and 200mg/kg results in significant reduction in serum hepatic enzymes and liver lipid peroxide which were increased by carbon tetrachloride. There was significant increase in serum total protein, albumin and liver GSH, SOD and CAT when compared to those in rats treated by carbon tetrachloride. The antioxidant activity of Camellia sinensis (100 and 200mg/Kg) were comparable with the effects of vitamin E (100mg/Kg). Histopathological
changes (congestion of central vein, centrilobular necrosis and sinusoidal congestion) induced by carbon tetrachloride were reduced to a moderate extent in Camellia-sinesis-treated rats. The result suggest that Camellia sinensis protects the liver from carbon-tetrachloride-induced damage and its anti-oxidant property.

Ramesh et al. (2009) observed the efficacy of green tea catechins (GTC from the plant Camellia sinensis), with epigallocatechin gallate (EGCG), as the major component related to hepatic oxidative abnormalities in atherosclerotic rats. When male albino Wistar rats were fed an atherogenic diet for 30 days and then treated with saline for 7 or 15 days, there was a significant decline in hepatic mean activities of antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase and glutathione-S-transferase), and non-enzymatic antioxidants (reduced glutathione, vitamins C and E) while there was a significant elevation in the mean level of hepatic malondialdehyde (MDA), in comparison to the values noted in control rats fed a normal diet. In addition, a concomitant increase in the activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) was noted, when compared to the values in control rats. Following intraperitoneal administration of GTC (100mg/kg) for 7 or 15 days to rats fed the atherogenic diet, significantly higher mean activities of enzymatic and non-enzymatic antioxidants and lower mean levels of MDA in hepatic tissue and lower mean activities of AST, ALT, ALP and LDH in serum were observed, compared to the values in the rats fed the atherogenic diet and treated with saline. Histopathological studies were performed to provide direct evidence of the atherogenic diet-induced hepatic changes and of the hepatoprotective effect of GTC. These results suggest that EGCG as a major component of green tea catechins may protect against the hepatic abnormalities occurring in Wistar rats fed an atherogenic diet.

Jing Gao et al. (2010) evaluated the effect of the chloroform extract of Terminalia catappa L. leaves (TCCE) on carbon tetrachloride (CCl₄)-induced acute liver damage and D-galactosamine (D-GalN)-induced hepatocyte injury. Moreover, the effects of ursolic acid and asiatic acid, two isolated components of TCCE, on mitochondria and free radicals were investigated to determine the mechanism underlying the action of TCCE on hepatotoxicity. In the acute hepatic damage test, remarkable rises in the activity of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (5.7- and 2.0-fold) induced by CCl₄ were reversed and significant morphological changes were lessened with pre-treatment
with 50 and 100 mg kg⁻¹ TCCE. In the hepatocyte injury experiment, the increases in ALT and AST levels (1.9- and 2.1-fold) in the medium of primary cultured hepatocytes induced by D-GalN were blocked by pre-treatment with 0.05, 0.1, 0.5 gL⁻¹ TCCE. It can be concluded that TCCE has hepatoprotective activity and the mechanism is related to protection of liver mitochondria and the scavenging action on free radicals.

Eesha et al., (2011) reported the hepatoprotective activity of *Terminalia paniculata* against paracetamol induced hepatic damage in rats. Paracetamol (2 g/kg) increased the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and the lipid peroxides. Treatment of Liv 52, silymarin and ethanolic extract of *Terminalia paniculata* (200 mg/kg) altered levels of biochemical marker and showed significant hepatoprotective activity. Ethanolic extract revealed the presence of phenolic compound and flavanoids. Our findings suggested that ethanolic bark extract of *Terminalia paniculata* possessed hepatoprotective activity in a dose dependent manner. *Terminalia paniculata* possesses hepatoprotective activity. It could be an effective and promising preventive agent against CPT induced hepatotoxicity.

Xu et al., (2012) analysed the crude polysaccharides from the flowers of tea plant (*Camellia sinensis*) (TFPS) were prepared with hot water and further fractionated on a DEAE-52 cellulose chromatography to afford three purified fractions of TFPS-1, TFPS-2 and TFPS-3. Then, their preliminary structures, antioxidant and antitumor activities in vitro and hepatoprotective activity in vivo were investigated. Compared with TFPS-2 and TFPS-3, TFPS-1 had relative higher content of sulfate and relative complicated monosaccharide composition. For hepatoprotective activity, crude TFPS significantly prevented the increase of serum alanine aminotransferase and aspartate aminotransferase levels, reduced the formation of malondialdehyde and enhanced the activities of superoxide dismutase and glutathione peroxidase in carbon tetrachloride-induced liver injury mice. The results suggested that TFPS should be a potent natural polymer with hepatoprotective activities.

2.3 ANTI-INFLAMMATORY ACTIVITY

Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemicals or microbiologic agents. It is widely distributed throughout the world. It is also known that anti-inflammatory effects can be elicited by a variety of chemical agents and that there is little correlation between their pharmacological activity and chemical structure (Sertie et al., 1990). This is associated with the complexity of the inflammatory
process, makes the use of different experimental models essential when contacting pharmacological trails.

Sagesaka et al. (1996) isolated the tea-leaf saponin, from the leaves of *Camellia sinensis* var. *sinensis*. Tea-leaf saponin inhibited rat paw edema induced by carrageenan in a dose dependent manner. Activation of hyaluronidase, one of the enzymes involved in inflammatory reactions, was inhibited by tea-leaf saponin. It was also found that tea-leaf saponin antagonized the action of leukotrien D4, one of the chemical mediators of inflammatory reactions. Any symptom of toxic reaction was not observed when tea-leaf saponin was administered orally to mice at a dose of 2000 mg/kg. The results strongly recommend that tea-leaf saponin have both have high anti-inflammatory and antimicrobial activity.

Akihisa et al. (1997) identified the triterpene alcohol constituents from the seeds of *Camellia japonica* L. and this led to the isolation of twenty-seven triterpene alcohols of which seven were novel naturally occurring compounds, tirucalla-5,7,24-trien-3 beta-ol (1), lemmaphylla-7,21-dien-3 beta-ol (2), isocupehol (3), isotirucallol (4), (24R)-24,25-epoxybutyrospermol (5) and its 24S-epimer (6), and isoaglaiol (7). The inhibitory effects of 3, 4, a mixture of 5 and 6, a mixture of 7 and its 24S-epimer (aglaiol), and eight known triterpene alcohols isolated in this study were evaluated in ear inflammation in mice induced by 12-O-tetradecanoylphorbol-13-acetate (TPA). The 50% inhibitory dose of these triterpenes for TPA-induced inflammation (1 microgram per ear) was 0.2-0.9 mg/ear. Hence the results suggest that triterpene from *C. japonica* have the anti-inflammatory activity.

A novel 3,4-seco-triterpene alcohol, named sasanquol, was isolated from the nonsaponifiable lipid of sasanqua oil from the seeds of *Camellia sasanqua*. Its structure was established to be 3,4-seco-Dβ-friedobacchara-4,21-dien-3-ol by spectroscopic methods. This is the first example of naturally occurring triterpene with a Dβ-friedobaccharane skeleton. The 50% inhibitory dose of this compound against 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced ear inflammation (1 microgram per ear) in mice was 0.4 mg per ear (Akihisa et al., 1998).
Lin et al. (1999) isolated the punicalagin and punicalin from leaves of *Terminalia catappa* L., which is evaluated for the anti-inflammatory activity in carrageenan-induced hind paw edema in rats. After evaluation of the anti-inflammatory effects, the edema rates were increased by carrageenan administration and reduced by drug treatment. After 4 hr of carrageenan administration, the best effect group was the punicalagin (10 mg/kg) treated group (inhibition rate was 58.15%), and the second was the punicalagin (5 mg/kg)-treated group (inhibition rate was 39.15%). However, even if the anti-inflammatory activity of punicalagin was the same as punicalin at the 5 mg/kg dose, the inhibition effect from larger doses of punicalagin was increased, but there was a decrease with a larger dose of punicalin. The data showed that both punicalagin and punicalin exert anti-inflammatory activity, but treatment with larger doses of punicalin may induce some cell damages.

Sur et al. (2001) reported the two groups of saponins, TS-1 and TS-2, from tea root extract (TRE) which tested for antiinflammatory activity and in vitro antioxidant activity. Both TS-1 and TS-2 inhibited carrageenan-induced paw edema in rats. The antioxidant activity of these compounds was evaluated using the xanthine-xanthine oxidase system. The study indicated that the previously observed antitumour activity of TRE might be mediated through scavenging of free radicals by saponins and their antiinflammatory activity.

Chattopadhyay et al. (2001) stated that methanol-water (1:1) extract of dried tea (*Camellia sinensis*) root extract (TRE) possess anti-inflammatory, analgesic and antipyretic activities at 1/10th of its LD50 dose of 100 mg/kg i.p. It was found that TRE inhibited the arachidonic acid-induced paw edema in rats which indicated that TRE produced the anti-inflammatory activity by inhibiting both the cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism. TRE also enhanced peritoneal cell count and the number of macrophages in normal mice. It is plausible that the saponins present in TRE may be responsible for antiinflammatory activities.

Fan et al. (2004) studied the antiinflammatory activity of *Terminalia catappa* ethanolic extract leaves using 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced ear edema in acute and chronic models. A bioassay-oriented fractionation procedure showed that the activity concentrates in the chloroform fraction. Ursolic acid (1) and 2α,3β,23-trihydroxyurs-12-en-28-oic acid (2), isolated from the chloroform fraction, exhibited strong
antiinflammatory activities. The results suggest that the triterpenic acids 1 and 2 are responsible for the antiinflammatory activity of *T. catappa* leaves.

Antibacterial bioassay-guided fractionation of an ethyl acetate root extract of *Terminalia sericea* led to the isolation of anolignan B. The isolated compound was further tested for anti-inflammatory activity using the cyclooxygenase enzyme assays (COX-1 and COX-2) and for potential mutagenic effects using the Ames test. In the anti-inflammatory assays, anolignan B showed activity against both COX-1 (IC(50) = 1.5 mM) and COX-2 (IC(50) = 7.5 mM) enzymes. No potential mutagenic effects were observed in the Salmonella microsome assay (TA98). Isolation of anolignan B from *Terminalia sericea* as well as the antibacterial and anti-inflammatory activities observed in this study has not been reported previously (Eldoen et al., 2006).

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*. Xylene-induced ear edema, acetic acid-induced vascular permeability and lambda-carrageenan-induced paw edema were used to investigate anti-inflammatory activity, and acetic acid-induced writhing and hot-plate tests were used to determine analgesic effect. Oral administration of theacrine (8-32 mg/kg) induced dose-related anti-inflammatory and analgesic effects. On the other hand, oral caffeine administration (8-32 mg/kg) did not show an inhibitory effect on the inhibition of inflammatory response or cause analgesia. Additionally, the result of the acute toxicity test showed that the LD(50) of theacrine was 810.6 mg/kg (769.5-858.0 mg/kg). The data obtained suggest theacrine possessed analgesic and anti-inflammatory activities.

Byrav et al. (2011) evaluated the effect of green tea in experimentally induced inflammatory bowel disease in animal model. The animals were divided into five groups (n = 6): Group I-Vehicle (ethanol), group II-TNBS + ethanol, group III-green tea-treated group was divided into two sub-groups on the basis of different doses: group IIIA-TNBS + green tea (35 mg/kg), group IIIIB-TNBS + green tea (70 mg/kg), group IV-TNBS + sulfasalazine (360 mg/kg), group V-TNBS + sulfasalazine (360 mg/kg) + green tea (least effective dose found in group III). After completion of 2 weeks of treatment, the rats were killed under ether anesthesia by cervical dislocation for assessment of intestinal inflammation and histological analysis. The study showed that green tea alone and in combination with sulfasalazine reduced inflammatory changes induced by tri nitro benzene sulfonic acid in rats. The results
concluded that a combination of green tea extract with sulfasalazine showed greater efficacy than single drug treatment.

The application of tea seed extract (TSE) has been widely investigated because of its biological activities. Two flavonoltriglycosides in TSE-camelliaside A (CamA) and camelliaside B (CamB) were subjected to hydrolysis in the presence of two commercial enzyme complexes, namely Smash and Mash. Mash hydrolyzed only the xylosyl moiety of CamB, and the main product was kaempferoldiglycoside (nicotiflorin, NF). On the other hand, Mash induced the hydrolysis of both CamA and CamB, and kaempferolmonoglycoside (astragalin, AS) was found to be a main product. Pure AS with > 96% purity was prepared by enzymatic hydrolysis of TSE using Mash, and the chemical structure of AS was confirmed by (1)H- and (13)C-nuclear magnetic resonance analyses. The prepared pure AS showed anti-inflammatory activities by significantly inhibiting cellular nitric oxide (IC(50) = 363 µg mL(-1)), prostaglandin E(2) (IC(50) = 134 µg mL(-1)) and interleukin-6 production (IC(50) = 289 µg mL(-1)) by lipopolysaccharide-stimulated RAW 264.7 cells. It was concluded that pure astragalin can be prepared by enzymatic partial hydrolysis of TSE and employed as an anti-inflammatory material (Lee et al., 2011).

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. In the search for mechanisms of EGCG-mediated melanoma cell suppression, we found that NF-κB was inhibited, and that reduced NF-κB activity was associated with decreased IL-1β secretion from melanoma cells. Since inflamasomes are involved in IL-1β secretion, we investigated whether IL-1β suppression was mediated by inflamasomes, and found that EGCG treatment led to downregulation of the inflamasome component, NLRP1, and reduced caspase-1 activation. Furthermore, silencing the expression of NLRP1 abolished EGCG-induced inhibition of tumor cell proliferation both in vitro and in vivo, suggesting a key role of inflamasomes in EGCG efficacy. This paper provides a novel mechanism for EGCG-induced melanoma inhibition: inflamasomedownregulation→decreased IL-1β secretion→decreased NF-κB activities→decreased cell growth. In addition, it suggests inflamasomes and IL-1β could be potential targets for future melanoma and anti-inflammatory therapeutics.
Azimi et al., (2012) focused on plants currently used and those with a high potency for the future development of anti-acne products. Studies on cell lines revealed that flavonoid, alkaloid, essential oil, phenol and phenolic compound, tannin, xanthone and xanthone derivative, and the bisnaphthiquone derivative are effective in treatment of acne. Animal studies showed that diterpene acid, phenylpropanoid glycosides, acetoside and flavonoids have anti-inflammatory activity. Eleven human studies revealed that *Camellia sinensis* has 5α-reductase inhibitory and anti-inflammatory activities. In addition to the standardization of this herb as anti-acne agents may help to find new sources of therapy for acne.

2.4. ANTIBACTERIAL ACTIVITY

Increase of antibacterial resistance is a global growing problem. Isolation of microbial agents less susceptible to regular antibiotics and recovery of increasing resistant isolates during antibacterial therapy is rising throughout the world. 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 increased shown that higher plants which represent a potential source of novel antibiotic prototypes. However, plants such as *Camellia* sp and *Terminalia* sp have enormous source of antimicrobial activity.

Shetty et al., (1994) investigated that extracts of Black tea, Japanese green tea, China tea or Coffee inhibited the growth of various bacteria causing diarrhecal diseases. Tea or coffee also showed bactericidal activity against *Vibrio cholerae*, *Salmonella typhimurium* and *Salmonella typhi*.

Vijaya et al., (1995) analysed the antibacterial effect of compounds extracted from a *Camellia sinensis* L. and the methanol extract of *Euphorbia hirta* L. were studied against dysentery causing *Shigella* spp. using the Vero cell line. Cytotoxicity studies of the extracts were performed using the cell line and the non-cytotoxic concentration of the extract was tested for antibacterial activity against the cytopathic dose of the pathogen. These extracts were found to be non-cytotoxic and effective antibacterial agents.

Sote and Wilson et al., (1995) tested the aqueous extracts from 8 plants used for tooth cleaning in Nigeria to inhibit the growth of five periodontopathic bacteria, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Eikenella*
corrodens and Campylobacter rectus. Extracts of all the plants except that of Massularia acuminata exhibited varying growth inhibitory potentials on the microorganisms. Extract of Terminalia glaucescens showed the widest spectrum of activity, inhibiting the growth of all the tested bacteria except P. gingivalis. These findings corroborate other studies that the plants possess antiplaque properties and suggest that they may be useful tools in preventive dentistry in poor developing countries.

Trees of the genus Terminalia have long been used in the traditional medicine of Kenya (East Africa). In an ethnopharmacological approach, extracts of the stem bark of Terminalia spinoso were investigated for antibacterial and antifungal activity. The extracts were active against Helicobacter pylori, with the following minimum inhibitory concentrations (MIC): MIC 50 of 1.25 mg/l, MIC 90 of 250 mg/l, and MIC range of 62.5-500 mg/l. Yeasts of the genus Candida showed a similar susceptibility, Fabry et al., (1996).

Yam et al.,(1997) stated that aqueous extracts of teas (Camellia sinensis) of different types and from various sources inhibited a wide range of pathogenic bacteria, including methicillin-resistant Staphylococcus aureus. Tea extracts were bactericidal to staphylococci and Yersinia enterocolitica at well below 'cup of tea' concentrations. Activity was confined to one of four fractions obtained from a green tea extract by partition chromatography. Testing of pure tea compounds and closely related chemicals suggested that the antibacterial activity of extracts of green tea can be explained by its content of epigallocatechin, epigallocatechingallate and epicatechingallate. In black tea extracts, theaflavin and its gallates are additional antibacterially active components.

The World Health Organisation (WHO) has recommended that all member states actively promote native medicines in their country. Ten Indian medicinal plants were screened for antibacterial activity specific to enteropathogens. Diffusion and dilution methods were used to measure the antibacterial activity. Allium sativum, Camellia sinensis, and Chamaecyschinta showed higher activity when compared to the rest. They had a minimum bactericidal concentration (MBC) of < 100 micrograms/ml and gave inhibition zones of more than 2 cm. Among the pathogens studied, Vibrio cholerae and Shigella flexneri were found to be highly susceptible to the plant extracts, (Vijaya & Ananthan, 1997).
Rasheed and Haider (1998) isolated the bacteria from saliva and teeth of cariogenic patients and identified by a variety of morphological and biochemical tests. Extracts of green tea strongly inhibited *Escherichia coli*, *Streptococcus salivarius* and *Streptococcus mutans*. The antibacterial effect of green and black tea extracts were compared with those of amoxicillin, cephadrine and eugenol. The result shows that Antibacterial activity of *Camellia sinensis* extracts effective against dental caries.

Perumal Samy et al. (1998) selected a total of 34 plant species belonging to 18 different families, selected on the basis of folklore medicinal reports practised by the tribal people of Western Ghats, India, were assayed for antibacterial activity against *Escherichia coli*, *Klebsiella aerogenes*, Proteus vulgaris, and *Pseudomonas aerogenes* (gram-negative bacteria) at 1000-5000 ppm using the disc diffusion method. Of these 16 plants showed activity; among them, *Terminalia arjuna* and *Vitex negundo* showed significant antibacterial activity against the tested bacteria. (Perumal Samy et al., 1998).

Shiota et al. (1999) found that epicatechin gallate, a constituent of an extract of tea leaves (green tea) markedly lowered the minimum inhibitory concentration (MIC) of oxacillin and other beta-lactams, but not of other antibacterial agents tested, in strains of methicillin-resistant *Staphylococcus aureus*. The antibacterial action of epicatechin gallate plus oxacillin was a bactericidal one.

Hamilton et al. (1999) analysed that component of aqueous extracts of green tea (*Camellia sinensis*), known to reverse methicillin-resistance in *staphylococci*, causes extensive morphological changes in methicillin-resistant but not in methicillin-sensitive *Staphylococcus aureus*. Clumps of partly divided cocci, consisting of up to 14 individuals, with thickened internal but normal external cell walls were seen by electron microscopy in cultures of methicillin-resistant *S. aureus* grown in the presence of the active principle. The morphological changes observed were consistent with selective inhibition of penicillin-binding proteins.

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 *Anogeissus leocarpus*. These compounds were analysed by GC-MS, IR, 1D and 2D-NMR, and also as acetates.
Antimicrobial effect of the glycoside on *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans* show that it possesses growth inhibitory effect at various concentrations.

The potential presence of naturally occurring antimicrobials in petals of *Camellia japonica* L., a member of the tea family, was investigated against food borne pathogens in microbiological media and food. Petals of the camellia flower (*C. japonica* L.) were extracted with methanol and fractionated into basic, acidic, and neutral fractions. The acidic fraction (equivalent to 1.0 g of raw sample per disk) produced an inhibitory zone of 14 to 19 mm (diameter) in a disk assay against the pathogens *Salmonella typhimurium* DT104, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Staphylococcus aureus* on agar plates (Kim et al., 2001).

Malekzadeh et al. (2001) stated that water extracts of black myrobalan showed significant antibacterial activity and had a minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 125 and 150 mg/l, respectively. The extract was active after autoclaving for 30 min at 121 degrees C. Plant powder (incorporated in agar) gave higher MIC and MBC values (150 and 175 mg/l, respectively). Water extracts of the black myrobalan at a concentration of 1-2.5 mg/ml inhibited urease activity of *H. pylori*. The results show that black myrobalan extracts contain a heat stable agent(s) with possible therapeutic potential. Other bacterial species were also inhibited by black myrobalan water extracts.

Gupta et al. (2002) studied the effect of methanol extract of the dried fruit powder of *Terminalia pallida* was evaluated for antimicrobial activity. The methanol extract of *T. pallida* showed a broad spectrum of antibacterial activity.

Silva et al. (2002) evaluated the antibacterial effect of *Terminalia macroptera* leaf (Tml) extract against nine reference and clinical *Neisseria gonorrhoeae* strains, including penicillin- and tetracycline-resistant and susceptible strains. Tml possesses anti-*N. gonorrhoeae* activity against all of the strains and the minimum inhibitory concentrations (MIC) were between 100 and 200 microg ml(-1). They used a liquid-liquid partition method to divide the Tml extract into five fractions and determined the anti-*N. gonorrhoeae* activity of each of the fractions. All of the fractions showed antibacterial activity. The most active one was identified as the diethyl ether fraction and had MIC values of between 25 and 50 microg ml(-1) against all of the strains.
Katerere et al. (2003) isolated from four pentacyclic triterpenes from *Combretum imberbe* Engl. & Diels, of which two are novel glycosidic derivatives of 1alpha,3beta,23-trihydroxyolean-12-en-29-oic acid (hydroxyimberic acid). *Terminalia stuhlmannii* Engl. & Diels stem bark yielded two glycosides of hydroxyimberic acid, one of which is reported for the first time. The structures of the isolated compounds were elucidated by spectroscopic methods. Several of the compounds had antibacterial activity, imberic acid showing particularly potent activity against *Mycobacterium fortuitum* and *Staphylococcus aureus*.

Stapleton et al. (2004) investigated that aqueous extracts of Japanese green tea (*Camellia sinensis*) are able to reverse beta-lactam resistance in methicillin-resistant *Staphylococcus aureus* (MRSA). Minimum inhibitory concentration (MIC) values for oxacillin were reduced from 256 and 512 to 1-4 mg/l, respectively, in the presence of these polyphenols. In addition, (+)-epigallocatechin gallate (EGCG) had a moderate capacity to modulate oxacillin activity against *S. aureus* BB568, but none against EMRSA-16. ECG, CG and EGCG increased the sensitivity of EMRSA-15 to oxacillin. The gallate moiety was essential for the oxacillin-modulating activity of ECG, as both (+)-epicatechin and (+)-epicatechin-3-cyclohexylearboxylate were unable to reverse resistance to oxacillin. Gallic acid and three alkyl gallates (methyl gallate, propyl gallate, and octyl gallate) did not modulate beta-lactam resistance in MRSA. Octyl gallate exhibited direct antibacterial activity against *S. aureus* BB568.

Simonetti et al. (2004) demonstrated that green tea (*Camellia sinensis*) increased antimicrobial activity against bacteria and fungi when used in combination with butylated hydroxyanisole (BHA). Glycolic extract taken from green tea showed only limited activity against *Streptococcus mutans* and no activity against *Candida albicans* and certain strains of *Escherichia coli*. BHA, at non inhibitory concentrations, increased the microbicidal activity of green tea against 10(10) *S. mutans* (p<0.01), non-susceptible *E. coli* (p<0.01) and *C. albicans* (p<0.01). Green tea in combination with BHA reduced the hydrophobicity of *S. mutans* (p<0.01) and greatly inhibited (p<0.001) the formation of hyphae in *C. albicans*.

Ethanic extracts and some fractions from 10 Indian medicinal plants, known for antibacterial activity, were investigated for their ability to inhibit clinical isolates of beta-lactamase producing methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *S. aureus* (MSSA). Synergistic interaction of plant extracts with certain antibiotics was also evaluated. The MRSA test strains were found to be multi-drug resistant and also
exhibited high level of resistance to common beta-lactam antibiotics. These strains produced beta-lactamases, which hydrolyze one or other beta-lactam antibiotics, tested. The extract of the plants from *Camellia sinensis* (leaves), *Delonix regia* (flowers), *Holarrhena antidysenterica* (bark), *Lawsonia inermis* (leaves), *Punica granatum* (rind), *Terminalia chebula* (fruits) and *Terminalia bellerica* (fruits) showed a broad-spectrum of antibacterial activity with an inhibition zone size of 11 mm to 27 mm, against all the test bacteria. The extracts from the leaves of *Ocimum sanctum* showed better activity against the three MRSA strains. On the other hand, extracts from *Allium sativum* (bulb) and *Citrus sinensis* (rind) exhibited little or no activity, against MRSA strains. The antibacterial potency of crude extracts was determined in terms of minimum inhibitory concentration (MIC) by the tube dilution method. MIC values, of the plant extracts, ranged from 1.3 to 8.2 mg/ml, against the test bacteria. Further, the extracts from *Punica granatum* and *Delonix regia* were fractionated in benzene, acetone and methanol. Antibacterial activity was observed in acetone as well as in the methanol fractions. In vitro synergistic interaction of crude extracts from *Camellia sinensis*, *Lawsonia inermis*, *Punica granatum*, *Terminalia chebula* and *Terminalia bellerica* was detected with tetracycline. Moreover, the extract from *Camellia sinensis* also showed synergism with ampicillin. TLC of the above extracts revealed the presence of major phytoconstituents, like alkaloids, glycosides, flavonoids, phenols and saponins. TLC-bioautography indicated phenols and flavonoids as major active compounds (Aqil et al., 2005).

Neyestani *et al.* (2005) observed that gallic acid in black tea extract and epigallocatechin and epigallocatechin gallate in green tea extract are present in the highest concentrations, respectively. At concentrations of 25 mg/mL, both black and green teas after 5 and 7 hours completely inhibited *E. coli* growth. Gallic acid at concentrations of 5, 10, and 25 microg/mL after 7, 5 and 3 hrs, respectively, inhibited bacterial growth. Both black and green tea extracts had either synergistic or antagonistic effects at different concentrations on selected antibiotics, while GA showed a synergistic effect with all the antibiotics tested in a dose-dependent manner. The effect was more prominent with amikacin and sulfamethoxazole.

Eeldren *et al.* (2006) studied the ethyl acetate root extract of *Terminalia sericea* led to the isolation of anolignan B. In the antibacterial test, anolignan B showed activity against both Gram-positive and Gram-negative bacteria. The minimum inhibitory concentration
values obtained (MIC) ranged from 3.8 microg/ml against *Bacillus subtilis* (Gram-positive) to 31 microg/ml against *Escherichia coli* (Gram-negative). No potential mutagenic effects were observed in the *Salmonella microsome* assay (TA98). Isolation of anolignan B from *Terminalia sericea* as well as the antibacterial and anti-inflammatory activities observed in this study has not been reported previously.

Mbwambo *et al.* (2007) analysed that extracts of the stem bark, wood and whole roots of *T. brownii* exhibited antibacterial activity against standard strains of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, and *Bacillus anthracis* and the fungi, *Candida albicans* and *Cryptococcus neoformans*. Aqueous extracts exhibited the strongest activity against both bacteria and fungi. Extracts of the roots and stem bark exhibited relatively mild cytotoxic activity against brine shrimp larvae with LC50 values ranging from 113.75-4356.76 and 36.12-1458.81 microg/ml, respectively. The results strongly supports that traditional medicinal plant, *T. brownii* aqueous extracts very effective in the treatment of diarrhea, and gonorrhea.

Cho *et al.* (2008) evaluated the antibacterial effects of tea polyphenols (TPP) from Korean green tea (*Camellia sinensis*) against clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA). Characterization of the minimal inhibitory concentration (MIC) of oxacillin for 30 *S. aureus* strains isolated from patients treated with oxacillin identified 13 strains with an oxacillin MIC >or= 4 microg/mL as methicillin-resistant *Staphylococcus aureus* (MRSA) (range: 8 to 512 microg/mL), while 17 strains were methicillin-susceptible *Staphylococcus aureus* (MSSA) (range: 0.25-0.5 microg/mL). The MICs of TPP ranged from 50 to 180 microg/mL for both the MSSA and the MRSA strains. The MICs of oxacillin for each of the 13 MRSA strains were reduced between 8 and 128-fold when these strains were co incubated with sub-MIC (<or= 0.5x MIC) levels of TPP, demonstrating that the combination of TPP plus oxacillin was synergistic for all of the clinical MRSA isolates. Two-dimensional polyacrylamide gel electrophoresis identified 14 extracellular proteins of MRSA-13 down-regulated and 3 proteins up-regulated by exposure to TPP. These studies demonstrate that TPP can differentially stimulate the expression of various proteins in these bacteria and synergize the bactericidal activity of oxacillin for MRSA.
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. Cocoa polyphenol pentamers significantly reduce biofilm formation and acid production by *Streptococcus mutans* and *S. sanguinis*. In the same way, trigonelline, caffeine and chlorogenic acid occurring in green and roasted coffee interfere with *S. mutans* adsorption to saliva-coated hydroxyapatite beads. *Studies* carried out on green, oolong and black tea indicate that tea polyphenols exert an anti-caries effect via an anti-microbial mode-of-action, and galloyl esters of (-)-epicatechin, (-)-epigallocatechin and (-)-gallocatechin show increasing antibacterial activities.

Osterburg *et al.* (2009) identified that polyphenol, (-)-epigallocatechin-3-gallate (EGCG), from green tea (*Camellia sinensis*), had antimicrobial effects against multiresistant clinical isolates of *A. baumannii*. Standard microplate assays were performed to determine the MIC of EGCG for 21 clinical isolates of *A. baumannii*. MICs ranged from 0.078 to 0.625 mg/mL, with MIC(50) and MIC(90) of 0.312 mg/mL and 0.625 mg/mL, respectively. All of the isolates of *A. baumannii* tested were killed by EGCG. In time-kill assays, EGCG resulted in a 3-log reduction in CFU/mL of *A. baumannii* after 5 h of incubation with the polyphenol. Synergy between the commonly used topical agent 5% mafenide acetate (Sulfamylon) and EGCG was noted for one clinical isolate, and partial synergy was noted for three other isolates. These findings demonstrate that EGCG is an effective bactericidal agent against antibiotic-resistant *A. baumannii* clinical strains in laboratory settings. EGCG has previously been shown to be safe, and therefore may be an attractive addition for the treatment of cutaneous *A. baumannii* infections where high concentrations of the drug can be applied to the wound surface.

Shinde *et al.* (2009) reported the antibacterial activity of acetone, hexane, dichloromethane leaf extract of five *Terminalia* species (*Terminalia alata* Heyne ex Roth., *Terminalia arjuna* (Roxb.) Wt. and Am., *Terminalia bellerica* (Gaertn.) Roxb., *Terminalia catappa* L. and *Terminalia chebula* Retz.) were tested by Agar-well-diffusion method against human pathogens *E. coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. The Rf values and relative activities of separated compounds were tested. Hexane and dichloromethane extracts have shown more antibacterial components than the acetone extract indicating the non-polar character of the antibacterial compounds. The non-polar character of the
antibacterial compounds was confirmed from the Rf values. It indicated that
the antibacterial activity was not due to tannins. *Terminalia catappa* found to possess the
compounds which are more antibacterial. *Terminalia arjuna* and *T. catappa* plants were most
promising for isolating antibacterial compounds.

Tea (*Camellia sinensis*) has been known for its modulation of resistance of
methicillin-resistant *Staphylococcus aureus* (MRSA) to beta-lactam antibiotics in vitro. The
MICs of the ampicillin, cefazolin, amoxicillin, oxacillin, tea extract alone and tea extract in
combination with beta-lactams were determined. Proportions of tea extracts and amoxicillin-
tea extract combinations were administered to groups of mice enterally. The in vitro
experiment showed that the MICs of four beta-lactams were greatly decreased in the presence
of 0.25% tea extract. However, in an in vivo experiment, amoxicillin in combination with 5%
tea extract conferred a higher ED(50) than that of antibiotic alone. Green tea extract, alone or
in combination with amoxicillin, does not have protective benefits in MRSA-infected mice.
This study concluded that tea extract weakened the antibacterial effect of amoxicillin in
MRSA infected mice. Tea drinking is not recommended in combination with amoxicillin
treatment. (Peng et al., 2010).

Gordon *et al.*, (2010) found that epigallocatechin-3-gallate (EGCG) is the major
catechin in green tea, has been shown to have antimicrobial effects against a number of
bacterial pathogens. The invitro activity of this compound against 40 clinical isolates of *S.
maltophilia*. MIC(50/90) values (minimal inhibitory concentrations for 50% and 90% of the
organisms, respectively) were 256 mg/L when determined by agar dilution and 512 mg/L by
broth microdilution. MBC(50/90) values (minimal bactericidal concentrations for 50% and
90% of the organisms, respectively) were 512 mg/L. In time-kill assays, the bactericidal
activity of EGCG was analysed by viable colony counts as well as a colorimetric assay for
bacterial reduction of XTT. EGCG was slowly bactericidal at 4x MIC, with a 2.5 log
reduction in viable bacteria at 24h, EGCG has promising in vitro antimicrobial activity
against *S. maltophilia*.

Li *et al.*, (2010) determine the antibacterial activity of six kinds of natural herbs in
Yunnan on normal oral predominant bacteria in vitro. The six kinds of herbs were effective
to the oral predominant bacteria. For the ten kinds of cariogenic bacteria, the MIC of
*Caesalpinia sappan* lignum was between 5-10 mg x mL(-1), and the MIC of
the *Terminalia chebula* retz was between 10-20 mg x ml(-1). The result indicates that *T. chebula* shows high antibacterial activity than other herbs.

Naderi *et al.* (2011) examined and found that methanolic extract of green and black tea were effective against *Streptococcus mutans* (ATCC3566). Five different concentrations (50mg/ml, 100mg/ml, 200mg/ml, 300mg/ml and 400 mg/ml) of tea extracts were tested using the well assay method. The Iranian green and black tea had an antibacterial effect on 100 to 400 mg/ml concentrations. The minimum inhibitory concentration of green and black tea was 150 and 50 mg/ml, respectively. The mean diameter of inhibition zone were 9.5 mm and 10.9 mm for methanolic extract of green and black tea, respectively. Both Iranian non fermented (green tea) and fermented (black tea) have anti *Streptococcus mutans* activity in vitro. The anti *Streptococcus mutans* activity of black tea appears on a lower concentration than green tea.

Jebashree *et al.* (2011) tested the hexane, ethyl acetate, ethanol and methanol extracts of, *Terminalia chebula*, and *Achyranthes aspera* were against the dental caries causing bacteria *Streptococcus mutans* and fungus *Candida albicans* isolated from caries infected patients. The four extracts of *T. chebula* and *M. elengi* showed antibacterial activity against *S. mutans*. *M. elengi* extracts and ethanol extract of *T. chebula* did not show any antifungal activity against *C. albicans*. Except for the hexane extract of *A. aspera*, the other three extracts showed activity against the tested microbes.

Geeta *et al.* (2012) analysed the alkaloids extracted from different parts (leaf, stem, stem bark, and fruits) of *Terminalia chebula* and screened for antimicrobial activity against nine bacteria (*Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus, Bacillus subtilis, Raoultella planticola, Enterobacter aerogens, Agrobacterium tumefaciens*, and *Klebsiella pneumoniae*) and two fungi (*Aspergillus flavus*and *Aspergillus niger*) and one yeast (*Candida albicans*). Minimum inhibitory concentration, Minimum bactericidal/fungicidal concentration, and total activity of the extracts, against each sensitive test pathogen, were also evaluated. Alkaloids from all plant parts showed good antimicrobial activity against almost all the test microorganisms except *A. niger*, against which, none of the tested extracts showed activity. The largest zone of inhibition (IZ 20.75 mm) was observed against *P. aeruginosa*. The total activity of the leaf alkaloid was found to be the same and the highest (256.41ml/g) was against *E. aerogens* and *A. tumefaciens*. 
Mbata et al., (2012) studied the antibacterial activity of the methanol and aqueous extract of *Camellia sinensis* on *Listeria monocytogenes* using Agar-gel diffusion, paper disk diffusion and microbroth dilution techniques. The results obtained showed that methanol and water extract exhibited antibacterial activities against *Listeria monocytogenes*. The leaf extract produced inhibition zone ranging from 10.0 – 20.1 mm against the test bacteria. The methanol extracts of the test plant produces larger zones of inhibition against the bacteria than the water extract. The minimum inhibitory concentration (MIC) for the methanol and water leaf extract was 0.26 mg/ml and 0.68 mg/ml respectively.

Enzweiler, et al., (2012) investigated the potential antimicrobial activity of aqueous extracts of white tea (*Camellia sinensis*), which were obtained by infusing and decocting. The disk-agar diffusion assay was performed using disks saturated with aqueous solutions at concentrations of 1 mg mL\(^{-1}\) to 20 mg mL\(^{-1}\), against bacteria as *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. Results: Growth inhibition of *S. aureus* was observed in decocts at 10 mg mL\(^{-1}\) concentration. The results suggested that white tea have effective potential antimicrobial activity against gram positive bacteria.