5. DISCUSSION

Natural drugs have been a part of the evolution of human, healthcare for thousands of years. Nowadays nearly 88% of the global populations turn to plant derived medicines as their first line of defense for maintaining health and combating diseases. One hundred and nineteen secondary plant metabolites derived from plants are used globally as drugs, 15% of all angiosperms have been investigated chemically and of that 74% of pharmacologically active plant derived components were discovered. (Raja et al., 2009). Plants are rich in a wide variety of secondary metabolites such as tannins terpenoids, alkaloids, flavonoids, etc. which have been found in vitro to have medicinal properties. Pharmacological studies have accepted the value of medicinal plants as potential source of bioactive compounds (Biswa et al., 2002). Phytochemicals from medicinal plants serve as lead compounds in antimicrobial discovery (Chakravarthy, et al., 1985; Cohen, 2002). Literature reports revealed that natural materials areas sources of new antibacterial, antidiabetic and antioxidant agents. Different extracts from traditional medicinal plants were tested and some natural products were permitted as new drugs against chronic diseases.

Large numbers of plants belonging to different families have been studied for their therapeutic properties (Bowers, 1976; Cordell, 1981; Stuffness and Cordell, 1987; Mukhtar et al., 2002). However, plants such as Psidium guajava and Azadirachta indica belonging to Myrtaceae and Meliaceae, 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

Medicinal plants are sources of bioactive compounds and play a dominant role in the maintenance of human health. Reports available on green plants represent a reservoir of effective chemotherapeuticants, these are non-phytotoxic, more systemic and easily biodegradable (Vyas, 1999; Kaushik et al., 2002; Chaman Lal and Verma, 2006). 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
phytocompounds 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 Psidium guajava and Azadirachta indica have been reported. The result of the GC-MS analysis of Psidium guajava samples are presented in Table 2. Nearly 18 compounds were identified in the methanol extractions of Psidium guajava and they were namely Butane,1,1-diethoxy; Pentane, 1,1idiethoxy; 4H-Pyran-4-one,2,3-dihydro-3-5-dihydroxy-6-methyl;Copaeone; a-Caryophyllene; 1H-Benzocycloheptene, 2,4 a,5,6,7,.,8,9aoctahydro-3,5,5-trimethyl-9-methylenne- (4aS-cis)- Naphthalene, 1,2,3,4,4 a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-; 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-[S-(Z)]-Nerolidol; Caryophyllene oxide; Ledol tetracyclo [6.3.2.0 (2.5). 0 (1,8)] tridecane-9-ol, 4,4-dimethyl;Caryophyllene oxide; cis-Z- Bisabolene epoxide;4,4,8- Trimethyltricyclo [6.3.1.0(1,5)]dodecane-2,99diol; n-Hexadecanoic acid; Hexadecanoic acid, ethyl ester; phytol.

Similarly, Zakaria and Mohd (1999) reported that the chemical composition of essential oil isolated from P. guajava leaves with the main components such as a-pinene, b-pinene, limonene, menthol, terpenyl acetete, isopropyl alcohol, longicylene, caryophyllene, b-bisabolene, caryophyllene oxide, b-copanene, farnesene, humulene, selinene, cardinene and curcumene. In addition, the leaves contain triterpenic acids as well as flavonoids; avicularin and its 3–1–4–pyranoside with strong antibacterial action(Iwu.,1993 and Oliver–Bever Bep.1996), fixed oil 6%, 3.15% resin, and 8.5% tannin, and a number of other fixed substances, fat, cellulose, tannin, chlorophyll and mineral salts (Nadkarni and Nadkarni.,1999). Begum et al. [2002] have been isolated from the leaves of guavaonic acid, guavacoumaric acid, 2a–hydroxyursolic acid,
jacoumaric acid, isoneriucoumaric acid, asiaticacid, ilelatifol d and b–sitosterol–3–O–b–
dglucopyranoside. In mature leaves, the greatest concentrations of flavonoids were found which
was M yricetin (208.44 mg kg-1), quercetin(2883.08 mg kg-1), luteolin (51.22 mg kg-1) and
kaempferol (97.25 mg kg-1) (Vargas et.al2006). Two triterpenoids, 20b–acetoxy–2a, 3b–
dihydroxyurs–12–en–28–oic acid (guavanoic acid), and2a, 3b dihydroxy–24–p–z–
coumaroyleoxyurs–12–en–28–oic acid (guavacoumaric acid), along with six known compounds
2a–hydroxyursolic acid, jacoumaric acid, isoneriucoumaric acid, asiatic acid, ilelatifol d and b–
sitosterol–3–O–b–dglucopyranoside, have been isolated from the leaves of P.guajava.
Guajavolide (2a–, 3b–6b–, 23–tetrahydroxyurs–12–en–28,20b–olide, and guavenoi acid, were
isolated from fresh leaves of P . guajava.

Besides the present investigation on GC-MS analysis, totally 47 compounds identified
from the methanol fractions of the Azadirachta indica are presented in Table 3. The plant samples
revealed the synthesis of DI-Homoserine,2-Furanmethanol,2-Cyclopentene-1,4-dione,Propanoic
acid, 2-hydroxy-2-methyl, Butanoic acid, 4-hydroxy-4-Amino-4,5-dihydro-2(3H)-furanone,
1-Pyrrolidineethanamine amine 2, 4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, Glycerin,4(H)-
Pyridine, N-acetyl, Aziridine, 2-isopropyl-1,3-dimethyl-, trans, Butane, 1-(ethenylxy)-3-methyl,
2,3-Pentanedione, 4-methyl, 4H-Pyran-4-one,Butanamide,1,3,5-Triazine-2,4,6-triamine,4-(4-
M ethyl-piperazin-1-yl)-1,5,-dihydro-imidazol-2-one,A cetone,1-(4-imethylaminoethoxy)phenyl13-
Amino-2-oxazolidinone.

Likewise, Hawkins and Ehrlich., 2006 observed that the chemical composition of
essential oil isolated from Neem leaves was analyzed by GC/MS and thirty two compounds were
identified, which represented 84.98 % of total oil. The oil contains ketones, terpenes and phenolic
esters. A.indica is a source of terpenoids, which play an important role in wound healing . A
variety of triterpenoids and various non-terpenoidal constituents have been reported from the
neem. Non-terpenoidal includes hydrocarbons, aromatics, phenolics,comarins, isocoumarins,
flavones, fatty acids and their esters, sulides. Siddiqui et.al.,(2004) identified the twenty-seven
compounds were in non-polar to less polar fractions of Azadirachta indica A . Juss. (neem) which
showed pesticidal activity..The pesticidal activity of neem contains triterpenoids, neem oil and
fractions containing volatiles against a variety of house and crop insects and mosquitoes(Ali et
Moreover, Harikrishnan et al., (2010). Identified the chemical constituents of of methanolic leaf extracts from Azadirachta indica (neem) analyzed by gas chromatography- mass spectro photometry (GC- MS). A. indica had 24 constituents, were identified. Four major (high percentage) compounds were identified in A. indica: n- hexadecanoic acid (14.34%), phytol (19.96%), 9,12,15- octa- decatrienoic acid, (18.57%), and vitamin E (11.37%). The Azadirachta indica extract had a high level of antimicrobial activity against fish pathogens as indicated by zone of inhibition, minimum inhibitory concentration, and minimum bactericidal concentration.

The result of this study suggested that the presence of these phytochemical in, A. indica and P. guajava might be the reason for its antibacterial activity, anti-diabetic and anti-oxidant activity. A. indica contains more bioactive compound than P. guajava. 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. ANTIBACTERIAL ACTIVITY

Bacteria are single-celled microorganisms that can multiply quickly in your body and cause serious health consequences. There is still an urgent require to identify novel substances active against pathogens with higher resistance (WHO., 2001; Cragg et al., 1997). Lot of works reports antibacterial and phytochemical constituents of medicinal plants and their use for the treatment of microbial infections (both topical & systemic applications) as possible alternatives to chemically synthetic drugs to which many infectious microorganisms have become resistant. All through the last few years the pace of development of new antibacterial drugs has slowed down, while the prevalence of resistance (especially multiple) has increased astronomically (Malika et al., 2004; Hugo. and Russel 1984).

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). Literature information
and ethno-botanical records recommend that plants are the sleeping giants of pharmaceutical industry (Russel and Furr 1977) and provide natural source of antimicrobial drugs that provides novel compounds that may be engaged in controlling some infections globally. 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, flavonoids, 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.

*Albizia indica* and *Psidium guajava* is most frequently used traditional medicinal plant. Nearly all parts of the plant are endowed with medicinal property. For the period of the last few decades, apart from studies in the chemistry of *A. indica* and *P. guajava* compounds, considerable progress has been made in evaluating biological activity of phytochemicals and antibacterial for medicinal applications. In the modern age, the plant is considered as a valuable source of unique natural products for development of medicines against various diseases (Hostettmann and Hamburger 1991; Chakravarthy and Gode 1985). Due to traditional medicinal uses of *A. indica* and *P. guajava*, this study was conducted to ascertain its potentially antibacterial and pharmacologically activity.

In the present study, antibacterial activity of methanolic extracts of leaves from *A. indica* and *P. guajava*, was tested against important human pathogens including Gram positive and Gram negative bacteria by agar disc diffusion method. Antibacterial activity of leaves of both plants against different pathogens is shown in the Figures 5 and 6. Maximum antibacterial activity (inhibition zone in mm) was recorded against *K. aerogenes* (12.5 mm) followed by *S. aureus* (10 mm) *B. subtilis* (8.7 mm) and *P. aeruginosa* (7.8 mm) with the leaves extract of *A. indica*. On the other hand, leaf extract of *P. guajava* showed maximum inhibitory effect on *K. aerogenes* (11 mm), *P. aeruginosa* (8.6 mm) followed by *S. aureus* (8.5 mm), and *B. subtilis* (6.2 mm). Of the plants tested, activity was more with *A. indica* than *P. guajava*. Among the methanolic leaf
extracts, *A. indica* showed more activity against all the bacteria tested than that of *P. guajava*. Among the pathogens, *K. aerogenes* was more susceptible to leaf extracts of both plants followed by *S. aureus*, *B. subtilis*, and *P. aeruginosa*. (Table 4).

The antibacterial activity of *Azadirachta indica* might be due to presence of triterpenoids, phenolic compounds, carotenoids, steroids, valinoid, ketones and tetra-triterpenoids azadiractin. Earlier studies on Azadirachata claim that a spermicidal fraction of neem oil (NIM-76) is more effective as an antimicrobial agent as compared to the neem oil and its effect is less on *Escherichia coli* and *Klebsiella pneumonia* (Sairam et al., 2000). This data of present study correlates with the study of Maragathavalli et al., (2012) stated the antimicrobial activity in leaf extract of neem (*Azadirachta indica*) against human pathogenic bacteria. *K. aerogenes*, *S. aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, Bacillus pumilus. Antimicrobial activities of alcoholic extracts of neem leaves were used. Varying concentration of each extracts 200mg/ml, 150 mg/ml, 100mg/ml, 50mg/ml, 25mg/ml prepared by using disc diffusion method. When compared with gentamycin 200mg and gentamycin10mg, the methanol and ethanol extract shows maximum inhibition on Bacillus pumilus, Pseudomonas aeruginosa and S. aureus. Similarly, the result of methanolic extract of *A. indica* and *P. guajava* leaves shows that has an antibacterial activity against gram-positive and gram-negative bacteria. This finding is in agreement with the previous studies bark of *A. indica* and *P. guajava* (Rahim et al., 2010; Sharma et al., 2009). Vanka (2001) Studied the antibacterial effect of Neem mouthwash against salivary levels of streptococcus mutans and lactobacillus. While streptococcus mutans was inhibited by Neem mouthwashes, with or without alcohol as well as chlorhexidine, lactobacillus growth was inhibited by chlorhexidine alone. Hence, the result revealed that Neem extract inhibiting S. mutans.

Thakurta et al., (2007) evaluated the antibacterial and antisecretory activity of neem extract against *Vibrio cholerae*, a causative agent of watery diarrhea such as cholera. The methanol extract of neem leaf was tested for its antibacterial, antisecretory and antihemorrhagic activity against *Vibrio cholerae*. Neem extract showed antisecretory activity on *Vibrio cholerae* induced fluid secretion in mouse intestine with inhibition values of 27.7%, 41.1%, 43.3%, 57.0%, and 77.9% at doses of 100, 200, 300, 450 and 1800 mg/kg, respectively. The results obtained in this
study give some scientific support to the uses of neem employed by the indigenous people in India employed for the treatment of diarrhea and dreadful disease cholera.

Similarly, Gonçalves et al., (2008) screened the antimicrobial effect of essential oils and methanol, hexane, ethyl acetate extracts from guava leaves. The extracts were tested against diarrhea-causing bacteria: Staphylococcus aureus, Salmonella spp. and Escherichia coli. Strains that were screened included isolates from seabob shrimp, Xiphopenaeus kroyeri (Heller) and laboratory-type strains. The essential oil extract showed inhibitory activity against S. aureus and Salmonella spp. The strains isolated from the shrimp showed some resistance to commercially available antibiotics. These data support the use of guava leaf-made medicines in diarrhea cases where access to commercial antibiotics is restricted. In conclusion, guava leaf extracts and essential oil are very active against S. aureus, thus making up important potential sources of new antimicrobial compounds.

Rattanachaikunsopon and Phumkhachorn, (2007) observed the bacteriostatic effect of flavonoids isolated from leaves of Psidium guajava on fish pathogens. The antimicrobial activity against fish bacterial pathogens of flavonoids (morin, morin-3-O-lyxoside, morin-3-O-arabinoside, quercetin, and quercetin-3-O-arabinoside) isolated from the leaves of Psidium guajava was evaluated. The flavonoids were shown to have bacteriostatic effect on all of the tested bacteria.

Moreover, Mahfuzul Hoque et al., 2007 reported the antibacterial activity of guava (Psidium guajava) and neem (Azadirachta indica) extracts against 21 strains of foodborne pathogens were determined. Guava and neem extracts showed higher antimicrobial activity against Gram-positive bacteria compared to Gram-negative bacteria except for V. parahaemolyticus, P. aeruginosa, and A. hydrophila. None of the extracts showed antimicrobial activity against E. coli and Salmonella Enteritidis. The minimum inhibitory concentration (MIC) of ethanol extracts of guava showed the highest inhibition for L. monocytogenes JCM 7676 (0.1 mg/mL), S. aureus JCM 2151 (0.1 mg/mL), S. aureus JCM 2179 (0.1 mg/mL), and V. parahaemolyticus IFO 12711 (0.1 mg/mL) and the lowest inhibition for Alcaligenes faealis IFO 12669, Aeromonas hydrophila NFRI 8282 (4.0 mg/mL), and A. hydrophila NFRI 8283 (4.0 mg/mL). The MIC of chloroform extracts of neem showed similar
inhibition for L. monocytogenes ATCC 43256 (4.0 mg/mL) and L. monocytogenes ATCC 49594 (5.0 mg/mL). However, ethanol extracts of neem showed higher inhibition for S. aureus JCM 2151 (4.5 mg/mL) and S. aureus IFO 13276 (4.5 mg/mL) and the lower inhibition for other microorganisms (6.5 mg/mL). No significant effects of temperature and pH were found on guava and neem extracts against cocktails of S. aureus. The results of the present study suggest that guava and neem extracts possess compounds containing antibacterial properties that can potentially be useful to control foodborne pathogens and spoilage organisms.

Therefore results noticed in the study showed that the extract obtained from Psidium guajava and Azadirachta indica 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.

5.3. ANTI DIABETIC ACTIVITY

Diabetes mellitus (DM) is a chronic metabolic disorder, which can be classified into type I diabetes (insulin-dependent diabetes mellitus or IDDM) and type 2 diabetes (non-insulin dependent diabetes mellitus or NIDDM). The prevalence of diabetes is rapidly increasing in industrialized countries (King et al., 1998) and type 2 diabetes accounts for 90 per cent of the disease. The available oral hypoglycaemic agents (Ramachandran, 2001).

Diabetes mellitus is known from ancient time onwards and numerous medicinal plants are used to control diabetes in traditional medicine. In South India, many medicinal plants are traditionally used to treat DM. Some of them were scientifically verified (Subramoniam and Babu, 2003). Majority of plants used in folk have not been studied scientifically. The present investigation involving medicinal plants such as Psidium guajava and Azadirachta indica, which are commonly used for various ailments, is a scientific attempt to explore their anti-diabetic activity. The methanolic extracts of Psidium guajava and Azadirachta indica (200 mg/kg) showed a significant reduction in blood glucose level in alloxan induced diabetic rat on the 8th and 15th day when compared to control (Table 6).

The levels of blood glucose on 15th day were 108.12 ± 4.57, 90.01 ± 3.12, 100.10 ± 4.57, 89.01 ± 3.12 and 84.21 ± 6.23 in animals treated with P. guajava (100mg/kg and 200mg/kg),
A. indica (100mg/kg and 200mg/kg), and commercial drug, Glibenclamide respectively. The activity was better in A. indica than P. guajava. However, the group that received Methanolic extract of A. indica leaves showed maximum reduction in blood glucose level among the experimental groups.

Maruyama et. al. (1985) reported that the butanol soluble fraction prepared from 50 per cent ethanol extract from Psidium guajava fruit inhibited the increase of the plasma sugar level in alloxan induced diabetic rats.

Grover et al. (2002) evaluated some medicinal plants of India for anti-diabetic potential. They assessed some medicinal plants and Psidium guajava is one among those which has anti-diabetic activity. They administered the aqueous extract of leaves of 1gm/Kg for 30 days. It showed a significant reduction in blood glucose, urea, body weight, liver glycogen and serum cholesterol. These were estimated in alloxan induced experimental rats and they were contrasted with control and also with insulin management.

The effect of an aqueous leaf extract of P. guajava on myocardial injury was studied in the model of global ischemia followed by reperfusion. High-energy phosphates and malondialdehyde in the reperfused hearts were significantly reduced with the plant extract (Conde et al., 2003). In another study, aqueous leaf extract of P. guajava exhibited cardioprotective effects against myocardial ischemia-reperfusion injury in isolated rat hearts. Augmentation of endogenous antioxidants, maintenance of the myocardial antioxidant status and significant restoration of most of the altered hemodynamic parameters may have contributed to its cardioprotective effect (Yamashiro et al., 2003). The cardio-inhibitory actions in rats and guinea pigs of the aqueous leaf extract of P. guajava also appeared to be due to cholinergic involvement in the mechanism of action.

Ojewole (2005) evaluated the hypoglycemic and hypotensive effects of P. guajava leaf extract. They administered intravenously as P. guajava leaf extract of 50–800mg/Kg. According to variation in doses, there occurred a significant reduction in systemic arterial blood pressure and heart rates of hypertensive, dahl–salt–sensitive rats. While evaluating by using cholinergic mechanisms, the extract caused a hypotension in mammalian experimental animal model. Such as
these effects were done by the presence of some compounds such as astannins, polyphenolic compound guaija verin and also some other compounds.

Wang et al. (2007) showed in their study as there occurred a significant inhibition of alpha–glucosidase activity in small intestine and that inhibition causes slowing down the uptake of carbohydrates from digestion. The effects of guava extracts could be due to the different composition in phenolics compounds or other non–phenolic components. Quercetin, quercetin–derived glycosides, gallocatechin, gallic acid and ferulic acid have been identified from guava leaf extracts (Chen and Yen 2007; Matsuo et al., 1994). All phenolic constituents, except ferulic acid, markedly suppressed the formations of α–dicarbonyl compounds and advanced glycation end products. Some authors reported the medicinal value of all parts of P. guajava with mainly leaf extract having anti–hyperglycemic effect. In the streptozotocin induced diabetes rats, the leaf extract of P. guajava induced glucose utilization in liver tissues (Gutierrez et al., 2008; Shen et al., 2008).

Kazi Rafiq et al., (2009) calculated the effects of indigenous medicinal plants of Bangladesh on blood glucose level and neuropathic pain in streptozotocin induced diabetic rats. They determined the value of blood glucose and anti–diabetic activity of Psidium guajava in combination with another two more plants as in the form of polydrug. They gave fresh aqueous extracts of three plants as polydrug to normal and streptozotocin induced rats. Diabetes was induced by streptozotocin by intravenous as 65 mg/Kg for albino rats. The freshly prepared aqueous extract of leaves in polydrug combination was given orally to streptozotocin–induced rats till 8 weeks after the streptozotocin administration by injection at a dose of 500mg/Kg body weight per day.

Ju–Wen et al. (2009) evaluated the hyperglycemic effect of Psidium guajava leaf extract. They mainly assessed the glycation process of protein as by its active compound that causes inhibitory action on it. Due to increased blood sugar level, there occurs more protein glycation and so, early glycation products and glycation end products (AGEs) are said to be major complication in diabetic patients. Their study was done to inhibit such a glycation process in albumin/glucose ratio model and it was compared with the extract of polyphenon 60, a polyphenol product extracted from green tea and also with standard antiglycation agent.
aminoguanidine. Reports showed that P. guajava leaf extracts inhibition upon is calculated to be 95% at 50Lg/ml. Such as, the leaf extract of P. consists of many compounds and among it, the phenolic compound showed effective inhibition on glycation of albumin and mainly quercitin showed 95% inhibition at 100 mg/ml.

Moreover, Gutierrez et al., (2011) studied the hypoglycemic effects of hexane, chloroform and methanol extracts of leaves of A. indica were evaluated by oral administration in streptozotocin-induced severe diabetic rats. The chloroform extract exhibited significant inhibitory activity against advanced glycation end product formation with an IC(50) average range of 79.1 mg/ml. Azadirachta indica can improve hyperlipidemia and hyperinsulinemia in streptozotocin-induced diabetic rats and, therefore, A. indica can be potentially considered to be an antidiabetic-safe agent.

Similarly, Patel et al., (2009) evaluated the antihyperglycemic, antihyperlipidemic and antioxidant activities of Dihar, a polyherbal formulation containing drugs from different herbs viz., Azadirachta indica, Tinospora cordifolia and Psidium guajava in streptozotocin (STZ, 45 mg/kg iv single dose) induced type 1 diabetic rats. Treatment with Dihar (100 mg/kg) for 6 weeks produced decrease in STZ induced serum glucose and lipids levels and increased insulin levels as compared to control. Aqueous extract of unripe Psidium guajava fruit peel in streptozotocin (STZ) reduced blood glucose level significantly in diabetic rats was reported by Rai et al., (2010).

Presence of several flavonoids, terpenoids, and their glycosides have been reported in this plant and possess antidiabetic properties (Cheng and Yang 1983; Huang et al., 1999; Prashant Rai et al., 2009). Tannins and gallic acid are invariably present in all parts of the plant viz., leaves, fruit, etc. (Trivedi and Mishra, 1984, Nair and Subramanian, 1964). In Japan, Guava leaf tea containing the aqueous extract from guava has been approved as one of the foods for specified health uses and is also now commercially available. Preliminary phytochemical tests in Psidium guajava and Azadirachta indica demonstrated the presence of flavonoids, terpenoids, and their glycosides. This might be one of the reasons for the anti-diabetic activity of these plants as suggested by Prashant Rai et al., (2009). Thus, the present study reported that the leaf extract of Psidium guajava and Azadirachta indica has anti-diabetic activity and also prevents the diabetic complications.
5.4. ANTIOXIDANT ACTIVITY

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 decrease the capability of such productive systems resulting in disturbances of the redox equilibrium that is established in healthy conditions that scavenge species may be of great value in preventing the onset and/or propagation of disease (Willet, 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 worldwide health care programmes and there is resurgence of interest in herbal medicines for treatment of various ailments. The efficacy of methanolic extracts of Psidium guajava and Azadirachta indica leaves on antioxidant activity induced by streptozotocin in rats. The enzymatic antioxidant parameters such as SOD, CAT, GPX, GSH and non-enzymatic antioxidant parameters Vit E and C were determined in this study (Table 8).

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 streptozotocin treated rats and its high level in ethanol with extracts of P. guajava and A. indica administered group which exhibited the effect of these plants. The SOD content in serum and plasma more significant in A. indica than P. guajava.

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 streptozotocin treated rats and its high level in methanolic extracts of P. guajava than A. indica administered group which exhibited the effect of these plants. The CAT content in serum and plasma more significant in methanolic leaves extract of A. indica.
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 Streptozotocin treated rats and its subsequently elevated in ethanol with extracts of P. guajava than A. indica administered group also expose the effect of these plants. The GPx content in serum more significant in A. indica than other extracts.

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 sulphohydryl 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 streptozotocin treated rats and its subsequently elevated in ethanol with extracts of P. guajava and A. indica administered group also expose anti-lipidperoxidative effect of these plants. The effect is more significant in A. indica than other extracts.

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 per oxidation (Karagezian and Gerorkian, 1989). In the present investigation, a
significant reduction in vitamin E and C content was noticed in streptozotocin induced rat that may be due to the reduced availability of glutathione which has been utilized in detoxification process. Contrary to this, streptozotocin induced rat with P.guajava and A. indica leaves treated animals showed an increased in the level of Vitamin E and C. Among the treatments, A. indica exhibited significant increase over P.guajava.

This study reported for the first time that A. indica has antioxidant activities than P.guajava. . Further studies may reveal the exact mechanisms of action responsible for the antioxidant activities. This study has highlighted the methanolic leaves extracts of P.guajava and A. indica could be a potential new natural source for antioxidant activity.