2. REVIEW OF LITERATURE

2.1. PHYTOCHEMICAL ANALYSIS

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from them, many based on their use in traditional medicine. Sixty per cent of the world population and 80% of the population in developing countries rely on traditional medicine for curing many diseases. The natural products form an integral part of human life from ancient civilizations to the current century and more than half of the drugs in the market are natural products or derivatives of them. Medicinal plants play a significant role in modern medicine. For modern medicine, bioactive compounds involved in creating novel modern drugs. The effective bioactive compounds elucidated from gcms analysis and docking studies. Among the medicinal plants azadirachta indica and psidum gujava rich in bioactive compound and their analysis are follows:

Mahmoodin a new limonoid, has been isolated from Azadirachta indica (neem) oil, along with seven known tetranortriterpenoids, azadirone, epoxyazadiradione, nimbin, gedunin, azadiradione, deacetylnimbin, and 17-hydroxyazadiradione. A new protolimonoid, naheedin, has been obtained from the neem fruits along with azadirachtol. Their structures have been elucidated through chemical and spectral analyses including gcms and nmr studies. The absolute configuration of 1 was established by comparison of its cd spectrum with those of the known tetranortriterpenoids. Mahmoodin showed significant antibacterial activity against various Gram-positive and Gram-negative organisms. Four hydrocarbons, icosane, docosane, 2-methyltricosane, and docosene, have also been identified by gc-ms of the EtOH extract of the fruit coats. Only docosane has earlier been reported from neem, while the remaining three are unreported from this plant (Siddiqui et al., 1992)

Idstein et al., (1995) extracted the volatile acids by pentane/dichloromethane (2 + 1) from tropical fruit pulps were identified and determined by capillary gas chromatography (HRGC) and combined capillary gas chromatography-mass spectrometry using EI- and CI mode (HRGC-EI/CIMS). Fifty one acids were identified in guava (P. guajava, L.). (E)-cinnamoic acid
(0.4 mg/kg) and (Z)-3-hexenoic acid (0.2 mg/kg) were determined as major constituents in guava.

A variety of triterpenoids and various non-terpenoidal constituents have been reported from the neem. Non-terpenoidal includes hydrocarbons, aromatics, phenolics, coumarins, isocoumarins, flavones, fatty acids and their esters, sulfides. 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 al., 1996; Ascher, 1997; Sharma et al., 1998).

Li et al., (1999) analyzed the constituents of essential oils from the leaves of Psidium guajava Linn by GC-MS qualitatively and quantitatively. Sixty compounds of the essential oils were identified at rate 90.56%. The major components were caryophyllene (18.81%), copaene (11.80%), [1αR-(1α alpha, 4a alpha, 7 alpha, 7a beta, 7b alpha)]-decahydro-1,1,7-trimethyl-4-methylene-1H-cycloprop[e] azulene (10.27%), eucalyptol (7.36%).

Paniandy et al., (2000) stated the chemical composition of Guava fruit (Psidium guajava Linn.). Guava fruit extract contains Vitamin C, vitamin A, iron, calcium, Manganese, phosphoric, oxalic and malic acids, saponin combined with oleanolic acid. Morin-3-O-α-L-lyxopyranoside and morin-3-O-β-L-arabopyranoside, flavonoids, guaijavarin, Quercetin. Essential oil contains hexanal, 2-hexenal, 2,4-hexadienal, 3-hexenal, 2-hexenal, 3-hexenyl acetate and phenol, while β-caryophyllene, nerolidol, 3-phenylpropyl acetate, caryophyllene oxide, pentane-2-thiol, 3-penten-2-ol and 2-butenyl acetate, 3-hydroxy-2-butano3-methyl-1-butanol, 2,3-butanediol, 3-methylbutanoic acid, (Z)-3-hexen-1-ol, 6-methyl-5-hepten-2-one, limonene, octanol, ethyl octanoate.

Pino et al., (2001) isolated the volatile compounds from strawberry guava fruit by simultaneous steam distillation-solvent extraction according to Likens-Nickerson. Compounds were identified by capillary GC-MS and sensorially characterized by sniffing GC. Two hundred and four compounds were identified in the aroma concentrate, of which ethanol, alpha-pinene, (Z)-3-hexenol, (E)-beta-caryophyllene, and hexadecanoic acid were found to be the major constituents. The presence of many aliphatic esters and terpenic compounds is thought to contribute to the unique flavor of the strawberry guava fruit.
Begum et al., (2002) characterize the Triterpenoids from strawberry guava leaves. Three pentacyclic triterpenoids including one new guajavanoic acid (2) and two known obtusinin (1) and goreishic acid I (3) have been isolated from the leaves of *Psidium* guajava. The new constituent 2 has been characterized as 2alpha-hydroxy-3beta-p-E-coumaroyloxyurs-12,18-dien-28-oic acid through 1H-NMR and 13C-NMR (broad band and DEPT). This is the first report of isolation of compound obtusinin (1) and goreishic acid from the genus *Psidium*.

Pino et al., (2002) observed the Volatile compounds from *Psidium* salutare fruits by simultaneous steam distillation-solvent extraction according to the Likens-Nickerson procedure. Compounds were identified by capillary GC and GC-MS. One hundred and fifty compounds were identified in the aroma concentrate, from which limonene, myrcene, and alpha-pinene were found to be the major constituents in the fruit.

Jordán et al. (2003) studied the aromatic profile in commercial guavas and identified a total of 51 components as the principal components in guava essence and fresh fruit puree by GC-MS. In the GC-MS analyses, totals of 43 and 48 aroma active components were detected by the panelists in commercial essence and fruit puree, respectively. Principal differences between the aromas of the commercial guava essence and the fresh fruit puree could be related to the presence of acetic acid, 3-hydroxy-2-butanone, 3-methyl-1-butanol, 2,3-butanediol, 3-methylbutanoic acid, (Z)-3-hexen-1-ol, 6-methyl-5-hepten-2-one, limonene, octanol, ethyl octanoate, 3-phenylpropanol, cinnamyl alcohol, \( \alpha \)-copaene, and an unknown component. (E)-2-hexenal seems more important to the aroma of the commercial essence than that of the fresh fruit puree.

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 determined by WHO method against *Anopheles stephensi* Liston. These identifications were basically made through GC-EIMS and were further supported by other spectroscopic techniques, including 13C NMR, UV and FTIR as well as retention indices. Thus sixteen n-alkanes, 1-16; three aromatics 2,6-bis-(1,1-dimethylethyl)-4-methyl phenol (17), 2-(phenylmethylene)-octanal (20), 1,2,4-trimethoxy-5-(1Z-propenyl)-benzene (27); three benzopyranoids 3,4-dihydro-4,4,5,8-tetramethylcoumarin (18), 3,4-dihydro-4,4,7,8-tetramethylcoumarin-6-ol (19), 1,3,4,6,7,8-
hexahydro-4,6,7,8,8-hexamethyl-cyclopenta[g]-2-benzopyran (22); one sesquiterpene methyl-3,7,11-trimethyl-2E,6E,10-dodecatrienoate (21); three esters of fatty acids methyl 14-methyl-pentadecanoate (23), ethyl hexadecanoate (24), ethyl 9Z-octadecenoate (25) and one monoterpeno 3,7-dimethyl-1-octen-7-ol (26) were identified. Except 6, 8, 24 and 25 all these compounds were identified for the first time from the pericarp and fifteen of these, 1-3, 7, 9, 10, 17-23, 26, 27, are hitherto unreported previously from any part of the tree. Thus the result indicates that this tree is a rich source of various natural products.

Hassimotto et al., (2005) reported that guavas are often included among superfruits, being rich in dietary fiber, vitamins A and C, folic acid, and the dietary minerals, potassium, copper and manganese. Having a generally broad, low-calorie profile of essential nutrients, a single common guava (P. guajava) fruit contains about four times the amount of vitamin C as an orange. Although the strawberry guava (P. littorale var. cattleianum), notably containing 90 mg of vitamin C per serving, has about 25% of the amount found in more common varieties, its total vitamin C content in one serving still provides 100% of the Dietary Reference Intake for adult males. Guavas contain both carotenoids and polyphenols – the major classes of antioxidant pigments – giving them relatively high potential antioxidant value among plant foods.

Guava (Psidium guajava L. cv. Chung-Shan-Yueh-Pa) is a cultivar used for juice processing in Taiwan because of its aroma. Volatile compounds were isolated from guava fruit by simultaneous steam distillation and solvent extraction according to the Likens-Nickerson method. Compounds were identified by capillary GC-MS and sensorily characterized by GC-sniffing. A total of sixty five compounds were identified. The major constituents identified in the guava fruits were: α-pinene, 1,8-cineole, β-caryophyllene, nerolidol, globulol, C6 aldehydes, C6 alcohols, ethyl hexanoate and (Z)-3-hexenyl acetate. The presence of C6 aldehydes, C6 alcohols, ethyl hexanoate, (Z)-3-hexenyl acetate, terpenes and 1,8-cineole is thought to contribute to the unique flavor of the guava fruit. (Hsin-Chun Chen et al., 2006).

Pino et al., (2007) analysed the guava (Psidium guajava L.), which has unique and quince-banana like flavor, is an economically important subtropical fruit in many tropical countries on all seasons. Volatile constituents from three Colombian varieties of guava fruits: Coronilla (commonly named guayaba común), Palmira ICA-1 (commonly named guayaba pera)
and Glum Sali (commonly named guayaba manzana) were isolated by headspace-solid phase microextraction using 100 mm polydimethylsiloxane fibers and analyzed by gas chromatography-mass spectrometry. Ninety-seven compounds were identified in the present study, 19 of them for the first time as volatile constituents of guava. Each variety has an atypical composition, characterized by a specific ratio between the main components and classes of substances. Palmira ICA-1 and Coronilla varieties had higher amounts of volatile compounds than Glum Sali variety, particularly esters; while in Glum Sali variety, predominant were hexanal, 2E-hexenal, and acids. Major volatiles in all varieties were either 3Z-hexenyl acetate, 3-phenylpropyl acetate, (E)-cinnamyl acetate, and hexanal.

Steinhaus et al., (2008) studied the aroma-active compounds in pink-fleshed Colombian guava using GC-MS analysis. The volatiles present in fresh, guavas (Psidium guajava, L.), variety regional rojo, were carefully isolated by solvent extraction followed by solvent-assisted flavor evaporation, and the aroma-active areas in the gas chromatogram were screened by application of the aroma extract dilution analysis. The results of the identification experiments in combination with the FD factors revealed 4-methoxy-2,5-dimethyl-3(2 H)-furanone, 4-hydroxy-2,5-dimethyl-3(2 H)-furanone, 3-sulfanylhexyl acetate, and 3-sulfanyl-1-hexanol followed by 3-hydroxy-4,5-dimethyl-2(5 H)-furanone, (Z)-3-hexenal, trans-4,5-epoxy-(E)-2-decenal, cinnamyl alcohol, ethyl butanoate, hexanal, methional, and cinnamyl acetate as important aroma contributors. Enantioselective gas chromatography revealed an enantiomeric distribution close to the racemate in 3-sulfanylhexyl acetate as well as in 3-sulfanyl-1-hexanol. In addition, two fruity smelling diastereomeric methyl 2-hydroxy-3-methylpentanoates were identified as the (R,S)- and the (S,S)-isomers, whereas the (S,R) - and (R,R)-isomers were absent. Seven odorants were identified for the first time in guavas, among them 3-sulfanylhexyl acetate, 3-sulfanyl-1-hexanol, 3-hydroxy-4,5-dimethyl-2(5 H)-furanone, trans-4,5-epoxy-(E)-2-decenal, and methional were the most odor-active.

Psidium guajava L. is a valuable farm fruit plant having many medicinal uses. Previously its budding leaves (PE) were shown to contain huge amounts of soluble polyphenolics (SP) including (in mg/g) gallic acid (348), catechin (102), epicatechin (60), rutin (100), quercetin (102), and rutin (100) which was detected by gcms analysis and this
compounds exhibit potent anticancer activity. However, reconstitution of these polyphenolics recovered only 40% of the original bioactivity (Chen et al., 2009).

Harikrishnan et al., 2010. Identified the chemical constituents of decoction (individual) and concoction (mixed) of ethanolic leaf extracts from Azadirachta indica (neem) and Ocimum sanctum (tulsi) were analyzed by gas chromatography-mass spectro photometry (GC-MS). Decoctions of A. indica and O. sanctum had 24 and 33 constituents, respectively. Mixed together, 26 compounds 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%). Three major compounds were identified in O. sanctum: phenol,2-methoxy-3-(2-propenyl) (15.32%), 9,12,15¬-octadecatrienoic acid, (16.94%), and 9,12,15-octadecatrienoic acid, methyl ester, (22.05%). Three major compounds were identified in the mixed extract: n-exadecanoic acid (16.58%), phenol,2-methoxy-3-(2-propenyl) (20.62%), and 9,12,15-octadecatrienoic acid, (25.98%). Four of the compounds in the mixed extract were new: eudesma-4(14),11-diene (0.18%), 6-azabicyclo[3.2.1]octane (0.51%), cyclohexane,1-ethyl-1-methyl-2,4-bis(1-methylenyl)-, 15-Elemen (0.77%), and globulol (1.45%). The mixed extract had a high level of antimicrobial activity against fish pathogens as indicated by zone of inhibition, minimum inhibitory concentration, and minimum bactericidal concentration.

Pino and Queris (2011) characterized the odor-active compounds in guava wine. The volatile compounds of guava wine were isolated by continuous solvent extraction and analyzed by GC-FID and GC-MS. A total of 124 volatile constituents were detected, and 102 of them were positively identified. The composition of guava wine included 52 esters, 24 alcohols, 11 ketones, 7 acids, 6 aldehydes, 6 terpenes, 4 phenols and derivatives, 4 lactones, 4 sulfur-compounds, and 5 miscellaneous compounds. The aroma-active areas in the gas chromatogram were screened by application of the aroma extract dilution analysis and by odor activity values. Twelve odorants were considered as odor-active volatiles: (E)-β-damascenone, ethyl octanoate, ethyl 3-phenylpropanoate, ethyl hexanoate, 3-methylbutyl acetate, 2-methyltetrahydrothiophen-3-one, 2,5-dimethyl-4-methoxy-3(2H)-furanone, ethyl (E)-cinnamate, ethyl butanoate, (E)-cinnamyl acetate, 3-phenylpropyl acetate, and ethyl 2-methylpropanoate.
The traditional guava variety cultivated in Israel, 'Ben Dov', emits a very strong odour, whereas some newly bred varieties have a mild odour. In this study the aroma profile composition of the high-aromatic 'Ben Dov' variety was compared with those of four new low-aromatic varieties. Overall, using gas chromatography/mass spectrometry, a total of 30 aroma volatiles were detected in fresh ripe guava fruit: 15 of them were specifically detected only in the high-aromatic 'Ben Dov' variety, 13 were detected in both the high- and low-aromatic varieties and two were detected only in the new low-aromatic varieties. Interestingly, 11 out of the 15 volatiles specifically detected in 'Ben Dov' were esters that contribute sweet, tropical and fruity notes. In contrast, ten out of 13 detected terpenes and two detected aldehydes, contributing green, spicy, herbal and woody notes, were common to both the high- and low-aromatic varieties. Based on these findings, it is concluded that accumulation of esters is the main reason why the traditional 'Ben Dov' guava variety emits such a strong tropical fruity odour. In contrast, the newly bred low-aromatic guava varieties did not synthesise esters at all and thus lacked fruity aromatic notes. Overall, the results of this study point out the important role of esters in forming tropical fruity guava odours. (Porat et al., 2011)

Ryu et al., (2012) evaluated the anticancer effects of guava leaf extracts and its fractions. The chemical compositions of the active extracts were also determined. Analysis of guava leaf hexane fraction (GHF) by gas chromatography and gas chromatography-mass spectrometry tentatively identified 60 compounds, including \(\beta\)-eudesmol (11.98%), \(\alpha\)-copaene (7.97%), phytol (7.95%), \(\alpha\)-patchouline (3.76%), \(\beta\)-caryophyllene oxide (CPO) (3.63%), caryophylla-3(15),7(14)-dien-6-ol (2.68%), (E)-methyl isoeugenol (1.90%), \(\alpha\)-terpineol (1.76%), and octadecane (1.23%). Overall, these findings suggest that guava leaf hexane fraction provide a source of potential therapeutic compounds for both the prevention and treatment of cancer.

### 2.2. Antibacterial Activity

Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Most of the drugs today are obtained from natural sources or semi synthetic derivatives of natural products and used in the traditional systems of medicine. Thus it is a logical approach in drug discovery to screen traditional natural products. The use of plant extracts and phytochemicals both with known antimicrobial properties is of great significance, in
the past few years a number of investigations have been conducted world wide to prove antimicrobial activities from medicinal plants (Alonso-Paz et al., 1995;). For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. According to World Health Organization (Santos et al., 1995) medicinal plants would be the best source to obtain a variety of drugs. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are a part of the essential oils (Jansen et al., 1987) as well as tannin (Saxena et al., 1994). There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections. Among the medicinal plants Azadirachta Indica and Psidium gujava rich in antibacterial activity and their review are follows:

Siddiqui et al., (1992) isolated a new limonoid compound mahmoodin from Azadirachta indica (neem) oil, along with seven known tetranortriterpenoids, azadirone, epoxyazadiradione, nimbin, gedunin, azadiradione, deacetylnimbin, and 17-hydroxyazadiradione. Mahmoodin showed significant antibacterial activity against various Gram-positive and Gram-negative organisms.

Rabe and van Staden (1997) observed the crude extracts from 21 South African medicinal plants, traditionally used for ailments of an infectious or septic nature, were screened for in vitro antibacterial activity using the agar diffusion and dilution methods. Almost all the activity exhibited was against Gram-positive bacteria, with 12 of the 21 plant species tested showing some activity against Bacillus subtilis. The highest activity was found in the methanol extracts from Psidium guajava and Warburgia salutaris. The majority of the antibacterial activity was present in the methanolic, rather than the aqueous extracts.

Fabry et al., ..(1998) analysed the antibacterial activity of east african medicinal plants such as Ximenia caffra (roots), Azadirachta indica (stem bark and leaves), and Spilanthes mauritiana (roots and flowers) were tested against 105 strains of bacteria from seven genera
(Staphylococcus, Enterococcus, Pseudomonas, Escherichia, Klebsiella, Salmonella, Mycobacterium). The minimum inhibitory concentration reached by 50% (MIC50%) and 90% (MIC90) of the strains for the extracts of X. caffra, and A. indica (stem bark) ranged from 0.13-8 mg/ml and from 0.5 to > 8 mg/ml, respectively. Their minimum bactericidal concentration by 50% (MBC50%) and MBC90% were all between 0.5 and > 8 mg/ml. A. indica (leaves), and S. mauritiana (roots and flowers) had MIC and MBC values > or = 8 mg/ml. Mycobacteria were not inhibited at extract concentrations of 0.5-2 mg/ml. It is concluded that plant extracts with low MIC and MBC values may serve as sources for compounds with therapeutic potency.

Das et al., (1999), prepared aqua neem, an emulsified product from the neem (A. indica) kernel was tested against four pathogenic bacteria of fish (i.e. Aeromonas hydrophila, Pseudomonas fluorescens, Escherichia coli and Myxobacteria spp.) to test its efficacy as an antibacterial agent. Growth inhibitory property of the product at 10, 15 and 20 ppm has been noticed and recorded. The percentage reduction of bacterial cell population was noted to be maximum on 9th day at 20 ppm concentration (i.e. 70.14%, 74.15% and 61.75% for A. hydrophila, P. fluorescens and E. coli respectively) with the only exception of myxobacteria which showed maximum reduction percentage (63.90%) on 15th day. Among all the bacteria tested A. hydrophila, P. fluorescens and Myxobacteria spp. exhibited maximum sensitivity to Aquaneem in terms of percentage reduction of bacterial cell population in comparison to E. coli.

Tona et al., (1999) stated the forty six aqueous extracts from 38 medicinal plant species belonging to different families were selected on the basis of their traditional medicinal use as antidiarrhoeic agents. They were submitted in a broad biological screening including antibacterial, antiamoebic and antispasmodic activities. The results of the testing have indicated that 37 extracts (80.43%), 33 (71.74%) and 32 (69.54%) exhibited some level of antibacterial, antiamoebic and antispasmodic activity respectively. Only very few plant extracts (17.39%) would act as antidiarrhoeic agents by a triple pronounced antibacterial, antiamoebic and antispasmodic action. They include aqueous extracts from leaves of Psidium guajava and Tithonia diversifolia.
Sairam (2000) investigated the NIM-76 from neem oil and its antimicrobial action against certain bacteria, fungi and Polio virus were tested. The NIM-76 inhibited growth of various pathogens tested including Escherichia coli and Klebsiella pneumoniae and it also exhibited antifungal activity against Candida albicans and antiviral activity against Polio virus replication in vitro cell lines. It also protected mice from systemic candidiasis as revealed by enhanced % survival and reduced colony forming units of C. albicans in various tissues. This shows that NIM-76 has a potent broad spectrum anti-microbial activity.

Vanka (2001) studied the antibacterial effect of Neem mouthwash against salivary levels of streptococcus mutans and lactobacillus has been tested over a period of 2 months. Also its effect in reversing incipient carious lesions was assessed. While streptococcus mutans was inhibited by Neem mouthwashes, with or without alcohol as well as chlorhexidine, lactobacillus growth was inhibited by chlorhexidine alone. The initial data appears to prove its effect in inhibiting S. mutans and reversing incipient carious lesion.

Arima and Danno (2002) isolated the four antibacterial compounds from leaves of guava (Psidium guajava L.), and the structures of these compounds were established on the basis of chemical and spectroscopic evidence. Two new flavonoid glycosides, morin-3-O-alpha-L-lyxopyranoside and morin-3-O-alpha-L-arabopyranoside, and two known flavonoids, guajavarin and quercetin, were identified. The minimum inhibition concentration of morin-3-O-alpha-L-lyxopyranoside and morin-3-O-alpha-L-arabopyranoside was 200 microg/ml for each against Salmonella enteritidis, and 250 microg/ml and 300 microg/ml against Bacillus cereus, respectively.

Alzoreky and Nakahara (2003) extracted edible plants from China, Japan, Thailand and Yemen were screened for their antibacterial activity against Bacillus cereus, Staphylococcus aureus, Listeria monocytogenes, Escherichia coli and Salmonella infantis. Buffered methanol (80% methanol and 20% PBS) and acetone extracted inhibitory substances against tested bacteria from 16 plants, as revealed by the disc assay. The minimum inhibitory concentrations (MICs) of extracts determined by the agar dilution method ranged from 165 to 2640 mg l(-1). The most sensitive microorganism to extracts from Azadirachta indica, Cinnamomum cassia, Rumex nervosus, Ruta graveolens, Thymus serpyllum and Zingiber officinale was B. cereus, with MIC
of 165 to 660 mg l\(^{-1}\). The result reflects that Azadirachta indica plant has high potential against the most sensitive organisms.

Thangavel et al., (2006) investigated a comparative study on the effect of plant extracts with the antibiotics on organisms of hospital origin. Thirty five plants belonging to twenty families were studied for their antimicrobial activity. Among the plants tested, 43% showed antimicrobial activity. Fifteen plants belonging to 10 families exhibited activity against gram positive bacteria and gram negative bacteria. Four plants namely Azadirachta indica, Garadenia jasminoides, Magnifera indica, and Wrightia tinctora showed an appreciable activity against the gram positive bacteria and seven plants against gram negative organisms. The inhibitory percentage of the leaf extracts against various pathogens were observed to be Staphylococcus aureus (40%), E.coli (28%), Shigella sp (25%), Salmonella sp (22%), Pseudomonas aeruginosa and Bacillus subtilis (20%), Klebsiella pneumoniae and Proteus vulgaris (17%), Vibrio cholera (14%) and Corynebacterium diphtheriae (11%). The results suggested that the leaf extracts of various plants has significant antibacterial activity against the tested microorganisms.

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. Azadirachta indica extract had significant antibacterial activity against the multi-drug-resistant Vibrio cholerae of serotypes O1, O139 and non-O1, non-O139. The minimum inhibitory concentration reached by 50% (MIC\(_{50}\)) and 90% (MIC\(_{90}\)), and minimum bactericidal concentration for the extract were 2.5, > 5, and 10 mg/ml, respectively. 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. Oral administration of the extract inhibited hemorrhage induced by Vibrio cholerae in mouse intestine at a dose > or = 300 mg/kg. 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.

Rattanachaikunsopon and Phumkhachorn., (2007) observed the bacteriostatic effect of flavonoids isolated from leaves of Psidium guajava Ion 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 l was evaluated. The flavonoids were shown to have bacteriostatic effect on all of the tested bacteria.

Chea et al., (2007) screened the 27 plant species used in the traditional medicine of Cambodia for in vitro antibacterial and antifungal activities. Thirty-three methanolic extracts were tested against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Mycobacterium smegmatis and Candida albicans. Screened by disk diffusion assay, the extracts showed antimicrobial activity especially on Gram-positive bacteria. None of the crude methanolic extracts showed activity against P. aeruginosa. Twenty-five selected extracts were evaluated using a micro-dilution test. Azadirachta indica (bark) and Harrisonia perforata (roots and stem) exhibited a bactericidal effect against M. smegmatis at 250 microg/ml.

The antibacterial activity of guava (Psidium guajava) and neem (Azadirachta indica) extracts against 21 strains of foodborne pathogens were determined--Listeria monocytogenes (five strains), Staphylococcus aureus (four strains), Escherichia coli O157:H7 (six strains), Salmonella Enteritidis (four strains), Vibrio parahaemolyticus, and Bacillus cereus, and five food spoilage bacteria: Pseudomonas aeruginosa, P. putida, Alcaligenes faecalis, and Aeromonas hydrophila (two strains). 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 O157:H7 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 faecalis 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 L. monocytogenes and 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. (Mahfuzul Hoque et al., 2007)

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.

Sharma et al., (2009) reported that seventeen Indian folklore medicinal plants were evaluated antibacterial activity of aqueous, ethanol and acetone extracts against 66 multidrug resistant isolates of major urinary tract pathogens (Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Enterococcus faecalis) by disc diffusion method. Ethanol extracts of Terminalia chebula and Ocimum sanctum exhibited antibacterial activity against Klebsiella pneumoniae. Ethanol extract of Azadirachta indica and Ocimum sanctum exhibited antibacterial activity against Enterococcus faecalis. The results support the folkloric use of these plants in the treatment of urinary tract infections by the tribals of Mahakoshal region of central India.

Rahim , et al., (2010) investigated that crude aqueous mixture and water soluble methanol extract from leaf and bark of Psidium guajava, a tropical fruit guava of the family Myrtaceae, showed strong antibacterial activity against multidrug-resistant V. cholerae O1. The in vitro minimum inhibitory concentration of the crude aqueous mixture and water soluble methanol extract, which was bactericidal against 10(7) CFU/mL of V. cholerae was determined to be 1,250 microg/mL and 850 microg/mL, respectively. The antibacterial activity of P. guajava was stable at 100 degrees C for 15-20 min, suggesting nonprotein nature of the active
component. The growth of V. cholerae in rice oral rehydration saline (ORS) was completely inhibited when 10 mg/mL (wt/vol) of crude aqueous mixture was premixed with the ORS in a ratio of 1:7 (vol. extract/vol. ORS). P. guajava, which is widely distributed in Bangladesh, thus offers great potential for use in indigenous, herbal medicine for controlling epidemics of cholera.

Dhiman et al., (2011) examined the chemical composition and in vitro antimicrobial potential of methanolic extract of Psidium guajava Linn (Myrtaceae). The inhibitory effect of methanolic extract of P. guajava was tested against three bacterial and two fungal strains by using the paper disc diffusion method. The methanolic extract exhibited antibacterial activity against E. coli with minimum inhibitory concentration, 0.78 µg/ml, minimum bactericidal concentration of 50 µg/ml, and appreciable antifungal activity with minimum inhibitory concentration of 12.5 µg/ml. Preliminary phytochemical analysis of methanolic extract revealed the presence of antimicrobial compounds such as flavonoids, steroids, and tannins, which may contribute for the antimicrobial action of P. guajava and this extract was found to be bacteriostatic and fungistatic in action.

Maragathavalli et al., (2012) stated the antimicrobial activity in leaf extract of neem (Azadirachta indica) against human pathogenic bacteria. E. coli, Staphylococcus 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 gentamycin 10mg, the methanol and ethanol extract shows maximum inhibition on Bacillus pumilus, Pseudomonas aeruginosa and Staphylococcus aureus in an asending order.

2.3. ANTIMICROBIAL ACTIVITY

Diabetes is one of the major degenerative disease in the world today. It is a major risk factor for the development of cardiovascular disease. About 70-80% of deaths in diabetic patients are due to vascular disease. In particular, hyperglycemia, the primary clinical manifestation of diabetes, is thought to contribute to diabetic complications by altering vascular cellular metabolism, vascular matrix molecules and circulating lipoproteins. For instance hyperglycemia increases diacylglycerol levels and activates protein kinase C activity in the aorta.
of streptozotocin (stz.) induced diabetic rats. Thickening of the basement membranes in renal
glomeruli and peripheral capillaries has been observed in stz. induced diabetic rats (Olgemoller
et al., 1993; Inoguchi et al, 1994) and hyperlipidemia is a feature of drug induced diabetes in
rats.

Azadirachta indica (Meliaceae) popularly known as neem is an indigenous plant widely
available in India and Burma. Different parts of this plant have been reported to have antiseptic,
wound healing and skin disease curing activity. Studies conducted in our laboratory reveals that
water soluble portion of alcoholic extract of leaves of Azadirachta indica possesses significant
antiinflammatory, antiserotonin, antifertility and hepatoprotective activity. Significant
hypolipidemic activity in rats fed on atherogenic diet and antihyperglycemic as well as
hypotensive activity have also been reported by us (Chattopadhyay R.R., 1995) Significant blood
sugar lowering effect of A. indica in alloxan and streptozotocin induced diabetic rats have also
been reported by several workers. It is well documented that cardiovascular disease induced by
hyperglycemia is associated with alterations in serum lipid profiles (Laakso M, 1996,
Chattopadhyay R.R., 1997).

Chattopadhyay (1999) analysed the blood sugar lowering activity of four important
medicinal plants (Azadirachta indica, Gymnema sylvestre, Catharanthus roseus and Ocimum
sanctum) were carried out against normal and streptozotocin-induced diabetic rat models. The
plant extracts decreased the blood sugar level in varying degrees. Blood sugar lowering unit
(BLU) of activity of each leaf extract and tolbutamide was calculated by ED50 values. Statistical
analysis revealed significant (P < 0.05) variation among the treatments as well as doses with
regard to their blood sugar lowering capacity. A. indica leaf extract was found to have the most
potent blood sugar-lowering activity than C. roseus, G. sylvestre and O. sanctum.

Khosla et al., 2000 observed the Hypoglycaemic effect of Azadirachta indica seed oil
in alloxan induced diabetic rabbits. Hypoglycaemic effect was comparable to that of
glibenclamide. Pretreatment with A. indica leaf extract or seed oil administration, started 2 weeks
prior to alloxan, partially prevented the rise in blood glucose levels as compared to control
diabetic animals. The data suggests that A. indica involved in controlling the blood sugar level
and plausible anti diabetics effects observed in diabetic rabbits.
Hypoglycaemic effect was observed with Azadirachta indica when given as a leaf extract and seed oil, in normal as well as diabetic rabbits. The effect, however, was more pronounced in diabetic animals in which administration for 4 weeks after alloxan induced diabetes, significantly reduced blood glucose levels. Hypoglycaemic effect was comparable to that of glibenclamide. Pretreatment with A. indica leaf extract or seed oil administration, started 2 weeks prior to alloxan, partially prevented the rise in blood glucose levels as compared to control diabetic animals. The data suggests that A. indica could be of benefit in diabetes mellitus in controlling the blood sugar or may also be helpful in preventing or delaying the onset of the disease. (khosla et al., , 2001)

Kausik Biswas et al., (2002) reviewed that Neem (Azadirachta indica A. Juss) is perhaps the most useful traditional medicinal plant in India. Each part of the neem tree has some medicinal property and is thus commercially exploitable. During the last five decades, apart from the chemistry of the neem compounds, considerable progress has been achieved regarding the biological activity and medicinal applications of neem. It is now considered as a valuable source of unique natural products for development of medicines against various diseases and also for the development of industrial products. This review gives a bird’s eye view mainly on the biological activities of some of the neem compounds isolated, pharmacological actions of the neem extracts, clinical studies such as antidiabetic ,anti cancer etc.,and plausible medicinal applications of neem along with their safety evaluation.

Gupta et al., (2004) analysed the effect of petroleum ether extracts of kernel (NSK) and husk (NSH) of neem (Azadirachta indica A. Juss, Meliaceae) seeds on the prevention of oxidative stress caused by streptozotocin (STZ) was investigated. Diabetes mellitus was induced in adult male Wistar rats after administration of STZ (55 mg/kg b.wt., i.p., tail vein). The effect of NSK (2 gm/kg, b.wt.) and NSH (0.9 gm/kg, b.wt.) orally for 28 days was investigated in diabetic rats. Insulin-treated diabetic rats (6 U/kg, i.p., 28 days.) served as positive control. Diabetic rats given normal saline served as diabetic control. Rats that neither received STZ nor drugs served as normal control. Serum creatine phosphokinase (CPK) increased in diabetic rats was significantly decreased on insulin, NSK, and NSH treatments. The decrease in activities of superoxide dismutase (SOD) and catalase (CAT) and increase in lipid peroxidation (LPO) of
erythrocytes as observed in diabetes was regained after insulin, NSH, and NSK treatments. However, there was insignificant improvement in SOD, CAT, and LPO of kidney on NSK and NSH treatment. In spite of increased CAT and SOD activities in liver and heart, LPO was also increased in diabetic rats. Insulin, NSH, and NSK treatments significantly protected animals from cardiac damage but not hepatic. Results suggest that NSH and NSK prevent oxidative stress caused by STZ in heart and erythrocytes.

Oh et al., (2005) screened the medicinal plants for inhibition of protein tyrosine phosphatase1B (PTP1B), an extract from Psidium guajava (Myrtaceae) leaves exhibited significant inhibitory effect on PTP1B. Thus, its antidiabetic effect on Lepr(db)/Lepr(db) mice was evaluated. Significant blood glucose lowering effects of the extract were observed after intraperitoneal injection of the extract at a dose of 10mg/kg in both 1- and 3-month-old Lepr(db)/Lepr(db) mice. In addition, histological analysis of the liver from the butanol-soluble fraction treated Lepr(db)/Lepr(db) mice revealed a significant decrease in the number of lipid droplets compared to the control mice. Taken together, it was suggested that the extract from Psidium guajava leaves possesses antidiabetic effect in type 2 diabetic mice model and these effect is, at least in part, mediated via the inhibition of PTP1B.

Hsieh et al., (2005) studied the anti-LDL glycative agents were investigated using aqueous extracts of Psidium guajava L. (PE), Toona sinensis Roem. (TE), Momordica charantia L. (ME) and Graptopetalum paraguayanum E. Walther (GE). Concentrations of extracts 0.01-0.625 mg/mL, low density lipoprotein (LDL; 100 microg protein/mL) and inducers glucose (400 mM) and glyoxal (2.5 mM) were incubated at 37 degrees C. Evaluation parameters involved the thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD), relative electrophoretic mobility (REM), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capability and total polyphenolic content. Results for anti-TBARS efficiency (in%) were P. guajava (75.77),, when induced by glucose; 36., respectively, by glyoxal. The lag times for CD formation (in min) were: 289 PE and TE, respectively, comparing to the control (45). REM was 1.6 with respect to PE (0.1 mg/mL) compared to the control (4.2). PE at 0.01 mg/mL effectively inhibited with 63.45% efficiency on AGEs induced by glucose. We conclude that PE virtually is a potent antiglycative agent, which can be of great value in the preventive glycation-associated cardiovascular and neurodegenerative diseases.
Wang et al., (2005). illuminated the role of water-soluble, 650 ml/L edible alcohol and 950 ml/L edible alcohol-soluble extracts of wild Psidium guajava leaves in Panzhihua Area in decreasing blood glucose. High-level blood glucose models were made by use of male Kunming mice given intraperitoneal injection of glucose, subcutaneous injection of adrenaline and intraperitoneal injection of streptozotocin (STZ), respectively. Blood glucose concentration was measured after oral administration (gastrogavage) of the soluble extracts of Psidium guajava leaves, respectively. Three extracts resisted the rise of blood glucose level induced by exogenous glucose and adrenaline to various degrees. The extracts of water, 650 ml/L alcohol and 950 ml/L alcohol significantly decreased the blood glucose level in STZ-induced diabetic mice by 36.3%, 33.5% and 31.3% respectively. Furthermore, among three extracts, water-soluble extract showed little influence on the growth of mice. Finally the result concluded that water-soluble, 650 ml/L edible alcohol and 950 ml/L edible alcohol-soluble extracts of wild Psidium guajava leaves in Panzhihua area may have different hypoglycemic potential.

Waheed et al., (2006) investigated the clinical hypoglycemic effect of seeds of Azadirachta indica in Type-2 diabetes mellitus. After assaying fasting plasma and urinary glucose, 10 patients of type-2 diabetes mellitus with no previous medication, 10 patients of type-2 diabetes mellitus taking oral hypoglycemic agents with history of inadequate control and six control subjects were given low (0.5 g tid) and high (2 g tid) doses of powdered part, aqueous extract and alcoholic extract of Azadirachta indica for 14 days. On 15th day blood and urine samples for glucose were taken. Based on results obtained it was found that Azadirachta indica has significant hypoglycemic activity in high dose and can be successfully combined with oral hypoglycemic agents in type-2 diabetic patients whose diabetes is not controlled by these agents.

Mukhtar et al., (2006) analyzed the antidiabetic properties of an ethanol extract of the stem bark of Psidium guajava, an indigenous medicinal plant used to control diabetes in Indian System of Medicine. The anti-hyperglycaemic activity of this plant on blood glucose levels of normal, normal glucose loaded (SGTT) and alloxan-induced hyperglycaemic rats was evaluated. The results showed that ethanol stem bark extract exhibited statistically significant hypoglycaemic activity in alloxan-induced hyperglycaemic rats but was devoid of significant hypoglycaemic effect in normal and normal glucose loaded rats (SGTT).
Rai et al., (2007) determined the glycaemic potential of \textit{P. guajava} fruit peel extract on blood glucose level (BGL) of normal and streptozotocin induced sub-diabetic rats during fasting blood glucose (FBG) and glucose tolerance test (GTT). The diabetic and sub-diabetic models showed hyperglycaemic effect from a single oral administration of variable doses of \textit{P. guajava} fruit peel extract. The maximum rise of 26.51 per cent was observed in BGL from a dose of 400 mg/kg bw exactly after 8 h of administration in normal rats whereas the maximum rise of 90.7 per cent was observed with the same dose of 400 mg/kg bw after 2 h of glucose administration in sub-diabetic rats. The hyperglycaemic effect of \textit{P. guajava} fruit peel suggests that the diabetic patients should peel off the guava fruits before consuming. However, it can also be useful in controlling hypoglycaemia occasionally caused due to excess of insulin and other hypoglycaemic drugs.

Shen et al., (2008) investigated the effect of aqueous and ethanol soluble solid extracts of guava (\textit{Psidium guajava} L.\textit{inn.}) leaves on hypoglycemia and glucose metabolism in type 2 diabetic rats. Low-dose streptozotocin (STZ) and nicotinamide were injected into Sprague-Dawley (SD) rats to induce type 2 diabetes. Acute and long-term feeding tests were carried out, and an oral glucose tolerance test (OGTT) to follow the changes in plasma glucose and insulin levels was performed to evaluate the antihyperglycemic effect of guava leaf extracts in diabetic rats. The results of acute and long-term feeding tests showed a significant reduction in the blood sugar level in diabetic rats fed with either the aqueous or ethanol extract of guava leaves (p < 0.05). Long-term administration of guava leaf extracts increased the plasma insulin level and glucose utilization in diabetic rats. The results also indicated that the activities of hepatic hexokinase, phosphofructokinase and glucose-6-phosphate dehydrogenase in diabetic rats fed with aqueous extracts were higher than in the normal diabetic group (p < 0.05). On the other hand, diabetic rats treated with the ethanol extract raised the activities of hepatic hexokinase and glucose-6-phosphate dehydrogenase (p < 0.05) only. The experiments provided evidence to support the antihyperglycemic effect of guava leaf extract and the health function of guava leaves against type 2 diabetes.
Rai et al., (2009) evaluated the glycaemic potential of aqueous extract of Psidium guajava unripe fruit peel on blood glucose level (BGL) of normal and streptozotocin induced mild and severely diabetic rats as an extension of our previous work carried out on Psidium guajava ripe fruit peel. The aqueous extract of P. guajava unripe fruits was prepared. Male 6-8 wk old albino Wistar rats were selected for the experiments. Diabetes was induced by streptozotocin infection. Blood glucose levels were measured by glucose oxidase method. Antihyperglycaemic activity of the extract was assessed in mild and severely diabetic rats. The maximum fall of 21.2 per cent (P<0.01) and 26.9 per cent (P<0.01) after 3 h of glucose administration during glucose tolerance test (GTT) was observed in BGL from a dose of 400 mg/kg, identified as the most effective dose, in normal and mild diabetic rats respectively. In severely diabetic rats the maximum fall of 20.8 and 17.5 per cent in fasting blood glucose (FBG) and post prandial glucose (PPG) levels, and 50 per cent (P<0.01) in urine sugar levels was observed with the same dose. Haemoglobin level increased by 5.2 per cent (P<0.05) and body weight by 2.5 per cent (P<0.05) after 21 days treatment. The result reflects that normal, mild and severely diabetic rat models had shown hypoglycaemic as well as antidiabetic effect of the unripe guava fruit peel aqueous extract.

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 Curcuma longa in streptozotocin (STZ, 45 mg/kg iv single dose) induced type 1 diabetic rats. STZ produced a significant increase in serum glucose, cholesterol, triglyceride, very low density lipoprotein, low density lipoprotein, creatinine, and urea levels in diabetic rat. 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. Dihar produced significant decrease in serum creatinine and urea levels in diabetic rats. There was a significant decrease in reduced glutathione, superoxide dismutase, catalase levels and increase in thiobarbituric acid reactive species levels in the liver of STZ-induced diabetic rats. Administration of Dihar to diabetic rats significantly reduced the levels of lipid peroxidation and increased the activities of antioxidant enzymes. The results suggest Dihar to be beneficial for the treatment of type 1 diabetes.
Soman et al., (2010) investigated the antioxidant as well as antiglycative potential of ethyl acetate fraction of guava leaves. Oral administration of the extract at different doses showed a significant decrease in blood glucose level. It also showed an improved antioxidant potential as evidenced by decreased lipid peroxidation and a significant increase in the activity of various antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase. Glycated hemoglobin as well as fructosamine which are indicators of glycation was also reduced significantly in treated groups when compared to diabetic control. In vitro studies also support the antioxidant as well as antiglycative potential of guava leaves.

Rai et al.,(2010) evaluated the hypolipidaemic and hepatoprotective effects of unripe Psidium guajava lfruit peel aqueous extract in streptozotocin (STZ) induced severely diabetic rats by assaying their triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL) cholesterol, alkaline phosphatase (ALKP), asperate amino transeferase (AST), alanine amino transferase (ALT) and creatanine (CRTN) levels. Severely diabetic albino Wister rats of same age group were treated orally once a day upto 3wk with a dose of 400 mg/kg bw of lyophilized extract. TG, TC, HDL, ALKP, AST, ALT and CRTN were estimated. LDL and VLDL cholesterol levels were calculated from the above measurements by using Friedwald formula. A significant decrease in TG (P<0.01), TC (P<0.01), HDL (P<0.001) VLDL (P<0.001) and LDL (P<0.01), ALKP (P<0.01), AST (P<0.05), ALT (P<0.05) and CRTN (P<0.001) levels were observed after 21 days treatment of aquous extract of raw fruit peel compared to pre treatment levels. The result conclude that extract showed significant hypolipidaemic activity in addition to its hypoglycaemic and antidiabetic activity.

Huang et al., (2011) reported the antihyperglycemic efficacy and mechanisms of action of P. guajava in streptozotocin (stz)-induced diabetic rats. After 4 weeks of P. guajava supplementation (125 and 250 mg/kg), P. guajava significantly restored the loss of body weight caused by stz and reduced blood glucose levels in a dose-dependent manner compared with that in diabetic control rats. Mechanistically, P. guajava protected pancreatic tissues, including islet β-cells, against lipid peroxidation and dna strand breaks induced by stz, and thus reduced the loss of insulin-positive β-cells and insulin secretion. Moreover, P. guajava also markedly inhibited pancreatic nuclear factor-kappa b protein expression induced by stz and restored the activities of antioxidant enzymes, including superoxide dismutase, catalase, and
glutathione peroxidase. The result conclude that P. guajava has a significant antihyperglycemic effect, and that this effect is associated with its antioxidative activity.

Gutierrez et al., (2011) studied the hypoglycemic effects of hexane, chloroform and methanol extracts of leaves of Azadirachta indica (AI) were evaluated by oral administration in streptozotocin-induced severe diabetic rats (SD). The effect of chronic oral administration of the extract for 28 days was evaluated in streptozotocin diabetic rats. Lipid peroxidation, glycogen content of liver and skeletal muscles, insulin, superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), oxidized glutathione (GSSG) levels were determined. In addition, advanced glycation end product formation (AGEs) was evaluated. The most active extracts were obtained with chloroform. Chloroform extract from AI shows increased levels of SOD, GSH, GSSG and CAT, hepatic glycogen content, glucose-6-phosphatase and insulin plasma levels, which also decreased the glucokinase (GK), lipid peroxidation and insulin resistance. 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 streptozocin-induced diabetic rats and, therefore, A. indica can be potentially considered to be an antidiabetic-safe agent.

Shrivastava et al., (2012) investigated the possible effect of Azadirachta indica leaf extract in high fat diet induced diabetic Charles Foster rats. The increased level of lipid peroxidation and altered levels of enzymatic (superoxide dismutase, glutathione peroxidase and catalase) and non-enzymatic (glutathione) antioxidants were seen in high fructose fed animals. The treatment with A. indica leaf extract significantly normalized the altered levels of lipid peroxidation and antioxidant status at 400 mg/kg b.w. dose. The A. indica leaf extract was also tested for in vitro inhibition of generation of superoxide anion and hydroxyl free radical in both enzymatic and non-enzymatic systems. The A. indica leaf extract was found to inhibit generation of superoxide anion and hydroxyl free radical significantly at 200 µg/ml concentration. Data of present study demonstrated that the A. indica leaf extract has both antidiabetic and antioxidant properties.
2.4. ANTIOXIDENT ACTIVITY

Azadirachta indica A. Juss, known as neem in vernacular, belongs to the family Meliaceae and is widely distributed in Asia, Africa and other tropical parts of the world (Sombatsiri et al., 1995). In Nepal, neem plants are distributed in the Terai (tropical) and the foothills (subtropical) of the country. Neem is a versatile medicinal plant, almost every part of which is being used in folklore and traditional systems of medicine for the treatment of a variety of human ailments. Neem oil, bark and leaf extracts have been therapeutically used as folk medicine to control diseases like leprosy, intestinal helminthiasis, respiratory disorders, constipation, and skin infections. However, apart from these uses, there are several reports on the biological activities and pharmacological actions based on modern scientific investigations, such as anti-inflammatory and, antioxidant, etc.

The effects of free radicals on human beings are closely related to toxicity, disease and aging (Maxwell, 1995). Most living species have an efficient defense system to protect themselves against the oxidative stress induced by Reactive Oxygen Species (ROS). Recent investigations have shown that the antioxidant properties of plants could be correlated with oxidative stress defense and different human diseases including cancer, atherosclerosis and the aging process (Stajner et al., 1995). The antioxidants can interfere with the oxidation process by reacting with free radicals, chelating free catalytic metals and also by acting as oxygen scavengers. Many plants contain substantial amounts of antioxidants like vitamin C and E, carotenoids, flavonoids, tannins, etc. that can be used to scavenge the excess free radicals from human body. The intake in the human diet of antioxidant compounds, or compounds that ameliorate or enhance the biological antioxidant mechanism can prevent and in some cases, help in the treatment of some oxidative related disorders.

Rao et al., (1998) isolated antioxidant compound from Azadirachta seed kernels using high pressure liquid chromatography with a hydrophobic reverse-phase chromatography column. The eluted molecule had lambdamax at 224 and 272 nm and was a potent inhibitor of plant lipoxygenases. In in vivo studies of horse gram during germination, low levels of lipoxygenase activity and lipid peroxides were found upon treatment with the Azadirachta extract. The antioxidant property of Azadirachta indica first time reported in this study. Recent investigations have shown that the antioxidant properties of plants could be correlated with oxidative stress.
defense and different human diseases including cancer, atherosclerosis and the aging process (Sanchez-Moreno et al., 1999; Malencic et al., 2000).

Jiménez-Escrig et al., (2001) reported that Guava fruit (Psidium guajava L.) as a new source of antioxidant dietary fiber. The antioxidant activity of polyphenol compounds was studied, using three complementary methods: (i) free radical DPPH* scavenging, (ii) ferric reducing antioxidant power assay (FRAP), and (iii) inhibition of copper-catalyzed in vitro human low-density lipoprotein (LDL) oxidation. All fractions tested showed a remarkable antioxidant capacity, and this activity was correlated with the corresponding total phenolic content. A 1-g (dry matter) portion of peel contained DPPH* activity, FRAP activity, and inhibition of copper-induced in vitro LDL oxidation, equivalent to 43 mg, 116 mg, and 176 mg of Trolox, respectively. These results indicate that guava could be a suitable source of natural antioxidants. Peel and pulp could also be used to obtain antioxidant dietary fiber (AODF), a new item which combines in a single natural product the properties of dietary fiber and antioxidant compounds.

Yamashiro et al (2003) determined the effects of the aqueous extracts from Psidium guajava L. radical-scavenging activity on myocardial injury produced in albino rats. Quercetin is a major antioxidative components of P. guajava L., and it exerted beneficial effects. The results indicates that P. guajava L. shows a cardioprotective effects against myocardial ischemia-reperfusion injury in isolated rat hearts and radical-scavenging actions.

Masuda et al (2003) identified the simple detection method for a powerful radical scavenging compound in a mixture containing a large variety of compounds, such as the raw extract of edible plants, was developed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as the radical reagent. The method was applied to the antioxidant plant such as Psidium guajava, Citrus depressa, and Hypericum chinense. Among them, Psidium guajava shows the powerful antioxidant activity due to the presence of antiradical plant constituents.

Dried ground leaves of Psidium guajava L. (guava) were extracted by water and aqueous ethyl alcohol 50% (1:10) ratio, and the total phenolic content in the extracts was determined spectrophotometrically according to Folin-Ciocalteu's phenol method and calculated as gallic acid equivalent (GAE). Remarkably high total phenolic content 575.3 +/-15.5 and 511.6+/-6.2
mg of GAE/g of dried weight material (for ethanol guava leaf extracts and water guava leaf extracts, respectively) were obtained. The antioxidant activity of lyophilized extracts was determined at ambient temperature by means of a 2,2-diphenyl-1-picrylhydrazyl (DPPH*) colorimetry with detection scheme at 515 nm. The activity was evaluated by the decrease in absorbance as the result of DPPH* color change from purple to yellow. The higher the sample concentration used, the stronger was the free radical-scavenging effect. The results obtained showed that ascorbic acid was a substantially more powerful antioxidant than the extracts from guava leaf. On the other hand, the commercial guava leaf extracts and ethanol guava leaf extracts showed almost the same antioxidant power whereas water guava leaf extracts showed lower antioxidant activity. The parameter EC(50) and the time needed to reach the steady state to EC(50) concentration (T(EC(50))) affected the antiradical capacity of the sample. The antioxidant efficiency (AE) has been shown to be a more adequate parameter for selecting antioxidants than the widely used EC(50). This study revealed that guava leaf extracts comprise effective potential source of natural antioxidants. (Qian and Nihorimbere, 2004).

Sithisarn et al., (2005) assessed for antioxidant activity in leaves, fruits, flowers and stem bark extracts from the Siamese neem tree (Azadirachta indica A. Juss var. siamensis Valeton, Meliaceae) using the 1,1-diphenyl-2-picryl hydrazyl (DPPH) scavenging assay, total antioxidant activity and inhibition of lipid peroxidation in Chago K1 cancer cell culture by the thiobarbituric acid reactive substances (TBARS) method. The results showed that leaf aqueous extract, flower and stem bark ethanol extracts exhibited higher free radical scavenging effect on the DPPH assay with 50% scavenging activity at 26.5, 27.9 and 30.6 microg/ml, respectively. The total antioxidant activity of these extracts was found to be 0.959, 0.988 and 1.064 mM of standard trolox, respectively. At 100 microg/ml, the flower ethanol and leaf aqueous extracts significantly decreased malondialdehyde (MDA) levels (46.0 and 50.6%, respectively) by the TBARS method. The results suggest that extracts from leaf, flower and stem bark of the Siamese neem tree have strong antioxidant potential. This report supports the ethnomedical use of young leaves and flowers of this plant as a vegetable bitter tonic to promote good health.
Thaipong et al., (2005) evaluated the hydrophilic and lipophilic antioxidant activities of guava fruits. The hydrophilic antioxidant activity (AOAH) and the lipophilic antioxidant activity (AOAL); and their correlations with vitamin C, and total phenolic and beta-carotene contents in fresh guava fruits of one white flesh clone ('Allahabad Safeda') and three pink flesh clones ('Fan Retief', 'Ruby Supreme,' and an advanced selection) were studied. A ferric reducing antioxidant power assay was used to estimate both AOAH and AOAL from methanol and dichloromethane extracts, respectively. The white flesh clone, 'Allahabad Safeda,' showed higher levels of both AOAH [33.3 microM Trolox equivalents (TE)/g fresh weight (FW)] and AOAL (0.25 microM TE/g FW) than the pink flesh clones that ranged from 15.5 to 30.4 and from 0.12 to 0.13 microM TE/g FW for AOAH and AOAL, respectively. The AOAH was positively correlated with vitamin C (r = 0.92, p < 0.01) and total phenolic (r = 0.97, p < 0.01) but was negatively correlated with beta-carotene (r = -0.73, p = 0.03). The AOAL was not correlated with these antioxidants.

Koul et al (2006) observed the antioxidant and inhibitory effects of Azadirachta indica on DMBA-induced skin carcinogenesis in Balb/c mice. Male Balb/c mice were divided into four groups on the basis of their respective treatments wherein mice of Group I served as controls. For induction of skin tumors, mice of Group II and IV were injected sub-cutaneously with 7,12-dimethylbenz(a)anthracene (DMBA). Mice of Group III and IV were administered aqueous Azadirachta indica leaf extract (AAILE) thrice a week throughout the experiment. After 14 weeks of the first DMBA injection, Group II and IV mice developed tumors. In the tumor-bearing mice that received AAILE (Group IV), a significant reduction in mean tumor burden and tumor volume was observed. Glutathione (GSH) content and the activities of GSH-based antioxidant enzymes viz. glutathione peroxidase (GPx) and glutathione reductase (GR) increased significantly in the skin tissues of all the groups of mice when compared to control counterparts. Catalase activity was found to decrease significantly in the skin of mice, which received AAILE treatment only (Group III). Activity of super-oxide dismutase (SOD) decreased significantly in all the tumorous tissues (Group II and IV mice). The result indicates that Azadirachta indica have both antioxidant and inhibitory effects on DMBA-induced skin carcinogenesis in Balb/c mice.
Sithisarn et al (2006) investigated the antioxidant activity of the aqueous extracts of leaves of Siamese neem tree (Azadirachtaindica A. Juss var. siamensis Valeton) from several extracting and drying methods using 2,2-diphenyl-1-picrylhydrazyl (DPPH)-scavenging assay. The leaves of Siamese neem tree were extracted using percolation, decoction, maceration, soxhlet extraction, freeze drying or spray drying methods. The extract was tested for antioxidant activity using DPPH-scavenging assay. Thin-layer chromatography of the extract from decoction was also tested. The freeze drying method gave the highest yield (51.50%, w/w) of crude extract, while decoction gave the most effective DPPH-scavenging activity (EC(50): 31.4 microg/ml). Thin-layer chromatography analysis was used to screen the leaf extract obtained using decoction, and the chromatogram showed spots corresponding to quercetin and rutin flavonoids which exhibited antioxidant activities (EC(50): 2.29 and 34.67 microg/ml, respectively). The result reflects the siamese neem tree leaf extracts possessed free radical scavenging activity against the DPPH radical. The most active extract was obtained with the leaf decoction method. It showed antioxidant activity with EC(50) of 31.4 microg/ml.

Wang et al (2007) studied the antioxidative activities of the extracts from Psidium guajava Linn leaves (PGL). The PGL was submersed with distilled water, 65% ethanol and 95% ethanol respectively. The 3 extracts were obtained after the solutions were filtered, concentrated and dried. The scavenging rate to hydroxyl radicals and inhibiting rate to lipid peroxidation were analyzed for the 3 extracts. The extracts from distilled water, 65% ethanol and 95% ethanol respectively showed effects on scavenging hydroxyl radicals and inhibiting lipid peroxidation in the dose-dependent manner, had 50% effective concentration (EC50) on scavenging hydroxyl radicals of 0.63, 0.47 and 0.58g/L, had EC50 on inhibiting lipid peroxidation of 0.20, 0.035, 0.18g/L and had total flavonoids contents of 3.28, 30.71 and 55.98g/kg respectively. The aquatic and the ethanol extracts from PGL possess the potential antioxidative activities in the study. The flavonoids may be one of their antioxidative components.

Marquina et al (2008) determined the composition and antioxidant capacity of the guava (Psidium guajava L.) fruit, pulp and jam. In this work, free acidity, pH, ash, nitrogen and water, the total polyphenol content and the antioxidant capacity of the peel, the shell and the pulp of the fresh fruit and the processed guava pulp and jam. The highest phenolic content was found in the
guava skin la (10.36 g/100 g skin) and the lowest in the jam (1.47 g/100 g jam), in dry weight. The antioxidant capacity of the skin was 10 times higher than that of the pulp, and the jam was twice that of the shell.

Manikandan et al. (2008) evaluated the chemopreventive potential of Azadirachta indica (neem) leaf fractions based on in vitro antioxidant assays, and in vivo inhibitory effects on 7,12-dimethylbenz[a]anthracene (DMBA)-induced hamster buccal pouch (HBP) carcinogenesis. In addition we also identified the major constituents in neem leaf fractions by HPLC. Analysis of the free radical scavenging activities and reducing potential of crude ethanolic extract (CEE), ethyl acetate fraction (EAF) and methanolic fraction (MF) of neem leaf revealed a concentration-dependent increase in antioxidant potential that was in the order EAF>MF>CEE. Administration of neem leaf fractions reduced the incidence of DMBA-induced HBP carcinomas at a lower concentration compared to the crude extract. Chemoprevention by neem leaf fractions was associated with modulation of phase I and phase II xenobiotic-metabolising enzymes, lipid and protein oxidation, upregulation of antioxidant defences, inhibition of cell proliferation and angiogenesis, and induction of apoptosis. However, EAF was more effective than MF in terms of antiproliferative and antiangiogenic effects, and expression of CYP isoforms. The greater efficacy of EAF may be due to higher content of constituent phytochemicals as revealed by HPLC analysis. The results of the present study suggest that the antioxidant properties of neem leaf fractions may be responsible for modulating key hallmark capabilities of cancer cells such as cell proliferation, angiogenesis and apoptosis in the HBP carcinogenesis model.

Chandra et al (2008) reported the protective potential of some herbal hypoglycemic agents on antioxidant status and levels of metal ions in streptozotocin-induced diabetic rats. Furthermore, in vitro antioxidant activity of the herbs was also evaluated. Oral treatment of diabetic rats with Allium sativum, Azadirachta indica, and Ocimum sanctum extracts (500 mg/kg of body weight) not only lowered the blood glucose level but also inhibited the formation of lipid peroxides, reactivated the antioxidant enzymes, and restored levels of GSH and metal ions in the animal model. The herbal extracts (50-500 microg) inhibited the generation of superoxide anions (O(2)(-)) in both enzymatic and nonenzymatic in vitro systems. These preparations also inhibited the ferrous-sodium ascorbate-induced formation of lipid peroxides in RBCs. The in vivo and in vitro protective effects of the above-mentioned herbal drugs were also compared.
with that of glibenclamide. On the basis of our results, this study conclude that the above-
mentioned herbal plants not only possess hypoglycemic properties, but they also decrease
oxidative load in diabetes mellitus. Therefore, we propose that long-term use of such agents
might help in the prevention of diabetes-associated complications.

Sultan et al (2009) stated the effects of four extracting solvents [absolute ethanol, absolute methanol, aqueous ethanol (ethanol: water, 80:20 v/v) and aqueous methanol (methanol: water, 80:20 v/v)] and two extraction techniques (shaking and reflux) on the antioxidant activity of extracts of barks of Azadirachta indica and leaves of Aloe barbadensis were investigated. The tested plant materials contained appreciable amounts of total phenolic contents (0.31-16.5 g GAE /100g DW), total flavonoid (2.63-8.66 g CE/100g DW); reducing power at 10 mg/mL extract concentration (1.36-2.91), DPPH(.) scavenging capacity (37.2-86.6%), and percent inhibition of linoleic acid (66.0-90.6%). Generally higher extract yields, phenolic contents and plant material antioxidant activity were obtained using aqueous organic solvents, as compared to the respective absolute organic solvents. Although higher extract yields were obtained by the refluxing extraction technique, in general higher amounts of total phenolic contents and better antioxidant activity were found in the extracts prepared using a shaker.

Akinmoladun et al., (2010)evaluated the antioxidant and free radical scavenging capacities of some Nigerian indigenous medicinal plants. Methanolic extracts of Alstonia boonei, Cassia alata, Newbouldia laevis, Spondias mombin, Globimetula cupulatum, Chromolaena odorata, Securidaca longepedunculata, Ocimum gratissimum, and Morinda lucida-widely used in ethnomedicine, were assessed for phytochemical constituents and antioxidant and free radical scavenging activities using seven different antioxidant assay methods. Phytochemical screening gave positive tests for steroids, terpenoids, and cardiac glycosides, alkaloids, saponins, tannins, and flavonoids contained in the extracts. P. guajava contained the highest amount of total phenolics (380.08 +/- 4.40 mg/L gallic acid equivalents), and the highest amounts of total flavonoids were found in the leaf extracts of C. alata (275.16 +/- 1.62 microg/mL quercetin equivalents [QE]), C. odorata (272.12 +/- 2.32 microg/mL QE), and P. guajava (269.72 +/- 2.78 microg/mL QE). Percentage 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was highest in S. mombin (88.58 +/- 3.04%) and P. guajava (82.79 +/- 2.84%) and compared with values obtained for ascorbic acid and gallic acid These results
suggest that the methanolic extracts of *P. guajava* plant parts possess significant antioxidant and radical scavenging activities that may be due to the phytochemical content of the plants and as such make them potential candidates as natural chemoprophylactic agents. In addition, multiple assay methods should be used in comparing antioxidant capacities of samples to have a reliable result.

Norshazila et al., (2010) stated that the antioxidant levels of seeds of guava (*Psidium guajava*), mango (*Mangifera indica* L.) and papaya (*Carica papaya* L.). Seeds are among byproducts from the processing of fruits based products. Instead of discarding seeds as waste, seeds with high potential as antioxidants could be utilised for commercial purposes. TPC assay showed that mango seeds had the highest TPC (i.e. 32 ± 0.001 mg GAE) followed by guava seeds (i.e. 20 ± 0.001 mg GAE) and papaya seeds (8 ± 0.003 mg GAE). For DPPH assay, IC50 data showed that mango seed extract scavenged 50% DPPH radicals at the lowest concentration (0.11 ± 0.01 mg/mL) followed by the positive control BHA (0.13 ± 0.01 mg/mL), guava seed extract (0.26 ± 0.01 mg/mL) and papaya seed extract (0.34 ± 0.01 mg/mL). The results indicated that mango and guava seeds showed the highest antioxidant level than papaya seeds.

Soman et. al., (2010) investigated the antioxidant as well as antiglycative potential of ethyl acetate fraction of guava leaves. Oral administration of the extract at different doses showed a significant decrease in blood glucose level. It also showed an improved antioxidant potential as evidenced by decreased lipid peroxidation and a significant increase in the activity of various antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase. Glycated hemoglobin as well as fructosamine which are indicators of glycation was also reduced significantly in treated groups when compared to diabetic control. In vitro studies also support the antioxidant as well as antiglycative potential of guava leaves.

Khan et al., (2011) profiled the antioxidant activity of green tea and guava leaf using HPTLC densitometry methods. Ten marker compounds have been resolved using silica gel 60 F(254) plates, toluene/acetone/formic acid (5:4:1 v/v/v) for markers 1-6, and toluene/ethyl acetate/formic acid/methanol (3:3:0.8:0.2 v/v/v/v) for markers 7-10 as the mobile phases. The high-performance thin layer chromatography densitometry was performed at wavelengths of 282
and 285 nm for the markers 1-6 and 7-10, respectively. Potent antioxidant activity and the presence of phenolics and flavan-3-ols has been observed for the guava leaf extracts suggestive of its use as an alternate economical source of antioxidants than green tea and the well-established food additive nutraceutical agent.

Huang et al., (2011) investigated the antihyperglycemic and antioxidant efficacy and mechanisms of action of Psidum gujava in streptozotocin (STZ)-induced diabetic rats. After 4 weeks of PG supplementation (125 and 250 mg/kg), Psidum gujava significantly restored the loss of body weight caused by STZ and reduced blood glucose levels in a dose-dependent manner compared with that in diabetic control rats. Mechanistically, Psidum gujava protected pancreatic tissues, including islet ß-cells, against lipid peroxidation and DNA strand breaks induced by STZ, and thus reduced the loss of insulin-positive ß-cells and insulin secretion. Moreover, Psidum gujava also markedly inhibited pancreatic nuclear factor-kappa B protein expression induced by STZ and restored the activities of antioxidant enzymes, including superoxide dismutase, catalase, and glutathione peroxidase. We conclude that Psidum gujava has a significant antihyperglycemic effect, and that this effect is associated with its antioxidative activity.

Choudhary and Swarnkar (2011) examined the total phenolics, flavonoids and vitamin C content vis-a-vis antioxidant activities were assayed in leaves and stem bark of Azadirachta indica, Cassia fistula, Mangifera indica, and Tamarindus indica using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and superoxide radical scavenging method. The DPPH radical scavenging activity positively correlated with the total phenolic content in both stem bark and leaf. Superoxide radical scavenging activity increased with increasing flavonoid contents. However, the vitamin C content could not be correlated with DPPH and superoxide radical scavenging capacity.

Psidium cattleianum J. Sabine (Myrtaceae) is a traditional medicinal plant in French Polynesia. The leaves and roots possess many medicinal properties. These effects may be correlated with the presence of antioxidant compounds. Seven flavonoids along with a benzoic acid were isolated from the leaves of P. cattleianum. The compounds indicated strong antioxidant and radical-scavenging activities in ALP, DPPH(·), ABTS(·-) and ORAC
assays. This study demonstrates that the leaves of *P. cattleianum* possess main compounds with interesting antioxidant and radical-scavenging activities, as clarified by four biological assays. Our findings may justify the use of these leaves in the traditional medicine of French Polynesia. Among the total eight known compounds, reynoutrin and luteolin were isolated for the first time from the genus *Psidium*.

Gull *et al.*, (2012) reported the levels of total phenols and vitamin C as well as antioxidant potential at three different ripening stages (un-ripe, semi-ripe and fully-ripe) of guava (*Psidium guajava* L.) fruit collected from three different geographical regions of Pakistan (Islamabad, Faisalabad and Bhakkar). The antioxidant potential of guava fruit extracts was assessed by means of different in-vitro antioxidant assays, namely inhibition of peroxidation in linoleic acid system, reducing power and radical scavenging capability. Overall, fruit at the un-ripe stage (G1) exhibited the highest levels of TPC, TFC, reducing power and DPPH radical scavenging activity, followed by the semi-ripe (G2) and fully-ripe (G3) stages. On the other hand, vitamin C content increased as the fruit maturity progressed, with highest value seen at the fully-ripe stage (G3) followed by the semi-ripe (G2) and un-ripe stage (G1). The concentration of vitamin C in fruits varied as: Faisalabad (136.4-247.9 mg 100 g⁻¹), Islamabad (89.7-149.7 mg 100 g⁻¹) and Bhakkar (73.1-129.5 mg 100 g⁻¹). The results showed that different stages of maturation and geographical locations had profound effects on the antioxidant activity and vitamin C contents of guava fruit.

Shrivastava *et al.*, (2012) investigated the possible effect of *Azadirachta indica* leaf extract in high fat diet induced diabetic Charles Foster rats. The increased level of lipid peroxidation and altered levels of enzymatic (superoxide dismutase, glutathione peroxidase and catalase) and non-enzymatic (glutathione) antioxidants were seen in high fructose fed animals. The treatment with *A. indica* leaf extract significantly normalized the altered levels of lipid peroxidation and antioxidant status at 400 mg/kg b.w. dose. The *A. indica* leaf extract was also tested for in vitro inhibition of generation of superoxide anion and hydroxyl free radical in both enzymatic and non-enzymatic systems. The *A. indica* leaf extract was found to inhibit generation of superoxide anion and hydroxyl free radical significantly at 200 μg/ml concentration. Data of present study demonstrated that the *A. indica* leaf extract has both antidiabetic and antioxidant properties.