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A SYSTEMIC REVIEW ON

ANTIOXIDANT AND HEPATOPROTECTIVE EFFECT OF PSIDIUM GUAJAVA

LEAF AND FRUIT EXTRACT

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A SYSTEMIC REVIEW ON ANTIOXIDANT AND HEPATOPROTECTIVE EFFECT OF PSIDIUM GUAJAVA LEAF AND FRUIT EXTRACT

A senior paper submitted to the Department of Medical Biochemistry, University of Addis Ababa in partial fulfillment of the requirements for the degree of Master of Science in Medical Biochemistry.

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As Senior Paper advisor(S), we here by certify that we have read and evaluated this senior paper prepared under our guidance, by TESFAHUN MOLLA entitled: antioxidant and hepatoprotective effect of psidium guajava leaf and fruit extract

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LIST OF ABBREVIATIONS

ACP:	Acid phosphatase
ROS:	Reactive oxygen species
TRAP:	Total radical trapping antioxidant potential
β -GAL:	β -galactosidase
β -NAG:	β -N-acetyl galactoseaminidase
TEAC:	Total equivalent antioxidant capacity
GAE/g)::	Gallic Acid Equivalent per gram
FRAP:	Ferric reducing antioxidant power
DPPH:	2,2-diphenyl-1-picrylhydrazyl
TAA:	Thioacetamide
ABTS:	2, 2-Azinobis (3-ethylbenzothiazoline-6-sulfonate)
APAP:	Acetaminophen
BHT:	Butylated hydroxyl toluene
PMS:	PhenazineMethosulfate
NBT:	Nitrobluetetrazolium
AAPH:	2, 2-Azo-bis, 2-amidinopropane dihydrochloride
BHA:	Butylatedhydroxyanisole
NAPSQI:	N-acetyl-p-benzoquinone imine
NAPQI:	N-acetyl-p-benzoquinone imine
GP _x :	Glutathione peroxidase
GLE:	Guajava leaf extract
QCT:	Quercetin

RNS: Reactive nitrogen species
GST: Glutathione-S transferase
MDA: Malondialdehyde
SOD: Superoxide dismutase
CAT: Catalase
ORAC: Oxygen radical absorbance capacity
IC50: Concentration in mg/ml of extract that inhibits 50% of DPPH radicals

ABSTRACT

Background: Plant derived natural products such as flavonoids, terpenoids, carbohydrates, tannins, saponins, steroids, proteins, amino acids and Vitamin C have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and hepatoprotective activity. *Psidium guajava* (Myrtaceae) is one of such plants in folk medicine that has been used for the management of various disease conditions. Various parts of the plant has been used in traditional medicine .Antioxidants from medicinal plants play an important role in inhibiting and scavenging radicals, thus providing protection to human against infection and degeneration diseases.

Objective: To review the antioxidant and hepatoprotective activity of *psidium guajava*.

Methods: Computerised literature search was conducted using PubMed, Medline and Google search engine using different key words for studies investigating the antioxidant and hepatoprotective activities of *Psidium guajava* using the following as heading terms, *psidium guajava*, antioxidant, hepatoprotectie, Liver injury, oxidative stress. In addition, the reference lists of the retrieved articles helped me to find further articles, relevant to the present review that was not revealed through the searching procedure.

Result: Out of a total of 152 articles on guava resulted 19 studies which were related to antioxidant and hepatoprotective activity of leaf and fruit extract were included in this review.

These were then categorized into six groups; 1) Six studies on in vitro antioxidant studies with leaf extracts, 2) four studies on in vitro antioxidant studies with fruit extract, 3) one in vivo study on antioxidant with leaf extract in rat, 4) one in vivo study on antioxidant with fruit extract in rat, 5) one study antioxidant fruit extract in human and 6) six in vivo studies on hepatoprotective with leaf extract in rat.

Conclusion: The antioxidant activity of *Psidium guajava* may be due to inhibition of the formation of radicals, scavenging of the formed free radicals, reducing of oxidized intermediates and the presence of the phenolic compounds. It also acts as promising hepato protective which is correlated directly with its ability to reduce activity of serum enzymes and enhance

antioxidant defense status. The phenolic content in Psidium guajava extract played a significant role on the antioxidant and hepatoprotective activity.

KEYWORDS: *guava, Psidium guajava, Antioxidant activity; Radical scavenging; Phenolic compound; Hepatoprotective activity; Carbon tetra chloride; Paracetamol.*

1. INTRODUCTION

Psidium Guajava is called Zeytuna in Ethiopia, guayaba in Spanish speaking countries and goiaba in Brazil; guava is a common shade tree or shrub in door-yard gardens in the tropics. It belongs to family of Myrtaceae, genus: *psidium*, species: *guajava* and common names of the plant are guava, goiaba, guayaba etc. (Vyas *et al.*, 2010). It is originated in the tropical South America and grows widely in Bangladesh, India, Thailand, Brazil, Florida, and West Indies, California and also in several other countries (Mittal *et al.*, 2010). It is native to and widely distributed in Mexico and Central America. However, the plant is cultivated today from the West coast of Africa to the Pacific regions, including India and China, with varieties originally introduced over the past 300 years from the United States. Generally, guava plant has spread widely throughout the tropics because it thrives in a variety of soils, propagates easily, and bears fruit relatively quickly. The guava berry is an important tropical fruit that is mostly consumed fresh. (Joseph *et al.*, 2011). Guava is rich in antioxidant compounds and contains a high level of ascorbic acid. Both myricetin and apigenin are antioxidants and they were reported to be contained in high amount in *Psidium Guajava*. It plays a vital role in fulfilling the vitamin C deficiency among the people of the Indian country since 100 g of fruit contains about 260 mg of vitamins ,which is 2–5 times higher than the fresh orange (Rahman *et al.*, 2003). The wood is hard and tough, used as posts for rural house buildings (Mittal *et al.*, 2010). The pharmacological actions and the medicinal uses of aqueous extracts of guava leaves in folk medicine including the treatment of various types of gastrointestinal disturbances such as vomiting, diarrhea, inhibition of the peristaltic reflex, gastroenteritis, spasmolytic activity, dysentery, abdominal distention, flatulence and gastric pain (Lutterodt, 1992). These extracts have also been indicated to cause disturbances of the central nervous system: insomnia, convulsions and epilepsy. Bronchitis, asthma attacks, cough, pulmonary diseases could also be treated with guava teas and could also be useful as anti-inflammatory and homeostatic agent. Moreover, aqueous extracts of guava leaves were described to be effective against a number of microbial strains and anti-rotavirus activity (Mittal *et al.*, 2010).

Traditional uses: *Psidium guajava* is an important food crop and medicinal plant in tropical and subtropical countries which is widely used like food and in folk medicine around the world. A survey of the literature shows *Psidium guajava* is mainly known for its antispasmodic and antimicrobial properties in the treatment of diarrhea and dysentery. It has also been used extensively as a hypo-glycaemic agent. Many pharmacological studies have demonstrated the ability of this plant to exhibit antioxidant, hepato-protective, anti-allergy, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, anticough, antidiabetic, anti-inflammatory and antinociceptive activities, supporting its traditional uses (Gutierrez *et al.*, 2008).

1.1. Photochemistry

Psidium Guajava is an important food crop and medicinal plant in tropical and subtropical countries and is widely used like food and in folk medicine around the world. This aims a comprehensive of the chemical constituents and clinical uses of *Psidium guajava*. Different pharmacological experiments in a number of *in vitro* and *in vivo* models have been carried out. Also have been identified the medicinally important phyto-constituents as indicated in Table 1.1 below.

Table 1.1.
Chemical composition of various parts of Guava (*Psidium guajava* Linn.) (Mittal et al., 2010)

Parts	Constituents
Fruit	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- α -L-arabopyranoside, flavonoids, guaijavarin, Quercetin, Myricetin and apigenin. 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-butenylacetate, 3-hydroxy-2-butano-3-methyl-1-butanol, 2,3-butanediol, 3-methylbutanoic acid, (Z)-3-hexen-1-ol, 6-methyl-5-hepten-2-one, limonene, octanol, ethyloctanoate (pink guava fruit), fiber, fatty acid.
Leaves	α -pinene, β -pinene, limonene, menthol, terpenyl acetate, isopropyl alcohol, longicyclene, caryophyllene, β -bisabolene, caryophyllene oxide, β copanene, farnesene, humulene, selinene, cardinene and curcumene, mallic acids, nerolidiol, β -sitosterol, ursolic, crategolic, and guayavolic acids, cineol, quercetin, 3-L-4-4-arabinofuranoside (avicularin) and its 3-L-4-pyranoside (Essential oil), resin, tannin eugenol, caryophyllene (1a α -, 4a α -, 7 α -, 7a β -, 7b α -)]-decahydro-1H-cycloprop[e] azulene, Guajavolide (2 α -, 3 β -, 6 β -, 23-tetrahydroxyurs-12-en-28,20 β -olide; 1) and guavenoic acid (2 α -, 3 β -, 6 β -, 23-tetrahydroxyurs-12,20(30)-dien-28-oic acid, triterpene oleanolic acid, triterpenoids, flavinone-2 2'-ene, prenil, dihydrobenzophenanthridine and cryptonine.
Bark	polyphenols, resin and crystals of calcium oxalate
Root	Tannin, leukocyanidins, sterols, Gallic acid, carbohydrates, salts, tannic acid
Seed	Proteins, starch, oils, phenolic, flavonoid compounds, flavonol glycoside, quercetin-3-O- β -D-(2''-Ogalloylglucoside)-4'-O-vinylpropionate
Twigs	Calcium, magnesium, phosphorous, potassium, sodium, fluoride, copper, iron, zinc, manganese, and lead. Flavonoids, sesquiterpene alcohols and Acid triterpenoids

1.2. Caloric and nutrient values of *psidium guajava*

Guava is one of the most popular, nutritionally rich fruit as indicated in (Table 1.2), with unique flavor, taste, and health promoting qualities making it a common in new functional foods category often called “super fruits”. Guavas are low in calories and fats but contains several

vital vitamins, minerals and antioxidant poly-phenolic and flavonoid compounds that play important role in prevention of cancers, anti-aging, immune booster etc. (Kamath et al., 2008)

Table 1.2. Nutrient value per 100g of Guava fruit (Kamath et al., 2008)

Calories	77-86g
Moisture	2.8-5.5g
Crude fiber	0.9-1.0g
Protein	0.1-0.5g
Fat	0.43-0.7g
Ash	9.5-10g
Carbohydrate	9.1-17mg
Calcium	17.8-30mg
Phosphorous	0.30-0.70mg
Iron	200-400 I.U
Carotene (Vitamin A)	0.046mg
Thiamin(vitamin B1)	0.03-0.04mg
Riboflavin(vitamin B2)	0.6-1.068mg
Niacin	40 I.U.
Vitamin B3	35 I.U
Ascorbic acid (vitamin C)	228mg

2. LITRATURE REVIEW

2.1. *Antioxidant activity of psidium Guajava*

Oxidative stress depicts the existence of products called free radicals and reactive oxygen species (ROS), which are formed under normal physiological conditions but become deleterious when not being eliminated by the endogenous systems. In fact, oxidative stress caused by an imbalance between the generation of reactive oxygen species and endogenous antioxidant systems. ROS are major sources of primary catalysts that initiate oxidation *in vivo* and *in vitro* and create oxidative stress which results in numerous diseases and disorders such as cancer, cardiovascular disease, neural disorders, Alzheimer's disease mild cognitive impairment, Parkinson's disease, alcohol induced liver disease, ulcerative colitis, ageing, and atherosclerosis. Oxygen derived free radicals such as superoxide anions; hydroxyl radicals and hydrogen peroxide are cytotoxic and give rise to tissue injuries. Excessive amount of ROS is harmful because they initiate bimolecular oxidation which leads to cell death and creates oxidative stress. In addition, oxidative stress causes inadvertent enzyme activation and oxidative damage to cellular system (Chanda and Dave, 2009).

Free radical is a chemical compound which contains an unpaired electron spinning on the peripheral layer around the nucleus. The family of free radicals generated from the oxygen is called ROS which cause damage to other molecules by extracting electrons from them in order to attain stability. ROS are ions, atoms or molecules that have the ability to oxidize reduced molecules. ROS are various forms of activated oxygen, which include free radicals such as superoxide anion radicals and hydroxyl radicals, as well as non-free radicals) and singlet oxygen (Halliwell, 1995). In the body free radicals are derived from two sources: endogenous sources, e.g. nutrient metabolism, ageing process etc and exogenous sources e.g. tobacco smoke, ionizing radiation, air pollution, organic solvents, pesticides, etc (Buyukokuroglu *et al.*, 2001).

When oxygen traps single electron, it becomes unstable and thus very reactive, since it generates harmful chain reactions against many biological molecules. The extreme toxicity of oxygen is related to its high capability of generating free radicals and in turn destroying many major biological molecules. They can attack on lipids and proteins and destroy membranes. ROS can damage DNA and lead to mutation and chromosomal damage. Oxidized cellular thiols

abstract hydrogen atoms from unsaturated fatty acids to initiate the peroxidation of membrane lipids (Valko et al., 2006). ROS can attack various substrates in body and contribute to development of chronic diseases. For example, oxidatively modified low density lipoprotein (LDL) has been hypothesized to be a causative agent in the development of cardiovascular diseases (Touyz, 2004).

Exogenous chemicals and endogenous metabolic processes in human body produce free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death. Superoxide anion radicals increase under stressed conditions such as heavy exercise, certain drugs, infection and various disease states. During normal metabolic processes, human body generates more than 2 Kg of O_2^- per year (Evans and Halliwell, 1999).

Cells are equipped with different kinds of mechanisms to fight against ROS and to maintain the redox homeostasis of cell. For example, antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) play important roles in scavenging the free radicals and preventing cell injury. Molecules such as vitamin C and E inhibit lipid peroxidation in cell. When the mechanism of antioxidant protection becomes unbalanced in human body, antioxidant supplement may be used to help reduce oxidative damage (Bergendi et al., 1999).

2.1.1. Natural sources of antioxidants

Medicinal plants are an important source of antioxidants (Rice, 2004). Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of certain diseases such as cancer, heart diseases and stroke (Prior and Cao, 2000). The secondary metabolites like phenolics and flavonoids from plants have been reported to be potent free radical scavengers. They are found in all parts of plants such as leaves, fruits, seeds, roots and bark (Mathew and Abraham, 2006).

There are many synthetic antioxidants in use. It is reported, however, that they have several side effects, such as risk of liver damage and carcinogenesis in laboratory animals (Gao et al., 1999, Williams et al., 1999, Ito et al., 1983, Osawa and Namiki, 1981). There is therefore a need for more effective, less toxic and cost effective antioxidants. Medicinal plants appear to have

these desired comparative advantages, and hence the growing interest in natural antioxidants from plants.

Psidium Guajava is one of this plants where the plant parts, are used commonly are fruits and leaves. Guava is rich in tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, lectins, vitamins, fiber and fatty acids. Guava fruit is higher in vitamin C than citrus (80 mg of vitamin C in 100 g of fruit) and contains appreciable amounts of vitamin A as well. Guava fruits are also a good source of pectin a dietary fiber. The leaves of guava are rich in flavonoids, in particular, quercetin. Much of guava's therapeutic activity is attributed to these flavonoids. The flavonoids have demonstrated antibacterial activity. Quercetin is thought to contribute to the antidiarrhea effect of guava; it is able to relax intestinal smooth muscle and inhibit bowel contractions. In addition, other flavonoids and triterpenes in guava leaves show antispasmodic activity (Vyas et al., 2010).

2.1.2. Evaluation of antioxidant activity

A great number of *in vitro* methods have been developed to measure the efficiency of natural antioxidants either as pure compounds or as plant extracts. *In vitro* methods, can be divided into two major groups: 1) Hydrogen atom transfer reactions like Oxygen Radical Absorbance Capacity (ORAC), Total radical trapping antioxidant potential (TRAP) and β carotene bleaching; 2) Electron transfer reactions like trolox equivalent antioxidant capacity (TEAC), Ferric reducing antioxidant power (FRAP), α , α -diphenyl- β -picryl-hydrazyl radical scavenging assay (DPPH), Superoxide anion radical scavenging assay, Hydroxyl radical scavenging assay, Nitric oxide radical scavenging assay and Total phenol assay. These methods are popular due to their high speed and sensitivity. However, it is essential to use more than one method to evaluate antioxidant capacity of plant materials because of the complex nature of phytochemicals (Chanda and Dave, 2009).

2.2. Hepatoprotective activity of *Psidium Guajava*

Liver is an important organ responsible for the metabolism, bile secretion, elimination of many substances, blood detoxifications, synthesizes and regulates essential hormones. Liver diseases have become a worldwide problem and are associated with significant morbidity and mortality.

The principal causative factors for the liver diseases in developed countries are excessive alcohol consumption, and viral-induced chronic liver diseases while in the developing countries the most frequent causes are environmental toxins, parasitic disease, hepatitis B and C viruses, and hepatotoxic drugs (certain antibiotics, chemotherapeutic agents, high doses of paracetamol, carbon tetrachloride (CCl₄), thioacetamide (TAA) etc. Chronic liver cirrhosis and drug induced liver injury accounting ninth leading cause of death in western and developing countries (Saleem et al., 2010). In the absence of reliable hepatoprotective drugs in modern medicine, a large number of herbal preparations have become increasingly popular for the treatment of liver disorders (Stickel and Schuppan, 2007).

A single drug is unlikely to be effective for all types of severe liver diseases. An effective formulation using indigenous medicinal plants, with proper pharmacological experiments and clinical trials may thus provide a satisfactory alternative. Polyherbal therapy is a pharmacological principle that has the advantage of producing maximum therapeutic efficacy with minimum side effects, and according to the World Health Organization (WHO), 80% of the world population uses plant-based remedies as their primary form of health care. The WHO is establishing definitive guidelines for clinical research methodologies and the appraisal of effectiveness of traditional medicines. There is also an increasing interest in natural antioxidants (e.g., phenols), present in medicinal and dietary plants, that might help prevent oxidative damage. Natural antioxidants may be effective treatments for several pathologies not only as conventional hydrogen-donating compounds (antiradical activity), but more importantly, may exert modulatory effects via actions on antioxidant, drug metabolizing and repairing enzymes as well as modulating as signaling molecules in important cascades for cell survival (Shakya and Shukla, 2011).

Increased mitochondrial stress and the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been implicated in acetaminophen (APAP) induced hepatotoxicity. Numerous medicinal plants and their formulation are used for the treatment of liver disorders in ethnomedical practice and traditional system of medicine in India (Shakya and Shukla, 2011).

In India, numerous medicinal plants are used for the treatment of liver disorders. One of the plants used traditionally is guava plant, *Psidium guajava* Linn (Myrtaceae). It is believed in folklore that the water decoction of the leaves of this plant can cure jaundice within three days. It is used widely in Mangalore district of Karnataka. *Psidium guajava* contains a number of chemical constituents, which are reported to possess antibacterial (Caceres et al., 1993), antidiarrheal (Maruyama et al., 1985), antimycobacterial (Malcolm and Sofowora, 1969), antihyperglycemic (Maruyama et al., 1985), antimalarial (Gessler et al., 1994), cytotoxic (Villareal et al., 1992) and antioxidant activities (Qian and Nihorimbere, 2004).

The anti-oxidant activity is due to presence of a number of constituents; the major ones are caryophyllene oxide, caryophyllene and a number of tannins (Ross, 2005). Since antioxidants are known to reduce the development of chemically induced liver damage, the effect of water decoction of the leaves of the plant *Psidium guajava* has been evaluated for hepatoprotective activity (Roy et al., 2006). Therefore Hepatotoxicity is one of the very common ailments resulting in to a serious debilities ranging from severe metabolic disorders to even mortality. Hepatotoxicity in most cases is due to free radicals. Free radicals generated by the metabolism of toxicants initiate the toxicity cascade. In view of severe undesirable side effects of synthetic agents, there is a growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity. Traditional medicine is widespread and plants still presents a large source of natural antioxidants that might serve as leads for the development of novel drugs. Several anti-inflammatory, digestive, anti-necrotic, neuroprotective, and hepatoprotective drugs have recently been shown to have an antioxidant and/or anti-radical scavenging mechanism as part of their activity. Conventional medicine is now pursuing the use of natural products such as herbs to provide the support that the liver needs on a daily basis. Herbal drugs are frequently considered to be less toxic and free from side effects than synthetic drugs (Sangeetha and krishnakumari, 2010).

3. OBJECTIVES

General objective

- ❖ To review the hepatoprotective and antioxidant activity of *Psidium Guajava*

Specific objectives

- To evaluate the hepatoprotective activity of p.guajava in acute experimental liver injury induced by paracetamol and CCl₄.
- To review the antioxidant activity and free radical-scavenging effects of extracts from guava leaves and dried fruit.
- To determine the effects of guava (*Psidium guajava*) consumption on total antioxidant status.
- To evaluate the hepatoprotective activity of different extracts (petroleum ether, chloroform, ethyl acetate, methanol and aqueous) of *P. guajava* in acute experimental liver injury induced by carbon tetrachloride and paracetamol

4. METHODS

Computerised literature search was conducted using PubMed, Medline and Google search engines using different for studies investigating the antioxidant and hepatoprotective activities of *Psidium guajava* using the following as heading terms, psidium guajava, antioxidant, hepatoprotective, liver injury, oxidative stress. In addition, the reference lists of the retrieved articles helped to find further articles, relevant to the present review that were not revealed through the searching procedure.

4.1. Inclusion criteria

A literature search was conducted on Pubmed, Medline and google for research articles relating guava (*Psidium guajava*) to antioxidant and hepatoprotective activity, in order to determine any potential antioxidant and hepatoprotective activity. A total of 152 articles on guava resulted, of which the 19 studies which were related to antioxidant and hepatoprotective activity of leaf and fruit extract were included in this review.

4.2. Data abstraction

Data from each of 19 articles fulfilling the inclusion criteria (that met the selection criteria) were abstracted.

5. RESULTS

Effects of different extracts of *Psidium guajava* on antioxidant enzyme activities and malondialdehyde (MDA) concentration are shown in the table 5.1. There was a significant difference in the antioxidant enzyme activities in the control and treated group with the extracts of *P. guajava* in all studies. MDA level was also significantly different. Table 5.2 shown the antioxidant activity of *Psidium guajava* by DPPH free radical scavenging method expressed as IC50. As indicated in the table 5.2 *Psidium guajava* has moderate antioxidant activity compared to the standard in both studies. Results of the scavenging activity of plant extracts on hydrogen peroxide, superoxide and DPPH are shown in Table 5.3. It could be seen from table 5.3 that plant extract scavenged H₂O₂ and superoxide more effectively than BHA, whereas BHA scavenged DPPH more effectively than plant extract. The effect of *Psidium guajava* on serum ALT, AST, ALP, bilirubin levels and liver weight are shown in table 5.4 and as shown in the table the values for ALT, AST, ALP, bilirubin and liver weight in all studies are different for hepatotoxic group and groups treated with the plant extract.

Table 5.1. Effects of different extracts of *psidium guajava* on antioxidant enzyme activities and malondialdehyde (MDA) concentration.

studies	Study subject characteristics	Plant part used & extraction solvent	Parameters measured	Control group	Treatment group	P value
Nor and Yatim, 2009	Hypertensive rat	Fruit puree	GPX GR	2171.8±65.9 ^a 126.1±14.2 ^a	2594.6±82.8 ^d 148.8±13.2 ^d	P<0.05
Rahmat A et al., 2006	Human(male)	Fruit consumption	GPX GR	466.32 ± 145.61 85.06 ± 26.00	451.10 ± 179.56 80.15 ± 35.94	P<0.05

			Total antioxidant	1.35 + 0.21	1.78 + 0.22*	
Akanji <i>et al.</i> , 2009	Rat	Ethanollic leaf extract	GSH MDA	0.80 ± 0.12*** 13.87 ± 0.53***	2.38 ± 0.14** 5.86 ± 0.38**	P<0.05
Osman <i>et al.</i> , 2011	Rat	Ethanollic leaf extract	GSH SOD CAT	5.97±0.34 ^c 14.86±2.57 ^c 4.88±0.06 ^b	8.01±1.05 ^{ab} 38.68±1.46 ^b 15.41±0.31 ^a	P<0.05
Tajua <i>et al.</i> , 2010	Rat	Aqueous extracts of leaves	GSH GPX SOD CAT	15.74±1.13 13.21±1.77 47.53±0.93 14.30± 1.01	22.30±1.20 17.16±0.84 63.81±1.51 23.40 ± 1.22	P<0.05

Table 5.2. Antioxidant activity of *Psidium guajava* by DPPH free radical scavenging method expressed as IC50.

Studies	Plant part used and extraction solvent	Reference (IC50 value µg/mL.)	<i>P. Guajava</i> extracts (IC50 value µg/mL.)	P value
Vyas <i>et al.</i> , 2010	Hydroalcoholic leaf extract	Ascorbic acid(25.8± 0.204)	45.5± 0.044	P<0.05
Qian and Nihorimbere, 2004	Ethanollic guava leaf extract	Ascorbic acid(50±0.5)	53±2.0	–

Table 5.3. Radical scavenging activity of *Psidium Guajava* expressed by decrement in absorbance.

Study	Plant part & extraction solvents	Types of radical & absorbance maximum	Concentration (mg/ml)	Reference (BHA)	<i>P.guajava</i>	P value
Ogunlana, 2008	Leaf extract of methanol	DPPH(517nm) H ₂ O ₂ (230 nm) O ₂ ⁻ (560 nm)	0.16 0.0543 0.25	0.126±0.001 0.024±0.003 0.142±0.001	0.282±0.026 0.014±0.002 0.011±0.000	—

Table 5.4. Effect of *Psidium guajava* on serum ALT, AST, ALP, bilirubin levels and liver weight.

studies	Study subject characteristics	Plant part used & extraction solvent	Parameters measured	Only PCM or CCl ₄ treated group	Treatment group (<i>P.guajava</i> + PCM or CCl ₄)	P value
Osman <i>et al.</i> , 2011	Rat treated with CCl ₄	Ethanollic leaf extract	ALT(u/m L) AST(u/m L)	94.25±9.05 ^a 198.56±3.79 ^a	55.05±6.03 ^b 82.25±9.38 ^b	P<0.05
Roy and Das, 2010	Rat treated with CCl ₄	Methanolic leaf extract	ALT(u/L) AST(u/L)	384.00±25.02 ^a 642.20±31.01 ^a	16.72±4.09 ^{**} 151.74±13.95 ^{***}	^a P<0.001 ^{***} p<0.001 ^b p<0.05

			ALP(u/L)	749.60±36.25 ^a	488.82±10.01 ^{***}	
			Serum bilirubin(mg/dl)	1.56±0.22 ^a	0.26±0.05 ^{***}	
			Liver weight(g/100gm body weight)	3.78±0.30 ^b	2.99±0.12 [*]	
Tajua <i>et al.</i> , 2010	Rat treated with PCM	Aqueous extracts of leaves	ALT(IU/l)	84.25±1.84 [#]	56.93±1.83 [*]	*P<0.05
			AST(IU/l)	125.73±2.61 [#]	77.58±1.56 [*]	
			ALP(IU/l)	114.72±3.01 [#]	68.40±1.42 [*]	
			Bilirubin	4.84±0.64 [#]	1.75±0.05 [*]	
Uboha <i>et al.</i> , 2010	Normal male rat	Leaf aqueous extract	ALT(IU/L)	9.02 ± 2.10	8.86 ± 2.00 [*]	*P<0.05
			AST(IU/L)	7.12 ± 2.18	6.94 ± 1.86 [*]	
			ALP(IU/L)	94.20 ± 4.82	93.72 ± 5.06 [*]	

Roy <i>et al.</i> , 2006	PCM treated rat	Aqueous extract	ALT(U/L)	384.00±25. 02 ^a	86.94±18.94 ***	^a P<0.001 *** p<0.001
			AST(U/L)	642.20±31. 01 ^a	178.92±8.07 ***	
			ALP(U/L)	749.60±36. 38 ^a	338.32±39.8 0***	
			Serum bilirubin(mg/gl)	1.56±0.22 ^a	0.56±0.04***	
			Liver weight(g /100gm body weight)	4.40±0.14 ^a	2.88±0.02***	
Roy and Das , 2010	PCM treated rat	Methanolic leaf extract	ALT(U/L)	378.25 + 28.45 ^a	132.25 + 7.73***	^a P<0.001 ^b p<0.01
			AST(U/L)	645.61 + 25.38 ^a	237.48 + 15.39***	^c p<0.05 ** p<0.01
			ALP(U/L)	758.66 + 30.58 ^a	479.46 + 7.52***	*** p<0.001
			Serum bilirubin(mg/dl)	1.68 + 0.20 ^b	0.35 + 0.09**	
			Liver weight (g/100g m body weight)w eight)	4.09 + 0.33 ^c	3.13 + 0.07**	

6. DISCUSSION

Evidence suggests that compounds especially from natural sources are capable of providing protection against free radicals. Many solvents are used for extraction of bioactive compounds from the plants. The solvents used vary in their polarity. The most commonly used solvent is methanol. This suggested that the polarity of the active components in guava leaves was lower than water. Since the polarity of methanol was slightly lower than water, this was considered to be one of the most suitable solvents for extraction (Tachakittirungrod et al., 2007), similar observation was obtained in (okonogi et al., 2007). Traditionally, water is used for extraction but it is next to methanol. The use of non polar solvents is comparatively less indicating that the active constituents are soluble in polar solvents only. Generally, any part of the plant can be used for antioxidant studies but most commonly used part is leaf followed by fruit (Chanda and Dave, 2009).

6.1. Antioxidant activity of *Psidium Guajava*

6.1.1. In vitro antioxidant studies - *Guava* leaf extracts

In the study conducted in Taiwan (Yin and Chin, 2007), to determine the antioxidant activity in a linoleic acid system. The antioxidant activities of extracts from guava, using the thiocyanate method (Mitsuda et al., 1996), has been determined and compared with that of polyphenon 60, which is a commercial polyphenol product extracted from green tea. Comparison of antioxidant activity of the extracts from guava at various concentrations is shown in Table 6.1.

Table 6.1. (Yin and Chin, 2007)

Antioxidant activities of extracts from leaves and dried fruit of guava

Sample concentration ($\mu\text{g/ml}$)	Peroxidation (%) ^a							
	Shi Ji Ba	Shui Jing Ba	Tu Ba	Hong Ba	Guava tea A	Guava tea B	Dried fruit	Tea polyphenon 60
50	9.3 \pm 0.4	14.3 \pm 1.9	13.0 \pm 2.6	15.6 \pm 1.1	20.6 \pm 2.9	17.8 \pm 2.6	49.9 \pm 2.7	17.8 \pm 1.8
100	3.8 \pm 0.1	4.0 \pm 0.2	4.4 \pm 0.4	5.0 \pm 0.3	5.7 \pm 1.3	4.7 \pm 0.2	11.6 \pm 0.8	10.1 \pm 1.1
150	4.5 \pm 0.1	4.1 \pm 0.1	4.6 \pm 0.1	4.9 \pm 0.2	3.8 \pm 0.1	3.7 \pm 0.2	7.3 \pm 0.3	11.3 \pm 0.4
200	4.7 \pm 0.2	4.8 \pm 0.4	4.8 \pm 0.1	4.5 \pm 0.1	3.4 \pm 0.2	4.5 \pm 0.1	4.8 \pm 0.2	13.1 \pm 0.4
500	9.0 \pm 0.3	8.0 \pm 0.1	8.7 \pm 0.3	8.9 \pm 0.1	6.3 \pm 0.2	6.2 \pm 0.2	0.7 \pm 0.1	19.5 \pm 1.2

^a Peroxidation (%) = [(absorbance of sample at 500 nm)/(absorbance of control at 500 nm)] \times 100. A low peroxidation (%) indicated a high antioxidant activity.

In all samples, the extracts from Shi Ji Ba showed a stronger antioxidant activity than did other samples at 50 µg/ml. However, the extracts from guava leaves and dried fruit all exhibited 95% inhibition at a concentration of 200 µg/ml. Except for the dried fruit extracts, the other samples were found to have weaker antioxidant activity when the concentration was over 200 µg/ml. The polyphenon 60 showed strong significant prooxidant effects and the maximum antioxidant activity was weaker than those of extracts from guava leaves and guava tea at a concentration of 100 µg/ml. The extracts from dried fruit of guava displayed weaker antioxidant activity than did other samples at 50 µg/ml. However, there was no significant difference in antioxidant activity ($P > 0.05$) between dried fruit of guava and other guava extracts at a concentration of 200 µg/ml. In the above results, the results indicated that 94.4–96.2% of linoleic acid oxidation was inhibited by the addition of guava leaf and guava tea extracts at a concentration of 100 µg/ml. The guava dried fruit extracts exhibited weaker antioxidant effects than did the leaf extracts (Yin and Chin, 2007). The chromatogram data in this study indicated that guava extracts contained phenolic acids, such as ferulic acid, which appeared to be responsible for their antioxidant activity.

The study in India by Vyas and his colleague (Table 5.3) investigated antioxidant activity of *Psidium Guajava* leaf extract by DPPH free radical scavenging method using ascorbic acid as standard. The extract of *Psidium Guajava* leaf extract was found to possess strong antioxidant activity (Vyas et al., 2010). This activity of *Psidium Guajava* extract may be attributed to their free radical-scavenging ability. The extent of antioxidant activity of *P. Guajava* extract was found significant as compared to standard. The antioxidant activity was expressed as IC₅₀; it is the measure of concentration in µg/ml of extract that inhibits 50% of DPPH radicals. IC₅₀ value for *Psidium Guajava* linn leaves extract was found to be 45.5 µg/ml. Thus *Psidium Guajava* linn leaves possess moderate antioxidant activity as compared to the standard, ascorbic acid (Table 6.2). In the same study the reducing power of *Psidium Guajava* linn leaves extract was studied using potassium ferricyanide reduction method, the amount of Fe²⁺ complex was then monitored by measuring the formation of Pearl's Prussian blue at 700 nm. As shown in (Table 6.3) the reducing power of the test drug extract of *Psidium guajava* increased with increase in concentration. Increased absorbance of the reaction mixture indicated the increased reducing

power, thus it is clear that *Psidium Guajava* linn leaves extract possess significant reducing power.

Table 6.2. Antioxidant activity by DPPH method (Vyas et al., 2010)

S.No.	Sample	IC50 value (µg/mL)
1.	<i>P. Guajava</i> extract	45.5±0.044
2.	Ascorbic acid(standard)	25.8±0.204

The data are expressed as mean value ± SD (n=3). All values are significant at P<0.05. Calculated using Graph Pad (ANOVA).

Table 6.3. Reducing power method (Vyas et al., 2010)

S.No.	Concentration(µg/mL) <i>P.guajava</i> extract	<i>P.Guajava</i> Absorbance	Ascorbic acid Absorbance
1.	20	0.483±0.002	0.33±0.001
2.	40	0.704±0.012	0.47±0.014
3.	60	0.815±0.01	0.62±0.200
4.	80	0.901±0.2	0.74±0.001
5.	100	0.982±0.06	0.82±0.010

The data are expressed as mean value ± SD (n=3). All values are significant at P<0.05. Calculated using Graph Pad (ANOVA)

Ferric reducing ability of plasma (FRAP) reducing power was also determined as a mechanism of antioxidant action of guava leaf extract (Tachakittirungrod et al., 2007). The principle of the FRAP method is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous colored form in the presence of antioxidants. The reducing power property indicates that the antioxidant compounds are electron donors and can reduce the oxidized intermediates of the lipid peroxidation process. Figure 6.1 shows the reducing power of guava leaf extracts from different solvents. All extracts had reducing power but not at the same level. The result clearly indicated that the methanol extract of guava leaves had the highest reducing power with the equivalent concentration (EC) value of 3.65 ±0.038 mM/mg extract which is due to its polarity effect to extract most of the active ingredients. This was much higher than that of Butylated

hydroxyl toluene (BHT) but a little lower than quercetin (QCT). BHT and QCT were used as a Reference. The ethyl acetate and butanol extracts showed lower activity with EC values of 1.15 ± 0.132 and 1.36 ± 0.032 mM/mg extract, respectively, which were about 31–37% less than that of the methanol extract. The lowest reducing property was obtained from the hexane fraction. From this point of view, it was confirmed that the methanol fraction of guava leaves possessed highest level of the potent antioxidant compounds. According to its high EC value, it could be considered that compounds in the methanol fraction were good electron donors and could terminate oxidation chain reactions by reducing the oxidized intermediates into the stable form. Therefore the results demonstrated that the mechanism of antioxidant action of guava leaf extracts was free radical scavenging and reducing of oxidized intermediates. The phenolic content in guava leaf fraction played a significant role on the antioxidant activity via reducing mechanisms. Like alpha-tocopherol (vitamin E), phenolic compounds contain chemical structural elements that may be responsible for their antioxidant activities.

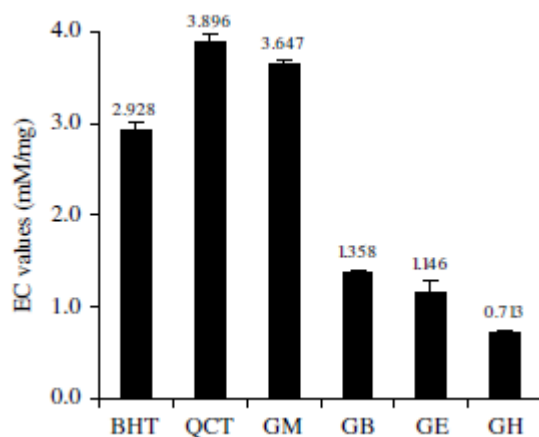


Figure 6.1. Reducing power of Guava leaf extracts from Methanol (GM), Butanol (GB), Ethyl acetate (GE) and Hexane (GH) in comparison with those of butylated hydroxyl toluene (BHT) and Quercetin (QCT). (Tachakittirungrod *et al.*, 2007)

In the study mentioned above three free radicals were used to assess the potential free radical-scavenging activities of guava extracts, namely 2, 2-Azinobis (3-ethylbenzothiazoline-6-sulfonate (ABTS^{•+}) radical, superoxide anion and peroxy radicals (Yin and Chin, 2007). The reduced ABTS is a peroxidase substrate which, when oxidized in the presence of H₂O₂ in a typical peroxidative reaction, generates a metastable radical with a characteristic absorption spectrum at maximum absorption value of 414 nm (Arnao *et al.*, 1996). The ABTS^{•+} radicals are

scavenged by antioxidants via the mechanism of electron-/hydrogen-donation and are assessed by measuring the decrease in absorption at 414 nm. In the superoxide anion-scavenging test, superoxide anion that is derived from dissolved oxygen through the Phenazine Metho sulfate (PMS/NADH) coupling reaction reduces Nitroblue tetrazolium (NBT) and increases absorption at 560 nm. The decrease in absorption at 560 nm with antioxidants indicates the consumption of superoxide anion. In the peroxy radical-scavenging assay, the ability of extracts from guava to scavenge peroxy radicals was measured by monitoring the loss of β -phycoerythrin fluorescence induced by 2, 2-Azo-bis, 2-amidinopropane dihydrochloride (AAPH). Thermal decomposition of AAPH leads to the formation of carbon-centred radicals which, under aerobic conditions, yield alkylperoxy radicals. These radical species can be detected by assay of fluorescent decomposition of β -phycoerthrin, a major pigment protein of sea algae. The absorption assay for antioxidants was based on oxidation of β -phycoerthrin molecules by peroxy radicals. The inhibitions of guava leaf extracts on the fluorescent decomposition of β -phycoerthrin, induced by AAPH, increased with concentration (2.5–10 lg/ml) up to a maximum, and then decreased at the concentration 25–50 lg/ml. This finding was similar to the results of the antioxidant activity assay in a linoleic acid system. The prooxidant effects of guava extracts at high concentrations may be correlated with phenoxy radical formed by the change of phenolic compounds with phenoxy radical reacted with β -phycoerythrin to participate in radical chain propagation.

Correlation analysis between the antioxidant properties and phenolic compounds of extracts from leaves and dried fruit of guava indicated that there was a linear relationship between antioxidant potency, free radical-scavenging ability and the content of phenolic compounds of guava leaf extracts (Yin and Chin, 2007). The calculated coefficients of correlations between antioxidant activity, scavenging effects on radicals and contents of phenolic compounds of guava extracts are shown in table 6.4. The antioxidant activity of guava extracts was significantly correlated with their scavenging effects on superoxide anion ($P < 0.01$), $ABTS^{\cdot+}$ radicals ($P < 0.01$) and peroxy radicals ($P < 0.05$). Therefore, the antioxidant activities of guava extracts may be due to their scavenging effects on radicals and blocking of the chain reaction in the peroxidation of linoleic acid. For scavenging effects on radicals, high correlations ($R = 0.921$ – 0.991) were observed between various radicals, indicating that these three methods

have satisfactory correlations for the examination of antioxidants. The antioxidant activity ($P < 0.05$) and scavenging effects on superoxide anion, $ABTS^{\cdot+}$ radicals and peroxy radicals ($P < 0.01$) of guava extracts was also well correlated with their contents of total phenolic compounds. The same trends were observed in the correlation of the content of gallic acid and the antioxidant activity and scavenging effects on superoxide anion. According to recent reports, a highly positive relationship existed between total phenolics and antioxidant activity in many plant species (Nabasree and Bratati, 2004, Dorman and Hiltunen, 2004).

In another study in China by Qian and Nihorimbere, as shown in table 5.2 antioxidant activity of different extracts from guava leaf according to the DPPH \cdot radical scavenging method was determined. The results in (figures 6.2 and 6.3) showed that the decrease in absorbance of the DPPH \cdot radical was due to its reduction by different antioxidants. Absorbance decreases as a result of a color change from purple to yellow as the radical was scavenged by antioxidant through donation of hydrogen to form the stable DPPH-H. The data showed that DPPH \cdot solution was bleached with all the samples tested. However, differences could be observed through different antioxidants used and their concentrations.

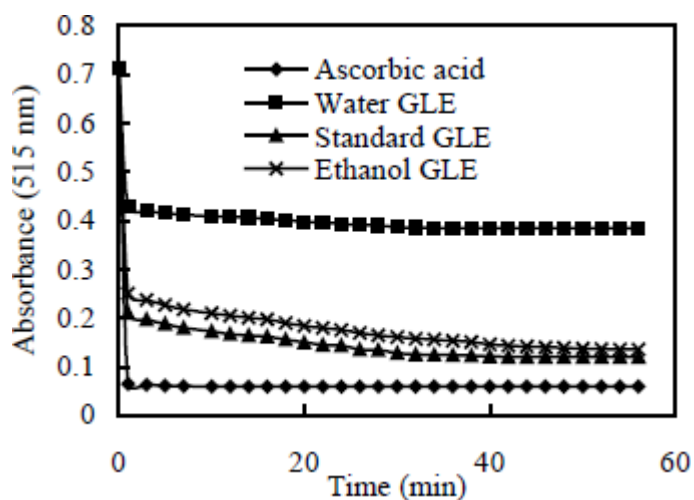


Figure 6.2. Time course of absorbance reduction of 4 ml of 6×10^{-5} mol/L 2,2-diphenyl-1-picrylhydrazyl (DPPH \cdot) in methanol and each tested sample (ascorbic acid, water GLE, ethanol GLE, standard GLE) at concentration: 0.25 mg/ml, by the decolorization reaction of DPPH \cdot radical from purple to yellow. GLE: guava leaf extracts (Qian and Nihorimbere, 2004).

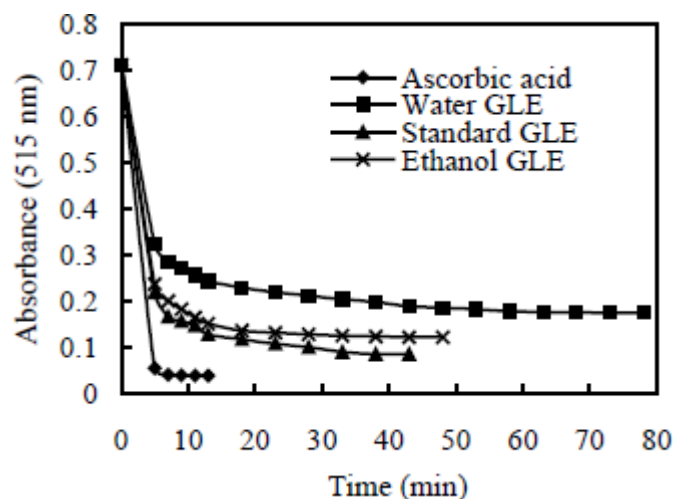


Figure 6.3. Time course of absorbance reduction of 4 ml of 6×10^{-5} mol/L 2,2-diphenyl-1-picrylhydrazyl (DPPH') in methanol and each tested sample (ascorbic acid, water GLE, ethanol GLE, standard GLE) at concentration:0.5 mg/ml, by the decolorization reaction of DPPH' radical from purple to yellow. GLE: guava leaf extracts. (Qian and Nihorimbere, 2004).

Table 6.4. Correlation between the antioxidant properties and phenolic compounds of extracts from leaves and dried fruit of *Guava* (Yin and Chin, 2007)

	Antioxidant activity ^a	Scavenging effects ^a			Phenolic compounds		
		Superoxide anion	ABTS ⁺ radicals	Peroxy radicals	Total	Gallic acid	Ferulic acid
Antioxidant activity	1.000						
Scavenging effects							
Superoxide anion	0.876*	1.000					
ABTS ⁺ radicals	0.880*	0.991*	1.000				
Peroxy radicals	0.853**	0.921*	0.944*	1.000			
Phenolic compounds							
Total	0.797**	0.927*	0.909*	0.875*	1.000		
Gallic acid	0.810**	0.759**	0.698	0.585	0.688	1.000	
Ferulic acid	0.685	0.763**	0.729	0.631	0.682	0.895*	1.000

^a The concentrations of guava extracts in the antioxidant activity, scavenging effects on superoxide anion, ABTS⁺ radicals and peroxy radicals used for correlation analysis were 50, 200, 10, 2.5 µg/ml, respectively.

* $P < 0.01$.

** $P < 0.05$.

Table 6.5 shows that ascorbic acid is superior inhibitor of DPPH' compared to guava leaf extracts. From (Table 6.5), ascorbic acid showed high percentage inhibition while standard guava leaf extracts, ethanol guava leaf extracts and water guava leaf extracts respectively, showed decreasing inhibition effect for all concentrations.

Table 6.5. Comparison of antioxidant activity of guava leaf extracts with ascorbic acid expressed as % inhibition (Qian and Nihorimbere, 2004)

Concentration (mg/mL)	Ascorbic acid	STD (GLE)	Ethanol (GLE)	Water (GLE)
0.1	35.76	31.69	27.62	18.23
0.25	91.58	83.02	75.87	46.14
0.4	92.42	85.69	80.92	47.12
0.5	94.38	87.79	82.74	75.13

GLE: Guava leaf extract, STD: Standard

For all the antioxidants, with higher the concentrations, the bleaching ability of the DPPH' solution was nearly complete. Ascorbic acid was substantially more active in all the concentrations used (Table 6.5) in comparison with different extracts from guava leaf. With high concentration of ascorbic acid, it was possible to observe nearly a 100% free radical scavenging effect. Ethanol GLE and standard GLE had nearly the same effect for the similar concentrations used. As for ascorbic acid, ethanol GLE, standard GLE, water GLE, a clear gradient activity was seen from the first to the last concentration used (Table 6.5). Ascorbic acid, known to act as antioxidant and different extracts from guava leaf showed good free radical-scavenging activity depending on the concentration used. The higher the concentration used the higher the free radical-scavenging effect. Total phenolic content values of standard GLE, ethanol GLE and water GLE were 598.25 ± 4 , 575.3 ± 15.5 and 511.6 ± 6.2 mg of gallic acid equivalent (GAE)/g of dry weight material, respectively. The average of total phenolic content of standard, ethanol and water GLE were significantly different at $P < 0.005$. Ascorbic acid, a potential antioxidant, was used as positive control of antioxidant activity compared with the extracts of guava leaf found to contain significant amounts of natural phenolics that are antioxidants. Guava leaf extracts (GLE) analysis revealed they contain different phenolic compounds such as 1-tannic acid; 2-procatechuic acid; 3-caffeic acid; 4-ferulic acid; 5-rutin trihydrate; 6-quercetin dihydrate (the HPLC profile was not reported). Due to the diversity and complexity of the natural mixtures of phenolic compounds in the different extracts of guava leaf, it is rather difficult to characterize every compound and assess or compare their

antioxidant activities (Zheng and Wang, 2001). This study showed that the amount of ethanol guava leaf extracts to total phenolic content was higher than that of water guava leaf extracts. Moreover, it can be seen from (Table 6.5) that ethanol GLE exhibited higher antioxidant activity (expressed in terms of percentage inhibition) than water guava leaf extracts for all the concentrations tested. Antioxidant activity of the extracts may also be attributable to unidentified substances or to synergistic interactions. Therefore guava leaf extracts showed potential antioxidant activity and can be used to extend the shelf life of foodstuffs, to reduce wastage and nutritional losses by inhibiting and delaying oxidation. The higher the sample concentration used, the stronger was the free radical scavenging effect. Increased intake of guava leaf extracts is therefore good for our health.

In another In vitro study by (Ogunlana , 2008), Assessment of the ability of the extract to scavenge the Reactive Oxygen Species (ROS), hydrogen peroxide, superoxide and the synthetic radical 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was determined with reference to the synthetic antioxidant Butylated hydroxyanisole (BHA) as indicated in (Table 5.2). Plant extract showed concentration- dependent scavenging activity on all reactive species used. Scavenging activity of plant extract on hydrogen peroxide and superoxide was more than that of BHA on same concentration. The remarkable mop-up potential of hydrogen peroxide and superoxide by the plant leaves could present a possible amelioration for the damages connected with hydroxyl radical to macromolecules by the following mechanisms among others:

Scavenging hydrogen peroxide, Scavenging superoxide anion radical and inhibiting the formation of hydroxyl radical from both hydrogen peroxide and superoxide as powered by Haber-Weiss reaction. Hydrogen peroxide only initiates lipid peroxidation weakly (Cohen and Heikkila, 1974). However, its ability to produce active oxygen species is due to its ability to generate highly reactive hydroxyl radical through the Fenton reaction (Namiki , 1990). The ability of plant extract to scavenge H_2O_2 could also reflect its ability to inhibit the formation of hydroxyl radical in vivo. However, BHA showed greater DPPH scavenging activity than plant extract. This may have been due to BHA's possession of a methoxy group which increases the accessibility of the radical centre of DPPH to BHA (Chin and Der, 1994). In the study in Thailand investigating Antioxidant Active Principles Isolated from *Psidium guajava* Grown in Thailand

(Okonogi et al., 2007). Antioxidant active compounds were isolated from methanol crude extracts of the leaves of Guava (*Psidium guajava* L.) grown in Thailand. The isolated compounds were screened for their *in vitro* antioxidant activity by a DPPH free radical scavenging assay. Results indicate that three isolated compounds contribute importantly to the antioxidant activity of guava leaves, providing a scientific basis for the use of this plant in traditional medicine. Their structures were determined on the basis of spectroscopic and chemical methods. The most active compound was found to be quercetin along with two flavonoid compounds, quercetin-3-*O*-glucopyranoside and morin. In this study; the comparative biological activity of three compounds isolated from guava leaves was evaluated as their antioxidant capacity to scavenge DPPH free radicals. It was found that all three isolated principles, quercetin, quercetin-3-*O*-glucopyranoside and morin had antioxidant activity but at different levels as with the IC₅₀ of 1.20±0.02, 3.58±0.05 and 5.41±0.20 µg/ml, respectively. It was clearly seen that quercetin is the most active principle in Thai guava leaves, followed by quercetin-3-*O*-glucopyranoside and morin, respectively. This result could be explained by the higher antioxidant activity being related to the greater number of hydroxyl groups on the flavonoid nucleus.

6.1.2. Human study - Guava fruit extracts

Human study in Malaysia (Rahmat et al., 2006), investigating the effects of guava (*Psidium guajava*) consumption on total antioxidant status as indicated in (Table 5.1) and lipid profile (total cholesterol, triglycerides, LDL cholesterol and HDL-cholesterol) in 28 normal male youth the study was carried out over nine weeks, which was divided into three phases, that is, baseline (one week), treatment (four weeks) and control (four weeks). There was a significant increase of total antioxidants during the treatment phase, compared to the baseline and control phases ($p < 0.05$). The increase in total antioxidant status was probably due to increased antioxidant consumption contributed from guava which has been reported to be high in antioxidant vitamins such as ascorbic acid, β-carotene, lutein, zeaxanthin and lycopene. There were trends of reduction for both glutathione peroxidase and glutathione reductase in the treatment phase as compared to baseline and control phases. However, the reduction was not statistically significant. The reduction of antioxidant enzymes was associated with decreased

oxidative stress and decrease in free radical activities, because antioxidant in plasma can act as a good indicator of oxidative stress in humans (Polidori et al., 2001). The consumption of guava, therefore, could result in improved antioxidant status. Thus, it could reduce the risk of disease caused by free radical activities.

6.1.3. In Vitro antioxidant Studies - Guava fruit Constituents

Study in Spain by Marquina and his colleagues (Marquina et al., 2008) measured the total polyphenol content and antioxidant capacity of the peel, shell, and pulp of the fresh guava fruit. Processed guava pulp and jam were also analyzed. The phenolic content was highest in the guava skin (10 times higher antioxidant capacity than pulp), and lowest in the jam.

In another Correlation study between Some Nutritional Components and the total Antioxidant Capacity, Measured with Six Different Assays in Eight Horticultural Crops (Corral et al., 2008) in vitro measurements of total antioxidant capacity (AOC) of guava and other fruit extracts was conducted. Total soluble phenolics, vitamin C, vitamin E, β -carotene, and total carotenoids were correlated with the total AOC of hydrophilic and lipophilic extracts from guava, avocado, mango, papaya, prickly pear fruit, and strawberry. Among the assays, hydrophilic extracts (HPEs) of guava had the highest AOC value, whereas those from the papaya and prickly pear fruit the lowest values.

In Similar study exhibited (Thaipong et al., 2005) at evaluating hydrophilic antioxidant activity (AOAH) and the lipophilic antioxidant activity (AOAL); and their correlations with vitamin C, and total phenolic and β -carotene contents in fresh guava fruits of one white flesh clone and three pink flesh clones Both white and pink flesh guavas showed high hydrophilic antioxidant activity and compounds for phenolic and vitamin C indicated that regular consumption of guava might be beneficial to health. That hydrophilic antioxidant activity, the major activity, had high correlations with both total phenolic and vitamin C indicated that the use of the total phenolic or vitamin C content to determine antioxidant activity level in guava fruit was feasible. Phenolic and vitamin C are the major contributors to the antioxidant activity of guava fruits, while the contribution of carotenoid is negligible.

In the study investigating guava Fruit (*Psidium guajava* L.) as a New Source of Antioxidant Dietary Fiber (Jiménez-Escrig et al., 2001), Pulp and peel fractions of *p.guajava* were tested, and

both showed high content of dietary fiber (48.55–49.42%) and extractable polyphenols (2.62–7.79%). 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.

6.1.4. In Vivo Antioxidant Studies - *Guava* leaf extract

In vivo antioxidant related study was one investigating the effects of the aqueous leaf extract of *P. guajava* on reduced glutathione(GSH) and malondialdehyde (MDA) concentrations in rats experimentally infected with *Trypanosoma brucei brucei* (Akanji *et al.*, 2009). In this study it was demonstrated that *T. brucei* infection resulted in high amounts of MDA and low concentrations of GSH as indicated in (Table 5.2).In the *T. brucei*-infected animals however the aqueous extract of *P. guajava* leaf was able to reduce the trypanosomosis associated lipid peroxidation as well as raise the level of GSH in the treatment group. It was speculated that the ability of the leaf extract of *P. guajava* to lower the MDA concentrations in the treatment group may be attributed to its antioxidant properties.

6.1.5. In Vivo Antioxidant Studies - *Guava* fruit extract

In another in vivo study(Nor and Yatim , 2009) aimed to determine the effects of pink guava puree supplementation on enzyme activities (Table 5.1), and kidney and liver function by carrying out tests on Spontaneous Hypertensive Rats (SHR). Twenty-four male SHR were divided into four groups [control, CG (distilled water); low dosage group, LDG (0.5 g/kg body weight); medium dosage group, MDG (1.0 g/kg body weight); high dosage group, HDG (2.0g/kg body weight)].The results of this study indicated that the specific activities of glutathione

peroxidase (GPx) was significantly higher in LDG (2332.5±81.8 U/l), MDG (2424.8±97.1 U/l) and HDG (2594.6±82.8 U/l) respectively, as compared to CG (2171.8±65.9 U/l). Significant differences were also seen in glutathione reductase (GR) activities among all treated groups [LDG (132.5±11.8 U/l), MDG (141.5±16.4 U/l), HDG (148.8±13.2 U/l) compared to CG (126.1±14.2 U/l)].The result, Therefore, indicated that administering pink guava puree to the HFD-induced obese rats significantly increased those antioxidant enzyme activities. The benefits of *Guajava* plant-based foods may be a consequence of bioactive phytochemicals found in these foods.

These findings therefore, suggested that the single ingestion of leaf or fruit extract can act as antioxidant via free radical scavenging and reducing oxidized intermediates, the phenolic content in guava leaf and fruit fraction played a significant role on the antioxidant activity.

6.2. HEPATOPROTECTIVE ACTIVITY OF *PSIDIUM GUAJAVA*

All studies reviewed under this section were in vivo hepatoprotective studies of *Guava* leaf extract.

Carbon tetrachloride (CCl₄) and Paracetamol (PCM) as a cause of Hepatotoxicity

Carbon tetrachloride and paracetamol converted into reactive oxidative metabolites by hepatic microsomal enzymatic system, which causes hepatotoxicity (Brent and Rumack, 1993).It is well documented that CCl₄ is biotransformed under the action of cytochrome P450 in the microsomal compartment of liver to trichloromethyl radical which readily reacts with molecular oxygen to form trichloromethyl peroxy radical. The hepatotoxic effects of CCl₄ are largely due to its active metabolite trichloromethyl radical and trichloromethyl peroxy radical. Both the radicals can bind covalently to the macromolecules and induce peroxidative degradation of the membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids .This leads to the formation of lipid peroxides followed by various changes in biochemical parameters (Sangeetha and krishnakumari, 2010).PCM overdose causes severe hepatotoxicity that leads to liver failure in both humans and experimental animals a small amount of PCM is metabolized together with cytochrome P450. As a result, N-acetyl-p-benzoquinone imine (NAPQI) or Nacetyl-p-benzosemiquinone imine (NAPSQI) appears in the body's system. Both these compounds are

very active chemical and their chemical structures indicate that they are capable of taking part in free radical reactions. Consequently, PCM overdose can lead to a number of unfavorable consequences, especially those affecting the liver. A large dose of this drug causes depletion of GSH level in the liver, because NAPQI reacts rapidly with glutathione, which consequently exacerbates oxidative stress in conjunction with mitochondrial dysfunction. Thus, the GSH depletion, especially occurring in acute hepatotoxicity, affects liver functions and leads to massive hepatocyte necrosis, liver failure or death (Tajua et al., 2010).

Toxicity study on *P.guajava*

The toxicology study was performed on the leaves of *Psidium guajava* L. calculating mean lethal dose in Swiss mice and alternative toxicology in Wistar rats. No deaths were observed in the toxicological results of the two experimental models in the dose range using up to 2g/kg body weight. Acute toxicity tests in rats and mice have proven the LD50 of guava leaf extracts to be more than 5g/kg. (Roy et al., 2006, Roy and Das, 2010, Gupta et al., 2011, Roy and Das, 2010).

6.2.1. Effect of *P.guajava* on serum enzymes and total bilirubin levels in hepatotoxicity

The study in India evaluated the hepatoprotective activity of *Psidium guajava* (PGJ) in acute experimental liver injury induced by paracetamol (PCM) as indicated in table (5.4) (Tajua et al., 2010). Serum transaminases, ALP and total bilirubin levels are susceptible to hepatotoxin and serve as markers of liver damage which promotes the release of such serum enzymes from hepatocytes into blood stream. The effects of pretreatment with *P.guajava* and *silymarin* on the PCM-induced elevation of serum enzymes AST, ALT, ALP and total bilirubin activities are shown in (Table 6.6). PCM induced elevation were found to be 125.73 ± 2.61 IU L⁻¹ in AST, 84.25 ± 1.84 IU L⁻¹ in ALT and 114.72 ± 3.01 IU L⁻¹ in ALP indicating that PCM have inflicted a significant damage to liver (Table 6.6)

Table 6.6. Effect of Psidium *guajava* on serum marker enzymes and total bilirubin level in PCM induced hepatotoxicity in rat. (Tajua et al., 2010).

SNo.	Groups	AST (IU/l)	ALT (IU/l)	ALP (IU/l)	Total bilirubin (mg/dl)
I	Control	76.52±1.37	55.46±2.35	63.61±1.09	1.51±0.15
II	PCM treated	125.73±2.61#	84.25±1.84#	114.72±3.01#	4.84±0.64#
III	PCM+Silymarin	79.78±1.59*	56.98±1.79*	70.40±1.48*	1.81±0.11*
IV	PCM+P.guajava	77.58±1.56*	56.93±1.83*	68.40±1.42*	1.75±0.05*

*Explanations: Each value represents the mean ± SD of six animals. #significant difference at P < 0.05 (BMRT) compared with the control. *significant difference at P < 0.05 (BMRT) compared with the PCM treated group.*

Pre-treatment of *P.guajava* 1 h prior to PCM administration significantly protected the elevation of transaminases, ALP and total bilirubin activities. The activities of AST, ALT and ALP in the high dose *P.guajava* plus PCM treated group were $77.58 \pm 1.56 \text{ IU L}^{-1}$, $56.93 \pm 1.80 \text{ IU L}^{-1}$ and $68.40 \pm 1.42 \text{ IU L}^{-1}$, respectively. Similarly, the activity of total bilirubin significantly ($P < 0.05$) decreased in *P.guajava* plus PCM treated group ($1.75 \pm 0.05 \text{ mg dl}^{-1}$) in comparison with the PCM induced hepatotoxic group ($4.84 \pm 0.16 \text{ mg dl}^{-1}$).

In this study, the plant *P.guajava* was observed to exhibit hepatoprotective effect as demonstrated by a significant decrease in the serum transaminases, ALP and total bilirubin level in rat induced with PCM hepatotoxicity. Moreover, the *P.guajava* enhanced the activities of antioxidant enzymes (GSH, GPx, SOD and CAT) against the PCM-induced hepatotoxicity in these animals as indicated in (Figure 6.4 (A-D)), suggesting that the reduction of oxidative stress in this scenario likely plays a role in the mechanism of its hepatoprotective effects. The liver is a major target organ for toxicity of xenobiotics and drugs, because most of the orally ingested chemicals and drugs first go to liver where they are metabolized into toxic intermediates. Various pharmacological or chemical substances are known to cause hepatic injuries such as paracetamol and CCl_4 (Gutiérrez et al., 2008). Excessive dose exposure to these hepatotoxins may induce acute liver injury characterized by abnormality of hepatic function and degeneration, necrosis or apoptosis of hepatocyte (Higuchi and Gores, 2003). With the increasing ingestion of drugs or exogenous chemicals, the possibility of liver injury will undoubtedly increase (Lee, 2003). At present, drug or chemical-induced liver injury has become

a major clinical problem. Much of attention should be paid to the mechanisms involving drug or chemical-induced liver injury. In addition, the search for effective therapeutical methods for the treatment of drug or chemical-induced liver injury is also very important (James *et al.*, 2003). With respect to PCM dependent hepatotoxicity it is generally accepted that P450-dependent bioactivation of PCM is a main cause of potentially fulminant hepatic necrosis upon administration or intake of lethal dose of PCM (Masubuchi *et al.*, 2005). In the course of acute liver failure, oxidative stress expressed by oxidant-antioxidant imbalance is profound in liver tissue. In recent studies PCM was found to induce substantial mitochondrial oxidative stress and peroxy nitrite formation (Knight *et al.*, 2001). This oxidative stress preceded cell injury by several hours (Jaeschke, 2003) and free radical scavengers attenuated PCM induced liver injury (Knight *et al.*, 2002). Serum transaminases (AST & ALT) and ALP activities have long been considered as sensitive indicators of hepatic injury and results indicate increased levels of serum transaminases was noted (Jain *et al.*, 2003). ALP has been attributed to the damaged structural integrity of the liver, because these are cytoplasmic in location and are released into circulation after cellular damage (Recknagel *et al.*, 1991). In the assessment of liver damage by PCM the determination of enzyme levels such as serum transaminases and ALP is largely used. Therefore, the marked release of transaminases and ALT into the circulation indicates severe damage to hepatic tissue membranes during PCM intoxication. In the present study, a single oral dose of PCM at 1g/kg caused a dramatic elevation in the serum transaminases and ALP activities (Ross, 2005), indicating an acute hepatotoxicity induced by administration of PCM (Table 6.6). High doses of PCM have been demonstrated to increase the serum levels of AST and ALT (Kozer *et al.*, 2003). The significantly decreased serum transaminases and ALP activities in the P.guajava and silymarin administered groups prior to PCM demonstrated its hepatoprotective effect.

Serum bilirubin is one of the most sensitive tests employed in the diagnosis of hepatic diseases. It provides useful information on how well the liver is functioning (Harper, 1961). Bilirubin, a chemical breakdown product of hemoglobin, is conjugated with glucuronic acid in hepatocytes to increase its water solubility. Bilirubin concentration has been used to evaluate chemically induced hepatic injury. Besides various normal functions liver excretes the breakdown product

of hemoglobin namely bilirubin into bile. Pre-treatment of *P.guajava* prevented severity of liver damage caused by PCM as evidenced by the low level of bilirubin in the serum (Table 5.4, 6.6). In another similar studies in rats (Roy et al., 2006, Roy and Das, 2010), hepatoprotective activity of methanolic extracts of *p.guajava* was evaluated in liver injury induced by both CCl₄ and PCM, the results were as indicated in the (Table 6.7).

Table 6.7. Effect of Silymarin and *p.Guajava* methanolic leaf extract (MT) on serum ALT, AST, ALP, bilirubin level and liver weight in CCl₄ and Paracetamol (PCM) induced acute liver injury in rats (Roy et al., 2006, Roy and Das, 2010).

[Values are mean ± SE from 6 animals in each group]

Treatment	Dose (po) (mg/kg)	ALT (U/L)	AST (U/L)	ALP (U/L)	Serum bilirubin (mg/dl)	Liver weight (g/100gm body weight)
Vehicle control	--	72.86±6.19	186.36±7.81	398.18±6.73	0.26±0.08	3.2±0.09
CCl ₄ control	--	384.00±25.02 ^a	642.20±31.01 ^a	749.60±36.25 ^a	1.56±0.22 ^a	3.78±0.30 ^b
CCl ₄ + Silymarin	100	57.22±2.81 ^{***}	205.46±6.12 ^{***}	408.02±5.58 ^{***}	0.28±0.03 ^{***}	3.10±0.06 ^{ns}
CCl ₄ + PGJ	250	60.10±7.70 [*]	572.66±43.16 ^{ns}	539.96±79.45 ^{***}	0.26±0.11 ^{***}	3.18±0.07 ^{ns}
CCl ₄ + PGJ	500	16.72±4.09 ^{**}	151.74±13.95 ^{***}	488.82±10.01 ^{**}	0.26±0.05 ^{***}	2.99±0.12 [*]

P values: ^a ≤0.001 vs. vehicle control, ^b ≤0.05 vs. vehicle control, ^{ns}>0.05, ^{*} ≤0.05, ^{**} ≤0.01, ^{***} ≤0.001 vs. CCl₄ treated control

After 48 hr of administration of both PCM and CCl₄, the serum levels of ALT, AST, ALP and bilirubin were markedly increased. *P.guajava* methanolic extracts (250mg/kg & 500mg/kg) significantly reduced the elevated serum levels of AST, ALT, ALP and bilirubin, the effect with 250mg/kg is less effective than the effect with 500mg/kg. Both PCM and CCl₄ induced the increase in liver weight, due to the blocking of secretion of hepatic triglyceride into the plasma. However the higher dose of the methanolic extract (500mg/kg) prevented the increase in liver weight when compared to hepatotoxin treated control. The hepatotoxic effects of both PCM and CCl₄ are largely due to generation of free radicals. Therefore drugs having antioxidant activity are effective in treating PCM and CCl₄ induced hepatotoxicity by scavenging the free radicals generated by the action of both PCM and CCl₄. Different extracts of *P.guajava* including methanolic extract reported to increase the reduction of DPPH (Qian and Nihorimbere, 2004).

Therefore the methanolic extracts of *P.guajava* Linn leaves showed good hepatoprotective activity in PCM and CCl₄ induced liver damage. The hepatoprotective activity may be due to the antioxidant effect of the plant. Similar hepatoprotective activity of aqueous extracts of *P.guajava* was also determined in the study designed to investigate the effects of aqueous extract of *Psidium guajava* leaves on the histology and biochemical indices of liver function as well as hematological indices in rats (Uboha et al., 2010). The results of this study suggested that aqueous extract of *Psidium guajava* leaves may be hepatoprotective, and not hepatotoxic, with hematopoietic potentials in both male and female rats. The photochemical report of this study suggested that the hepatocellular function-enhancing effect of the extract may result from the action of the various extracts' contents, especially the presence of flavonoids which have been reported to have antioxidative effects (Middleton, 1996).

In another comparative study as indicated in (Table 5.4) by (Roy and Das, 2010) designed to evaluate the hepatoprotective activity of different extracts (petroleum ether, chloroform, ethyl acetate, methanol and aqueous) of *P. guajava* in acute experimental liver injury induced by carbon tetrachloride and paracetamol. The effects observed were compared with a known hepatoprotective agent, silymarin (100 mg/kg). The methanolic extract of *Psidium guajava* leaves showed significant hepatoprotective activity when administered at doses of 200 mg/kg orally. The effect produced by the dose of methanolic extract of *P. guajava* leaves was similar to that produced by silymarin (100mg/kg), a well known hepatoprotective agent. Damage induced in the liver is accompanied by the increase in the activity of some serum enzymes. In the acute liver damage induced by different hepatotoxins, The anti-hepatotoxic action of *P. guajava* methanolic leaf extract was substantiated by significant attenuation of the increased levels of serum enzymes, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and bilirubin in carbon tetrachloride and paracetamol induced hepatotoxicity than the other extracts. The CCl₄ and PCM induced significant increase in liver weight was also observed in the study, which is due to blocking of secretion of hepatic triglycerides into the plasma. Silymarin and methanolic extract of *Psidium guajava* (200mg/kg) also prevented the increase of liver weight in rats. Therefore methanolic extract of leaves of *Psidium guajava* plant possesses

better hepatoprotective activity compared to other extracts. It was suggested in the study as the hepatoprotective activity may be due to the anti-oxidant effect of the plant.

In another study in Egypt (Osman *et al.*, 2011) the hepatoprotective effect of guava against carbon tetrachloride-induced hepatotoxicity in rats liver was evaluated (Table 5.4), a significant decrease in the total protein and serum albumin contents was observed in treated rats with carbon tetrachloride. However significant increase in the serum total protein content and albumin were noticed in the rats treated with ethanolic extracts or silymarin when compared with CCl₄-treated group and a significant increase in liver homogenate MDA level, activities of lysosomal enzymes (ACP, β -GAL and β -NAG) and activities of ALT, AST, GGT was observed in CCl₄ alone treated rats when compared with the normal control but the remarkably reduction in the homogenate MDA levels or lipid peroxidation, the activities of ALT, AST, GGT and lysosomal enzymes (ACP, β -GAL and β -NAG) were noticed in the rats treated with ethanolic extracts or silymarin and these were no significant difference when compared with normal control group. These results indicated that the active ingredient of the *P.guajava* extracts have an inhibitory effects on membrane permeability of lysosomes. It may be due to the effect of antioxidant compounds present on the ethanolic extracts on the constituents of phospholipids bilayers of the membrane of rat liver lysosomes. This inhibitory effect appeared to have stabilizing effect on the membrane fluidity.

6.2.2. Effect of *P.Guajava* on reduced glutathione (GSH), Glutathione

Peroxidase (GPx), superoxide dismutase (SOD) and Catalase (CAT) activities.

In the study (Tajua *et al.*, 2010) Effect of *P.guajava* on Reduced glutathione (GSH), Glutathione peroxidase (GPx), superoxide dismutase (SOD) and Catalase (CAT) activities was evaluated in the rats liver homogenate. The results was as indicated in the (Table 5.4) and figures below:-

Figure 6.4 A

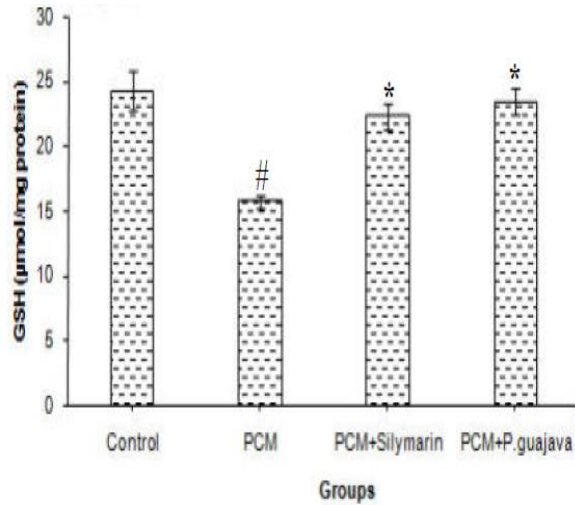


Figure 6.4 B

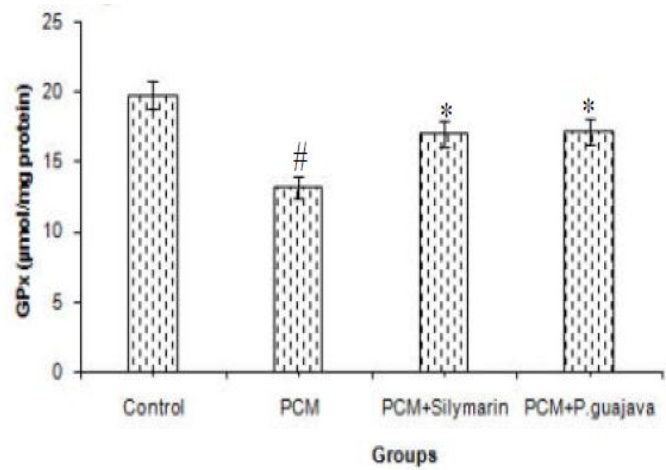


Fig 6.4 C

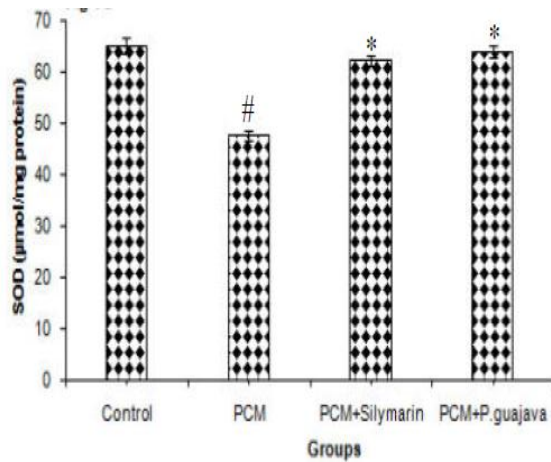


Figure 6.4 D

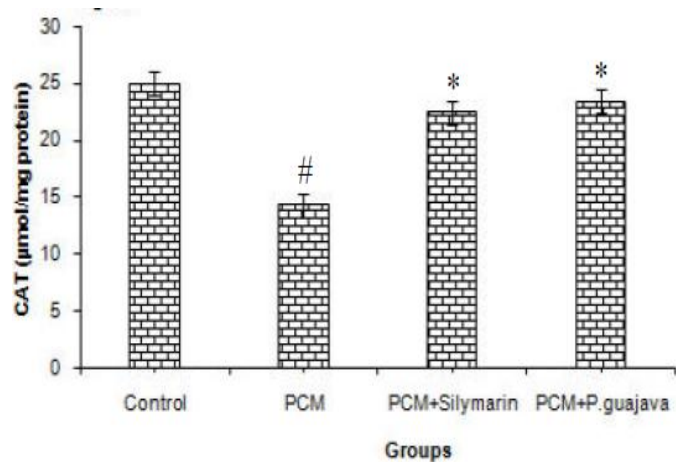


Figure 6.4(A-D) Hepatoprotective effect of *P.guajava* on liver. GSH (Figure 5.4A), GPx (Figure 5.4B), SOD (Figure 5.4C) and CAT (Figure 5.4D) level in PCM induced hepatotoxicity in rat. Each bar represents the mean \pm SD of six animals. # $P < 0.05$ Bonferroni's multiple comparison tests (BMRT)) significant difference from control. * $P < 0.05$ (BMRT)) significant difference from PCM treated group. (Tajua et al., 2010)

Reduced glutathione is a substrate for glutathione related enzymes, and a regenerator for alpha-tocopherol. Therefore, it plays an important role in the antioxidant defense system (Meister, 1991). It is well known that a large dose of PCM causes hepatic GSH depletion because NAPQI reacts rapidly with glutathione (Masubuchi et al., 2005), which consequently exacerbates oxidative stress in conjunction with mitochondrial dysfunction. The GPx present in the cells can catalyze this reaction. Depletion of GSH below a threshold value was associated

with a significant conversion of xanthine dehydrogenase to reversible xanthine oxidase, a superoxide radical generation reaction catalyzing enzyme (Cighetti *et al.*, 1993). In this study, the hepatic content of GSH and GPx were found to be decreased significantly in PCM intoxicated rats compared with the control rats (Figure 6.4A and 6.4B). However, concomitant administration with *P.guajava* significantly prevented the PCM induced depletion of hepatic GSH and GPx, indicating the antioxidant effect *P.guajava* and *silymarin* in PCM intoxicated rats (Yin and Chin, 2007). All evidence, including serum enzyme activity, GSH level and damage markers show that *P.guajava* diet could decrease PCM induced oxidative stress. Living tissues are endowed with innate antioxidant defense mechanisms, such as the presence of the enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (Qian and Nihorimbere, 2004). A reduction in the activities of these enzymes is associated with the accumulation of highly reactive free radicals, leading to deleterious effects such as loss of integrity and function of cell membranes (Sheela and Angusti, 1995). Antioxidant enzymes such as SOD and CAT are easily inactivated by lipid peroxides or reactive oxygen species, which results in decreased activities of these enzymes in PCM toxicity (Gao *et al.*, 2006). CAT is most abundant in the liver and is responsible for the catalytic decomposition of H₂O₂ to oxygen and water. SOD is an extremely effective antioxidant enzyme, and is responsible for catalytic dismutation of highly reactive and potentially toxic superoxide radicals to H₂O₂ (Reiter *et al.*, 2000). The antioxidant effects of *P.guajava* and *silymarin* on the PCM induced depletion of hepatic antioxidant enzymes are presented in (Figure 6.4C and 6.4D). The activities of SOD and CAT in the PCM group were significantly decreased when compared with the control group. The results strongly suggest that the significant decrease of hepatic CAT and SOD activities observed in rats treated with PCM may be largely affect due to increased free radical production caused by administration of PCM (Krishnakantha and Lokesh, 1993). In rats treated with *P.guajava*, however, the activities of these antioxidant enzymes were significantly higher than in the rats exposed to PCM alone. *P.guajava* is similarly rich in phytochemical and exhibits antioxidant capacity against oxidative stress (Qian and Nihorimbere, 2004). Hypopharyngeal gland protein of rat enhances the proliferation of primary cultured rat hepatocytes *invitro* and this protein was present in *P.guajava* (Yin and Chin, 2007). All evidence, including serum enzyme activity,

GSH level and damage markers show that a *P.guajava* diet could decrease PCM-induced oxidative stress.

In the similar study in Egypt (Osman *et al.*, 2011) levels of superoxide dismutase(SOD),Catalase(CAT), glutathione-S transferase(GST), reduced glutathione (GSH) and lipid peroxidation was evaluated.As indicated from the results (Table 5.4) in hepatoprotective period, treated rats with carbon tetrachloride showed a significant decrease in the GST,SOD,CAT and GSH activity. Treatment rats with guava leaves ethanolic extracts or silymarin significantly increased the GST, SOD, CAT and GSH activity when compared with CCl₄-treated group. Guava leaves ethanolic extract exerted a highly inhibitory effect on the decrease of GST, SOD, CAT and GSH activity .In the curative period as can be seen, the highest increase on homogenate GST, SOD, CAT and GSH activity was noticed in the rats treated with guava leaves ethanolic extracts with no significant difference when compared with normal group. This potential antioxidant activity may be due to the high content of phenolic compound in guava leaves ethanol extract that played a significant role on the antioxidant activity as stated by (Qian and Nihorimbere, 2004), the study investigating antioxidant power of phytochemicals from *Psidium guuuajava*.

Malondialdehyde(MDA),a stable metabolite of the free radical mediated lipid peroxidation cascade, was widely used as marker of lipid peroxidation.In the hepatoprotective period, a significant increase in liver homogenate MDA level was observed in CCl₄ alone treated rats when compared with the normal control group. However, CCl₄ induced elevation of the liver homogenate MDA levels were lowered significantly by the treatment of the rats with the guava leaves ethanolic extracts or silymarin when compared with CCl₄-treated group. The remarkably reduction in the homogenate MDA levels were noticed in the rats treated with ethanolic extracts or silymarin and these were no significant difference when compared with normal control group. In the curative period, the same trend was observed in treatment animals with guava leaves ethanolic extracts or silymarin significantly which reversed the elevation of hepatic MDA formation when compared with CCl₄-treated group. Also no significant effects were observed between the rats treated with guava leaves ethanolic extracts or silymarin and normal control.These results are in agreement with (Teselkin *et al.*, 2000),who demonstrated

that antioxidants prevent CCl₄ toxicity, particularly hepatotoxicity, by inhibiting lipid peroxidation.

Histopathological studies, showed paracetamol and CCl₄ to produce extensive vascular degenerative changes and centrilobular necrosis in hepatocytes. Treatment with *silymarin* and *P.guajava* extract produced mild degenerative changes and absence of centrilobular necrosis when compared with control. Liver sections from control rats showed normal lobular architecture and normal hepatic cells with a well-preserved cytoplasm, nucleus and nucleoli were defined (Figure 6.4A). Whereas rat treated with PCM showed marked regenerative activity in the form of binucleation, prominent nucleoli, nuclear enlargement, loss of nucleus, centrilobular necrosis, and kupffer cells were hyperplastic (Figure 6.4B). No significant morphological changes were noted in liver of animals given only *silymarin*, as compared to that of animals in the control group (Figure 6.4C). Treatment with *P.guajava* showed normal lobular structure with hardly ascertainable regenerative activity in PCM-challenged animals (Figure 6.4D), similar observation was observed in (Roy *et al.*, 2006, Roy and Das, 2010, Osman *et al.*, 2011, Roy and Das, 2010, Sambo *et al.*, 2009, Uboha *et al.*, 2010). An agreement was observed in the study mentioned above (Osman *et al.*, 2011) significantly increases were found in the activities of SOD and CAT enzymes when compared with CCl₄-treated group. The content of reduced glutathione and GST in all treatments generally decreased as compared with normal group except treatment rats with guava leaves ethanolic extracts, which cause significant increase in the reduced glutathione content when compared with other treatments during hepatoprotective and curative periods with no significant difference when compared with normal control group. This potential antioxidant activity may be due to the high content of phenolic compound in guava leaves ethanol extract that played a significant role on the antioxidant activity as stated by (Tachakittirungrod *et al.*, 2007).

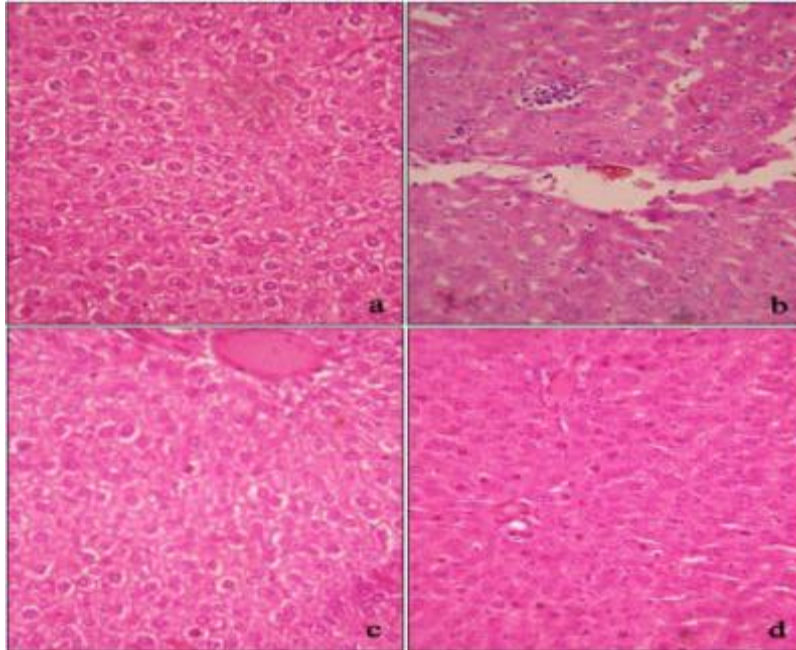


Figure 6.5 Effect of *P.guajava* against PCM induced hepatotoxicity in rat. (a) Normal histological appearance of the liver of control rat, (b) PCM induced hepatotoxicity in rat, (c) PCM + Silymarin pretreatment, (d) PCM + *P.guajava* pretreatment(Tajua et al., 2010).

Taken together, these findings, suggest that the aqueous, ethanolic and methanolic extracts from leaves of *p.guajava* exhibit hepatoprotective effect which was demonstrated by a significant decrease in the serum transaminases, ALP and total bilirubin level in rat induced with PCM and CCl_4 hepatotoxicity. Moreover, the *P.guajava* enhanced the activities of antioxidant enzymes (GSH, GPx, SOD and CAT) against the PCM and CCl_4 -induced hepatotoxicity in these animals, suggesting that the reduction of oxidative stress in this scenario likely plays a role in the mechanism of its hepatoprotective effects.

7. CONCLUSION

Polyherbal therapy is a pharmacological principle that has the advantage of producing maximum therapeutic efficacy with minimum side effects and, according to the World Health Organization (WHO).

Herbal drugs are frequently considered to be less toxic and free from side effects than synthetic drugs.

Phytochemical investigation of different extracts of *p.guajava* show that it contains carbohydrates, tannins, flavonoids, saponins, steroids, proteins, amino acids, reducing compounds, alkaloids, polyphenols, glycosides and alkaloids.

Many pharmacological studies have demonstrated the ability of this plant to exhibit antioxidant, hepato-protective, anti-allergy, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, anticough, antidiabetic, anti-inflammatory and antinociceptive activities, supporting its traditional uses.

Extracts of *p.guajava* act as antioxidant via free radical scavenging and reducing of oxidized intermediates the phenolic content in guava leaf fraction played a significant role on the antioxidant activity.

Guava consumption resulted in significant increase in the levels of total antioxidants and reduced oxidative stress, as indicated by decreased levels of antioxidant enzymes (glutathione peroxidase and glutathione reductase).

Ethanol extracts of fruit peels of *p.gajava* had high radical scavenging activities and also had high total phenolic content.

Guava (Psidium guajava) fruit is considered a highly nutritious fruit because it contains a high level of ascorbic acid (50–300 mg/100 g fresh weight).

P.guajava can be used as a safe, cheap, and effective alternative chemopreventive and protective agent in the management of liver diseases.

It exhibit hepatoprotective effect which was demonstrated by a significant decrease in the serum transaminases, ALP and total bilirubin level in rat induced with PCM and CCl₄

hepatotoxicity. Moreover, the *P.guajava* enhanced the activities of antioxidant enzymes (GSH, GPx, SOD and CAT) against the PCM and CCl₄-induced hepatotoxicity in these animals, suggesting that the reduction of oxidative stress in this scenario likely plays a role in the mechanism of its hepatoprotective effects.

8. RECOMENDATION

- ❖ Plant derived natural products such as flavonoids, terpenoids, carbohydrates, tannins, saponins, steroids, proteins, amino acids and Vitamin C etc should received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and hepatoprotective activity
- ❖ Much of attention should be paid to the medicinal values of p.guajava with special reference to its antioxidant and hepatoprotective activities.
- ❖ Guava fruit should be supplemented as an intervention mechanism for diseases associated with oxidative stress.
- ❖ Further studies need to confirm the potential active constituents and antioxidative and hepatoprotective activity of this active constituents of guava extracts, but more importantly, if such activity exists in the fruit, which is the part of the plant that is consumed.
- ❖ In these and other future studies, the amount of antioxidant tested should be compared to what is actually biologically available in the blood after the guava fruit is consumed.

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