J. Agric. Sci. Mansoura Univ., 30 (10): 6421 - 6441, 2005

BIOLOGICAL ACTIVITIES OF DIFFERENT EXTRACTS AND ESSENTIAL OIL FROM GUAVA (*Psidium guajava Linn*) LEAVES GORWING IN EGYPT

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ABSTRACT

Phytochemical investigation of different extracts from guava (*Psidium guajava* L.) leaves revealed the presence of carbohydrates and/or glycosides, tannins, saponinis, flavonoids, sterols and/or triterpenes and phenolic compounds while alkaloids and coumarins were absent. Also quantitative analysis of different extracts showed that crude ethanolic extract Et.1 contained more tannins, saponons, flavonoids and phenolic compounds (9.60%, 1.12%, 0.88% and 3.03%) than other extracts, also ethylacetate extract contained flavonoids and phenolic compounds (0.74% and 0.82%), respectively but aqueous extract contained moderate contents from these constituents (4.02% , 1.32%, 0.13% and 1.04%), respectively.

Essential oil of dried *P. guajava* L. leaves was extracted by steam distillation and analysed by Gas chromatography Mass Spectroscopy (GC-MS). Essential oil yield was 0.67 g/100g from dried leaves. Thirty two components were identified by GC-MS and the major constituents were limonene (15.22%), Junipene (9.58%), glaucine (9.03%), beta terpinyl acetate (8.70%) and D-nerolidol (8.58%).

The inhibitory effects of successive extraction of guava leaves with petroleum ether (PE), chloroform (Ch1), ethyl acetate (EA), ethanol 96% (Et.2), water (W), complete ethanol 70% (Et.1) separately and three concentrations of essential oil on lipid peroxidation induced by Fe^{++}/ascorbate in rat liver mitochondria and microsomes were determined. Also free radical scavenging activity of different extracts and essential oil were determined using 2,2 – diphenyl – 1 – picrylhydryzyl ($DPPH^{\bullet}$). The obtained results revealed that crude ethanolic extract Et.1 has high effect in protected rat liver mitochondria and microsomes from lipid peroxidation and was substantially more powerful antioxidant where the percentage of inhibition of lipid peroxidation in mitochondria and microsomes were (95.80% and 97.88% compared with rutin as standars (97.23% and 98.90%) and free radical scavenging using $DPPH^{\bullet}$ was (94.38% and rutin 95.69%). Et.1 and essential oil were evaluated for the protective effect against liver damage induced by carbon tetrachloride (CCl_4) in male albino rats. Rats were orally administered with 50 and 100 mg/kg body weight from ethanolic extract (Et.1) and essential oil for 14 days before CCl_4 challenge and 100 mg from Et.1 and essential oil for toxicity analysis without CCl_4 administration significantly damaged the liver as evident from very high activity of serum marker enzymes and glutathione – S – transferase. Also decrease total protein, albumin but increased bilirubin contents in the serum. Et.1 administration significantly restored the elevated activities of liver marker enzymes, increased total protein and albumin, content enhanced the antioxidant enzyme activity also decrease bilirubin content than essential oil which gave slight effect at the same time Et.1 and essential oil at 100 mg/kg body weight had no any toxic effect. The results of antitumor activity of Et.1 and essential oil on *Erhlisch ascites* carcinoma (EACC) cells indicated that essential oil had higher antitumor activity than Et.1 which the dead cells percentage were ranged from 87 – 100% but in Et.1 ranged from 76 – 95%.

Keywords: Guava, *Psidium guajava Linn*, leaves, extracts, essential oil, chemical composition, lipid peroxidation, $DPPH^{\bullet}$, free radicals, antioxidants, CCl_4, hepatoprotective, anticancer.

INTRODUCTION

Guava (*Psidium guajava linn*) belonging to the family of myrtaceae, has been used as health tea. Its leaf contains copious amounts of phenolic phytochemicals which inhibit peroxidation reaction in the living body, and therefore can be excepted to prevent various chronic disease such as diabetes, cancer, heart disease (Begum *et al.*, 2002). Furthermore, decreasing of free radicals has antioxidizing effect in the body, meaning that guava leaf polyphenols can prevent arterial sclerosis, thrombosis, cataract and inhibit senescence of the body and skin (Okuda *et al.*, 1982). Many people habitually take medicinal decoction of guava leaf for long for treatment of diarrhea and therefore, the safety of guava leaves have empirically been confirmed (Tona *et al.*, 2000). South American folk medicine uses tea from its leaves, leaf buds and flowers to treat intestinal colic and diarrhea. Also people in china use guava leaf as anti-inflammatory and haemostatic agent (Barocci *et al.*, 1999). Also guava leaf contains an essential oil rich in 1,8-cineol, D-limonene, caryophyll, eugenol, alpha pinene and myrcene (Ogunwande *et al.*, 2003) as well as essential oil has anti-microbial activity were previously studied by (Karawya *et al.*, 1999). In addition tannins, phenols, triterpenes and three flavonoids (quercetin, avicularin and guaijaverin) have been isolated from leaves (Begum *et al.*, 2004). Pharmacological study revealed that essential oil, aqueous, alcoholic and ethylacetate extracts of leaves exhibited a significant muscle relaxant activity on the smooth muscle especially that of the trachea as well as a significant anti-inflammatory activity, that explains the use of guava leaves in the Egyptian folk medicine for treatment of caught (Abdel-Wahab *et al.*, 2004).

Free-radicals are generated continuously in the body due to metabolism and disease (Yeum *et al.*, 2003). In order to protect themselves against free radicals, organisms are endowed with endogenous and exogenous defenses, yet these defense systems are not sufficient in crtical situations thus the active dietary constituents contributing to these protective effects are the antioxidants. Also administration of antioxidants reduces or eliminates the biochemical and pathological changes brough about by lipid peroxidation (Teoh *et al.*, 1992).

Humans are continuously exposed to different kinds of chemicals such as food additives, industrial chemicals, pesticides and other under-irable contaminants in the air, food and soil (Hasegawa *et al.*, 1995). Most of these chemicals induce a free radical-mediated lipid peroxidation leading to disruption of biomembranes and dysfunction of cells and tissues (Cho *et al.*, 2003). The free radical-mediated hepatotoxicity can be effectively managed upon administration of such agents possessing antioxidants (Attri *et al.*, 2000), free radical scavengers and anti-lipid peroxidation activities (Lim *et al.*, 2000). Apart from the natural antioxidant defense system, there are various synthetic antioxidants in use but these compounds have been reported to have various side effects. In this context natural compounds isolated from plants deserve significance.

Cancer is the second leading cause of death in the United States and in many other nations in the world. The prognosis for a patient with

metastatic carcinoma of the lung, colon, breast or prostate remains a concern and accounts for more than half of all cancer deaths (Galati and Brien, 2004). Thus plants from tropical regions are considered to be one of the potential sources of the screening of anti-cancer agents. Several Thai edible plants such as lemeon grass, Holy Basil and Sweet Basil possess antitumer activity. Also guava (*Psidium guajava* L.) leaf oil showed the highest anti-proliferative activity compared with vincristine on human mouse epidermal carcinoma and murine leukemia cell lines using *in vitro* assay.

Leaves are used by Egyptian traditional as cough depressant thus the present research was undertaken to investigate the nature of different constituents present in different extracts and, essential oil from guava leaves. Inhibitory effects of various extracts and essential oil on free radical scavenging activity and lipid peroxidation in rat liver mitochondria and microsomes induced by Fe^{++}/ascorbate model system, liver chemoprevention against toxicity induced by CCl_4 were studied. Also essential oil and ethanolic extract were tested as antitumor activity on *Erhlisch ascites* carcinoma (EACC) cell lines using *in vito* assay.

MATERIALS AND METHODS

Chemicals:-

Thiobarbituric acid (TBA), bovin serum albumin (BSA), 1-chloro-2,4-dinitro-benzene (CDNB), reduced glutathione, Dimethyl sulfoxide (DMSO), 2,2-diphenyl-1-picrylhydryzyl (DPPH) and carbon tetrachloride were obtained from Sigma Chemical Co. (St. Louis Mo.). all chemicals used for the study were of analytical grade.

Plant material:-

Leaves samples of guava (*Psidium guajava linn*) cultiver balady were collected in April (2003) from trees cultivated in Experimental Station of Faculty of Agriculture, Cairo university, Giza, Egypt. All samples were obtained from flowering plants, whole leaves were washed and dried in shade at room temperature for 5 – 7 days reduced to fine powder and kept for phytochemical study.

Plant extracts:-

500 g of the air – dried powdered leaves of *P. guajava* were extracted separately by percolation with ethanol (70%), then the extracts were filtered, the volume was reduced under vacuum and finally was freeze dried (Et.1).

A second plant samples from the same batch (500 g) were successively extracted at room temperature (3 × 96 h.) with petroleum ether (b.p 60 - 80°C) (PE), chloroform (Ch1), ethylacetate (EA), ethanol (96%) (Et.2) and water (W). The solvent was then removed from each extract by distillation under reduced pressure at a temperature not exceeding 50°C. then the yield percentage of each extract was determined using gravimetric method and calculated on dry weight based.

General phytochemical examinations:-

Preliminary phytochemical tests were carried out on the ethanolic extract and different extracts of guava leaves. Carbohydrates and/or glycosides were tested according to Lewis and Smith (1967), flavonoids were tested by Geissman (1962), saponins were tested by Shellard (1957), sterols and/or triterpens were tested by Fransworth *et al.* (1964), coumarins were tested by Feigle (1960) and tannins were tested by (Burns, 1971).

Determination of chemical constituents in different extracts of guava leaves:-

Chemical constituents of different extracts of guava leaves such as total tannins, total saponins, total flavonoids ad total phenolic compounds were determined as follows:

Total tannins content were determined according to (Burns, 1971), total flavonoids content were determined by (Zhuang *et al.*, 1992), total saponins were determined by using method of (Ebrahimzadeh and Niknam, 1998) and total phenolic compounds were determined according to Swain and Hillis (1959).

GC.MS analysis of dried guava leaves essential oil:-

Leaves samples were submitted to steam distillation for 3 h. then the oil was colleted and dried over anhydrous sodium sulphate, producing yield of 0.67% on dry weight.

Gas Chromatography-Mass Spectroscopy was used for identification of essential oil compounds according to Adams (1995). Essential oil of dried guava leaves analyzed as described by Mona Al-Shalaby and Hanaa Ali, 2001). Analytical GC/MS was carried out on a HP spectroscopy 6890 series with HP selective detector 5973, under the control of a HP chemstation version A02.12 data system. A carbowax capillary column, 50m * 0.53mm I.D., 1.5 m thickness (HP company, U.S.A) was used with helium as carrier gas (flow rate 1.5ml/min). Sample was injected using the split sampling technique, ratio 1: 50 with sample amount 1ul. Injectio port temperature 280°C. Column temperature was held at 40°C for 5min and then programmed at 3°C/min to 280°C and held these for 20min. Detector temperature: 300°C. Mass spectroscopy operating parameters were: electron ionization at 70 eV, accelerating voltage 10kV and scan M/z range 30-650. The identification of constituents was carried out by comparing retention time with those of authentic reference compounds, or peak-matching library research using the standard mass library (NIST Standard Mass Library).

Antioxidant activity of different extracts and essential oil of guava leaves:-

Animals:-

Young male Spargue-Dawley rats weighing 100-120 g were housed at 25±1.0°C with 60% relative humidity, illuminated for 12 h. a day starting at 7.00 a. m. and were given free access of food and water.

Preparation of mitochondria and microsomes in rat livers

Rats were killed by decapitation after fasting for 24 h. and their liver tissue was quickly removed. Microsomal and mitochondrial fractions were

isolated from liver tissue by the method of Pedersen *et al.* (1978) with slight modifications and the protein was determined by the method of Lowry *et al.* (1951).

Measurement of lipid peroxidation

A mixture of mitochondrial suspension (0.5 ml), containing 5 mg protein, 50 m*M* Hepes buffer (pH 7.4) (0.1 ml), 20 m*M* KCl (0.1 ml), 10 u*M* $FeSO_4$ (0.1 ml), 0.2 m*M* ascorbate (0.1 ml) and the indicated amounts of various extracts and essential oil of guava leaves were incubated at 37°C for 30 min in a final volume 1.0 ml (Haraguchi *et al.*, 2002). A mixture of microsomal suspension (10 µl), containing 100 µg protein, $FeSO_4$ (1.0 µ*M*) combined with ascorbic acid (500 µ*M*) and the indicated amounts of extracts and essential oil of guava leaves were incubated at 37°C for 30 min in a final volume 1.5 ml (Aboul – Enein *et al.*, 2003).

Determination of lipid peroxidation products

Lipid peroxidation was measured using the reaction with thiobarbituric acid, TBA (Buege and Aust, 1978 and Eriksson *et al.*, 1992). All reagents were prepared freshly and all reactions were carried out in triplicate, Rutin was used as a positive control, inhibition (I) of lipid peroxidation in percent was calculated by the following equation:

$$I\ (\%) = 100 \times (A_1 - A_2/A_1)$$

Where: A_1 was the absorbance of the control reaction (= full reaction, containing no test compound) and A_2 was the absorbance in the presence of the inhibitor.

Free radical – scavenging method:-

The antioxidant activity of different extracts and essential oil of guava leaves were measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH according to (Brand – Williams *et al.*, 1995) and the inhibition percent of the DPPH by the samples was calculated according to the formula of (Yen and Duh, 1994) as follows:

$$\%\ \text{Inhibition} = [(Ac\ (0) - Ac\ (t))\ /Ac\ (0)] \times 100$$

Where: Ac (0) was the absorbance of the control at t = 0 min and Ac (t) was the absorbance of the antioxidant at t, which varied with the different content.

Effect of crude ethanolic extract (Et.1) and essential oil at different concentrations against hepatotoxicity of carbon tetrachloride in rats:-

Experimental module:

Adult male albino rats weighting 170±10g were raised in the animal house of Toxicology Department, at Central Agricultural Pesticide Laboratory, Agriculture Research Central. The albino rats were kept under normal laboratory conditions for two weeks before the commencing the experiments. Animals were grouped as follows (six rats for each group)

Group I: control (10% DMSO).
Group II: CCl_4 + (10% DMSO).
Group III: Et.1 alone (100 mg/kg body weight)
Group IV: Essential oil alone (100 mg/kg body weight)
Group V: CCl_4 + Et.1 (50 mg/kg body weight, test 1)
Group VI: CCl_4 + Et.1 (100 mg/kg body weight, test 2)

Group VII: CCl_4 + essential oil (50 mg/kg body weight, test 3)
Group VIII: CCl_4 + essential oil (100 mg/kg body weight, test 4)

Et.1 and essential oil were given orally for 14 days every extract and essential were dissolved in a known volume of (10% DMSO). All the animals except group I, III and IV were administered a single oral dose of CCl_4 (1:1 in liquid paraffin at 2.0 g/kg body weight). 6 h. after the last dose of extract and essential oil. Rats were killed by decapitated after 24 h. from taken CCl_4. blood and tissue were collected for various examinations.

Biochemical parameters:

The following biochemical parameters were analyzed to evaluate the hepatotoxicity of Et.1 and essential oil by the methods given below.

Activities of glutamate oxaloacetate transaminase (AST), glutamate pyruvatr transaminase (ALT) were determined according to Reitman and Frankel (1957). Activity of alkaline phosphatase (ALP) was determined according to Empfehlungen (1971). Glutathione - S – transferase (GST) activity was determined in liver cytosolic fraction according to the method described by (Habig *et al.*, 1974), using 1– chloro- 2,4 dinitrobenzene (CDNB) as substrate.

Albumin was determined in serum according to Doumas *et al.* (1971) using colorimetric kits, and serum bilirubin contents were determined using commercially available kit according to Walter and Gerade (1970).

In vitro assay of antitumor activity:

Tumor cell lines adopted for this study were *Erhlisch ascites* carcinoma cells (EACC). The cell lines were cultured in 5% CO_2 incubator at 37 °C with a Roswen Park Mark Institute (PRMI) medium containing 10% fetal bovine serum (FBS). The assay for evaluating antitumor activity was performed following the procedure of Bennett *et al.* (1976). The level of crude ethanol extract Et.1 and essential oil antitumor action on the selected cell lines were in the range of 100 – 1000 ppm.

Statistical analysis:

The obtained data were statistically analyzed using the method of Snedecor and Cochran (1982) and LSD (Least squares difference) test was used to compare the significant differences between means of treatment (Waller and Duncan, 1969). Values are expressed as mean ± SE.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of different extracts of guava leaves:-

As shown in Table (1), the phytochemical study of the different extracts of guava leaves revealed the presence of carbohydrates and/or glycosides, tannins, saponins, flavonoids, sterols and/or triterpenes and phenolic compounds. Alkaloids and coumarins were absent in all extracts. Also after extraction with different solvents the chemical constituents were determined gravimetery, the results are shown in Table (2) and the yields

were 1.32%, 2.26%, 1.43%, 5.20%, 2.08% and 8.33% for successively extraction with pet-ether (60 - 80°C), chloroform, ethylacetate, ethanol 96%, water and complete ethanol 70% separately. It may be due to pet-ether and chloroform extract non polar compounds such as pigments, ethyl acetate extract, flavonoids compounds but ethanol extract those of polar and non polar compounds such as carbohydrates, phenolic compounds, tannins, saponins and flavonoids.

Table (1): Preliminary phytochemical screening of different extracts of guava leaves

Test	Pet-ether 60 - 80°C	Chloroform	Ethylacetate	Ethanol 96%	Water	Complete ethanol 70%
Carbohydrates and/or glycosides	-	-	-	++	+	++
Flavonoids glycosides	-	-	++	++	++	++
Saponins	-	-	±	±	+	+
Tannins	-	-	-	++	++	++
Sterols and/or triterpens	+	±	±	-	-	+
Alkaloids	-	-	-	-	-	-
Coumarins	-	-	-	-	-	-
Phenolic compounds	-	-	±	++	++	++

(++) Moderate amount (+) Low amount (-) Absent (±) Traces.

Table (2): Yield percentage of different extracts from guava leaves (based on dry weight):-

Solvent	Yield (Percentage %)
Pet-ether 60 - 80°C (PE)	1.32
Chloroform (Chl)	2.26
Ethyl acetate (EA)	1.43
Ethanol 96% (Et2)	5.20
Water (W)	2.08
Complete ethanol 70% (Et1)	8.33

Chemical analysis of different extracts of guava leaves:-

The obtained data in Table (3) show that the percentages of tannins and saponins compounds were found to be (8.30, 4.02, 9.60%), (0.26, 1.32, 1.12%) on dry weight based, respectively for successively extraction with ethanol 96%, water and extraction with ethanol 70% separately but percentage of flavonoids and phenolic compounds were found to be (0.74, 0.21, 0.13, 0.88%) and (0.82, 1.73, 1.04, 3.03%) on dry weight based respectively for successively extraction with ethyl acetate, ethanol (96%), water and complete ethanol 70% separarely. These results are in agreement with those obtained by Masuda *et al.* (2002) and Abdel-Wahab *et al.* (2004) who reported that percentage of tannins in complete ethanolic extract of

guava leaves was 8.3% and percentage of total flavonoids was 0.57% calculated as quercetin. Also Hopia *et al.* (1999) found that total phenolic content values of ethanolic and water extracts of guava leaves were 575.3 and 511.6 mg of gallic acid equivalent for 100g from dry weight material.

Table (3):Total tannins, saponins, flavonoids and phenolic compounds contents in different extracts of guava leaves :-

Solvent	Total tannins %	Total saponins %	Total flavonoids %	Total Phenolic compounds %
Pet-ether 60 - 80°C (PE)	–	–	–	–
Chloroform (Chl)	–	–	–	–
Ethyl acetate (EA)	–	–	0.74	0.82
Ethanol 96% (Et2)	8.30	0.26	0.21	1.73
Water (W)	4.02	1.32	0.13	1.04
Complete ethanol 70% (Et.1)	9.60	1.12	0.88	3.03

*** All determinations based n dry weight.**

Chemical composition of dried guava leaves essential oil:-

Chemical composition of guava leaves essential oil was analyzed by GC – MS and the results are summarized in Table (4). The essential oil yield in the dried leaves was 0.67% g/100g sample after steam distillation. 32 volatile components were identified (100%). Particularly the major constituent was limonene (15.22%), junipene (9.58%), glaucine (9.03%), beta terpinyl acetate (8.70%) and D – nerolidol (8.58%).

The other main compounds were beta–bisbolene (4.18%), beta–bisabolol (4.18%), α-humulene (3.32%), 1.8–cineole (2.36%), benzeneacetic acid 2-hydroxy (2.31%), Z-citral (1.92%), delta – 9 – tetra-hydrocannabivannic (1.78%), aromadendrene (1.41%), ethanone – 1– phynyl (1.43%), boldine (1.40%), crassanine (1.32%), germacrene (1.21%),α–copaene (1.17%), bowdensine (1.09%) and trans caryophyllene (1.0%). 11 compounds were identified as traces constituents less than 1% such as beta himachalene (0.94%) and 5 – octadecyne (0.81%). These results are not agreement with those abtained by Silva *et al.* (2003) who found that oil from *P. guajava* containing α – pinine (23.95%), 1.8 – cineole (21.4%) and β – bisabolol (9.2%). These differences may be due to the climatic conditions of the growth of guava trees which could widely influence qualitative composition of the oil, but ogunwande *et al.* (2003) showed that the composition of the voltial oil obtained from the hydrodistillation of *P. guajava* L. leaves growing in Nigeria was contained limonene and β – carophyllene as a major compounds.

Effect of successive extracts and essential oil of guava leaves on mitochondrial and microsomal lipid peroxidation·-

The obtained data in Table (5) show that Et.1 of guava leaves gave the highest inhibition values of 95.80% and 91.50% at the concentration of 1.0 mg/ml and 0.5 mg/ml, respectively for mitochondria lipid peroxidation. The same extract (Et.1) gave 97.88% and 94.25% inhibition values at the concentration of 1.0 mg/ml and 0.5 mg/ml, respectively for microsomal lipid peroxidation.

Table (4): GC – MS analysis of dried guava leaves essential oil:-

Compounds	Rt	Percentage %
Beta – myrcene	5.08	0.51
Limonene	5.69	15.22
Beta terpinyl acetate	5.78	8.70
1,8 - cineole	6.08	2.36
Ethanone – 1– phenyl	15.81	1.43
3- cyclohexene – 1,1 - dimethanol	18.21	6.12
junipene	18.36	9.58
α-humulene	20.17	3.32
Methanone, cyclopropyl – 3- pyrdinyl	21.34	0.81
5 – octadecyne	21.51	0.81
Beta – bisabolene	22.25	4.18
Beta - himachalene	22.48	0.94
Cis – gammabisabolene	22.84	0.75
Trans caryophyllene	23.21	1.0
Boldine	24.57	1.40
Crassanine	25.93	1.32
Benzeneactic acid 2 – hydroxy	28.46	2.31
Cycloheptene, 5 – bromo	29.24	0.96
D – nerolidol	30.44	8.58
Glaucine	30.95	9.03
α – copaene	31.85	1.17
β – bisabolol	32.60	4.18
Globulol	33.18	0.46
Germacrene	33.57	1.21
Z-citral	34.05	1.92
Bowdensine	34.18	1.09
Aromadendrene	34.57	1.41
Cis – L – carvylacetate	35.18	0.59
Valeraldehyde, dimethyl hydrazone	37.05	0.56
Delta – 9 – tetra hydrocannabivarinic	39.56	1.78
Albuterol – N – butylboronate	43.36	0.71
Demeton – O - methyl	46.91	0.79
Unknown	—	4.98

This may be due to Et.1 contain high content from flavonoids, tannins and phenolic compounds which have antioxidant effects than other extracts, also ethanol extracts (Et.1 and Et.2) have a protective effect against Fe^{++}/ascorbate model system. This may be due to its high content from flavonoids (quercetin, kampferol, quercetrin, apigenin 7.0 glucoside and luteolin 7.0 glucoside) resulted in the inhibition of lipid peroxidation (Kawagoe and Nakagawa, 2000). But the essential oil gave the lowest inhibition values of 18.88% and 15.51% for 1.0 mg/ml and 0.5 mg/ml, respectively for mitochondria lipid peroxidation and the same trend was shown in microsomes lipid peroxidation. This may be due to essential oil contain low content from

antioxidant compounds such as monoterpenes as shown in Table (4) which have low antioxidant effects. These data are in harmony with those obtained by Lachman *et al.* (2000) who found that rutin is an important anti – lipoperoxidant agent and has also a strong scavenger of hydroxyl and superoxide radicals. Also Sudheesh and Vijayalakshmi (2005) showed that fractions rich in flavonoids obtained from the fruits of *Punica granatum* extract gave potential anti-peroxidative effect.

Table (5): Inhibitory effects of successive extracts and essential oil of guava leaves on Fe++/ascorbate model system – induced lipid peroxidation in mitochondria and microsomes rat liver:-

Sample		Inhibition (%)[a]	
		Mitochondria	Microsomes
Rutin			
(Standard)	1.000 mg/ml	97.23	98.90
	0.500 mg/ml	93.20	95.10
	0.250 mg/ml	81.89	84.27
P.E.			
	1.000 mg/ml	23.52	26.15
	0.500 mg/ml	20.12	21.90
	0.250 mg/ml	12.06	13.33
Ch1			
	1.000 mg/ml	42.66	42.77
	0.500 mg/ml	36.10	37.46
	0.250 mg/ml	26.22	29.83
E.A			
	1.000 mg/ml	66.20	68.18
	0.500 mg/ml	54.11	57.23
	0.250 mg/ml	38.50	40.40
Et.2			
	1.000 mg/ml	82.33	85.16
	0.500 mg/ml	79.15	80.08
	0.250 mg/ml	62.27	70.66
W			
	1.000 mg/ml	79.00	82.18
	0.500 mg/ml	68.50	69.40
	0.250 mg/ml	49.77	53.80
Et.1			
	1.000 mg/ml	95.80	97.88
	0.500 mg/ml	91.50	94.25
	0.250 mg/ml	67.95	75.13
Essential oil	1.000 mg/ml	18.88	21.24
	0.500 mg/ml	15.51	18.14
	0.250 mg/ml	13.22	14.89

Inhibition (%) was expressed as the absorbance of sample compared with the absorbance of control. All values are mean of 3 replicates.

Based on these experimental results, it is suggested that the inhibitory effects of Et.1 and Et.2 extracts of guava leaves showed to have the highest inhibition value on Fe^{++}/ascorbate in mitochondria and microsomes might be partly due to action of free radical scavengers.

Antioxidant activity of different extracts and essential oil from guava leaves according to the DPPH radical scavenging method:-

The results in (Table 6) show that the decrease in absorbance of DPPH• radical was due to its reduction by different antioxidants. Rutin (standard) is superior inhibitor of DPPH• compared to guava leaf extracts and gave high percentage inhibition 92.40% and 95.69% for 50, 100 µg/ml while Et.1 and Et.2 and (W) extracts respectively, showed decreasing inhibition effect for all concentrations than other extracts and essential oil. From these data it can be seen that water and ethanolic extracts of guava leaves showed good free radical- scavenging activity dipending on the concentration used. The higher concentration used the higher free radical-scavenging effect. These may be due to ethanolic and water extracts found to contain significant amount of natural phenolic compounds such as tannic acid, procatechuic acid, caffeic acid, ferulic acid, rutin trihydrate and quercetin dihydrate. These results are in good agreement with those obtained by Zheng and Wang (2001) who reported that natural mixtures of phenolic compounds has high antioxidant activity. Based on these experimental results, it is suggested that guava leaf extracts comprise effective potential source of natural antioxidants.

Table (6): Free radical scavenging activity of successive extracts and essential oil from guava leaves:-

Sample	DPPH• radical decolouration (µg/ml)		
	10	50	100
Rutin (Standard)	48.6	92.40	95.69
P.E.	18.56	27.80	30.60
Ch1	23.60	29.98	44.70
E.A	26.78	35.60	60.44
Et.2	42.30	83.80	90.10
W	31.20	65.40	78.81
Et.1	62.30	90.53	94.38
Essential oil	13.11	21.60	24.88

* Comparison of antioxidant activity of different extracts and essential oil of guava leaf with rutin expressed as % inhibition.

** All values mean of 3 replicates.

Effect of crude ethanolic extract (Et.1) and essential oil at different concentrations against hepatotoxicity of carbon tetrachloride in rats:-

The crude ethanolic extract (Et.1) and essential oil of guava leaves were evaluated for the protective effect against liver damage induced by carbon tetrachloride (CCl_4) in male albino rats.

Rats were orally administered with 50 and 100 mg/kg body weight from ethanolic extract (Et.1) and essential oil for 14 days before CCl_4 challenge and 100 mg from Et.1 and essential oil alone for toxicity analysis without CCl_4 administration. The results (Table 7, 8, 9) show that CCl_4 administration significantly damage the liver as evident from very high activities of serum and liver marker enzymes. It also increased the activity of glutathione-S-transferase as antioxidant enzyme status of the animals, and

billirubin content and decreased total protein in the serum, but treatment with Et.1 and essential oil for 14 days before CCl_4 treatment offered considerable protection to liver as evidenced from the levels of biochemical parameters as follows:

Activity of marker enzymes:

The effects of Et.1 and essential oil on liver marker enzymes are give in Table (7): Acute CCl_4 administration significantly increased the level of liver injury marker enzymes like ALT, AST and ALP. Administration of Et.1 and essential oil at (50 and 100 mg/kg body weight) decreased the activities of these enzymes in the serum compared with control positive. Rats treated with Et.1 and essential oil at dose 100 mg/kg body weight alone showed no change in the activity of these enzymes compared to the normal animals (negative control).

Table (7): Effect of crude ethanolic extract Et.1 and essential oil from guava leaves at different concentrations on liver marker enzymes:-

Groups	AST IU/l	ALT IU/l	ALP Iu/l
Control	34.55 ± 0.26	23.67 ± 0.19	39.85 ± 0.09
CCl_4	53.14 ± 0.50	30.81 ± 0.11	45.79 ± 0.12
100 mg Et.1	33.82 ± 0.10	23.1 ± 0.52	38.92 ± 0.12
100 mg essential oil	33.94 ± 0.03	22.88 ± 0.07	38.99 ± 0.56
Test 1	47.29 ± 0.41	27.42 ± 0.33	41.21 ± 0.46
Test 2	45.17 ± 0.48	26.18 ± 0.47	39.38 ± 0.36
Test 3	49.42 ± 0.33	28.96 ± 0.02	42.81 ± 0.11
Test 4	47.66 ± 0.20	27.11 ± 0.51	41.86 ± 0.08
LSD at 5%	0.991	1.02	0.89

Each value represent mean ± SE

Effect on antioxidant enzyme (GST), total protein, albumin and bilirubin contents:-

Tables (8, 9) show the effect of Et.1 and essential oil on the glutathione – S – transferase levels in the rat liver. Administration of CCl_4 increased activity of GST enzyme significantly when compared to normal rats, while prophylactic administration of Et.1 and essential oil showed an decrease in the activity of this enzyme and Et.1 extract was more effective than essential oil due to high content of antioxidant compounds, when Et.1 and essential oil were administered alone (100 mg/kg body weight) there were a slight but not so significant increase in the GST activity. CCl_4 administered group showed elevated increase in serum bilirubin and a significant decrease in total protein (Table 9). The treatment with Et.1 and essential oil increased total protein, albumin and bilirubin contents in the serum compared with control positive (CCl_4) and Et.1 increased these contents than essential oil thus has low antioxidant compounds. Et.1 and essential oil alone treated groups did not show any significant change

compared to normal rats. These may be due to LD_{50} of the tested extract showed that ethanolic extract is not toxic in doses up to 2.05 g/kg body weight but essential oil not toxic in doses up to 0.62 g/kg body weight (Abdel Wahab *et al.*, 2004).

These results showed that the prophylactic treatment with Et.1 and essential oil of guava leaves before CCl_4 treatment offered considerable protection to liver as evidenced from the levels of biochemical parameters. (AST, ALT and ALP). 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.

It well known that necrotizing agents like CCl_4 produce sufficient injury to hepatic parenchyma to cause large increases in bilirubin content (Plaa and Hewitt, 1982) Et.1 prevented severity of liver damage caused by CCl_4 as evidenced the low level of bilirubin in the serum. In the liver it has been shown that toxicity of CCl_4 mediated by the Cyt P450 dependent mixed oxidase – mediated biotransformation product, trichloromethyl free radical (CCl_3) and subsequent derivative CL_3COO. These free radical combines with the cellular lipid and proteins to produce lipid peroxidation, measured through its catabolite malonadyaldhyde (MDA), resulting in structural changes of endoplasmic reticulum and other biomembranes and loss of metabolic activity leading to liver damage (Kadiiska *et al.*, 2000). Various studies showed that the antioxidative activity or inhibition of free radicals is important in the protection against CCl_4 – induced liver lesions (Johnston and Kroening, 1998). Damage the liver cells cause leakage of cellular enzymes in to serum. A significant rise in AST, ALT could be taken as an index of liver damage (Drotman and Lawhorn, 1978) GST, a phase II enzyme, confers protection against toxic chemicals by activity metaboloizing in to less toxic compound (Wallig *et al.*, 1998). In the present study pretreatment with Et.1 showed decreased activity of GST enzyme compared to CCl_4 – treated rats indicating the potentiality of Et.1 to act as an antioxidant by preventing the peroxidation damage caused by CCl_4 not essential oil because it has low content from antioxidant compounds. Total protein is done as a routine test to evaluate the toxicological nature of various chemicals. The decreased total protein in CCl_4 – treated groups is due to the damage caused the liver by CCl_4. The ability of Et.1 to maintain the total protein may be due to the non – toxic antioxidant constituents present in the extract.

Preliminary phytochemical analysis of Et.1 showed the presence of flavonoids. Flavonoids are natural products having significant biological activities including antiradical and antioxidant properties Minato *et al.* (2003). Also flavonoids has inhibitory effect on microsomal Cyt P450 enzyme or on lipid peroxidation Et.1 may be interfering with the Cyt P450 and ultimately hindering the biotransformation of CCl_4 At the same time Et.1 may also process antioxidant activity with inhibited the deleterious effect of free radicals generated by CCl_4 influencing the membrane rigidity by preventing inhibiting the membrane peroxidation.

These data are in harmony with those obtained by Oh *et al.* (2004) who found that onitin and onitin 9 – 0 - glucoside and kaempferol – 3 – 0 glucoside isolated from *Equisetum arvense* has hepatoprotective activities on

tacrine – induced cytotoxicity in human liver. Also Peres *et al.* (2000) found that quercetin administration at a dose of 150 µ/mol/kg body weight markedly reduces liver injury as shown by smaller increases in plasma AST, ALT and ALP.

Also *Artemisia scoparid thumb. a* perennial herb and a significative source of rutin previously, is indigenous to Pakistan and has been traditionally used in liver damage (Janbaz *et al.*, 2002).

Table (8): Effect of crude ethanolic extract Et.1 and essential oil from guava leaves at different concentrations on glutathione – s transferase activity µm/min/mg protein:-

Groups	Glutathione – s transferase activity µm/min/mg protein
Control	1.06 ± 0.03
CCl_4	1.44 ± 0.02
100 mg Et.1	1.18 ± 0.04
100 mg essential oil	1.10 ± 0.06
Test 1	1.38 ± 0.02
Test 2	1.27 ± 0.03
Test 3	1.40 ± 0.04
Test 4	1.30 ± 0.05
LSD at 5%	0.12

Each value represent mean ± SE

Table (9): Effect of crude ethanolic extract Et.1 and essential oil from guava leaves at different concentrations on total protein, albumin and serum bilirubin content:-

Groups	Total protein g/dl	Albumin g/dl	Bilirubin Mg/dl
Control	6.36 ± 0.37	3.47 ± 0.31	0.674 ± 0.02
CCl_4	5.05 ± 0.55	2.81 ± 0.11	0.833 ± 0.04
100 mg Et.1	6.39 ± 0.35	3.54 ± 0.27	0.645 ± 0.03
100 mg essential oil	6.26 ± 0.43	3.32 ± 0.45	0.618 ± 0.05
Test 1	5.72 ± 0.16	3.03 ± 0.56	0.708 ± 0.03
Test 2	5.89 ± 0.06	3.22 ± 0.45	0.666 ± 0.05
Test 3	5.35 ± 0.38	2.96 ± 0.02	0.726 ± 0.00
Test 4	5.42 ± 0.33	3.01 ± 0.57	0.684 ± 0.02
LSD at 5%	1.07	1.17	0.10

Each value represent mean ± SE

Effect of crude ethanolic extract Et.1 and essential oil from guava leaves at different concentrations on *Erhlish ascites* carcinoma (EACC) cells:-

The effect of ethanolic extract Et.1 and essential oil of guava leaves as antitumor are recorded in Table (10) which shown that incubation of tumor cells with ethanolic extract and essential oil reduced the viability of those cells and the dead cells were increased with increased concentration from

ethanolic extract or essential oil, the addition of 100 ppm ethanolic extract reduced the viability from 100% to reach 86, 77, 51, 24 % respectively. On the other hand 100 ppm essential oil reduced the viability from 100% to reach 65, 49, 28, and 13% respectively. Finally the addition of 1000 ppm ethanolic extract and essential oil produced the highest reduction in the viability of tumor cells. But generally the ethanolic extract was lower than essential oil may be due to the occurrence of nutrients in these ethanolic extract which activate the tumor cells more than essential oil which contain antioxidant compounds such as limonene (monoterpenes) and 1,8 – cineole. From the results of viability study as shown in Table (10), it s clear that ethanolic extract and essential oil from guava leaves have antitumor activity against *Erhlisch ascites* cells and essential oil had the strongest effect compared with ethanolic extract.

Table (10): Effect of ethanolic extract Et.1 and essential oil from guava leaves at different concentrations on *Erhlisch ascites* carcinoma (EACC) cells:-

Time (min)	Control A		Ethanolic extract Et.1							
	V%	D%	100 ppm		200 ppm		500 ppm		1000 ppm	
			V%	D%	V%	D%	V%	D%	V%	D%
30	100	0	86	14	81	19	75	2	65	35
60	99	1	77	23	69	31	63	37	54	46
90	98	2	51	49	46	54	35	65	15	85
120	94	6	24	76	15	85	11	89	5	95
Time (min)	Control B		Essential oil							
	V%	D%	100 ppm		200 ppm		500 ppm		1000 ppm	
			V%	D%	V%	D%	V%	D%	V%	D%
30	100	0	65	35	61	39	57	43	44	56
60	96	4	49	51	40	60	28	72	24	76
90	92	8	28	72	21	79	6	94	0	100
120	89	11	13	87	9	91	0	100	0	100

Where D% number of dead cells.
V% number of viable cells.

These results are in agreement with those obtained by Zanden *et al.*, 2004 and Galati and Brien (2004) who found that plant phenolics and flavonoids has antioxidant, anti – inflammatory and anti – carcinogenic effects and their chemopreventive activity in animal. *In vivo* experiments showed that flavonoids has ability to inhibit phase I and induce phase II carcinogen metabolizing enzymes that initiate carcinogenesis, also inhibite the promotion stage of carcinogenesis by inhibiting oxygen radical forming enzymes or enzymes that contribute to DNA synthesis or act as ATP mimics and inhibit protein kinases that contribute to proliferative signal transduction. Also, flavonoids may prevent tumor development by inducing tumor cell apoptosis by inhibiting DNA topisomerase II and P^{53} downregulation or by causing mitochondrial toxicity which initiates mitochondrial apoptosis. In this respect Rietjens *et al.* (2005) showed that flavonoids may interfere in several for the steps that lead to the development of malignant tumors, including protecting DNA from oxidative damage, inhibiting carcinogen activation and activating carcinogen detoxifying systems. Also, the main flavonoids in guava

leaves are myricetin and apigenin. Myricetin is good antioxidant and a potent anticarcinogen and anti mutagen (Miean and Mohamed, 2001).

At the same time Mansoroi *et al.* 2005 showed that guava (*Psidium guajava* L.) leaf gave the highest anti – proliferative activity with the IC_{50} value of 0.0379 mg/ml (4.37 times more potent than vincristine) may be due to high content from monoterpene derivatives such as limonene and citral.

CONCLUSION

1- As a results of this study crude ethanolic extract (Et.1 from guava leaves) containing hight amount from tannins, saponinis, flavonoids, phenolic compounds than other extracts (P.E, Ch1. E.A, Et.2 and W).
2- The essential oil from dried leaves contain 32 voltaile components, the major constituent were limonene, junipone, glaucine, beta terpinyl acetate, D – nerolidol, beta – bisabolol, α-humulene and 1, 8 – cineole.
3- Et.1 has high effect in protected rat liver mitochondria and microsomes from lipid peroxidation and free radical scavenging activity than other extracts and essential oil.
4- Et.1 found to be effective in protecting liver injury caused by the administration of CCl_4 than essential oil.
5- Antitumor activity of Et.1 and essential oil on *Erhlisch ascites* carcinoma (EACC) cells showed that essential oil was more effective than Et.1.
6- The previous photochemical study indicated that leaves extracts from *P. guajava* L. as well as the essential oil were safe for use as antioxidant, antitumor and protective liver from damage which induced by CCl_4.

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J. Agric. Sci. Mansoura Univ., 30 (10), October, 2005

التأثيرات البيولوجية لكل من الزيت الطيار والمستخلصات المختلفة لأوراق الجوافة النامية في مصر

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قسم الكيمياء الحيوية – كلية الزراعة – جامعة القاهرة

تعتبر أوراق الجوافة من المصادر الهامة للحصول على المركبات الثانوية كالتانينات والفينولات والفلافونيدات ولها عدة استخدامات في الطب الشعبي في عديد من البلدان كمصر حيث يستخدم المستخلص المائي للأوراق في علاج السعال حيث أن له تأثير مضاد لتقلصات العضلات اللاإرادية للقصبة الهوائية وذلك للمحتوى العالي من الفلافونيدات وأيضاً يستخدم لعلاج حالات الإسهال حيث إن مضاد للميكروبات المسببة للإسهال ولهذا تم إجراء عمليات استخلاص متعاقبة للأوراق باستخدام مذيبات متدرجة في القطبية مثل ألأثير البترولي ، الكلورفورم، خلات الأيثايل، الأيثانول ٩٦٪ والماء وأيضاً تم إجراء استخلاص بالأيثانول ٧٠٪ بمفرده وتم إجراء تحليل وصفى وكمي للمركبات الموجودة في كل مستخلص على حده أوضحت النتائج احتواء المستخلص الكحولي والمائي وخلات الأيثايل على كل من التنينات والفينولات والفلافونيدات والصابونين.

ولمعرفة تأثير هذه المركبات كمضادات لعملية أكسدة اللبيدات في كل من الميتوكوندريا والميكروسوم لكبد الفئران ثم إجراء عملية أكسدة اللبيدات بنظام الحديدوز – اسكوربيات وأيضا تم تقدير عملية التقليل من تكون الشقوق الحرة باستخدام مركب ٢،٢ داى فيثايل بكريل هيدرازيل وقد وجد أن المستخلص الكحولي بمفرده له تأثير مضاد للأكسدة عالي مقارنة بباقي المستخلصات وللمقارنة تم استخلاص الزيت الطيار من الأوراق بعملية التقطير البخاري فوجد أن محتواه في الأوراق ٠,٦٧٪ ووجد أيضا محتواه العالي من التربينات الأحادية كالليمونيين و ١، ٨ سينونول وأيضاً وجد أن الزيت الطيار له تأثير منخفض كمضاد للأكسدة.

وأيضاً لمعرفة تأثير المستخلص الكحولي والزيت الطيار فقط على منع عمليات التدهور التي تحدث للكبد داخل الفئران تم إجراء عملية سمية للكبد باستخدام رابع كلوريد الكربون ومعاملته بالمستخلص الكحولي والزيت الطيار قبل المعاملة برابع كلوريد الكربون فوجد أن المستخلص الكحولي له تأثير واضح على تقليل الآثار السامة لرابع كلوريد الكربون بالمقارنة بالزيت الطيار.

ولمعرفة تأثير المستخلص الكحولي والزيت الطيار كمضاد للأورام السرطانية فتم أجراء تجربة معملية باستخدام خلايا ارليش السرطانية فوجد إن الزيت الطيار له تأثير أعلى من المستخلص الكحولي ومن هذا يتضح أن أوراق الجوافة مصدر من المصادر الهامة للحصول على المواد الطبية الهامة بكميات عالية سواء كمضادات أكسدة وأيضا استخدامها في منع الآثار الضارة الحادثة للكبد نتيجة لعمليات التلوث المختلفة وأيضا المكونات الكيميائية للأوراق تستخدم كمواد مضادة للأورام.