Antioxidant, Antimicrobial, and Anticarcinogenic Properties of Egyptian Guava Seed Extracts

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Abstract: Guava seeds waste not yet used for any beneficial purpose, while the proximate composition of defatted guava seeds meal contain 11.52% protein, 0.54% oil and 79.62% crude fiber. Extraction of total phenolic compounds (TP) from guava seed meal was optimized using ethanol, methanol, acetone or iso-propanol, at different meal:solvent (M:S) ratios. Extraction TP from meal by using 80% acetone was higher than those extracted by other solvents, where TP reached to 91.05 mg TP/100g meal at 1:20 (M:S ratio). Two methods were carried out to determine antioxidant activity of guava seeds meal, the first was Free Radical Scavenging Activity (FRSA) using Diphenyl picrylhydrazyl (DPPH). All extracts at 1:15 and 1:20 M:S ratios exhibited greater scavenging activity than those extracted at 1:10 M:S ratio. The same trend was also observed by using the second method of β- carotene bleaching assay, where the ratios of 1:15 and 1:20 M:S gave extracts characterized with its higher antioxidant activity (99.17-80.93%). The Highest AOA was achieved with acetone extract at 1:15, M:S ratio reaching 99.17%. Eight chosen guava meal extracts were tested for their antimicrobial activity (AMA) against five bacterial strains. The bacterial strains included: *E.coli* 0157:H7 ATCC 51659, *Staphylococcus aureus* ATCC 13565, *Bacillus cereus* EMCC 1080, *Listeria monocytogenes* EMCC 1875 and *Salmonella typhimurium* ATCC25566. The overall results indicated that, different bacteria species were affected by guava seed meal extracts. One chosen guava seed meal phenolic extract namely: 80% ethanol at 1:15 M:S ratio extract has been evaluated as a chemo-preventive agents. This was established by testing the ethanolic guava meal extract (EGME) for any cytotoxic activity against some types of human tumor cell. The obtained results indicate that, EGME possess potential anticarcinogenic properties. Chlorogenic acid, caffeic acid, ferulic acid and O-coumaric acid were identified in EGME at 1:15 (M:S) ratio by using HPLC. The results showed that, guava seed is a very promising source of bioactive compounds. It is a very potential source of antioxidant, antimicrobial, and anticarcinogenic compounds.
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1. Introduction

Guava (*Psidium guaijava L.*) is an important tropical and semitropical fruit mainly consumed fresh. The fruit is a berry which contains a fleshy pericarp and seed cavity with fleshy pulp and numerous small seeds. Egyptian guava is yellow in color and owing to its abundance and reasonable price it is eaten by all sectors of the society. The main guava fruits products are beverages, juices, and canned slices. The seeds are the waste product of these industries and are not yet used for any beneficial purpose. Much work has been reported on the biological activity of guava peel, flesh and leaves (Sanda *et al*, 2011; Labibah 2009; Gutiérrez *et al*. 2008). On the other hand, little attention has been given to the tons of guava seeds produced each year. The seeds are thrown in landfills which may present environmental load. Guava seeds have been reported to contain 14% oil, 15% protein and 13% starch (Burkill, 1997). The concentration of total extractable phenolics in guava seed powder using different solvents ranged from 834.83mg $100g^{-1}$ in the water extract to 1.10mg 100g $^{-1}$ (Azouz and Kishk 2007). Michael et al. (2002a) detected ten phenolic and flavonoid compounds in guava seeds.

A free radical is an atom missing an electron. Free radicals are unstable and seek to bond to other atoms to reach stability. They do so by stealing their electrons from other atoms, and in doing so they can cause damage to cells or to DNA contained within the cells. Free radicals are thought to play a part in the ageing process, in some autoimmune diseases, and in the development of cancer, heart disease, osteoporosis and many others (Florence 1995; Pham-Huy et al. 2008). Free radicals are produced through a number of normal internal functions of the body as well as when the body is subjected to certain toxic environmental pollution. The body can normally cope with low levels of free radicals, but high levels needs substances from outside the body to fight them. Antioxidants are substances that combat or neutralize free radicals before excessive damage occurs to our body cells.

Phytochemicals are substances present in the plant kingdom. Among these phytochemicals phenolic compounds are the most common in nature. Phenolic compounds exhibit a wide range of physiological properties, such as antioxidant, anti-allergenic,

anti-artherogenic, anti-inflammatory, anti-microbial, anti-thrombotic, cardioprotective and vasodilatory effects (Benavente-Garcia, 1997; Manach, et al. 2005; Puupponen-Pimia et al., 2001; Samman et al.1998). Phenolic compounds have attracted much interest recently because *in vitro* and *in vivo* studies suggest that they have a variety of beneficial biological properties which may play an important role in the maintenance of human health. Their significance in the human diet and their antimicrobial activity has been recently established (Ramesh and Pattar, 2010). The antimicrobial activity of several essential oils has been attributed to the presence of phenolic compounds (Tsao and Zhou, 2000; Lambert et al, 2001).

Extraction of phenolic compounds from plant material is influenced by various parameters such as solvent polarity, particle size, extraction procedures and conditions. The impact of the extraction of phenolic compounds on the analysis has often been overlooked as substantial variations in the extraction procedures and solvents are documented in the recent literature (Naczk and Shahidi 2004; Antolovich et al 2000). Several solvents such as methanol, ethanol, acetone, water, ethyl acetate and, to a lesser extent, propanol, dimethyl formamide, dimethyl sulfoxide and their combinations have been used for the extraction of different classes of phenolic compounds (Naczk and Shahidi, 2004; Antolovich et al., 2000; Majors, 1995; Vrhovsek et al., 2004; Parejo et al., 2004).

Seeds as the by-product of the fruit processing industry have been reported to contain phenolic compounds (Jasna et al.2009, Packer et al., 2010 and El-Bedawey et al., 2010). Seeds that were found to contain phenolic compounds include: citrus seeds (Bocco et al. 1998), apricot kernel (Yiğit et al. 2009) and mango seed (Maisuthisakul 2008).

Thus the aim of the present study was to add value to a waste product (guava seeds).This will be achieved through the evaluation of the biological activity of guava seeds in order to use them as a sustainable source for the pharmacological and food industries. The phenolic extracts of guava seeds will be examined for their antioxidant, antimicrobial and anticarcinogenic activity.

2. Materials and Methods

Guava (*Psidium guaijava* L.) seeds are the crop of summer 2010. The seeds were supplied by the Egyptian Fruit Company (Best), 6th October, Egypt. The seeds were cleaned, washed in running water; air dried, and then ground using a Wiley Mill. The ground seeds were defatted in a soxhlet apparatus with n-hexane to remove the oil. Defatted ground seeds (meal) were spread to dry at room temperature and ground to pass through a 60 mesh screen and saved for further work.

Microorganisms (*E.coli* 0157:H7 ATCC 51659, *Staphylococcus aureus* ATCC 13565, Bacillus cereus EMCC 1080, *Listeria monocytogenes* EMCC 1875 and *Salmonella typhimurium* ATCC25566) were obtained from the Microbiological Resources Center (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University.

Extraction of phenolic compounds using organic solvents: In this experiment the effect of 80% methanol, 80% ethanol, 80% acetone and 80% iso-propanol on the extraction of total phenolic compounds from guava seeds meal was tested. Experiments were carried out at room temperature, at a meal : solvent ratios of 1:10, 1:15, and 1:20 w/v. A single extraction was carried out for 30 min. using an electric stirrer. The extract was filtered and then subjected to rotary evaporation (BUCHI- Germany) until almost dryness (5ml). After which, analytical methods were carried out on different crude phenolic extracts of guava meal.

Analytical methods:

Moisture, oil, protein, ash and crude fibre contents of guava seeds and meal were determined according to A.O.A.C. (2005).

Total phenolic compounds were determined by the Folin Ciocalteu method according to (Hung et al. 2002) and measured as gallic acid equivalent.

Antioxidant activity was determined by two methods: Free radical scavenging activity (DPPH) assay according to (Kuda et al. 2005) and by the β-carotene/ linoleic acid method described by (Al-Shaikhan et al., 1995).

HPLC analysis was carried out according to (De Leonardis et al, 2005) using an HPLC system – HP1100 (Agilent Technologies, Palo Alto, CA, USA), equipped with an auto-sampler, quaternary pump and diode detector. Identification was carried out according to standard phenolic compounds.

Antimicrobial activity for different extracts was tested against five pathogenic bacterial strains using the disc diffusion method as described by Kotzekidou et al. (2008).

Anticarcinogenic activity was determined in the National Cancer Institute (Biology Department) on Intestinal Carcinoma cell line (CACO) by measurement of potential cytotoxicity of the phenolic extract which was carried out by the Sulfo-Rhodamine-B stain (SRB) assay, according to the method of (Skehan et al., 1990). Cell line Carcinomas were Liver Carcinoma Cell Line (HEPG2), Larynx Carcinoma Cell Line (HEP2), Colon Carcinoma Cell Line (HCT), Cervical Carcinoma Cell Line (HELA), Breast Carcinoma Cell Line (MCF7), Intestinal carcinoma cell line (CACO).

Statistical analysis: The results are presented as average and standard deviation (SD). All results were evaluated statistically using analysis of variance according to (McClave & Benson 1991).

3. Results and Discussion

Table (1) gives the proximate composition of guava seed and guava defatted seeds (meal). Protein content in the guava seeds and meal was 8.22 and 11.52%, respectively, while the oil content was 13.63 and 0.54%, respectively. Guava seeds and meal contain very high levels of crude fibre, 68.23% in the seed and 79.62% in the meal. Prasad and Azeemodin (1994) reported that, the moisture, oil, protein, crude fibre and ash contents of guava seeds were 4.1, 16.0, 7.6, 61.4 and 0.93%, respectively, while the defatted guava seeds meal were 1.5, 0.4, 9.0, 73.0 and 1.1%, respectively. Moreover, Burkill (1997) stated that, guava seeds contain 14% oil, 15% protein and 13% starch.

Table 1. Proximate composition of guava seed and meal

Constituents (%)	Guava Seeds	Defatted Guava Seeds (Meal)
Protein	8.22 ± 0.28	11.59 ± 0.71
Oil	13.63 ± 0.53	0.54 ± 0.52
Ash	1.09 ± 0.61	1.21 ± 0.36
Fiber	68.23 ± 0.45	79.62 ± 0.84
NFE	8.83 ± 0.75	7.04 ± 0.69

Values in table are given on moisture free basis ± Standard deviation. NFE= Nitrogen free extract.

Phenolic Compounds from Guava Seeds Meal

Since the solubility of phenolics in general is governed by their chemical nature which may vary from simple to very highly polymerized substances. Polar organic solvents are most effective in solubilizing phenolic compounds from plant materials (Goli et al. 2005; Turkmen 2006). It thus seemed advisable to first examine the type of solvent suitable for optimum extraction of phenolic compounds from guava seed meal. In this context 80% methanol, 80% ethanol, 80% acetone and 80% iso-propanol were investigated.

Table (2) indicates the total phenolic contents (TP) that extracted from guava seed meal by using different solvents, at different Meal: solvent (M:S) ratios. Statistical analysis showed a significant difference ($p<0.05$) between the amount of phenolic compounds that extracted by the four investigated solvents. A significant difference was also clear between the three investigated M:S ratios when using the examined solvents each individually. Acetone at 1:20 M:S ratio extracted the highest amount of TP from guava meal to reach 91.05 mg TP/100g guava meal. This was followed by methanol at 1:20 M:S ratio extracting 79.56 mg TP/100g guava meal. While, ethanol and iso-propanol at the three investigated M:S ratios extracted 42.48-24.5 and 14.54-34.63 mg TP/100g guava meal, respectively. Azouz and Kishk (2007), reported that, chloroform extracted 1.10 mg TP/100g from guava seed powder. Also, Castro-Vargas et al. (2010) extracted phenolic fraction from guava seeds using supercritical carbon dioxide and co-solvents, the obtained yield reached to 0.380 and 1.738% (w/w) by using supercritical and the soxhlet procedures, respectively. Norshazilla et al. (2010) found Malaysian guava seeds to contain 20 mg phenolic/100g seeds as gallic acid equivalents. This controversy in results is due to difference in genotypes.

Results of Table (2) showed that, acetone extracted more TP probably because acetone polarity is more suitable than the three other solvents. Kim et al. (2007) developed a method of designing solvents for the optimal extraction of bioactive ingredients from mulberry leaves using an alcohol-water binary solvent. From their study they reported that the extraction efficiency of the bioactive ingredients was correlated with the solvent polarity. This finding is in agreement with our results. Taha et al. (2011) studied for the optimization of phenolic compounds and chlorogenic acid extraction from sunflower meal. They found that 80% acetone extracted maximum phenolics and chlorogenic acid when applying conventional, microwave-assisted, and ultrasound assisted extractions. Sun et al. (2006) prepared oat groat phenolic extracts using acetone, methanol and hexane. The acetone extracted highest amount of phenolic compounds.

Table 2. Total Phenolic Compounds Extracted From Guava Seed Meal (mg/100g) Using Different Solvents and Different Meal: Solvent Ratios

Solvents	1:10	1:15	1:20	LSD
Acetone	47.48 $^{3/a}$± 0.50	64.58 $^{2/b}$± 0.52	91.05 $^{1/a}$ ± 0.47	0,9989
Methanol	19.52 $^{3/c}$± 0.50	75.80 $^{1/a}$± 0.75	79.56 $^{2/b}$± 0.51	1,2015
Ethanol	24.5 $^{3/b}$± 0.50	39.64 $^{2/c}$± 0.50	42.48 $^{1/c}$± 0.55	1,0397
iso-Propanol	14.54 $^{3/d}$± 0.45	28.49 $^{2/d}$± 0.50	34.63 $^{1/d}$± 0.55	1,0046
LSD at 5% level	0,9197	1,0912	0,9898	---

Different numbers in each row indicates significant differences of extraction ratios at ($P<0.05$) for each solvent.

Different letters in each column indicates significant differences between solvents at ($P<0.05$) for each extraction ratio.

Antioxidant activity of Guava meal phenolic extracts

All plant phenolic classes have the structural requirements of free radical scavengers and have potential as food antioxidants (Jayathilankan et al. 2007). The antioxidant activity (AOA) of phenolic compounds may result from the neutralization of free radicals initiating oxidation processes or from the termination of radical chain reactions. Also AOA of phenolic compounds is due to their high tendency to chelate metals. In this investigation two different in vitro methods have been used for the determination of the antioxidant activity of the extracts: The DPPH free radical scavenging activity (FRSA) and Inhibition of β-carotene co-oxidation in a linoleate model system.

DPPH (1,1-Diphenyl-2-picryl-hydrazyl) free radical scavenging activity method is widely used in antioxidant activity studies. In this method the reduction of alcoholic DPPH solutions in the presence of a phenolic compound which have been reported to be potent hydrogen donators to the DPPH radical (Von Gadow et al., 1997) because of their excellence structural chemistry (Rice-Evans et al., 1997).

Table 3. Radical Scavencing Activity of Guava Meal Phenolic Extracts as Measured by the DPPH Method.

Solvents	Extraction ratio	Inhibition %			
		25 µl	50 µl	100 µl	R^2
Acetone	1:10	82.32 ^{c}± 0.30	86.57 ^{d}± 0.51	88.30 ^{e}± 0.36	0.9441
	1:15	92.28 ^{a}± 0.28	92.54 ^{b}± 0.50	93.24 ^{bc}± 0.24	0.9846
	1:20	92.44 ^{a}± 0.41	93.47 ^{a}± 0.50	94.22 ^{a}± 0.25	0.9918
Methanol	1:10	71.25 ^{g}± 0.69	77.53 ^{h}± 0.50	81.15 ^{h}± 0.74	0.9765
	1:15	89.90 ^{b}± 0.60	91.49 ^{c}± 0.50	92.81 ^{cd}± 0.17	0.9971
	1:20	89.59 ^{b}± 0.37	92.28 ^{b}± 0.30	92.57 ^{d}± 0.25	0.8222
Ethanol	1:10	73.58 ^{f}± 0.52	80.51 ^{f}± 0.45	84.33 ^{f}± 0.25	0.9700
	1:15	78.62 ^{e}± 0.4	92.60 ^{b}± 0.40	93.24 ^{bc}± 0.24	0.7800
	1:20	80.57 ± 0.45 ^{d}	93.40 ^{a}± 0.39	93.47 ^{b}± 0.50	0.7500
iso-Propanol	1:10	56.56 ^{h}± 0.45	79.72 ^{g}± 0.63	83.49 ^{g}± 0.50	0.8527
	1:15	69.51 ^{I}± 0.45	83.40 ^{e}± 0.36	92.60 ^{cd}± 0.40	0.9848
	1:20	73.28 ^{f}± 0.29	92.34 ^{b}± 0.39	92.81 ^{cd}± 0.18	0.7680
LSD at 5% level	- - -	0,7643	0,7858	0,6378	- - -

Different letters in each column indicates significant differences between solvents at ($P<0.05$) for each extraction ratio.

R^2 Correlation coefficient (linear)

DPPH activities of the four extracts at 1:10,1:15, and 1:20 M:S ratios of guava seed meal at three different concentrations (25µl, 50µL and 100µl) are represented in Table (3). Statistical analysis between treatments at different M:S ratios at each phenolic concentration is demonstrated in the same table. Correlation coefficient (R^2) of phenolic concentration and each M:S ratio for every solvent ranged between 0.99 to 0.75. Highest FRSA (94.22%) was achieved by TP extract resulting from acetone at 1:20 M:S ratio and 100µL of the extract. This result is in agreement with Taha et al. (2011) and Sun et al. (2006). At 25µL phenolic concentrations all FRSA values were below 90% except for acetone extract at both 1:15 and 1:20 M:S ratio, where FRSA reached 92%. Meal : Solvent ratio of 1:10 with all the tested solvents and at all the examined concentration resulted in inferior FRSA values than 1:15 and 1:20. Generally, all TP extracts resulting from acetone, methanol, and ethanol at 1:15 & 1:20 M:S ratios with TP concentration 50µl and 100µL exhibited FRSA ranging between 91-94%. Norshazilla et al. (2010) studying the antioxidant activity of guava, mango and papaya seeds reported the seed extracts to possess 93-96% FRSA at concentration of 1mg/ml. Phenolic compounds generally exhibit significant scavenging effects against DPPH free radical (Michael et al 2002 b).

The second method used for the determination of the antioxidant activity of guava seeds meal was the β-carotene bleaching assay. In this method, oxidation of linoleic acid releases linoleic acid peroxides as free radicals that oxidizes β-carotene resulting in discoloration, thus decreasing the absorbance value (Talcott et al. 2000).

Results of the antioxidant activity (AOA) of guava meal extracts as measured by the β-carotene bleaching assay are represented in Table (4). Statistically there is a significant difference (at $p<0.05$) between AOA of the four solvent extracts, at 1:10, 1:15, and 1:20 M:S ratio. The exception was both ethanol and iso-propanol extracts of guava seeds meal at 1:15 M:S ratio that showed no significant difference

between them. Results showed significant difference between the three M:S ratios for each solvent. The four investigated solvents at both 1:15 and 1:20 M:S ratios gave extracts with high AOA ranging between 99.17 – 80.93%. At M:S ratio 1:10 the AOA was much lower 55.82 - 81.37%. Highest AOA was achieved with acetone extract at 1:15, M:S ratio reaching 99.17%. There is a negative correlation between phenolic content and antioxidant activity. Antioxidant activity of seed extracts could not be predicted on the basis of its total phenolic content only (Ordonez et al 2005). Tawaha et al. (2007) suggested that the negative correlation between TP and AOA may be due to the TP that does not necessarily all the antioxidants that may be present in an extract, for instance betalain that contains both phenolic and non-phenolic structures.

Table 4. Antioxidant Activity of Guava Meal Extracts at Different Meal:Solvent Ratios as Measured by the β-Carotene Bleaching Method.

Solvents	**1:10**	**1:15**	**1:20**	**LSD at 5%**
Acetone	73.59 $^{3/b}$± 0.41	99.17 $^{1/a}$± 0.59	97.88 $^{2/a}$± 0.34	0,9147
Methanol	55.82 $^{3/d}$± 0.52	88.41 $^{2/c}$± 0.52	92.6 $^{1/c}$± 0.36	0,9468
Ethanol	70.77 $^{3/c}$± 0.61	97.46 $^{1/b}$ ± 0.83	80.93 $^{2/d}$± 0.50	1,3622
iso-Propanol	81.37 $^{3/a}$± 0.77	97.00 $^{1/b}$± 0.5	94.44 $^{2/b}$± 0.67	1,3215
LSD at 5% level	1,1447	1,1826	0,9228	- - -

Different numbers in each row indicates significant differences of extraction ratios at (P<0.05) for each solvent.

Different letters in each column indicates significant differences between solvents at (P<0.05) for each extraction ratio.

Antimicrobial activity of guava meal phenolic extracts

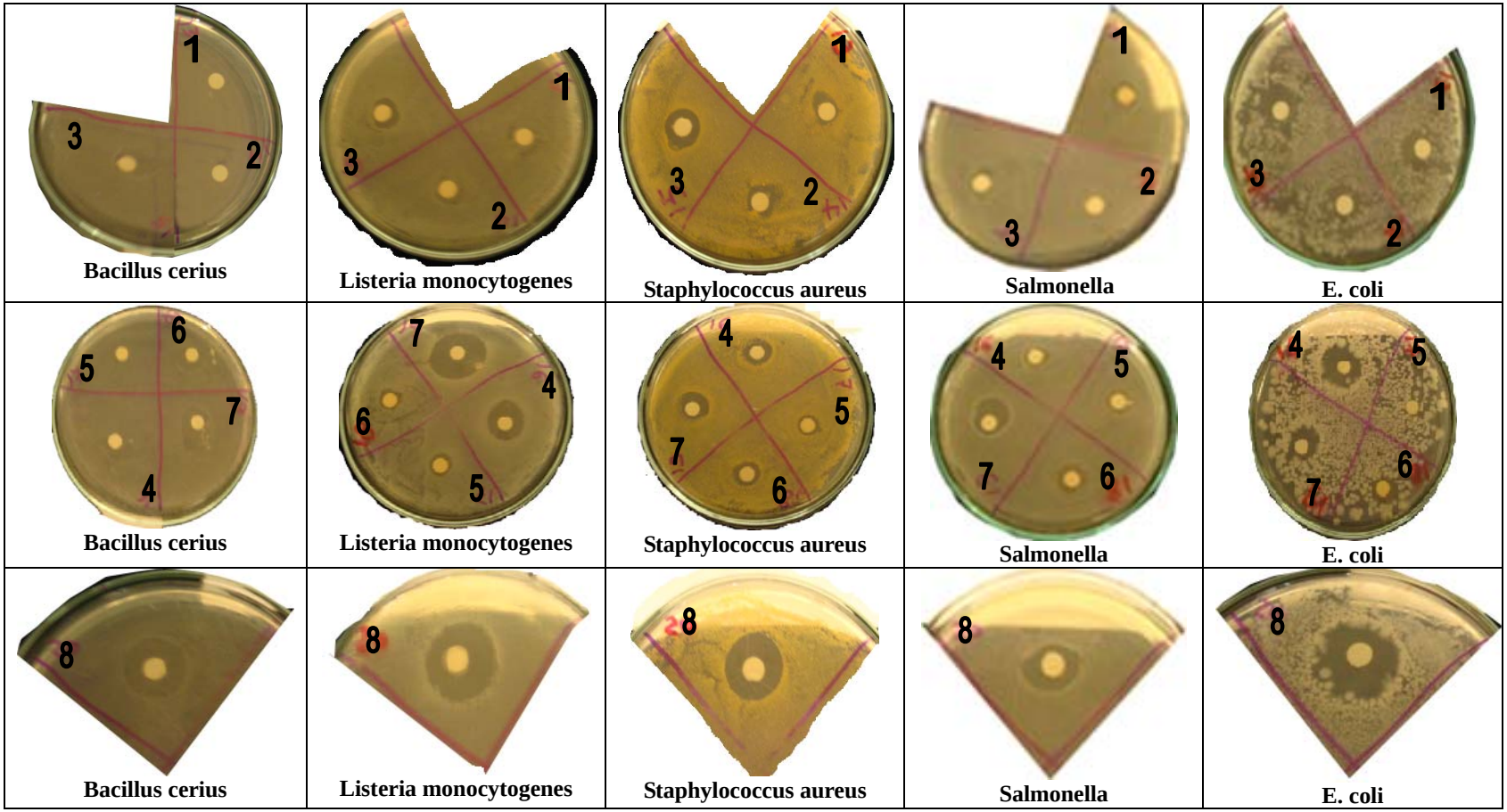
Phenolics were the most important compounds with antibacterial activity, and that among several phenolics gallic acid was identified as the most active compound for inhibition of bacteria tested (Shoko et al., 1999). Thus, it seemed worthwhile to evaluate the chosen phenolic extracts as antimicrobial agents. Eight chosen phenolic guava seed meal extracts were examined for their antimicrobial activity (AMA) against five bacterial strains using the disc diffusion method. The chosen phenolic extracts were resulting from 80% acetone, methanol, ethanol and iso-propanol, at 1:10 and 1:15 M:S ratios.

Table (5) and Figure (1) revealed that, all the guava meal phenolic extracts exhibited different levels of antimicrobial activities against five tested pathogenic bacteria, at both M:S ratios investigated. An exception was observed with the guava meal methanolic extract at 1:20 (M:S), which had a negative effect on *Bacillus cereus* and *Salmonella typhimurium.* Highest inhibition of bacillus cereus and *E.coli* was achieved with isopropanol guava extract at 1:20 M:S ratio (zone inhibition diameter 18 mm and 18.6 mm), respectively. Highest zone of inhibition 22.3 mm diameter was achieved with isopropanol, 1:15 (M:S) against *Listeria monocytogenes.* Ethanol guava meal extract at 1:20 (M:S) resulted in highest inhibition of *Staphylococcus aureus* and *Salmonella typhimurium* with inhibition zone diameters 17 mm and 12.3 mm, respectively.

Table 5. Effect of different guava meal extract at two M:S ratios on the gross inhibition of the five tested pathogenic bacteria strains.

Samples	Strains/ inhibition zone diameter (mm)				
	B.c	**Lis**	**St**	**Sa**	**E0157**
Meal:Methanol					
1:15	11	11.3	11.6	9.6	15
1:20	- - -	9.3	11.5	- - -	10.3
Meal:Eethanol					
1:15	13.6	11.3	14.3	11	15
1:20	15.6	16.6	17	12.3	17.7
Meal: Acetone					
1:15	17	9	10	12	11.3
1:20	15.6	10.3	10.6	11.6	10.5
Meal: Isopropanol					
1:15	12.3	22.3	14.5	16	14.5
1:20	18	15	15.8	13	18.6

B.c (*Bacillus cereus* EMCC 1080), Lis (*Listeria monocytogenes* EMCC 1875), St (*Staphylococcus aureus* ATCC 13565), Sa (*Salmonella typhimurium* ATCC25566) and E. O157 (*E.coli* o157:H7 ATCC 51659).



- 1: Meal: Methanol (1: 15). 2: Meal:Methanol (1:20). 3: Meal:Eethanol (1 : 15) 4: Meal: Eethanol (1: 20).
- 5: Meal: Acetone (1: 15). 6: Meal: Acetone (1 : 20) 7: Meal: Isopropanol (1: 15) 8: Meal: Isopropanol (1: 20).

Figure 1. Antimicrobial activity of extracted defatted guava seed meal by using some solvents, against five pathogenic bacterial strains.

The overall results indicated that, different bacteria species exhibit different sensitivities towards phenolics. In the present work Gram-positive and Gram-negative microorganisms were affected by the phenolic extracts of guava seed meal. Estevinho et al (2008) reported that, the susceptibility of bacteria to phenolic compound and Gram reaction appears to have influence on growth inhibition. The inhibitory effect of phenols could be explained by interactions with the cell membrane of microrganisms and is often correlated with the hydrophobicity of the compounds (Sikkema *et al.*1995; Weber and De Bont 1996). Phenolic compounds could have an activating or inhibiting effect on microbial growth according to their constitution and concentration (Rauha *et al*., 2000; Reguant *et al*., 2000; Alberto *et al*., 2001, 2002; Estevinho *et al*., 2008; Rodríguez Vaquero *et al*., 2010).

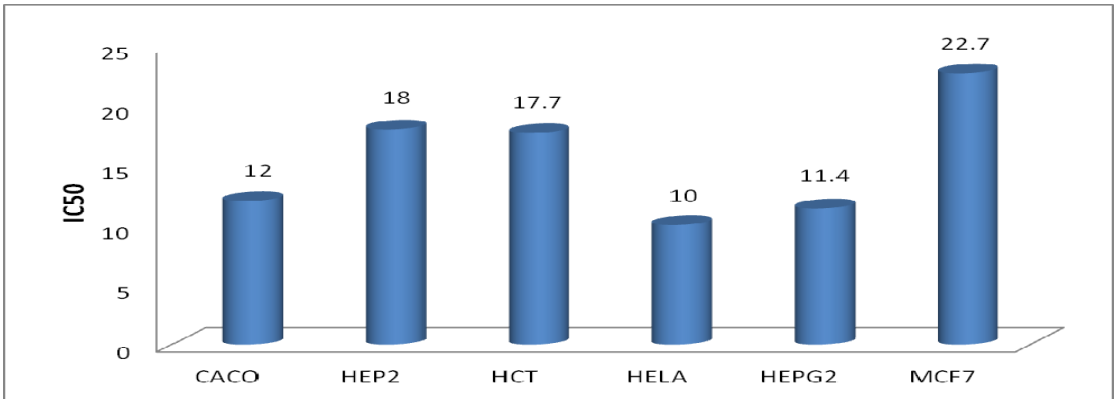
No work was reported on the antimicrobial potential of guava seed extracts. Yet the extracts of guava leaves were reported to inhibit the growth of several pathogenic bacteria (Arima and Danno 2002; Sanda et al. 2011)

Anticarcinogenic activity of guava meal phenolic extract

This evaluation was carried out in the National Cancer Institute, Biology Department, Cairo. The experiment was done by the Sulfo-Rhodamine-B stain (SRB) assay. One chosen guava seed meal phenolic extract (ethanol at 1:15 M:S ratio) has been evaluated as a chemo-preventive agents. This was established by testing the ethanolic guava meal extract (EGME) for any cytotoxic activity against the following human tumor cell lines: Liver Carcinoma Cell Line (HEPG2); Larynx Carcinoma Cell Line (HEP2); Colon Carcinoma Cell Line (HCT); Cervical Carcinoma Cell Line(HELA); Breast Carcinoma Cell Line(MCF7); Intestinal carcinoma cell line (CACO).

Figure (2) represent the effect of EGME on the human carcinoma cell lines tested and the results are indicated by the IC_{50}, which is the dose of the compound (guava phenolic extract) which kills surviving cells up to 50%. The smaller the concentration or dose the more effective is the compound. Looking at Figure (2), the following could be observed:

- That EGME was most effective on Cervical Carcinoma Cell Line (HELA) with a IC_{50} = 10 µg/ml. This means that at this dose of EGME 50% of the surviving cells were killed.
- Liver Carcinoma Cell Line (HEPG2) and Intestinal carcinoma cell line (CACO) needed 11.4 µg/ml and 12.0 µg/ml of EGME to reach IC_{50}, respectively.
- This was followed with Colon Carcinoma Cell Line (HCT) and Larynx Carcinoma Cell Line (HEP2) with 17.7 and 18 µg/ml of EGME to reach IC_{50}, respectively.
- Breast Carcinoma Cell Line (MCF7) needed the highest dose of EGME 22.7 µg/ml to reach IC_{50}.



Intestinal = CACO Larynx = HEP2 Colon = HCT
Cervical = HELA Liver = HEPG2 Breast = MCF7

Figure 2: Anticarcinogenic effect of ethanolic extract of guava seed meal on different carcinoma cell lines.

The only work reported on the use of guava seed extract as anticarcinogenic agent was reported by (Salib and Michael 2004) where they found that the acetone extract of guava seeds showed moderate activity against Ehrlich ascites carcinoma cells and Leukemia P338 cells.

These results indicate that EGME possess potential anti carcinogenic properties, but as recommended by the Biology Department –National Cancer Institute-Cairo further pharmacological investigations in vitro and in vivo are required to confirm the activity of the tested EGM extract.

HPLC analysis of the ethanolic guava seed meal extract

The ethanolic extract (ethanol at 1:15 M:S ratio) of guava seed meal was subjected to HPLC. Figure (3) shows the separation a large number of compounds of which four phenolic acids were identified. The phenolic acids were identified according to their retention time in comparison with authentic samples. The identified phenolic acids were: chlorogenic acid (5.934 min), caffeic acid (6.858 min), ferulic acid (8.424 min), and O-coumaric acid (9.583).

Michael *et al*. (2002a) isolated ten phenolic and flavonoid compounds including one new compound from guava seeds. Among the phenolic and flavonoid components were: ellagic, gallic, and caffeic acid, methyl gallate, 8,3'-dimethoxy gossypetin, quercitin 3-o-glucoside with the aglycones kaempferol, quercitin and myricetin along with the natural acylated flavonol glycoside.

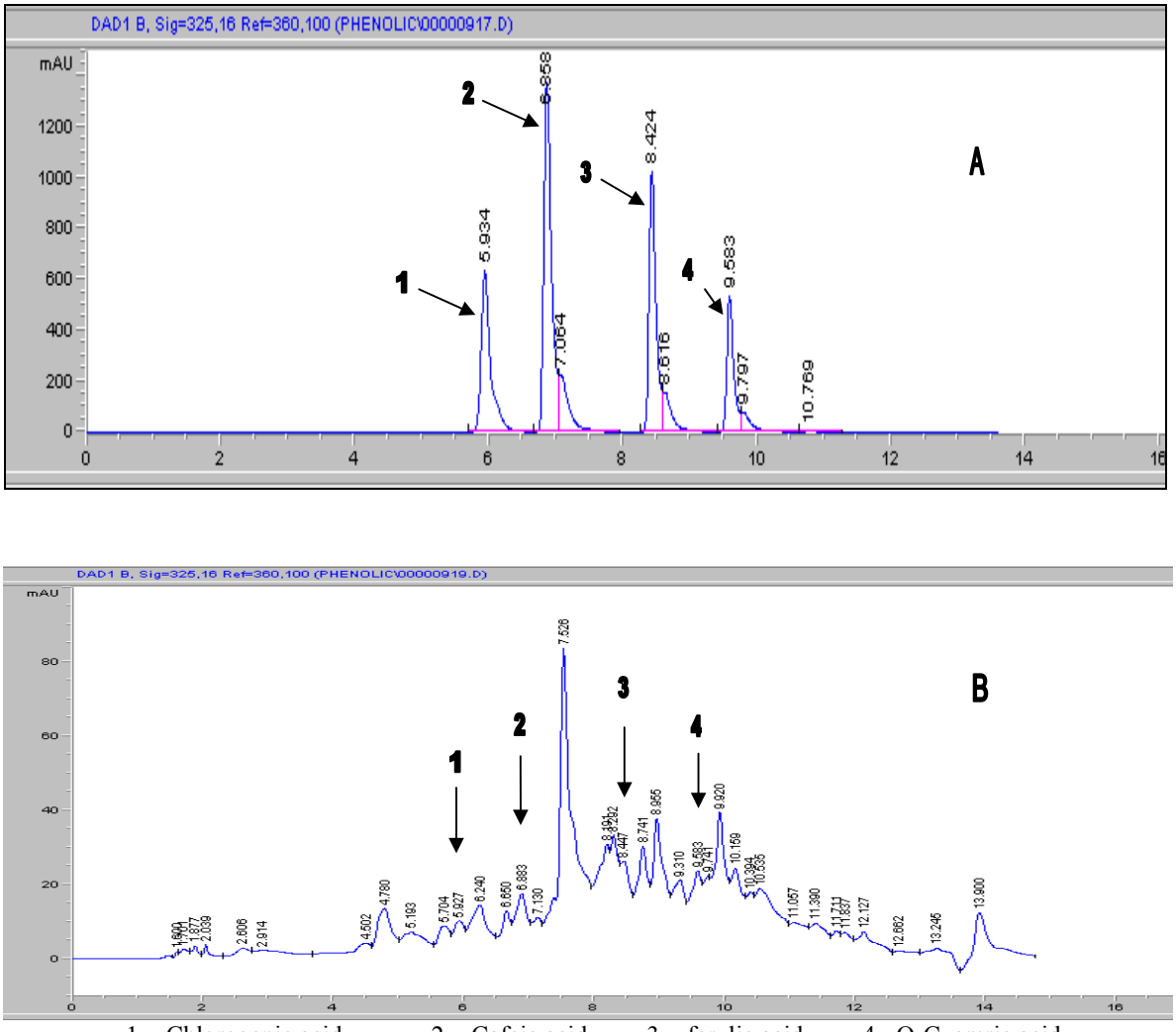


Figure 3. HPLC chromatogram of four standard phenolic compounds (A) and ethanolic extract of guava seed meal (B).

Conclusion

Guava seed meal is a very promising source of bioactive compounds. It is a very potential source of antioxidant, antimicrobial, and anticarcinogenic compounds.

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