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Research Article
A Comparative Evaluation of Microwave and Conventional Soxhlet Extraction Methods for the Antioxidant, Hypoglycemic and Hypolipidemic Potentials of Jordanian *Psidium guajava* Raw Fruit Peel Extracts

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Abstract

Background: *Psidium guajava* L. (Myrtaceae) is one of the medicinal plants in Jordan that have been reported in the folk medicine for different indications and few of them have been experimentally and/or clinically determined. **Objective:** The current study aims to explores the aqueous extracts obtained from Jordanian premature *P. guajava* fruit peel (PGFP), using two different methods for extraction. Also to assess these extracts different pharmacological activities as well as their phytochemistry. **Materials and Methods:** Two different methods for extraction were used, Microwave-Integrated Soxhlet (MIS) and Conventional Soxhlet (CS) extraction methods. The TLC analysis was used to optimize each extraction method and for the preliminary phytochemical screening. The HPLC-MS/MS analysis allowed the identification and quantification of the phenol compounds in the PGFP two extracts. For each extract type, the antioxidant activity was evaluated using the ABTS method. In addition, the hypoglycemic and hypolipidemic potentials for each extract were also evaluated on normoglycemic and streptozotocin-treated diabetic rats. Statistical analysis was performed using the one-way analysis of variance (ANOVA) in order to reveal significant ($p<0.05$) differences between the tested animal groups. **Results:** The CS extract showed ellagic acid as the major constituent, followed by gallic, P-coumaric, furelic acids, quercetin and ascorbic acid in order of their percentage content (total phenol content). Regarding the MIS extract, ascorbic acid was detected as the major compound followed by gallic, P-coumaric acids and quercetin. Moreover, MIS extract showed to contain higher level of total phenol compounds. Comparison of pharmacological studies showed more potent effects of CS as antioxidant and hypolipidemic extract, where the MIS extract showed higher hypoglycemic effect. **Conclusion:** The presented data suggest that PGFP can be used for their antioxidant, hypolipidemic and hypoglycemic effects, if the extraction methods were optimized for the required pharmacological effect. In this study, it was correlated that, significant hypolipidemic effect of unripe guava to high content of ellagic acid in its fruit peels and antioxidant action of the active extract. This effect for ellagic acid seems to be mediated by metabolic pathway by which it acts as antilypolytic agent to reduce the consequences of diabetes mellitus, such as atherosclerosis rather than antidiabetic effect.

Key words: *Psidium guajava*, fruit peel, hypoglycemic, hypolipidaemic, microwave, extraction, phenol

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Different plant parts of *Psidium guajava* L. are commonly used as medicinal crude drugs[1]. The fruits are considered as a highly nutritious, because it is a rich source for tannins, phenols, triterpenes, flavonoids, volatile oils, saponins, carotenoids, lectins, fibers, fatty acids, ascorbic acid, as well as carotenoids and its derivatives[2]. In addition, guava fruit also contains sugar, vitamins A and B, proteins and mineral salts, mainly iron, calcium, phosphorus and pectic substances (pectin), polyphenolic compounds, ellagic acid, guiajaverin and quercetin[3,4]. The relative and the absolute composition for these plant secondary metabolites may vary, depending on the origin of the plant, growing conditions, sample pretreatment, extraction temperature and solvent and the extraction method[5].

Potential pharmacological activities of the extract from the fruit, leaf, bark or roots of *P. guajava* include antimicrobial[6], antispasmodic[7], antioxidant and hepatoprotective[8], antihypertensive[9] and hypoglycemic activities[10] in human and in animal models, indicating the immense potential of this plant in treatment of many diseases[11]. Several studies investigated the antihyperglycemic and antidiabetic effect of different parts of *P. guajava*. In particular, *P. guajava* leaves have attracted attention as a folk medicine for diabetes in Japan East Asia and Africa[12,13]. Furthermore, the aqueous extract of *P. guajava* leaves improved diabetes symptoms such as hyperglycemia, nephropathy and insulin resistance in diabetic animal models[14]. This was confirmed by clinical trials using guava leave tea[15]. Moreover, long-term clinical trials in prediabetic and type 2 diabetic patients[16] have shown that ingestion of *P. guajava* leaves tea significantly reduced the serum levels of total cholesterol, triglycerides in patients with hypercholesteremia and hypertriglycemia. The ethanolic extract of the stem bark of *P. guajava* exhibited significant hypoglycemic activity in alloxan-induced hyperglycemic rats with no effect in normal rats[17]. Oral administration of juice from ripe *P. guajava* fruit was shown to cause significant hypoglycemia in normoglycemic and STZ-treated diabetic rats[10]. Recently, although many studies[18,19] showed hypolipidemic and antihyperglycemic effects of aqueous extract of lyophilized unripe guava fruit peel in diabetic rats, they could not correlate the observed activities to the chemical composition of the plants.

In a study by Masadeh *et al.*[20] indicates that ethanolic extracts from a number of medicinal plants grown in Jordan such as *P. guajava*, might be a valuable source for extraction and/or isolation of new antibacterial drugs acting against *S. aureus*, *E. faecalis*, *E. coli* and *S. typhi*. In another study by Masadeh *et al.*[21], they also showed that the ethanol extracts of Jordanian *P. guajava* exerted a potential cytotoxiciy against *H. pylori*. Up to our knowledge, no phytochemical studies have been performed on unripe *P. guajava* fruit peels of Jordanian origin (PGFP). Also, all the above performed studies were conducted using the leaves of *P. guajava* and were only investigating the plants antimicrobial activities.

This study aimed to treat this shortage of data, therefore, Microwave-Integrated Soxhlet (MIS) and Conventional Soxhlet (CS) extraction methods were used to extract PGFP. In addition, for each extract (ext) the antioxidant effect (using ABTS assay method), blood glucose level and lipid profile changes were evaluated in STZ induced diabetic rats, in order to correlate the studied pharmacological activities with PGFP extract phenol content.

MATERIALS AND METHODS

Plant material: Unripe fruits of *P. guajava* obtained from the local market in Amman city, capital of Jordan, which was collected from Jordan valley during the autumn season (September-November, 2013) were used. A voucher specimen of *P. guajava* was compared with that deposited in the Herbarium of the University of Jordan (code ASU August, 2013; voucher No. RI 3/2013).

The raw fruit were peeled off with a knife and the thin greenish peel was cut into small pieces and deposited in freezer at 20°C in the laboratories of the Faculty of Pharmacy, Applied Science University, Jordan for later use.

Extraction

Conventional Soxhlet extraction (CS ext): The peel pieces were mechanically crushed and 30 g were continuously extracted for ~8 h in 150 mL distilled water with total 3-4 extraction cycles. The CS ext was filtered and concentrated in rotatory evaporator at 35±5°C under reduced pressure to obtain semisolid material. The concentrated extract was lyophilized and stored at 4°C until used.

Microwave-Integrated Soxhlet extraction (MIS ext): The MIS ext was performed inside a microwave oven (Sharp [M-340B], Thailand). This oven has a frequency of 2450 MHz with a maximum delivered power of 1000 W. A convenient hole was opened on the microwave oven. The hole was enclosed with PTFE to prevent any leakage of the inner heat, the dimensions of the PTFE-coated cavity are 30×30×20 cm. A clevenger apparatus was settled in the hole and connected with sample

flask. A cooling system outside of the microwave cavity condensed the distillate continuously. The temperature was controlled with the aid of a shielded thermocouple inserted directly into the sample container at 78°C inside the oven and at -1°C for the cooling system. The MIS procedure was performed at atmospheric pressure.

The peel pieces were mechanically crushed and 60 g were loaded in MIS with 50 mL distilled water. The cooling system was seated for extraction time was 20 min cycle^{-1}. This cycle was done in triplicate and then the total extracts were combined, filtered and lyophilized, then stored at 4°C until used.

ABTS antioxidant assay: The principle of the antioxidant 2, 2¢-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assay is based on the method described by Jeong *et al.*[22] with modifications. The antioxidant activity of PGFP extracts was determined by its ability to scavenge the liberated ABTS•+radicals. A calibration curve was established using Troloxä, a water-soluble vitamin E analog, serves as a standard antioxidant. Different trolox concentrations were prepared with its linear equation for assay calculation using the method described in Sigma antioxidant kits. A 25 µL 3% H_2O_2 and 10 µL of PGFP extracts and 20 µL of previously prepared myoglobin solution were incubated for 5 min at room temperature with the addition of 150 µL ABTS substrate working solution. Finally, 100 mL of stop solution was added, followed by determining the absorbance of radical cation spectrophotometrically at 405 nm. Results were expressed as (mg dry extract mL^{-1}). All analyses were run in duplicate and mean values was calculated.

Chromatographic analysis

TLC analysis: In aim to determine the optimal time required for each method of extractions, pre-coated Thin Layer Chromatography (TLC) analysis for PGFP extracts obtained at different time intervals were subjected to qualitative chemical screening for the identification of various classes of active chemical constituents such as carbohydrates, tannins, saponins, cardiac glycosides, steroids, flavonoids and alkaloids. Phytochemical analysis was done using three different chromatographic systems, ethyl acetate:formic acid:glacial acetic acid:water (100:11:11:24), ethanol:water (50:50), isopropanol:ethyle acetate:water (70:10:20) were all developed on TLC plates (Silica gel 60F-254) with the adsorbent layer thickness of 0.25 mm (E-Merck) were used. Detection of the separated spots was done according to the standard methods[23].

HPLC-MS/MS analysis: Chromatographic separation of PGFP extracts were performed on a reversed phase thermohpersil-keystone, DBS Hypersil C8 column (150×4 mm, 5 µm) using an agilent 1200 LC system (Agilent, Santa Clara, CA, USA) equipped with degasser (G1379B), binary pump (G1312A) along with autosampler (G1367B). The autosampler was maintained at 6°C and programmed to draw 20 µL of sample for chromatographic separation.

An isocratic mobile phase of deionized water: 0.01%TEA/methanol (40:60, v/v) was applied at a flow rate of 1.0 mL min^{-1}. The column temperature was kept at 27°C. The total analytical run time was 6.0 min for each sample. Detection was carried out on an AB Sciex (Applied Biosystem/MDS SCIEX, Foster city, CA, USA) API-3200 Q-Trap mass spectrometer, equipped with a turboionspray interface operated in negative ion mode (ESI). Quantification was performed using Multiple Reactions Monitoring (MRM) method. Instrument parameters optimized were collision-activated dissociation gas (CAD): Medium flow; curtain (CUR) gas: 24 psi, nebulizer gas (gas1): 30 psi, heater gas (gas 2): 25 psi, ion spray voltage: -4500 V, source temperature: 550°C. Compound dependent voltage parameters were as listed in Table 1. System control and data analysis were performed by AB Sciex analyst software (version 1.5). The expected phenolic compounds were chosen based on previous studies[11,24-27].

All samples were re-dissolved with known sufficient amount water and then completed with water and methanol with a final diluent composition of (1:3, water: methanol). The dilution process (volume of diluent needed) depends on the produced retention times, where there should be a match in retention times between the standards and the extracted analysts.

Determination of phenol compounds content: Quantification of the detected phenol compounds was performed using area under the peak of the isolated ions and the established one point calibration of the corresponding standard compounds. Analyte final concentrations were determined as (mg kg^{-1}) fresh weight of plant material. All samples were analyzed in triplicates.

Table 1: Expected phenol compounds in PGFP with their MS, MS-MS and dependent voltage parameters

Analytes	MS (m/z)	MS-MS fragment (m/z)	DP	EP	CE	CXP
Ellagic acid	300.6	200.9	-64	-5	-42	-1
Gallic acid	168.8	124.9	-50	-5	-20	-2
Quercetin	300.9	150.9	-50	-5	-27	-1
P-coumaric acid	162.7	118.8	-33	-5	-20	-2
Ferulic acid	192.9	133.9	-35	-6	-23	-2
Ascorbic acid	174.9	114.9	-37	-5	-17	-2

The total content of phenol compounds in each extract was calculated as the sum of concentrations of each compound. The percentage of each phenol compound was calculated as its relative content to the total content of phenol compounds in each extract. The log p-value (the logarithm of the partition coefficient between two phases) for each detected compound was calculated using Marvin view (Version 5.1.4), Chem Axon Ltd., Budapest, Hungary.

Diabetic rats and experimental design

Induction of diabetes on rats: Experiments were performed with 6-8 weeks old, healthy, male Albino Wistar rats, (weighting 200-250 g) obtained from the Applied Science University animal house, Amman, Jordan. All animals were housed under standard environmental conditions (25±2°C temperature, 50±5% humidity with a 12:12 h dark and light cycle) and maintained with free access of water and a standard laboratory diet (carbohydrates 30%, proteins 22%, lipids 12% and vitamins 3%). The study followed the international ethics standards for the care and use of laboratory animals.

Diabetes was induced in the rat by intraperitoneal (i.p.) injection of a freshly prepared streptozotocin (STZ) (Sigma-Aldrich, USA) solution (45 mg kg^{-1}/0.5 mL acetate buffer 0.1 M, pH 4.5) to each group of overnight-fasted rats for every other day during a week. Animals were allowed to drink 5% glucose solution for 24 h after STZ administration to prevent the drug induced hypoglycemic effect. Diabetes was identified by measuring fasting plasma glucose levels one week after injection of the last dose of STZ using eye vein blood sample. The STZ-treated animals was considered as diabetic when the fasting glucose levels[28] are 150-300 mg dL^{-1}.

Experimental design: Diabetic rates were selected and divided into 6 groups of 8 rates each, then treated for 4 weeks as follow:

- **Group 1 (Negative control):** Received 1 mL of oral vehicle (distilled water only)
- **Group 2 (Positive control):** Received a single daily oral dose of 3 mg kg^{-1} of glibenclamide solution as a standard oral hypoglycemic agent starting at 7th day of last dose of STZ treatment
- **Groups 3-6:** Starting at the 7th day of last dose of STZ treatment, received a single daily oral dose of plant extracts with the following concentrations
- Groups 3 and 4 received 200 and 400 mg kg^{-1} of MIS ext and groups 5 and 6 received 200 and 400 mg kg^{-1} of CS ext. The doses of the extracts were determined from preliminary short-term pilot study with a range of variable doses in our laboratory. Vehicle, glibenclamide and plant extracts were given orally by gavage as a single daily treatment for 4 weeks. Animal body weight (BWt) was estimated initially and at the end of the 4th week

Estimation of blood glucose and lipid profile: Fasting blood glucose (FB), triglyceride (TG), Total Cholesterol (TC) and High Density Lipoproteins (HDL) were determined using spectrophotometric assay on eye vein blood serum and utilizing the standard kit (Linear chemicals®, Spain). Absorbencies were read using a UV-Vis spectrophotometer (SPUV-26, SCO TECH®, Germany). Low Density Lipoprotein (LDL) was calculated using Friedewald *et al.*[29] equation.

Statistical analysis: Data are expressed as Mean+Standard Error (SE) of the mean. Statistical analysis was performed using the one-way analysis of variance (ANOVA). If the overall F-value was found statistically significant ($p>0.05$), further comparisons among groups were made according to *post hoc* Turkey's test. All statistical analysis was performed using SPSS version 20.

RESULTS

Comparison between the MIS and CS extraction methods: Table 2 describes the differences between the two exts obtained using different extraction methods. The percentage yield of the extraction (with respect to the starting fresh plant material) using the CS extraction method was approximately twice the yield obtained using the MIS method. Nevertheless, MIS method was more time and energy efficient.

Antioxidant activity: The antioxidant action for the CS ext was superior to the MIS ext using the ABTS method with percentage of difference in IC_{50} of 9.36% (Table 2).

TLC analysis: In the preliminary phytochemical study, TLC analysis for each ext type showed the presence of carbohydrates, saponins, amino acids, tannins, volatile oils, alkaloids and flavonoids in each ext with different composition (Table 2).

Table 2: Effect of extraction methods (CS and MIS) on the physical characterizations, chemical composition and antioxidant activity of PGFP extracts

Extract characters	CS method	MIS method
Wight of fresh plant material/cycle	30 g	60 g
Extraction time	8 h	1 h
Averaged percentage of yield	4.8%	2.5%
Extraction temperature	90-100°C	78-88°C
Lyophilized extract color	Caramel brown powder	White to ivory powder
Antioxidant activity: IC_{50} (mg dry extract mL^{-1})	0.2970	0.3248
Tannins	++	++
Saponins	+	+
Cardiac glycosides	-	-
Carbohydrates	+++	++
Flavonoids	++	+++
Alkaloids	+	+
Amino acids	++	++
Volatile oils	++	++

-: Absent, +: Slightly present, ++: Moderately present and +++: Highly present

Table 3: Log p-value, yield (mg kg^{-1}) and percentage content for each detected phenol compound in the PGFP extracts using CS and MIS extraction methods

		CS		MIS	
Metabolites	Log p-value#	mg kg^{-1}*	%**	mg kg^{-1}*	%**
Ellagic acid	2.32	12.1	30.86	ND	-
Quercetin	2.16	2	5.10	0.7	0.35
P-coumaric acid	1.83	8.3	21.17	3	1.50
Ferulic acid	1.67	7.2	18.36	ND	-
Gallic acid	0.72	8	20.40	26.4	13.26
Ascorbic acid	-1.91	1.6	4.08	169	84.88
Total		39.2	-	199.1	-

#Calculated using Marvin view (version 5.1.4), Chem Axon Ltd., Budapest, Hungary, *Yield for the metabolite determined as mg kg^{-1} of fresh weight of plant material, **Percentage of metabolite to the total sum of phenols in the extract and ND: Not detected

HPLC-MS/MS analysis: The profile of the phenol compounds in each PGFP ext was determined using HPLC-MS/MS analysis, showed six of the expected phenol compounds in the CS ext, while only four compounds were determined in the MIS ext (Table 3).

Comparison of phenol compounds content between the two exts: Table 3 showed the content of phenol compounds in each ext using the HPLC-MS/MS method. Ellagic acid was found as the major compound in the CS ext with 30.86% of total phenol content, followed by p-coumaric acid, gallic acid, ferulic acid, quercetin and ascorbic acid at low concentrations. On the contrary, ascorbic acid was found as the major compound in the MIS ext with a very high content of 84.88%, followed by gallic acid, while quercetin and p-coumaric acid were found at very low concentrations, approximately less than half their levels in the CS extract.

In addition to the qualitative and quantitative content differences in the individual phenol compounds, the MIS ext showed to contain approximately 5 times higher content of total phenols than its content in the CS ext.

Pharmacological studies

Effect of exts on blood glucose: At the end of the 4th week of treatment, the MIS ext treated groups showed potential hypoglycemic effect in STZ diabetic rats in a dose dependant manner (Table 4). Although, this effect of MIS ext was less than the effect of glibenclamide, it was more pronounced than the CS ext effect.

Effect of exts on body whight: The body weight for all the experimental animal groups showed a pronounced reduction compared to their initial body weight (Table 4) except for the glibenclamide treated group were an increase in the animal body weight was found. While the MIS ext treated groups resulted in body weight loss not very different from the effect of the untreated control group, the CS ext treated groups showed a significant prevention of sever body weight loss ($p>0.05$), when compared to the untreated control group.

Effect of exts on blood lipid profile: Both exts showed dose dependent hypolipidemic effect as shown by the pronounced reduction in TC and TG serum levels (Table 4). The most significant treatment was for the higher dose of the CS ext ($p<0.05$), compared to the other treated and the untreated animal groups.

In general, an increase in the serum levels of HDL was reported with all animal groups treat with both exts, in a dose dependent manner compared to the untreated control group, which didn't show any detectable changes. This increase was more pronounced than the effect of glibenclamide, when using the MIS ext at the higher dose. On the contrary, only the higher dose of CS ext showed a significant reducation effect on the LDL serum level ($p<0.05$), compared to the other treated and the untreated animal groups. This effect was more superior than the effect of the glibenclamide treated group.

Table 4: Effect of oral administration of the tested plant extracts on post treatments Fasting Blood Glucose Levels (FBGL), Total Cholesterol (TC), triglycerides (TG), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) and body weight (BWt) in STZ diabetic rats for duration of treatments of 4 weeks

Groups	INT FBGL	FBGL±SE	%†	TC±SE	%†	TG±SE	%†	HDL±SE	%†	LDL±SE	%†	BWt±SE	%†
G1 diabetic untreated	205.01	235.21±39.34	12.84	241.15±49.58	67	140.73±39.09	71	47.30±6.05	0	165.70±46.99	85	159.00±11.83	-28
G2 glibenclamide 3 mg kg^{-1}	200.98	105.78±18.79	-89.99	151.20±17.58	2	86.83±16.27	-3	47.09±8.09	23	86.74±14.38	-20	141.25±12.80	37
G3 MIS 200 mg kg^{-1}	148.99	152.61±12.62	2.43	206.28±43.34[a]	62	83.24±10.77	47	59.98±6.43	27	129.65±35.33[a]	80	149.29±11.57	-25.62
G4 MIS 400 mg kg^{-1}	191.29	166.95±7.78	-12.72	168.09±25.07	45	60.26±6.35	6	69.22±4.65	39	86.82±20.56	55	146.67±14.24	-26.05
G5 CS 200 mg kg^{-1}	185.79	280.59±34.37	51.03	199.28±42.49[a]	49	89.86±10.80	36	58.22±12.85	7	123.09±31.41[a]	61	146.88±10.35	-15.67
G6 CS 400 mg kg^{-1}	202.56	292.23±22.40	30.68	104.03±31.41*[a]	-89	70.80±9.59*	-71	38.74±5.37	25	51.13±7.73*[a]	-188	145.00±15.24*	-16.18

INT FBGL: Initial fasting blood glucose levels, at the 7th day of last dose of STZ treatment, the values are expressed as the Mean+SE of the mean, statistical analysis was performed using the one-way analysis of variance (ANOVA), †Percentage change in mean value after 4 weeks of treatment, *Significantly different the value of the G1 control rats at $p<0.05$ and [a]Significantly different the value of the other treated rats at $p<0.05$

DISCUSSION

Compared with glibenclamide, both MIS and CS aqueous exts of PGFP showed potential hypolipidemic effect in STZ induced diabetic rats, but with weaker hypoglycemic effect (for the MIS ext only). The effective dose was 400 mg kg^{-1} for 4 weeks of treatment with the exts. Accordingly, observations of this study confirm the data by Rai *et al.*[30]. Rai *et al.*[19] that suggested ethnomedical uses of the guava leaves and fruit in the management or control of type 2 diabetes mellitus complications.

Notably, significant hypolipidemic effect of CS ext ($p \leq 0.05$), which was more pronounced than the effect of glibenclamide and the MIS ext treated groups was accompanied with moderate reverse of weight loss. This hypolipidemic property of CS ext in STZ diabetic animal model could be considered as an advantage for this medicinal plant. These findings are consistent with Farinazzi-Machado *et al.*[31] who showed that animals treated with guava pulp juice had significantly lower body weight, glycemia, TC and TG levels and significantly augmented the levels of HDL compared to the control group. Moreover, cellular observations have been previously showed an increase in the activities of hepatic hexokinase and glucose-6-phosphate dehydrogenase in diabetic rats treated with CS-similar ethanol extract[14]. In a similar study by Guo *et al.*[32], indicated that guava leave extracts improved glucose metabolism and insulin sensitivity in the skeletal muscles of rats by modulating the insulin-related signaling.

Possible synergism effects between different metabolic compounds have been referred to be responsible for the pharmacological properties observed for medicinal plant extract[33]. Many of the previous studies attributed the hypolipidimic and hypoglycemic effects of guava to the presence of quercetin and its derivatives[11,34]. Differently, others suggested that other constitutes of guava extract such as tannins, flavonoids, saponins may be responsible rather than being caused by quercetin alone. In this study, it is found that the CS ext showed a potential hypolipedemic effect, which was less notably observed by the MIS ext. These findings can be largely attributed to the more complicated mixture of phenol compounds in the CS extract rather that to its total phenol content, which was detected at higher content in the MIS ext. Moreover, the CS ext content of quercetin was three times higher than its content in the MIS ext. These findings emphasizing on our above assumption of synergism effect between different compounds in the extract and agrees with the previous studies[35].

These observed variations in the pharmacological activities of the PGFP ext treated groups was largely influenced by the method of extraction used, as it is known that using microwave energy for extraction would increase the solubility of the more polar compounds[36,37]. In this study, MIS ext showed its major compound is ascorbic acid, which has the lowest log p-value (highest polarity) among the other detected phenol compounds in combination with gallic acid (with the second lowest log p-value). On the other side, the CS ext showed its major compound is ellagic acid, which has the highest log p-value, combined to ferulic acid (moderate polarities) both of them were not detected in the MIS ext.

Same findings was also presented by Deguchi and Miyazaki[15] who showed that compounds with phenol hydroxyl groups was involved in the hypoglycemic and hypolipidemic effects of guava leave extracts, suggested that the active compound was a polymerized polyphenol, which is composed of ellagic acid (found in CS ext as the major compound) and other low molecular weight polyphenols. In addition, many previous study findings[38-40] showed that the antioxidant action of plants extracts may alleviate the STZ induced toxicity and contribute to hypoglycemic and hypolipidemic effects. The superior antioxidant activity for the CS ext could also be correlated to its specific phenol composition rather than to their total content. These findings greed with the study done by Kilic *et al.*[41] were they found ellagic acid to be more potent antioxidant compound compared to butylated hydroxytoluene, butylated hydroxyanisole, α-tocopherol and ascorbic acid, when using the hydrogen peroxide scavenging method at equivalent concentrations.

In spite of the lack of any clinical evidences and based only on the current findings, the consumption of guava therefore, could result in improved antioxidant status and lipid profile in patients at height risk to be exposed to environmental free radicals and high intake of fatty food. Thus, patients are advised to use this plant material as a home remedy to reduce the risk of diseases, caused by free radical activities and high cholesterol in blood. Also, phyto-pharmaceutical industry are invited to consider developing of new dietary supplement, which is composed of a PGFP standardized extract that may be used as a preventive treatment for the complementary medicine approach.

The limitations of this study involved varied parameters. Of which, no other solvent for extraction were used except of water, which limit the extraction process for polar compounds only. That was because we attempted to find an economic, safe and taste accepted home remedy, which is approachable to local Jordanians residence, whom willing to adapt a new life style modifications including their food processing methods, in aim to improve their health status. Another limitation was that it couldn't identify many other chemicals other than the phenols in the extracts, in spite of the fact that the TLC analysis showed the presence of other phytochemicals in the extracts with variations. The reason was that the used chromatographic method depends on the presence of standards compounds of which many of them were not available.

CONCLUSION

The presented data suggest that PGFP can be used for their antioxidant, hypolipidemic and hypoglycemic effects, if the extraction methods were optimized for the required pharmacological effect. In this study, it was correlated that significant hypolipidemic effect of unripe guava to high content of ellagic acid in its fruit peels and antioxidant action of the active extract. This effect for ellagic acid seems to be mediated by metabolic pathway by which it acts as antilypolytic agent to reduce the consequences of diabetes mellitus such as atherosclerosis rather than antidiabetic effect. Therefore, further studies by *in vivo* models are still needed to confirm these potential pharmacological activities of this plant.

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