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Antimicrobial and antioxidant activities of *Psidium guajava* leaves growing in Egypt

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ABSTRACT

Psidium guajava is an important edible plant, widely used in folk medicine as anti-allergy, antiplasmodial, and anticough. In the current study the total phenolic & total flavonoid contents, antioxidant and antimicrobial activities of different extracts from Psidium guajava leaves were investigated using the reported methods. The n-butanol fraction demonstrated the highest phenolic content with 547.13, followed by 397.25, 324.26, 306.12, 216.21 for 90% methanol, ethyl acetate, 100% methanol, and 85% methanol, respectively, while the lowest value of 55.13 mg gallic acid equivalent (GAE)/ g dry extract was obtained for the diethyl ether fraction. SC_{50} values of the n-BuOH, 90% methanol, EtOAc, 100% methanol, and 85% methanol were 13.91, 19.09, 32.13, 38.26, and 43.26 µg/ml respectively for the DPPH assay compared to 8.0 of the positive standard ascorbic acid, whereas those of the total antioxidant capacity (TAC) were 436.02, 541.0, 412.13, 394.41, and 351.91mg ascorbic acid equivalent (AAE)/g dry extract respectively. Optical density (OD) values of the reducing power antioxidant activity (RPAA) were 0.873, 0.767, 0.712, 0.681, and 0.649 respectively, compared to 0.970 of the positive standard ascorbic acid. Furthermore, the tested extracts showed noticeable antimicrobial activities against the antibiotic resistant pathogens i.e., Staphylococcus aureus (G+ve bacteria) with inhibition zones between 8-12mm, Pseudomonas aeruginosa (G-ve bacteria) with inhibition zones between 7-11mm, Candida albicans (yeast) with inhibition zones between 8-13mm, and there is no any activities were recorded against Aspergillus niger (fungi).In, conclusion the antimicrobial and antioxidant activities may be attributed to the presence of phenolic compounds.

Keywords: *Psidium guajava*, Antimicrobial, Antioxidant, DPPH, Phytochemical screening.

INTRODUCTION

Infectious diseases are caused due to a complex interaction between the pathogen, host and the environment. The control of bacteria and fungi becomes complex because of the emergence of resistant bacteria and fungi to many conventional antibiotics[1].Reactive oxygen species (ROS) are formed during normal cellular metabolism, but when present in high concentration they become toxic as well as hurtful consequences like oxidative stress and cancer[2]. External antioxidants, like antioxidants extracted from plants, can be administrated in order to combat those radicals[3, 4].The use of medicinal plants in the world contributes significantly to primary health care. Many plants are used in the form of crude extracts, infusions or plasters to treat common infections. The medicinal value of plants attributed to certain chemical substances that produce a definite physiological action on the human body. The

most important among these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds [5]. *Psidium guajava* Linn.(Family Myrtaceae)usually known as guava and is native to South and Central America [6]. Different parts of the plant are used as antioxidant [7], and antimicrobial [8]. Numerous bioactive ingredients were isolated from the plant leaves *viz.*, tannins, triterpenes, flavonoids [9, 10].Therefore, the current study was undertaken to evaluate the antioxidant, and antimicrobial activities of methanol extract of *Psidium guajava* leaves as well as its derived fractions.

MATERIALS AND METHODS

Plant Material

The leaves of *Psidium guajava* (Myrtaceae) were collected from Zoological Garden, Giza, Egypt in January, 2014. Authentication of the plant was established by Eng. Teresa Labib, General Manager and head of plant Taxonomy in El-Orman Botanical Garden, Giza, Egypt. Voucher specimen was deposited at Laboratory of Medicinal Chemistry, Theodor Bilharz Research Institute. The fresh leaves were washed with clean water to remove debris and completely dried in shade place at room temperature and then powdered by electric mill.

Extraction and Fractionation

Extraction was done at room temperature by simple extraction method using different solvents namely, 100% Methanol, 90% Methanol, 85% Methanol, 70% Methanol and 50% Methanol. Dried powdered leaves (15 g) were mixed separately with 100 ml of each solvent in 500 ml conical flasks. The flasks were sealed tightly and kept for 24 hr. The supernatant was filtered using Whatman filter paper No.1 and evaporated using a rotary evaporator (Rotatory evaporator, Buchi, Switzerland) to obtain the crude dried extract. Large scale extraction was carried out via taking the plant powder (500 g), was soaked in (2L) of 90% methanol for one week at room temperature. The 90% methanol extract was defatted with diethyl ether and then fractionated by using different organic solvents; petroleum ether, methylene chloride, EtOAc and *n*-BuOH. Each fraction was filtered and then concentrated. The yield of each fraction was determined and kept in dark for analysis.

Phytochemical screening

Phytochemical screening for the secondary metabolites (alkaloids, tannins, sterols, saponins, glycosides, sterols/terpenes, sugars, flavonoids and phenols) was carried out by the reported methods [11-14].

Determination of total phenolic content

The total phenolic content of plant extracts was determined using Folin-Ciocalteu's reagent according to the reported methods [15, 16].

Determination of total flavonoids content

The content of flavonoids of each extract was determined according to the reported procedures [17].

Antioxidant assays

Free radical scavenging antioxidant activity

The free radical scavenging antioxidant activity was carried out according to the reported method [18, 19].

Determination of total antioxidant capacity

The antioxidant activity of plant extracts was determined according to phosphomolybdenum method, using ascorbic acid as standard [20, 21].

Reducing power antioxidant assay (RPAA)

The reducing power activity was evaluated according to the reported procedure [22].

Antimicrobial Activity

Disc agar plate method was used to evaluate the antimicrobial activity of ME as well as its derived sub-fractions according to the reported method [23, 24].

Statistical analysis

All data were presented as mean ± S.D. using SPSS 13.0 program [25].

RESULTS AND DISCUSSION

Phytochemical screening

Identification of the major chemical constituents of 90% methanol extract and its derived fractions of was carried out using the conventional standard procedures [26-28], to determine presence or absence of the different phytoconstituents viz., alkaloids(Mayer's and Draggendorff's), flavonoids (Shinoda test, Aluminum chloride and Potassium hydroxide), steroids and terpenoids (Salkowski and Libarman-Burchard's), tannins (Ferric chloride and Gelatin tests), saponins (Frothing and Hemolytic tests), anthraquinones (Borntrager's), carbohydrates (Molisch's and Barfoed's), and coumarins (Sodium hydroxide) tests. The results were evaluated by visual inspection as change in color or precipitation. Phytochemical screening showed the presence of certain secondary metabolites as viz., flavonoids, terpenoids, tannins, steroids, alkaloids, coumarins, anthraquinone, and carbohydrates. Chetia et al. (2014) reported the presence of tannins, flavonoids, terpenoids, steroids, glycosides, cardiac glycosides, phlobatannin, alkaloids and reducing sugars and absence of saponin & anthraquinone [29]. Moreover, our results are in agreement with the previous reports [30, 31].

Total Flavonoids and Total Phenolic Contents

The content of total phenolics in the extracts was estimated by FCR method in terms of µg GAE/mg extract, the results showed that the *n*-butanol exhibited high phenolic content with (547.13) followed by 90% methanol extract (397.25), ethyl acetate extract (324.26) and others. On the other hand, the diethyl ether contained lowest phenolic content. Furthermore, it was found that all the tested extracts *i.e.*,*n*-butanol and all ratios of methanol extracts are rich in flavonoids content but diethyl ether, petroleum ether and methylene chloride extracts have low content of flavonoids (Table 1). Regarding the literature; the dry guava extract showed high levels of phenolics (766.08 ± 14.52 mg/g), and flavonoids (118.90 ± 5.47 mg/g) [32]. Zahidahet al. (2013) reported that the pink guava leaves possessed a higher TPC (368.61 ± 25.85 mg/100 g GAE) [33]. Another study was carried out by Nantitanon et al., revealed that guava leaf extract showed high total phenolic content (TPC) [34].

Antioxidant activity

Free radical scavenging antioxidant activity

2,2'-diphenyl-1-picrylhydrazyl (DPPH) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerize, as would be the case with most other free radicals [26].The delocalization also gives rise to the deep violet color, characterized by an absorption band in methanol solution centered at about 517 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color [5].The antioxidant effect is proportional to the disappearance of $DPPH^{\bullet}$ in test samples. The Violet color generally fades or disappears when an antioxidant is present in the medium. Results were reported as SC_{50}, which is the amount of antioxidant necessary to decrease the initial $DPPH^{\bullet}$ concentration by 50%. The lower the SC_{50}, the higher is the antioxidant power.Our results revealed that the *P. guajava* had significant scavenging effects with increasing concentration in the range of 1-200 µg/ml when compared with that of ascorbic acid, the DPPH activity was found to increase in dose dependent manner. *n*-butanol extract give the highest activity (13.91 µg/ml) followed by 90% methanol (19.09 µg/ml), ethyl acetate extract (32.13 µg/ml), 100% methanol (38.26 µg/ml), 85% methanol (43.26 µg/ml), 70% methanol (59.12 µg/ml), methylene chloride (68.49 µg/ml), petroleum ether (71.61 µg/ml), and diethyl ether extract showed the least activity (80.76 µg/ml)(Table 1). The different extracts from leaves of *P. guajava* i.e., *n*-hexane, ethyl acetate, and methanol were tested as antioxidants. The antioxidant activity of ethyl acetate extract is 65.63% [35].The ethyl acetate, petroleum ether and methanol extracts were studied for their antioxidant activities at different concentrations (20, 40, 60, 80 and 100 µg/ml) using 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) free radical assay. The free radical scavenging potential of the methanol extract exhibited the maximum activity of 81.24% in 100 µg/ml concentration [36].It was reported that the bioactive ingredients viz., phenolic acid, flavonoids, and tannins have been proved to be responsible for the antioxidant activity [37-39].

Table 1: Total phenolic content (TPC) and total flavonoids content (TFC) of the different extracts of *P. guajava*

Sample	TFC (mgRE / g ext.)[a]	TPC (mgGAE / g ext.)[b]	DPPH SC_{50} [µg/ml][c]
100% MeOH	79.14 ± 1.41	306.12 ± 0.84	38.26 ± 1.10
90% MeOH	91.78 ± 1.04	397.25 ± 1.68	19.09 ± 0.89
85% MeOH	68.55 ± 1.24	216.21 ± 0.98	43.26 ± 0.51
70% MeOH	53.17 ±0.81	196.17 ± 1.13	59.12 ± 0.81
Diethyl ether	18.58 ± 2.16	55.13 ± 0.40	80.76 ± 0.46
Petroleum ether	29.19 ± 1.47	110.96 ± 1.78	71.61 ± 0.89
Methylene chloride	41.25±1.17	147.81 ± 1.17	68.49 ± 1.11
Ethyl acetate	88.48 ±2.01	324.26 ± 2.14	32.13 ± 0.86
n-butanol	99.13 ± 0.87	547.13 ± 1.31	13.91 ± 0.89

Results are expressed as mean values ± standard deviation (n = 3).
[a]TPC (total phenolic content) values are expressed as mg gallic acid equivalent/g extract (mg GAE/g ext.).
[b]TFC (total flavonoid content) values are expressed as mg rutin/g extract (mg RE/ g ext.).
[c]A higher DPPH radical-scavenging activity is associated with a lower SC_{50} value.

Total Antioxidant Capacity (TAC)

A wide range of assays can be used for assessment of the total antioxidant capacities of plant extracts. The total antioxidant capacity of *Psidiumguajava* extracts were measured spectrophotometrically through phosphomolybdenum method which is based on the reduction of Mo (IV) to Mo (V) and the subsequent formation of green phosphate/Mo (V) compound with a maximum absorption at 695 nm. A high absorbance value of the sample indicates its strong antioxidant capacity. This method is a quantitative one, since the antioxidant capacity is expressed as the number of equivalent of ascorbic acid [20]. The results in (Table 2) exhibited that, most tested extracts showed considerable antioxidant capacities of 436.02, 541.0, 412.13, 394.41, and 351.91 mg ascorbic acid equivalent (AAE)/g dry extract respectively for *n*-BuOH, 90% methanol, EtOAc, 100% methanol, and 85% methanol [40].

Reducing Power antioxidant assay (RPAA)

This method is based on the ability of substances, which have reduction potential, to react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form blue colored ferric ferrous complex $(Fe^{3+})_4[Fe^{2+}(CN^-)_6]_3$ that has an absorption maximum at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power of the sample, reducing power was reported as ascorbic acid equivalent per gm of dry sample. The reducing power is related to electron transfer ability of the plant extract. In this assay is used to measure the transferring capacity of Fe^{3+} to Fe^{2+}[41]. The Optical density (OD) values of the reducing power antioxidant assay (RPAA) were 0.873, 0.767, 0.712, 0.681, and 0.649 respectively for *n*-BuOH, 90% methanol, EtOAc, 100% methanol, and 85% methanol, and the remaining extracts showed low activity compared to 0.970 of the positive standard ascorbic acid at concentration 200 µg/ml(Table 2). Based on the results the *P. guajava* have an ability of transferring the Fe^{3+} into Fe^{2+}, and it minimizes the oxidative damage in the tissues.The reducing power antioxidant activity of ethanolic extract of *P. guajav*a leaves was evaluated and the results proved that the ethanolic extract of have an antioxidant potential on a concentration dependent manner compared to the ascorbic acid as a standard [42].

Table 2: Total antioxidant capacity and reducing power activity of the different extracts of *P. guajava* at concentration 200 µg/ml

Sample	Total antioxidant capacity (mg AAE /g ext.)	Reducing Power antioxidant assay (RPAA)
100% MeOH	394.41 ± 0.19	0.681
90% MeOH	541.00 ± 1.47	0.767
85% MeOH	351.91 ± 1.01	0.649
70% MeOH	297.18 ± 1.74	0.613
Diethyl ether	101.00 ± 0.79	0.191
Petroleum ether	169.22 ± 1.62	0.326
Methylene Chloride	192.29 ± 2.14	0.511
Ethyl acetate	412.13 ± 1.92	0.712
n- butanol	436.02 ± 1.92	0.873
Ascorbic acid		0.970

***In vitro* antimicrobial activity**

Numerous previous reports revealed that most parts of *Psidium guajava* grown in different regions around the world showed noticeable antimicrobial activities against certain pathogenic microbial stains [43-49]. Result of inhibitory efficacy of leaves extracts against four different microbial strains, i.e., *Staphylococcus aureus* (G+ve

bacteria), *Pseudomonas aeruginosa* (G-ve bacteria), *Candida albicans* (yeast) and *Aspergillus niger* (fungi) shown in (Table 3).the results revealed that the tested extracts showed noticeable antimicrobial activities against the antibiotic resistant pathogens *i.e., Staphylococcus aureus* (G+ve bacteria) with inhibition zones between 8-12mm, *Pseudomonas aeruginosa* (G-ve bacteria) with inhibition zones between 7-11mm, *Candida albicans* (yeast) with inhibition zones between 8-13mm, and there is no any activities were recorded against *Aspergillus niger* (fungi) comparing with (Penicillin G) as antibiotic with inhibition zones 25, 22, and 26mm against *Candida albicans*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* respectively. Chetia et al. (2014) reported the antibacterial activity of the ethanol extract against *B. cereus* &*S. epidermis* and methanol extract against *B. cereus, S. epidermis, E. coli, S. aureus, P. vulgaris*. Moreover, both of the ethanol and methanol extracts showed obvious antifungal activity against *C. albicans*, and there is no any activity was recorded against *P.crysogenum* [29].The antimicrobial activity of *P. guajava* was evaluated against two gram-negative bacteria *Escherichia coli* &*Salmonella enteritidis* and two gram-positive bacteria *Staphylococcus aureus* &*Bacillus cereus*. Both of methanol and ethanol extracts showed inhibitory activity against gram-positive bacteria, the methanol extract had a mean zones of inhibition of 8.27 &12.3 mm, and the ethanol extract had a mean zone of inhibition of 6.11 & 11.0mm against *B. cereus* and *S. aureus*, respectively and there is no any activity was reported against the gram-negative bacteria [50]. Also, *P. guajava* showed strong antimicrobial activity against *Candida albican* and *Enterococcus fecalis* [51]. Furthermore, the water and methanol extracts from the leaves of *P. guajava* potentially inhibited growth of pathogenic bacterial starins *i.e., Pasteurella multocida, Streptococcus suis, Escherichia coli* and *Salmonella typhimurium*, but the acetone extract was only active against *Streptococcus suis* and *Pasteurella multocida*[52]. Some authors have been reported on that the presence of certain chemical constituents in the tested extracts of *P. guajava* were responsible for their antimicrobial activities like; anthocyanins, alkaloids, flavonoids, tannins, and triterpenoids [53-56]. Two triterpenoids namely, betulinic and lupeol were isolated from *P. guajava* leaves showed antifungal and bacterial effect against certain pathogens [55]. Also, some flavonoidal compounds isolated from *P. guajava* namely, morin-3-Olyxoside,morin-3-*O*-arabinoside, quercetin-3-Oarabinoside, guaijavarin and quercetin were reported to showed antibacterial activities [57, 58].

Table 3: *In vitro* antimicrobial activity of *P. guajava* extracts

	Clear Inhibition zone (Θmm)[a]			
Sample	***Candida albicans***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	***Aspergillus niger***
100% MeOH	9	11	9	-
90% MeOH	8	10	12	-
85% MeOH	8	8	11	-
70% MeOH	13	8	8	-
Diethyl ether	-	-	-	-
Petroleum ether	-	-	-	-
Methylene Chloride	7	7	9	-
Ethyl acetate	7	9	10	-
n- butanol	9	9	12	-
Penicillin G	25	22	26	-

[a]*The results of samples against Staphylococcus aureus(G+ve bacteria); Pseudomonas aeruginosa(G-ve bacteria); Candida albicans(yeast); Aspergillus niger (fungus); (-); inactive. Samples were dissolved in 2ml methanol and 100 micro liters were poured in 1ml-diameter cup.*

CONCLUSION

On the basis of our current finding, *P. guajava* leaves might be a good source of naturally occurring antimicrobial and antioxidant agents due to the presence of certain bioactive secondary metabolites and active ingredients viz., flavonoids, tannins, anthraquinones, and alkaloids.

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