Available online at *www.ujpronline.com*
Universal Journal of Pharmaceutical Research
An International Peer Reviewed Journal
ISSN: 2831-5235 (Print); 2456-8058 (Electronic)

RESEARCH ARTICLE

ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF *PSIDIUM GUAJAVA.* (GUAVA) MEDICINAL PLANT LEAVES USED IN FOLK MEDICINE FOR TREATMENT OF WOUNDS AND BURNS IN HUFASH DISTRICT AL MAHWEET GOVERNORATE–YEMEN

Samir Ahmed Ali ALhaidari[1], Aziza M. Taj Al-Deen[1], Ali Gamal Al-Kaf[2], Fatima A. Al-Hadi[1], Qais Abdullah[1], Anas AL Mahbashi[1], Fouad A. Al-Dubai[3]
[1]Biology Department, Faculty of Science, Sana'a University, Yemen.
[2]Medicinal chemistry Department, Faculty of pharmacy, Sana'a University,Yemen.
[3]Biology Department, Faculty of science, Dhamar University, Yemen.

Article Info:

Article History:
Received: 1 February 2019
Reviewed: 13 March 2019
Accepted: 27 April 2019
Published: 15 May 2019

Cite this article:
ALhaidari SAA, Taj Al-Deen AM, Al-Kaf AG, Al-Hadi FA, Abdullah Q, AL Mahbashi A, Al-Dubai FA. Antimicrobial and antioxidant activity of *Psidium guajava* (guava) medicinal plant leaves used in folk medicine for treatment of wounds and burns in Hufash district Al Mahweet Governorate–Yemen. Universal Journal of Pharmaceutical Resear *Psidium guajava* ch 2019; 4(2): 6-12.
https://doi.org/10.22270/ujpr.v4i2.250

***Address for Correspondence:**
Samir Ahmed Ali ALhaidari, Biology Department, Faculty of Science, Sana'a University, Yemen.
E-mail: *samiralhaidari@gmail.com*

Abstract

Objective: *Psidium guajava* (PG) belongs to the family Myrtaceae that is believed to have active components that help to treat conditions like malaria, gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, and a number of other conditions. The objective of current study was phytochemical screening of chemical constituents of *Psidium guajava* extract.
Methods: In this study methanolic and aqueous extracts of one plant namely *Psidium guajava*, were screened for the presence of phytochemical constituents and tested for their antimicrobial and antioxidant activity.
Results: TLC tests conducted revealed Rf values in the leaves for alkaloids, Flavonoids, Tannins, Phenols and Saponins(0.96-0.97-0.99-0.97-0.99) respectively. The antimicrobial activity extracts against four bacterial isolates *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella* sp. and a single fungal isolate *Candida albicans* with concentrations (0.5 mg/ml, and 1,0 mg/ml) of the extract were added to the disc and respective solvent was used as negative control. The antioxidative activity of leaf was evaluated by using 1,1- diphenyl-2 picrylhydrazyl (DPPH), the results showed are 88.4%, highest from standard, ascorbic acid 87.5%.
Conclusion: The qualitative phytochemical analysis revealed the results showed presence of alkaloids, terpenoids, glycosides, resins, saponins, tannins, flavonoids, phenols, and amino acid were present in the methanol extract, with absence of glycosides, and amino acids in the aqueous extracts in leaves plant.
Keywords: Antimicrobial, antioxidative, phytochemical, *Psidium guajava*.

INTRODUCTION

Psidium guajava (PG) belongs to the family Myrtaceae, which is considered to have originated in tropical South America. Guava crops are grown in tropical and subtropical areas of the world like Asia, Egypt, Hawaii, Florida, Palestine and others. The genus *Psidium* comprises approximately 150 species of small trees and shrubs in which only 20 species produce edible fruits and the rest are wild with inferior quality of fruits[1]. *Psidium guajava* is a phytotherapic plant used in folk medicine that is believed to have active components that help to treat and manage various diseases. The many parts of the plant have been used in traditional medicine to manage conditions like malaria, gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, and a number of other condition[2]. *P. guajava* leaves extract has a wide spectrum of biological activities such as anticough, antibacterial, haemostasis, anti diarrhoeal and narcotic properties[3]. The leaves of guava contain an essential oil rich in cineol, tannins, triterpenes, flavonoids, resin, eugenol, malic acid, fat, cellulose, mineral salts, and a number of other fixed substances[4]. In a study that attempted to investigate antioxidant activity of *P. guajava* leaves extract by DPPH 2, 2- diphenyl-1-picrylhydrazyl) free radical scavenging method using ascorbic acid as standard, it was found that the extract of *P. guajava* leaves extract was found to possess strong antioxidant activity ,this

activity of *P. guajav*a extract may be attributed to their free radical-scavenging ability. The extent of antioxidant activity of *P. guajava* extract was found significant as compared to standard value for *P. guajava* linn leaves extract was found to be 45.5 µg/ml. Thus *P. guajava* linn leaves possess moderate antioxidant activity as compared as standard[5].

Table 1: R_f values of TLC solvent system for different extracts of *P. guajava*.

Phytochemical	Mobile phase	Confirmatory test	Extract	R_F Value
Alkaloids	Acetone: water: 26% ammonia (90:7:3)	Dragendorff reagent	1 ml HCL+9 ml water	0.96
Flavonoides	Chloroform: ethyl acetate (6:4)	Aluminum chloride reagent	70% ethanol	0.97
Tannins	Chloroform: ethyl acetate (6:4)	10% $FeCl_3$ reagent	25ml water	0.99
Phenols	Toluene: Acetone: formic acid (60:60:10)	10% KOH reagent	Methanol	0.97
Saponins	Ethyl acetate	Vanillin sulfuric acid reagent	Methanol	0.99

Table 2: Yields of *P. guajava* leaves extracts from methanolic and aqueous extracts.

M	Powder of plants	Amount of samples used (g)	Solvent	Volume of the solvent used (ml)	Extract yield/(g)*
1	*P. guajava*	100	Pure Methanol	400	32.40±0.08
2	*P. guajava*	100	distilled Water	400	27.2±0.06

Mean values of the yield are presented as mean ± SEM, Values are statistically, significant when $p \leq 0.05$.

Table 3: Phytochemical composition of the methanolic and aqueous leaves extracts of *P. guajava*.

Plant	*P. guajava L.*								
Chemical Compounds/ Solvents	Alkaloids	Terpenoids	Glycosides	Resins	Saponins	Tannins	Flavonoids	Phenols	Amino acids
Methanolic extract	+	+	+	+	+	+	+	+	+
Aqueous extract	+	+	-	+	+	+	+	+	-

Absence **(+)**, Presence **(-)**

. **Table 4: Antimicrobial activity of standard antibiotics discs against tested bacterial and fungal.**

	Inhibition zones diameter (mm) of tested antibiotic				
Antibiotic Organisms	AM (10 µg)	CIP (25 µg)	CF (30 µg)	PZ (75 µg)	PC (100 µg)
Staphylococcus aureus.	19	26	20	21	20
Escherichia coli.	17	28	18	20	19
Pseudomonas aeruginosa.	18	30	17	21	18
Klebsiella sp.	20	33	22	23	17
Candida albicans.	21	31	20	19	22

AM=Amoxycillin, CIP= Ciprofloxacin, CF=cefazllin, PZ=Cefoperazone, PC=piperacillin

MATERIALS AND METHODS

Samples extraction: The Samples of 100 g of the grinded powder were put in sterilized flasks together with 400 ml of pure methanol for methanolic extraction treatments, while for aqueous extraction treatments, samples of 100 g of grinded powder were put in sterilized flasks with 400 ml of distilled water each. All flasks were covered with transparent nylon and tin and then all were put on a rotary shaker machine for 24 hours. The filtration process for each sample was carried out using filter paper to obtain a pure solution. The evaporation process for each methanol solution and distilled water was conducted separately in the evaporator methanol and distilled water solution. The obtained extracts were kept in dark conditions in the refrigerator at 4°C until used in the experiment[6].

Qualitative tests

Phytochemical screening of plant extracts

The methanolic and aqueous extracts subjected to phytochemical screening were alkaloids, terpenoids, glycosides, resins, saponins, tannins, flavonoids, phenols, and amino acids.

Alkaloids: Dragendorff's test

In a test tube, 2-3 drops of Dragendorff's reagent was added to 0.1 ml of the extract, orange precipitate indicated the presence of alkaloids.

Terpenoids: Salkowski test

In a test tube 5 ml of extract was mixed in 2 ml of chloroform and then 3 ml of concentrated sulfuric acid was added to form a layer. A reddish brown coloration forms at interface.

Glycosides: Keller-Killani test

Concentrated sulfuric acid in a test tube and extract sample were mixed with glacial acetic acid containing

1 drop of Ferric chloride (1:1:1volume). A brown ring appears in the presence of glycosides.

Resins: Turbidity test

To 5 ml extract 5 ml distilled water was added, the occurrence of turbidity shows the presence of resins.

Saponins: Foam test

A 5 ml extract was shaken with 2 ml of distilled water. If foams are produced and persists for ten minutes this indicates the presence of saponins.

Tannins: $FeCl_3$ test

A 4 ml extract was treated with 4 ml $FeCl_3$, the formation of green colour was taken as positive for tannin.

Flavonoids: Shinoda test

Extract was mixed with magnesium ribbon fragments, and concentrated hydrochloric acid was added drop wise. Orange, red, pink, or purple coloration indicates the presence of flavonoids.

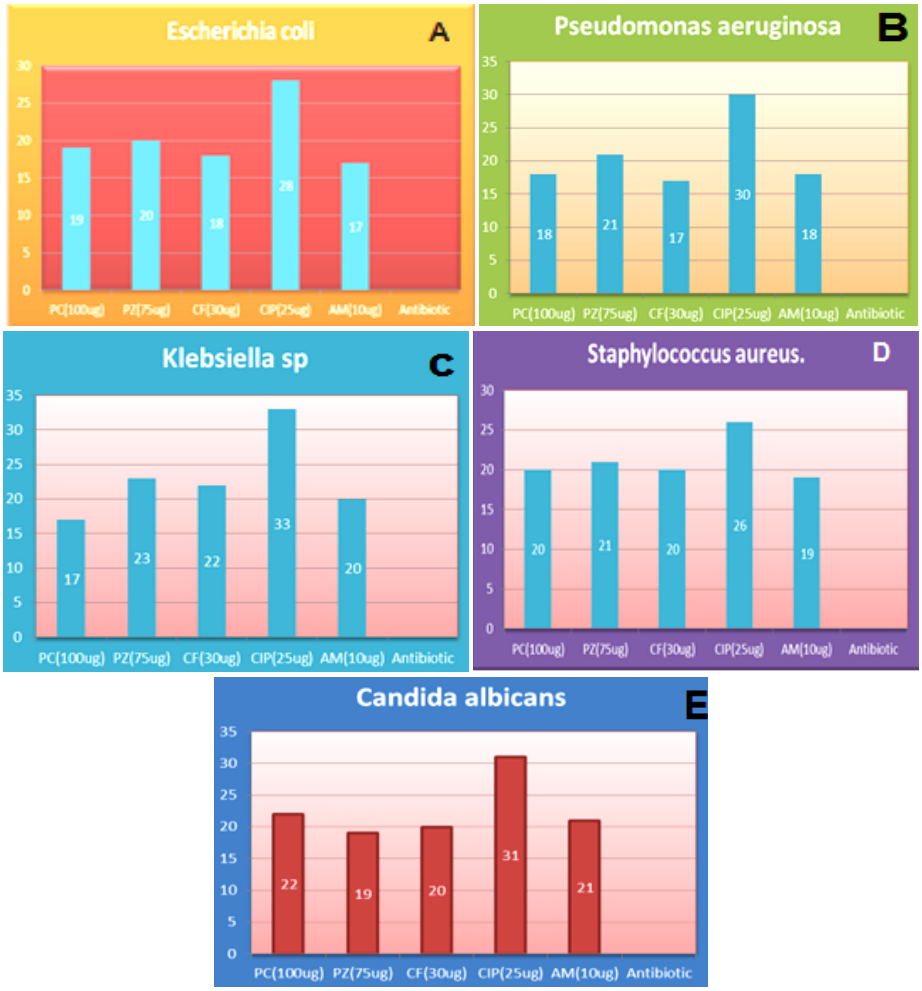


Figure 1: Antimicrobial activities (inhibition zones mm.) of standard antibiotics discs against tested bacterial and fungal.

Phenols: $FeCl_3$ test

Extract was mixed with 2 ml of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols.

Amino acids: Biuret test

Extracts and 1 drop 2% Copper sulphate solution and 1 ml 95% ethanol excess of potassium hydroxide were mixed. Pink or yellow color in ethanol layer appears.

Thin Layer Chromatography.

One gram of *P. guajava,* powder was boiled with of with solvent system made from 15 ml H_2SO_4 test for Alkaloids, 10 ml 70% ethanol test for Flavonoides and Saponins, 25 ml water test for Tannins and phenols in rounded flasks. The TLC plate was prepared as such : (Layer: silica gel layers 0.25 mm thickness, 10 cm length and 5 cm wide). The filtrate obtained was evaporated to dryness in a water bath at 37°C .The residue was dissolved by 0.2 ml methanol. The solution was used for spotting the TLC by capillary tube by only one centered spot. The TLC plate was put inside a saturated tank, and development was waited. When the mobile phase reaches two thirds of plate's length, the plate was lifted out from the tank and let to dry in air. The plate was examined by UV lamp at the wavelength 365 nm. The colors of florescence appeared and recorded. The plate was sprayed carefully reagent, and let to dry for 10 min. Then sprayed with solution and plate was examined under U.V. lamp at the wavelength 365 nm. The iodine was used as the visualizing agent to detect the spot.A meter rule was used to measure the distance moved by the solvent and distance moved by spot, from which the retention factor (R_f values) of the various spots was calculated[6].

Table 5: Antimicrobial activity of the methanolic extracts of leaves of (*P. guajava*) and standard antibiotics discs against tested bacterial and fungal.

Organisms	Zone of inhibition (mm)						
	Extract		Antibiotic				
	0.5 g/ml	1.0 g/ml	AM (10 µg)	CIP (25 µg)	CF (30 µg)	PZ (75 µg)	PC (100 µg)
Staphylococcus aureus.	18	17	19	26	20	21	20
Escherichia coli.	17	15	17	28	18	20	19
Pseudomonas aeruginosa.	15	14	18	30	17	21	18
Klebsiella sp.	14	15	20	33	22	23	17
Candida albicans.	15	17	21	31	20	19	22

Table 6: Antimicrobial activity of the aqueous extract of leaves (*P. guajava*) and standard antibiotics discs against tested bacterial and fungal.

Organisms	Zone of inhibition(mm) Antibiotic						
	0.5 g/ml	1.0 g/ml	AM (10 µg)	CIP (25 µg)	CF (30 µg)	PZ (75 µg)	PC (100 µg)
Staphylococcus aureus	13	15	19	26	20	21	20
Escherichia coli.	14	16	17	28	18	20	19
Pseudomonas aeruginosa.	11	15	18	30	17	21	18
Klebsiella sp.	14	16	20	33	22	23	17
Candida albicans	13	17	21	31	20	19	22

TLC was performed for alkaloids, flavonoids, tannins and phenols solvent system and confirmatory tests are shown in Table 2.

Calculation of RF of each spot was as follows

$$R_F = \frac{\text{Distance moved by solute from the origin}}{\text{Distance moved by solvent from the origin}}$$

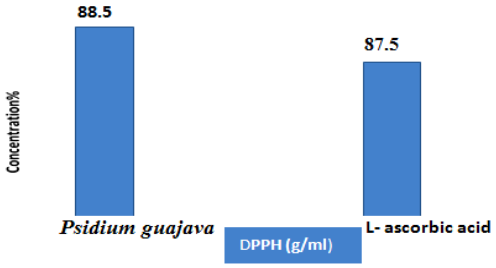


Figure 2: Antioxidant activities of the selected extracts and L-ascorbic acid using the (DPPH) free radical-scavenging assay.

Antimicrobial Activity of Plants extracts.

Microbial Cultures: Fresh plates of the four bacterial isolates *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella sp.* and a single fungal isolate *Candida albicans* were obtained from the National Center of Public Health Laboratories, Sana'a.

Media Use: The bacterial test were *spread* over the nutrient ager (56 g/1000 ML distilled water) was weight into separate flask and dispensed into distilled water make a total volume of 1 liter. Then the fungal test were spread over the sabouraud dextrose ager (65 g/1000 ML distilled Water) was weighted into separate flask and dispensed into distilled water to make a total volume of 1 liter. These powders were dissolved in distilled water and used for evaluation of their antibacterial and antifungal activities.

The mixture was heated in an electric water bath (GFC, 1083, Germany) until the Agar melted to form a homogenous solution. The prepared medium was separately transferred to Durum medium bottle and sterilized by autoclaving at 121°C for 30 minutes. The sterile medium was allowed to cool to about 45°C before being poured aseptically in an inoculation chamber (Ceslab England) in 15 ml portions, into sterile petri dishes to cool and gel into solids[8].

Antimicrobial activity assay: Two different concentrations (0.5 mg/ml, and 1.0 mg/ml) of the extract were added to the disc and respective solvent was used as negative control.

Table 7: Antioxidant activities of the selected extracts and L- ascorbic acid using the (DPPH) free radical-scavenging assay.

Particular	Antioxidant activity DPPH (g/ml)
L- ascorbic acid	87.5±0.05
P. guajava	88.4±0.20

Zone of inhibition: The bacteria plates were incubated at 37°C for 24 hrs while the fungal plates were incubated at for 72 hours, and observed for the zone of inhibition of growth. The zones were measured with a transparent ruler and the result recorded.

Determination of antioxidant activity

The scavenging ability of the natural antioxidants of the leaves towards the stable free radical DPPH was measured by the specific method[9]. The leaf extracts (20 µl) were added to 0.5 ml of methanolic solution of DPPH (0.3 mM in methanol) and 0.48 ml of methanol. The mixture was allowed to react at room temperature for 30 min. Methanol served as the blank and DPPH in methanol, without the leaf extracts, Served as the positive control. After 30 min of incubation, the discolouration of the purple colour was measured at 517 nm in a spectrophotometer**)**. The radical scavenging activity was calculated as follows:

Radical Scavenging Activity

(RSA %)=

$$\frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Statistical Analysis

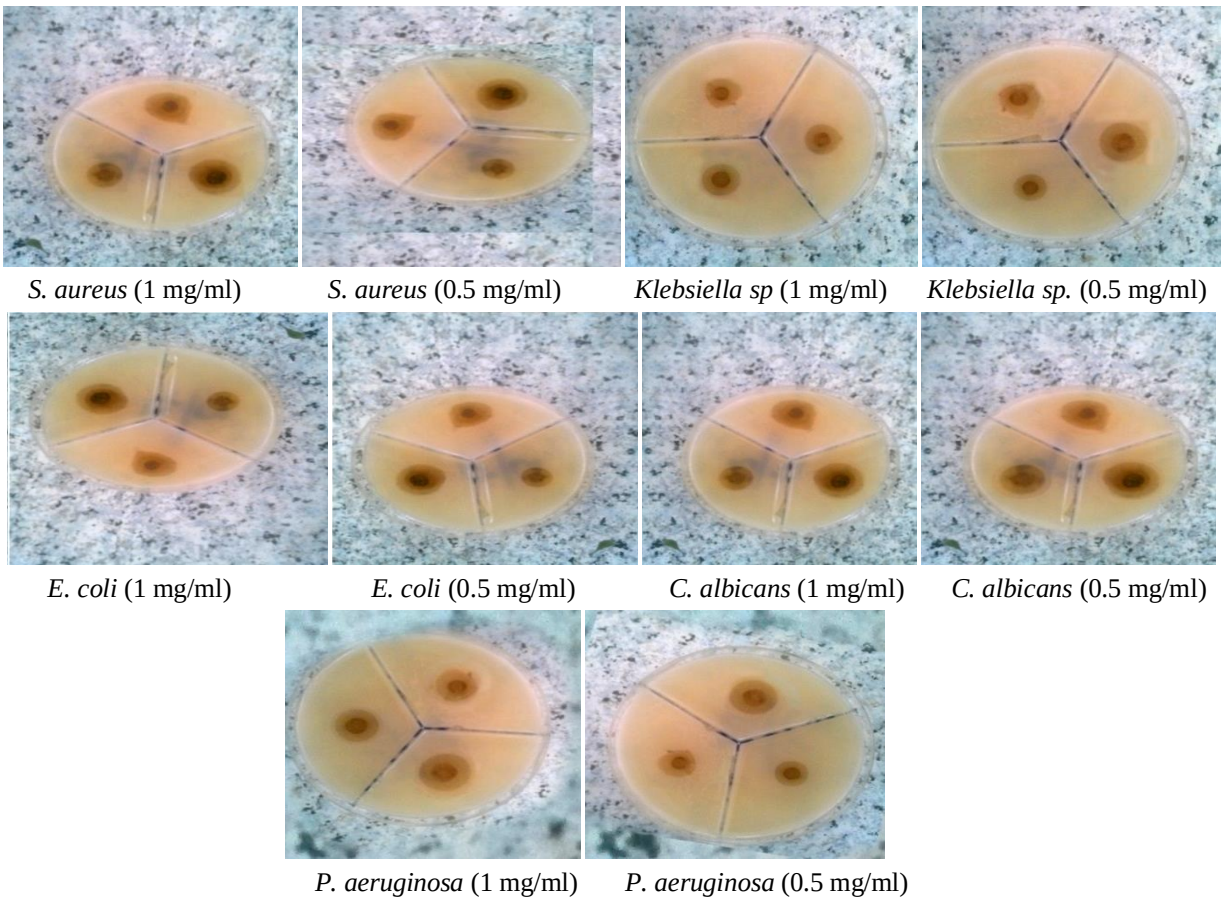
Analysis of variance was made for all data using (SPSS) version (25) computer program.

RESULTS AND DISCUSSION

In this study methanolic and aqueous extracts of one plants namely *P. guajava*, were screened for the presence of phytochemical constituents and tested for their microbial and antioxidant activity.

Yield from different solvents

Yield of methanolic extract of *P. guajava*, extracted with 100% methanol produced 32.40 g. While yield of distilled water extract of *P. guajava* produced 27.62 g.



S. aureus (1 mg/ml) *S. aureus* (0.5 mg/ml) *Klebsiella sp* (1 mg/ml) *Klebsiella sp.* (0.5 mg/ml)

E. coli (1 mg/ml) *E. coli* (0.5 mg/ml) *C. albicans* (1 mg/ml) *C. albicans* (0.5 mg/ml)

P. aeruginosa (1 mg/ml) *P. aeruginosa* (0.5 mg/ml)

Plate 1: Inhibition zones observed with leaves methanolic extracts *of P. guajava.*

Mean values of the yield are presented as mean ± SEM. Values are statistically significant when $p \leq 0.05$.

A similar investigation done in a study[10] revealed that aqueous extracts (16.35%) of *P. guajava* gave high yields than of methanolic extracts (14.22%), which is contrary to current findings. Similarly, a previous study also reported a 16.35% yield in aqueous extracts from *P. guajava*[11]. Yet the percentages of yields in both studies were less than of the present study.

Phytochemical composition of the methanolic and aqueous leaves extracts.

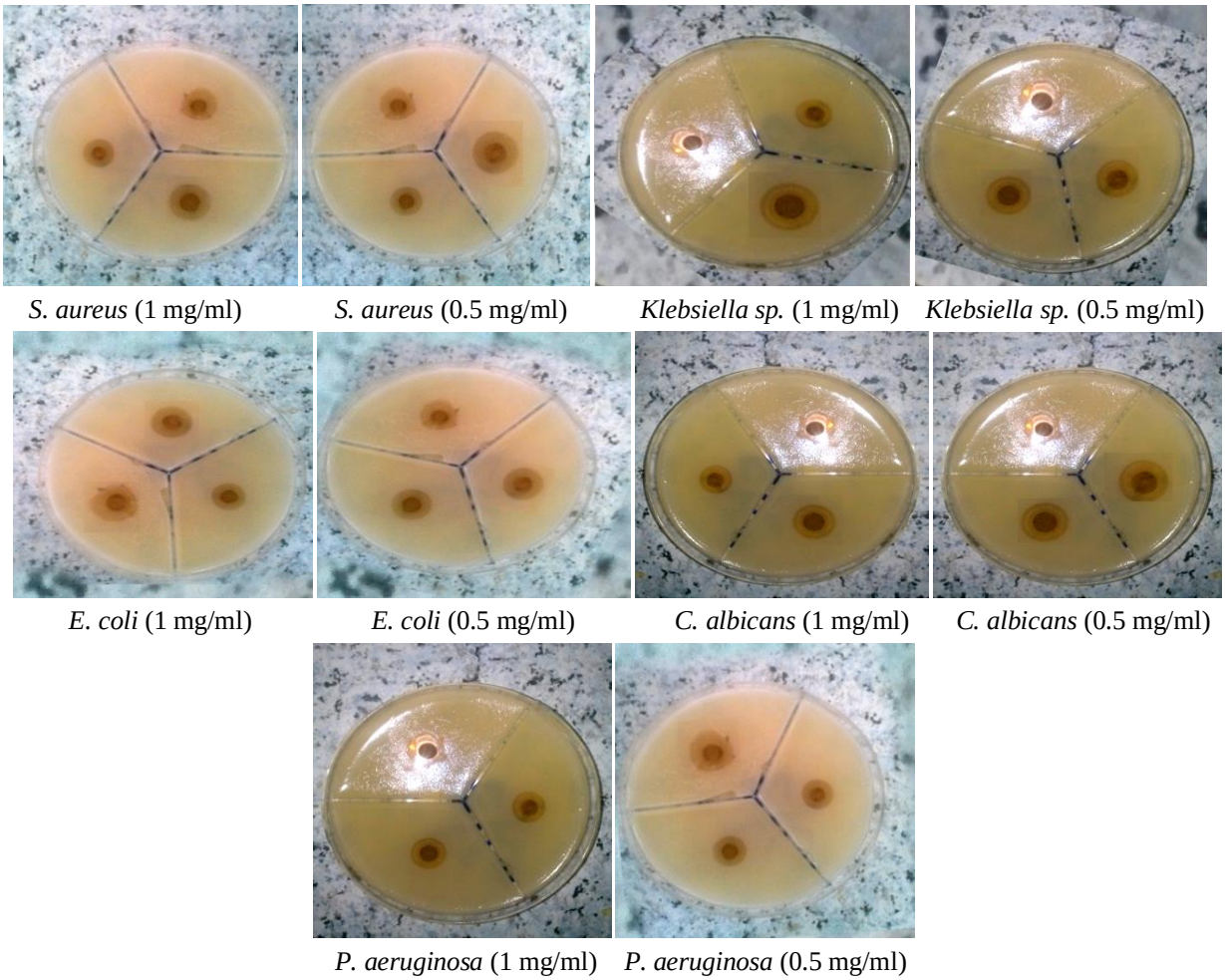
The summarized phytochemical screening of chemical constituents of *Psidium guajava* extract is shown in Table 3. The results revealed the presence of active compounds in the two different extracts. As the table shows, the methanol and aqueous extracts indicate the presence alkaloids, terpenoids, glycosides, resins, saponins, tannins, flavonoids, phenols, and amino acid were present in the methanol extract, with absence of glycosides, and amino acids in the aqueous extracts in all three plants. In a previous study**,** methanolic extracts of *Psidium guajava* revealed the presence of alkaloids, tanins, flavonoids and glucosides[12]. Similarly other study has showed the presence of tannins, saponins, flavonoids, alkaloids and phenols as in current study[13].

Thin Layer Chromatography (TLC)

Five secondary metabolites (alkaloids, flavonoids , tannins, phenols and saponins) were used for (TLC) thin layer chromatographic analysis. TLC tests conducted revealed R_f values in the leaves of *P. guajava* for alkaloids, Flavonoids, Tannins, Phenols and Saponins (0.96-0.97-0.99-0.97-0.99) respectively. In a study done through TLC profiling proved that different Rf values represent different chemical constituents present within methanol leaf extract of *P. guajava*[14]. There were six visible spots. The Rf values (spot 1, RF=0.98), (spot 2, RF=0.78), (spot 3, RF=0.62), (spot 4, RF=0.54) (spot 5, RF=0.32) and (spot 6 RF=0.19). Similar R_f values were in agreement with this investigation.

Antibacterial and antifungal activity of plants extracts

Antimicrobial activity of standard antibiotics discs against tested bacterial and Fungal are displayed in Table 4 and Figure 1. The results of the study indicated that control Antibiotics against bacteria and Fungi showed different inhibitory zones. Antibiotics activity of AM (10 µg), CIP (25 µg), CF(30 µg), PZ (75 µg) and PC (100 µg) against *S. aureus were* 19, 26, 20, 21, 20 mm; *E. coli* 17, 28, 18, 20, 19 mm; *Pseudomonas aeruginosa* 18, 30, 17, 21, 18 mm; *Klebsilla sp*. 20, 33, 22, 23, 17 mm, and *Candida albicans* 21, 31, 20, 19, 22 mm respectively.



S. aureus (1 mg/ml) *S. aureus* (0.5 mg/ml) *Klebsiella sp.* (1 mg/ml) *Klebsiella sp.* (0.5 mg/ml)

E. coli (1 mg/ml) *E. coli* (0.5 mg/ml) *C. albicans* (1 mg/ml) *C. albicans* (0.5 mg/ml)

P. aeruginosa (1 mg/ml) *P. aeruginosa* (0.5 mg/ml)

Plate 2: Inhibition zones observed with leaves aqueous extracts of *P. guajava*.

The antimicrobial activity of the methanolic extracts of *P. guajava* compared to the selected antibiotics against selected microorganism Table 5 and Plate 1 showed that all antibiotics gave higher inhibition zones than the two extract concentrations. Yet, the activity of the two concentrations was closest to Amoxycillin activity, but much lower than the resistant *S. aureus* and *Escherichia coli*. The antimicrobial activity of the aqueous extracts of *P. guajava* against selected microorganisms was less in activity compared to all the selected antibiotics Table 6 and Plate 2. This study showed that Ciprofloxacin (30 µg) gave the highest inhibition zone among all antibiotics with the selected organisms 26, 28, 30 mm against *S. aureus, E. coli, P. aeruginosa* respectively. In other study[15] Ciprofloxacin (25 µg) gave high diameter of inhibition zone which reached up 19, 23, 23mm against *Staphylococcus aureus, E. coli, Pseudomonas aeruginosa* respectively. Similar results were achieved in a previous study[16]. It was showed that the antimicrobial activity of the methanolic and aqueous extracts of *P. guajava* leaves achieved different diameters of the bacterial growth inhibition zone against *Klebsiella sp* and *E. coli*, while a study[16] has mentioned that, the extract of *P. guajava* leaves had no any activity against *Klebsiella sp* and *E. coli*[17] explained that the methanol extract of *P. guajava* leaves had an antibacterial activity with mean zones of inhibition of 12.3 mm, against *S. aureus*, while, in this study, high diameter of 17mm was achieved from methanol extract of *P. guajava* leaves Table 5 and Plate 1. In this study, the results from water extract of *P. guajava* leaves against *E. coli*. Showed that the diameter of inhibition zone reached up 14 mm Table 6 and Plate 2, these are similar result achieved by 18, who mentioned that, the antibacterial effects of water extracts from *P. guajava* (guava) leaves demonstrated mean exhibited zones of inhibition of 13.7 mm on *E. coli*.

Antioxidant activity

Results showed are 88.4%, highest from standard, ascorbic acid 87.5% (Table 11 and Figure 2). These results revealed that the value of the *P. guajava* leaves

extract was superior to the control (88.4%). Another study carried out by a previous study[19]. Results showed that the value of antioxidant activity in the guava extract was 94.4% at a concentration of 100 µg/ml, and the guava dried fruit extracts exhibited weaker antioxidant effects than did the leaf extracts. A study estimate the antioxidants in *P. guajava* leaves extract, showed a significant role of plant leaves as an antioxidant[20]. Similar results obtained another study[6] where the antioxidants reached 82% in the full concentration of the leaves extract.

CONCLUSIONS

The present study showed that *P. guajava* are rich sources of useful secondary metabolites, It is strongly recommended of using them for general medicinal purpose and specially for treat wounds and burns diseases. It is strongly recommended of using them for production of effective pharmaceutical compounds. It is noticeable that the leaves of *P. guajava* are very rich in antioxidant content and therefore are good sources and safe and economical.

ACKNOWLEDGEMENTS

The authors extend their thanks and appreciation to the Sana'a University, Yemen to provide necessary facilities for this work.

AUTHOR'S CONTRIBUTION

ALhaidari SAA: writing original draft, investigation. **Taj Al-Deen AM:** Writing, review, and editing. **Al-Kaf AG:** writing, review, supervision. **Al-Hadi FA:** writing, review, and editing. **Abdullah Q:** methodology, investigation, formal analysis. **AL Mahbashi A:** conceptualization, methodology, investigation. **Al-Dubai FA:** data curation, supervision. Final manuscript was read and approved by all authors.

DATA AVAILABILITY

Data will be made available on request.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

REFERENCES

1. Mani A, Mishra R, Thomas G. Elucidation of diversity among Psidium species using morphological and SPAR methods. J Phytol 2011 3(8): 53-61.
2. Abdelrahim SI, Almagboul AZ, Omer MEA, Elegami, A. Antimicrobial activity of *Psidium guajava* L Fitoterapia. J List Int J Microbiol 2002; 73(7-8), 713-715. *https://doi.org/10.4103/0973-1296.66939*
3. Ramírez JC, Cura CI, Da Cruz Moreira, *et al.* Analytical validation of quantitative real-time PCR methods for quantification of *Trypanosoma cruzi* DNA in blood samples from Chagas disease patients. The J Mol Diag 2015; 17(5), 605-615. *https://doi.org/10.1016/j.jmoldx.2015.04.010*
4. Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African J Biotech 2008; 7(12): 1797-1806. *https://doi.org/10.1186/1472-6882-6-11*
5. Keservani RK, Kesharwani RK, Vyas N, Jain S, Raghuvanshi R, Sharma AK. Nutraceutical and functional food as future food: A review. Der Pharmacia Lettre 2010; 2(1): 106-116.
6. Gokhale SB, Kokate CK, Purohit AP, Shah BN, Seth AK. Pharmacognostic studies of the *Lagenaria siceraria* (Molina) Standley. Int J Pharm Tech Res 2007; 2(1), 121-124.
7. Ciulci I. Methodology for the analysis of vegetable drugs. Chemical industries branch, Division of industrial operations". UNIDO Romania: 1994; 24, 26 and 67.
8. Tanko Y, Abdelaziz MM, Adelaiye AB, Fatihu MY, Musa KY. Effects of hydromethanolic leave extract of *Indigofera pulchra* on blood glucose levels of normoglycemic and alloxan-induced diabetic Wistar rats. Int J App Res Nat Prod 2008; 1(4), 13-18.
9. Puntawong S, Okonogi S, Pringproa K. *In vitro* antibacterial activity of *Psidium guajava* Linn. leaf extracts against pathogenic bacteria in pigs. Chiang Mai Univ J Nat Sci 2012; 11, 127-34. *https://doi.org/10.4103/0975-7406.80776*
10. Mensor LL, Menezes FS, Leitão GG, Reis AS, Santos TCD, Coube CS, Leitão SG. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytotherapy Res 2001; 15(2), 127-130. *https://doi.org/10.1002/ptr.687*
11. Gitika, Kumar M. Antibacterial activity of *Psidium guajava* L. leaves extracts against some gram-positive and gram-negative bacteria. Europ J Pharm Med Res 2016; 3(10), 261-266. *https://doi.org/10.4103/0973-1296.66939*
12. Kangogo KG, Kagira MJ, Maina N, Karanja MS. Qualitative phytochemical screening of camellia sinensis and *psidium guajava* leave extracts from Kericho and Baringo Counties. 2014; 5(3): 506-512. *https://doi.org/10.7897/2277-4343.076247*
13. Anbuselvi, S.,and Rebecca, J. Phytochemical biochemical antimicrobial activty of *psidium guajava* leaf extract. J Pharm Sci Res 2017; 9(12), 2431-2433. *https://doi.org/10.30574/gscbps.2017.1.2.0024*
14. Abubakar EMM. (2009). Antibacterial activity of crude extracts of *Euphorbia hirta* against some bacteria associated with enteric infections. J Med Plants Res 2009; 3(7):498-505.
15. Siti NS, Kamaruzaman S. Antibacterial activity of *Psidium guajava* L. Methanol leaf extract against plant pathogenic bacteria in the genera Pectobacterium and Xanthomonas. Int J Appl Biol Pharmac Technol 2012; 3, 246-252. *https://doi.org/10.4103/0975-7406.80776*
16. Bhalodia NR, Shukla VJ. Antibacterial and antifungal activities from leaf extracts of Cassia fistula l.: An ethnomedicinal plant". J advanced pharm tech research 2011; 2(2), 104. *https://doi.org/10.4103/2231-4040.82956*
17. Rabe T, Van Staden J. Antibacterial activity of South African plants used for medicinal purposes. J Ethnopharmacol 1997; 56(1), 81-87. *https://doi.org/10.1016/S0378-8741(96)01515-2*
18. Biswas B, Rogers K, Fredrick M, Dwayne Borde VU, Pawar DP, Shelar SR, Apturkar RM. Antimicrobial activity of some medicinal plants. Science Research Reporter;2013; 3(1): 33-37. *https://doi.org/10.1556/EuJMI.3.2013.4.6*
19. Adefuye A, Samie A, Ndip. *In-vitro* evaluation of the antimicrobial activity of extracts of *Bridelia micrantha* on selected bacterial pathogens. J Med Plant Res 2011; 5, 5116-5122.
20. Chen HY, Yen GC. Antioxidant activity and free radical-scavenging capacity of extracts from guava (*Psidium guajava* L.) leaves. Food Chem 2007; 101(2): 686-694. *https://doi.org/10.1016/j.foodchem.2006.02.047*
21. Mishra R., Tiwari P, Srivastava M, Singh CS, Ghoshal S. A Comprehensive Review On *Psidium guajava* Linn (Amaratafalam)". World 2007; 7, 8.