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Antimicrobial and Antioxidant Characteristics of Volatile Components and Ethanolic Fruit Extract of *Prosopis farcta* (Bank & Soland.)

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Abstract

The present investigation was conducted to study the volatile composition of essential oil, as well as the radical scavenging and antimicrobial characteristics of the ethanolic fruit extract of *Prosopis farcta* (Bank & Soland.). The fruit essential oil was analyzed by means of gas chromatography/mass spectrometry (GC/Mass). Twenty seven compounds were identified, which represented 97.3% of the total oil. Among the chemical components of the oil, the highest relative percentage was recorded for 9,12-octadecadienoic acid ethyl ester (35.11%). Palmitic acid (21.38%), cemberene A and nonanal (4.7%), and myristic acid (4.4%) were identified as the major constituents. *P. farcta* ethanolic fruit extract exhibited a high phenol content (61.55±0.07 mg gallic acid equivalent (GAE)/g of dry plant), while its total flavonoid content was found to be 17±0.08 mg quercetin equivalent (QE)/g of dry plant. Evaluation of antioxidant efficacy of ethanolic fruit extract was performed using various free radical scavenging assays including DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (Ferric Reducing Antioxidant Power), and ABTS+ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid). In DPPH scavenging assay, IC_{50} value of the ethanolic fruit extract of *P. farcta* was found to be 62.45±00 µg/mL. The extract also exhibited a significant ABTS+ free radical inhibition (IC50, 53.24±0.03 µg/mL) while displaying a moderate reducing ability (IC_{50}=121.43±0.57 µg/ml) in FRAP assay. Results of antimicrobial screening revealed the higher inhibitory effect of ethanolic fruit extract against the growth of *Staphylococcus aureus* and *Escherichia coli* both with MIC values of 16 µg/ml when compared with other tested bacteria. A relatively significant degree of anti-candidial activity was detected for *P. farcta* ethanolic fruit extract (MIC50, 32 µg/mL) among the tested fungal species.

Keywords: Antimicrobial, Antioxidant, Flavonoid, Phenol, *Prosopis farcta*.

1. Introduction

There has been an increasing interest in natural therapeutic agents of plant origin in order to produce new and effective herbal drugs (1). Medicinal herbs have been commonly used as alternative medicine for the treatment and prevention of various diseases such as diabetes, cancer, and infectious diseases (2). Many approaches have already been followed for screening extracts and essential oils in order to achieve new active components including antioxidant and antimicrobial agents.

Porosopis farcta, from the family Fabaceae, is a woody perennial and spiny shrub, which grows wild in various parts of southern Iran and is well adapted to warm climate and drought (3). Prosopis species is native to Asia and usually stands at 40 cm high and can reach 2-3 m in height in some places (4). *P. farcta* has been used for the treatment of a variety of disorders. The fruits of *P. farcta* have a specific pleasant aroma and provide a source of flavonoids, alkaloids, saponins, and tannins in addition to volatile compounds (5,6). The extract of the aerial parts of the plant exhibited variable degrees of antimicrobial activity as declared in

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a previous study (7). According to the literature, some other Prosopis species such as *P. cineraria*, *P. juliflora*, and *P. laevigata* have shown various pharmacological activities such as analgesic, antipyretic, antihyperglycemic, and antioxidant properties (8). Results of another research conducted on the essential oils extracted from different parts of *P. farcta* showed the presence of various compounds such as 3-hydroxy-β-damascone, methyl hexadecanoate, and phytol as major constituents (9). However the extract of dried fruit pod of another Prosopis species have revealed the presence of prosphylline, maslinic acid 3-glucoside, linoleic acid, and flavanone glycosides (10). The aim of present work was to characterize the chemical composition of the fruit essential oil and study the phenolic and flavonoid contents as well as the in vitro antimicrobial and antioxidant efficacy of *P. farcta* ethanolic fruit extract.

2. Materials and methods

2.1. Chemicals and reagents

Culture media, chemicals, solvents, and reagents were purchased from Merck Chemical Company (Damstadt, Germany). DPPH (2,2-diphenyl-1-picryl hydrazil), TPTZ (2,4,6-tris(2-pyridyl)-s-triazine), ABTS (2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonate), and quercetin were purchased from Sigma Aldrich (St. Louis, MO, USA).

2.2. Collection and authentication of plant

Fresh fruits of *P. farcta* were collected from Jahrom, southern Iran on August 2015. The plant material was identified by the plant taxonomist at the Dept. of Pharmacognosy, School of Pharmacy, Shiraz University of Medical Sciences. A voucher specimen (MPRCM-93-80) was deposited at the herbarium of Medicinal Plants Processing Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

2.3.Essential oil extraction

Pods were separated from the fruits (400 g), crushed in a grinder and subjected to hydrodistillation for 4 h using a Clevenger type apparatus. The essential oil was separated, dried over anhydrous sodium sulphate, filtered, and kept at 4 ºC pending GC/Mass analysis.

2.4. Preparation of ethanolic extract

The ground fruit pods (100 g) were macerated in ethanol 96% for 72 h at room temperature with occasional shaking. The extract was filtered and concentrated in a rotary evaporator under reduced pressure at 48 ºC, and the residue was dried in a speed vacuum concentrator and then freeze-dried to remove last traces of the solvent. The resulting extract was stored at -18 ºC until the chemical and pharmacological analysis.

2.5. GC/Mass analysis of essential oil composition

This analysis was performed on a gas chromatograph 7890A system coupled with a mass detector, 5975 C)Agilent technologies, USA). Fused-silica capillary column (HP-5MS, 5% phenyl methyl siloxane, 30 m×0.25 mm×0.25 µm, (Agilent Technologies, USA) was used. The oven temperature was programmed to rise from 60 to 280 ºC at a rate of 10 ºC/min and held at 280 ºC for 10 min. Helium was used as the carrier gas with a flow rate of 1 mL/min, and the interface temperature was 280 ºC. A 1 µL volume of the essential oil was injected in split mode (1:50), and the mass spectra were acquired in EI mode (70 eV) in a mass range of 30-600 m/z. Identification and quantification of essential oil components was based on the data obtained from Wiley nl7 library, calculation of KI values for each chemical component, and comparison with Adams values. Further comparisons of data were made with the KI and mass spectral data appeared in NIST and Pherobase databases as well as the values reported in the literature (11-13).

2.6. Determination of total phenolics

Total phenol content was estimated using the Folin-Ciocalteu colorimetric method (14). To prepare a calibration curve, concentrations of 6.25-200 mg/L (in methanol, water 50:50) of gallic acid, as a standard phenol, were prepared and 5 mL Folin-Ciocalteu (1:10) and 4 mL sodium carbonate 1M were added to 0.5 mL of each concentration. The mixture was centrifuged for 12 min at 4000 rpm, and the absorbance was measured at 765 nm after 15 min using a UV spectrophotometer T90

(PG Instruments, UK). The calibration curve equation was Abs=0.00469 Conc.+0.0070, R^2=0.9998. A concentration of 150 mg/L from ethanolic extract was prepared and the above-mentioned procedure was employed. Total phenol content was expressed as mg gallic acid equivalent (GAE)/g of dry plant material.

2.7. Determination of total flavonoid

This process was performed using aluminium chloride colorimetric method (15). Concentrations of 20-80 mg/L in methanol from quercetin were prepared as a standard flavonoid. Aluminium chloride (2%) in methanol was added to each concentration at the combination ratio of 1:1. Absorbance of the mixture was taken at 415 nm after 10 min by using a UV spectrophotometer T90 (PG Instruments, UK). The calibration curve equation was calculated using the following equation Abs= 0.02985 conc.+0.04811, R^2=0.9978. A concentration of 500 mg/L from ethanolic extract of fruit pod was prepared, and the above-mentioned method was employed. Total flavonoid content was expressed as mg quercetin /g of dry plant material.

2.8. DPPH free radical scavenging test

DPPH free radical scavenging activity of extract was evaluated using a previously reported procedure (16). As prescribed in the procedure, 200 µl of 100 mM methanolic solution of DPPH free radical was mixed with 20 µl of sample at different concentrations (6.25-400 µg/mL). The mixture was left in the dark for 30 min and the absorbance was measured at 490 nm using an Awarness Technology Stat Fax-2100 microplate reader (Awarness Technology, USA). A sample containing 20 µl of methanol and 200 µl of DPPH solution served as the control, while the blank contained an equal amount of extract in methanol. Samples contained 20 µL of methanol and 200 µL of DPPH solution and, while a mixture of 200 µL DPPH solution and 20 µL methanol without extract was taken as the control. Calculations for percentage inhibition of DPPH free radicals were made using the below equation below (Eq. 1):

$$\% \text{ of Inhibition } = 100 - \left[\left(\frac{Abs.\ test - Abs.\ blank}{Abs.\ control}\right) \times 100\right] \quad \text{(Eq. 1)}$$

2.9. $ABTS^+$ radical scavenging assay

$ABTS^+$ radical scavenging activity of the ethanoilc extract was determined by the procedure cited in by Re *et al* (17). $ABTS^+$ radical was freshly prepared by a mixture of equal amounts of ammonium persulfate solution (2.45 mM) and ABTS solution (7 mM) and stored in a dark condition for 16 h. This solution was diluted with ethanol 96% (1:1) to yield an absorbance of 0.7±0.02 at 734 nm and, then used as for assay. The Ssamples of extract at concentration of 12.5-200 (µg/ml) were prepared, and to each 25 µL, 475 µL ABTS+ was added. This mixture was under vortexed for 10 s and kept at room temperature for 6 min. Absorbance was then measured at 734 nm using an Epoch Eliza reader, (Biotek Co, USA). Ethanol 96% was used as the blank and ABTS solution was used as the control. Percentage inhibition of ABTS+ radical was calculated using the following equation (Eq. 2).

$$\% \text{ inhibition} = \left(1 - \left(\frac{Abs.test - Abs.blank}{Abs.control}\right)\right) \times 100 \quad \text{(Eq. 2)}$$

2.10. Ferric reducing antioxidant power (FRAP)

The FRAP assay was carried out using a procedure described by Benzie and Strain with slight modifications (18). Solutions of acetate buffer 300 mM pH 3.6, ferric chloride (III) 20 mM, and TPTZ 10 mM in 40 mM HCl were separately prepared. FRAP reagent was prepared by a 10:1:1 combination of the above solutions respectively. A 20 µL sample of P. farcta fruit pod extract concentrations (6.125-400 µg/mL) was mixed with 180 µL of FRAP reagent in a 96-well microplate and incubated at 37 °C for 10 minutes. Absorbance of the complex was measured at 593 nm using an Epoch microplate reader. The Ccontrol contained 20 µL methanol and 180 µL FRAP reagent without extract. FRAP value was calculated using the following equation (Eq. 3):

$$\text{FRAP Value } = \left(100 - \left(\frac{\text{Abs. control}}{\text{Abs. Sample}}\right) \times 100\right) \quad \text{(Eq. 3)}$$

2.11. Antimicrobial screening

2.11.1. Determination of minimum inhibitory concentration (MIC)

Antibacterial and antifungal activities of *P. farcta* ethanolic fruit pod extract were evalu-

ated against standard bacterial and fungal species and the standard antibiotics using broth microdilution method based on Clinical Laboratory Standard Institute M07-A8 (19). The microbial strains tested in this study were 2 Gram-positive bacteria (*Staphylococcus aureus*, PTCC-1431 and *Staphylococcus epidermidis* PTCC-1435), 2 Gram-negative bacteria (*Escherichia coli*, PTCC -1396 and *Klebsiella pneumonia*, PTCC-1053) and 2 fungal species (*Candida albicans*, PTCC-5027 and *Aspergillus niger*, PTCC-5154). Standard microbial strains were obtained from the Fungi and Bacteria Culture Collection Center (Tehran, Iran). The extract was tested at various concentrations ranging from of 0.5 to 256 µg/mL. A concentration range of two-fold serial dilution (from 4 to 512 µg/ml) was prepared in Muller-Hinton broth for the bacteria and in Sabouraud-dextrose agar for the fungi in a 96-well plate. The inoculum suspension (10 µl of a bacterial suspension from a 12 h culture containing 10^6 cfu/ml) was inoculated and incubated at 37 ºC for 24 h. The first well without turbidity, was considered as the macroscopic evaluation of minimum inhibitory concentration (MIC). Antimicrobial activity was performed by determining MIC values as the lowest concentration of the extract that completely inhibited visible growth. The negative control contained the nutrient broth only, while the positive control comprised of nutrient broth and the test organisms. The test was performed in triplicate. Ampicillin (Amp), gentamycin (Gen), and fluconazole (Flu) were used as standard antibiotics for comparing antimicrobial efficacy of *P. farcta* fruit extract and to verify the reproducibility of the results (20).

2.12. Statistical Analysis

IC_{50} values were calculated by linear regression. Data were expressed as mean±SD. Statistical differences between treatments were identified using one way ANOVA followed by Tukey post-test and significant differences between the mean values of controls and samples were determined using comparison test at a level of $P<0.05$.

3. Results

Extraction yield of the fruit essential oil of *P. farcta* was found to be 0.05% (v/w), while the percentage yield of its ethanolic extract was determined to be 8.23% (w/w).

3.1. Essential oil composition

As represented by the results of GC/MS analysis in Table 1 and Figure 1, among the chemical compositions of *P. farcta* fruit essential oil, palmitic acid, 9,12-octadecadienoic acid ethyl ester, cembrene A, myristic acid, farnesyl acetate, nonanal and α- terpinyl acetate were the major constituents. Therefore, fatty acids were found to have an appreciable contribution in the chemical composition of fruit essential oil (64.2%). Moreover, sesquiterpene, oxygenated monoterpene, and diterpene compounds were detected as other markers and found to comprise 13.8, 8.9, and 4.7% of the oil content, respectively (Table 1).

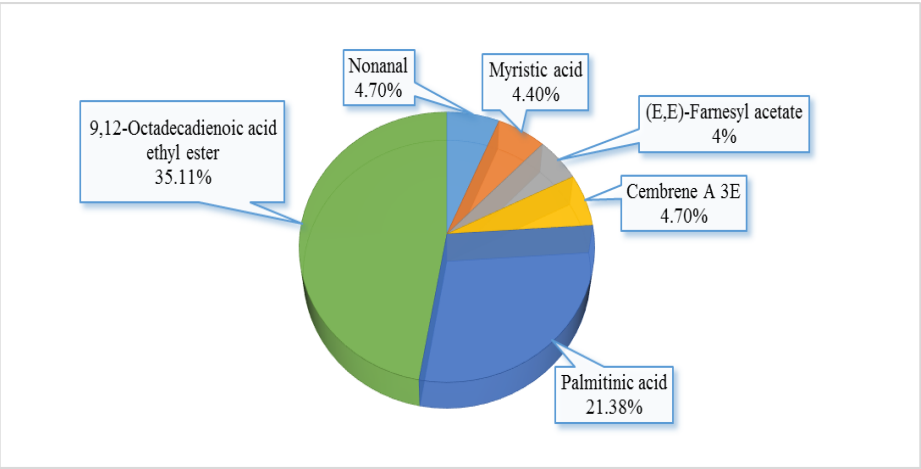

Figure 1. Major chemical constituents of the fruit essential oil of *P. farcta.*

Table 1. Chemical composition of fruit pod essential oil of *P. farcta*.

Compound	Area %	KI^c	KI^r
1,8-Cineole	0.5	1033	1031
n-Octanol	0.4	1068	1068
Linalool L	0.8	1100	1096
Nonanal	4.7	1106	1100
Decanal	0.7	1206	1201
β-Citronellol	0.9	1228	1225
Linalool acetate	2.2	1257	1254
α-Terpinyl acetate	3.5	1352	1349
Geranyl acetate	0.5	1384	1383
trans-Caryophyllene	0.7	1425	1428
Bicyclogermacrene	1.1	1502	1494
γ-Cadinene	0.5	1519	1513
Δ-Cadinene	1.6	1528	1530
Lauric acid	1.6	1567	1568
(+)-Spathulenol	0.5	1584	1576
Cedrol	1.9	1609	1604
β-Eudesmol	0.4	1657	1654
Neointermedeol	1.4	1662	1658
(Z,Z)-Farnesol	0.7	1697	1698
Myristic acid	4.4	1771	1780
(E,E)-Farnesyl acetate	4	1847	1846
Pentadecanoic acid	1.2	1866	1866
Cyclohexadecane	1	1880	1881
Palmitic acid, methyl ester	0.4	1926	1926
Cembrene A (3E)	4.7	1950	1948
Palmitinic acid	21.4	1984	1984
9,12-Octadecadienoic acid, ethyl ester	35.1	2164	2160
Unknown	2.7		
Total identified compounds	**97.3**		
Oxygenated monoterpenes	8.9		
Sesquiterpene hydrocarbons	4.9		
Oxygenated Sesquiterpenes	8.9		
Diterpene	4.7		
Fatty acid	64.2		
Others	5.8		

KI^c kovats retention indices of volatile constituents, which were experimentally calculated.

KI^r kovats retention indices of volatile constituents reported in the literature.

3.2. Total phenol and flavonoid

Table 2 shows total phenol and flavonoid contents of the ethanolic fruit extract of P. farcta and the IC_{50} values obtained from different free radical scavenging tests performed with fruit pod extract.

Table 2. Total phenol and flavonoid contents and antioxidant activity of *P. farcta* ethanolic fruit pod extract.

Sample	Total Phenol Content[1]	Total Flavonoid Content[2]	DPPH Assay IC_{50} (µg/ml)	FRAP Assay IC_{50} (µg/ml)	ABTS+ Assay IC_{50} (µg/ml)
P. farcta fruit pod extract	61.55±0.07	17±0.08	62.45±00	121.43±0.57	53.24±0.03
Quercetin	-	-	26.51±0.06	8.69±0.03	25.64±0.02

[1]mg GAE /g of dry plant material.

[2]mg QE /g of dry plant material.

Total flavonoid content of *P. farcta* ethanolic fruit pod extract was determined and found to be 17±0.08 mg quercetin equivalent (QE)/g of dry plant (Table 2).

3.3. Antioxidant screening

The results of antioxidant screening and the relevant IC_{50} values of the fruit extract obtained from DPPH, ABTS+ and FRAP assays are given in Table 2. The fruit extract showed IC_{50} values 62.45±00 and 53.24±0.03 µg/ml respectively against DPPH and ABTS+ free radicals. The FRAP value of ethanolic fruit extract was found to be 121.43±0.57 µg/ml (Table 2, Figure 2&3).

3.4. Antimicrobial screening

Antibacterial and antifungal potential of the fruit extract were assessed in terms of minimum inhibitory concentration (MIC) and the results were recorded (Table 3). The extract demonstrated antibacterial activity against both Gram-positive and Gram-negative bacteria. The MIC values for the extract against both *S. aureus* and *E. coli* were found to be 16 µg/mL, while the extract showed MIC values of 32 and 128 µg/mL, respectively, against *S. epidermidis* and *K. pneumonia* (Table 3). Among the tested fungal strains, the extract exhibiting MIC value of 32 µg/mL against *C. albicans*, while the MIC value was found to be 256 µg/mL when tested against *A. niger* (Table 3). The MIC values for the representative antibiotics against different test organisms are shown in Table 3.

4. Discussion

Results of the present study demonstrated the *in vitro* antioxidant and antimicrobial activity of *P. farcta* fruit extract. The results indicated that the extract from the fruits of *P. farcta* is rich in phenolic compounds, while it contains lower levels of flavonoids. Several phenolic compounds such as apigenin, luteolin, chalcone, and cinnamic acid derivatives have been previously identified from other *Prosopis* species (21, 22). Phenolic compounds are well-known for their antioxidant, antimicrobial, and various other therapeutic effects (23). A careful literature search revealed that some flavonoids have been detected in the seeds and other organs of *P. farcta* from different origins, some of which demonstrated *in vitro* and *in vivo*

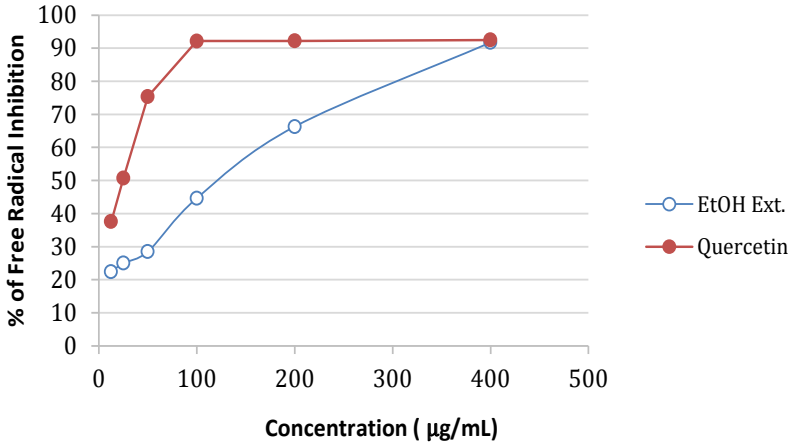

Figure 2. DPPH free radical inhibition of ethanolic fruit extract of *P. farcta* and quercetin.

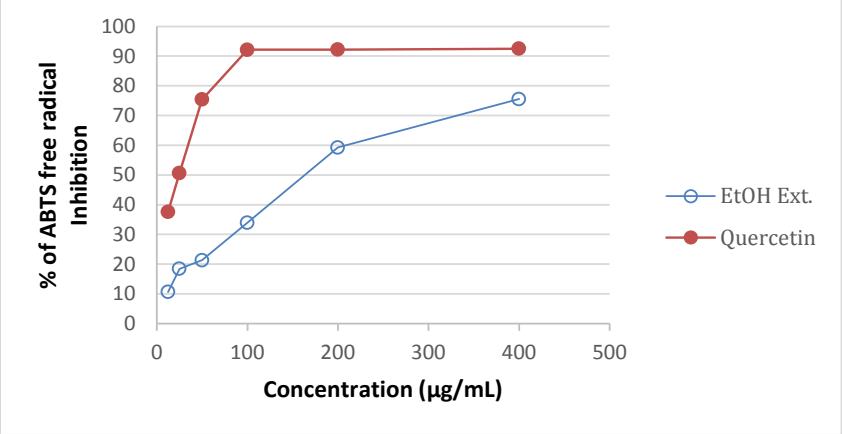

Figure 3. ABTS free radical inhibition of the ethanolic fruit extract of *P. farcta* and quercetin.

antioxidant and antimicribial activities in addition to several other therapeutic effects (24, 25).

The ethanolic fruit pod extract of *P. farcta* showed significant radical scavenging activity compared with quercetin, as a reference standard. The lowest IC_{50} value was detected for the extract against $ABTS^{+}$ free radical. On the other hand, the ABTS+ radical scavenging capacity of the extract was more prominent than DPPH free radical.

The high content of phenolic compounds were found as a remarkable characteristic of the *P. farcta* extract, as this group of compounds are well-documented for demonstration of antioxidant activities in biological systems (26). Previous studies have shown the relationship between antioxidant activity and phenolic content of plant extracts (27). The free radical inhibitory effect of the *P. farcta* fruit extract against DPPH and $ABTS^{+}$ free radicals in the present study, could be mainly attributed to the high phenolic content of the extract. Mechanistic studies declare that polyphenolic compounds are capable of donating hydrogen to free radicals in order to remove odd electrons thereby preventing further undesirable free radical chain reactions, which may lead to various kinds of health disorders (28).

The FRAP value obtained for the extract indicated a weak ferric reducing antioxidant property compared with quercetin, the reference standard. Hence, this conclusion may be derived that the compounds in the extract are lacking structural requirements for manifestation of remarkable reducing properties. FRAP is also a useful analytical tool for the assessment of antioxidants by measuring their oxidation-reduction potential. In FRAP assay, antioxidant agents or extracts can reduce the ferric-tripyridyltriazine (Fe^{+3}-TPTZ) complex formed during this test to an intense blue ferrous form (Fe^{+2}-TPTZ) at low pH conditions. Therefore, decrease in Fe^{+2} levels by an antioxidant ex-

Table 3. Antimicrobial activity of ethanolic fruit pod extract of *P. farcta*.

Type	Microorganism	Standard	Fruit Extract	Antibiotics		
			MIC(µg/ml)	Gen	Amp	Flu
Gram-positive bacteria	*Staphylococcus aureus*	PTCC-1431	16	8	4	-
	Staphylococcus epidermidis	PTCC-1435	32	8	16	-
Gram-negative bacteria	*Escherichia coli*	PTCC-1396	16	16	4	-
	Klebsiella pneumonia	PTCC-1053	128	16	8	-
Fungi	*Aspergillus niger*	PTCC-5154	256	-	-	16
Fungi	*Candida albicans*	PTCC-5027	32	-	-	16

MIC: Minimum Inhibitory Concentration

Amp: Ampicillin, Gen: Gentamicin, Flu: Fluconazole

(-): Not tested

tract or compound may serve as a significant indicator of its potential antioxidant activity (29).

4.1. Antimicrobial Screening

The extract demonstrated antimicrobial activities against both Gram-positive and Gram-negative bacteria and the tested fungal strains. The results were comparedwith the reference antibiotics, gentamycin, ampicillin, and the antifungal fluconazol. The highest antifungal effect was detected for the fruit extract against *C. albicans*, while *A. niger* was the least affected fungus by the ethanolic fruit extract (Table 3). The strongest antibacterial activity was observed for the extract against *S. aureus*, while the extract showed the lowest degree of antibacterial activity against *K. pneumonia* (Table 3).

The observed antimicrobial activity of ethanolic fruit extract of *P. farcta* was found to be concentration-dependent. The results of antimicrobial screening of the extract revealed its superior bactericidal activity against *S. aureus* and *E. coli* compared with those of *S. epidermidis* and the standard antibiotics. On the other hand, the extract showed a lower degree of antibacterial effect against *S. epidermidis*, while *K. pneumonia* was the least sensitive one among the tested bacterial strains. Previous research has shown that other *P. juliflora*, another *Prospois* species, demonstrated significant antimicrobial activity against various microorganisms; and the observed activity was attributed to the presence of bioactive compounds such as flavonoids and other phenolic compounds (30).

As evidenced by the results of this study, the fruit extract of *P. farcta* exhibited significant antimicrobial and antioxidant characteristics, therefore could be considered as a source of biologically active natural compounds. The phenolic compounds such as flavonoids and phenolic acids present in the fruit extract may be considered as the major components responsible for these activities. The difference in the results of *P. farcta* antibacterial screening against the tested Gram-positive and Gram-negative strains may be due to the fact that the active constituents of the extract may have different mechanisms of action. As an example, the hydrophobic properties of active chemical components of plant extracts can lead to interactions with the cell membrane. Flavonoid-cell membrane interactions is one of the interactions considered as the main possible mechanism for exerting the biological effects of phenolic compounds (31). Furthermore, the essential oil obtained from fruit pods of *P. farcta* is a rich source of volatile fatty acid esters, while containing the biologically active constituents like mono- and sesquiterpenes and diterpenes, some of which have applications in drug, cosmetic, and fragrance industries.

5. Conclusion

In the current study, essential oil obtained from fruit pods of *P. farcta* was found as a rich source of fatty acids with mono-, sesqui- and diterpenic compounds. Its fruit ethanolic extract showed effective antimicrobial and antioxidant characteristics. The results clearly showed the promising effect of *P. farcta* fruit extract against both Gram-positive and Gram-negative bacteria. Since there is a continuous rise in microbial resistance, the fruit extract of *P. farcta* may be considered as a suitable supplemental candidate to overcome this problem. Additionally, the role of individual components of this extract or their combinations with the usual antimicrobial agents could be another interesting subject for future investigations.

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Conflict of Interest

None declared.

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