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Investigation of antibacterial and antioxidant properties of three medicinal plants from Gaziantep, Turkey

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ABSTRACT: Current research aimed to reveal antibacterial and antioxidant properties of acetone and ethyl acetate extracts of *Phlomis armeniaca*, *Echinophora tenuifolia* subsp. *sibthorpiana* and *Moringa oleifera* plants obtained from herbalists in Gaziantep. Extracts of *P. armeniaca*, *E. tenuifolia* subsp. *sibthorpiana* and *M. oleifera* plants have antibacterial effect at varying degrees against test bacteria. Both ethyl acetate and acetone extracts of *P. armeniaca* plant exhibited higher antibacterial activity than studied other plant extracts. It was also found that the antioxidant activity increased with increasing concentrations. Since antioxidant and antibacterial activities were observed in almost all of the tested plant extracts, it was concluded that *P. armeniaca*, *E. tenuifolia* subsp. *sibthorpiana* and *M. oleifera* plants could be natural sources of antioxidant and antibacterial.

Keywords: Medicinal plant; Antibacterial activity; Bacteria; Antioxidant activity; Free radical.

1. INTRODUCTION

Infectious diseases are responsible for about 50,000 deaths worldwide each year. This situation has become even more serious with the increase of bacterial strains with multidrug resistance. Infectious illnesses influence every people. Due to solving the problem of resistance, researchers are in the race to find brand antibiotics [1]. Medicinal plants have some bioactive compounds such as coumarins, terpenoids, tannins, essential oils and alkaloids and they utilized as a starting point for antibiotic synthesis [2].

Imbalance with free radical activity and antioxidant activity cause oxidative stress. Antioxidants prevent oxidation which produce free radicals. They can start a chain reaction which produce more free radicals, leading to death of cells and tissues. Antioxidants convert these radicals into less reactive species [3]. Because of their natures, plants are known as natural antioxidant sources. They have many phytochemicals which reduce the risks of brain dysfunction, cardiovascular diseases, cataracts and cancers. When synthetic antioxidants and natural antioxidants are compared, synthetic antioxidants have many carcinogenic effects [4].

Phlomis armeniaca is a perennial plant which belongs to Lamiaceae [5]. It has many medicinal properties. It is an expectorant and provides healing by softening the chest in cough and bronchitis. It plays a role in curing respiratory tract mucosal infections. It is good for hoarseness. It is good for stomach aches caused by stomach cold and has diuretic properties [5].

Echinophora tenuifolia subsp. *sibthorpiana* is a perennial plant. The leaves and flowers of the plant are used. It is good for cold, cough, bronchitis and asthma. It also has gas-digesting, stimulating and stomach-relieving effects [6]. *E. tenuifolia* subsp. *sibthorpiana* is used both as a fungicide and added to pickles to give fragrance after the aboveground part of this plant is dried in Turkey, especially Isparta [7].

Moringa oleifera is a plant originating from India and generally grows in tropical climates. It is usually a small perennial tree. It is also called the miracle tree, because almost every part of the *M. oleifera* plant has a separate value [8]. In Malaysia, *M. oleifera* seeds are consumed as peanuts. The roots are edible like horseradish. The leaves can be eaten as greens, in salads, in vegetable dishes. In the Philippines, the leaves are used in soup, etc. Its seeds contain Ben oil which is used as a paint ingredient in painting and fine arts, as well as in the lubrication of sensitive machines like watches [9].

The target of the current research is to reveal antibacterial and antioxidant properties of ethyl acetate and acetone extracts of *Phlomis armeniaca, Echinophora tenuifolia* subsp. *sibthorpiana* and *Moringa oleifera.*

2. MATERIALS AND METHODS

2.1. Collecting of the plant materials

Phlomis armeniaca, Echinophora tenuifolia subsp. *sibthorpiana* and *Moringa oleifera* were bought from a herbal shop in Gaziantep, Turkey.

2.2. Preparation of the extracts

30 g of *P. armeniaca, E. tenuifolia subsp. sibthorpiana* and *M. oleifera* were extracted in a shaker for 24 h utilizing 300 ml acetone and ethyl acetate, separately. The extracts were filtered and residues were evaporated (40°C) with a rotary evaporator [10].

2.3. Antibacterial activity

2.3.1. Microorganisms

Ten bacteria were used in the study as follows: *Listeria monocytogenes* ATCC 7644, *Salmonella enterica* serovar *typhimirium* ATCC 14028, *Staphylococcus aureus* subsp. *aureus* ATCC 25923, *Bacillus cereus* 702 ROMA, *Yersinia pseudotuberculosis* ATCC 911, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* IMG 22, *Enterobacter aerogenes* CCM 2531, *Gordonia rubripertincta* (lab isolate) and *Proteus vulgaris* (lab isolate).

2.3.2. Disc diffusion method

The antibacterial properties of extracts of *P. armeniaca, E. tenuifolia subsp. sibthorpiana and M. oleifera* were determined with utilizing disc diffusion method. Each plant extract was dissolved in dimethyl sulfoxide (DMSO) at 30 mg/ml concentration. Acetone extracts and ethyl acetate extracts were studied in different petri dishes. 20 µl plant extracts added to the discs (5 mm diameter), separetely. 20 µl DMSO added to the disc for negative control. Gentamycine was used as positive control. Plates were incubated at 37°C overnight. Diameter of zones were measured with a ruler [11,12]. The tests were carried out two times.

2.3.3. Determination of Minimum Inhibition Concentration (MIC)

Acetone and ethyl acetate extracts were prepared 30 mg/ml concentration in DMSO. MIC values of the extracts were determined with 96 well plates by the method of Yiğit et. [13].

2.4. Antioxidant activity

2.4.1. Total Phenolic Content

The quantity of the total phenolic content was denoted as µg of gallic acid equivalent (GAE)/ml. The tests were carried out three times [14].

2.4.2. Total Flavonoid Content

The quantity of the total flavonoid content was denoted as µg of catechin equivalent (CE)/ml. The tests were carried out three times [15].

2.4.3. Total Antioxidant Capacity

The quantity of the total antioxidant capacity was denoted as µg of ascorbic acid equivalent (AAE)/ml. The tests were carried out three times [16].

2.4.4. 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity

Plant extracts were prepared at 250-1000 µg/ml concentrations. DPPH radical scavenging activity of the extracts was determined by the method of Brand-Williams et al. [17]. The tests were carried out three times. BHT and rutin were used as standards. The DPPH radical scavenging activity was calculated using the following equation:

DPPH radical scavenging activity (%) = [(A0 – A1) / A0] x 100

A0 is the absorbance of the control

A1 is the absorbance of the sample

2.4.5. Cupric reducing antioxidant capacity (CUPRAC)

CUPRAC activity of the extracts was studied by the method of Özyürek et al. Absorbance was measured at 450 nm. BHT was utilized as standard antioxidant substance [18].

3. RESULTS AND DISCUSSION

3.1. Antibacterial activity

Antibiotic resistance creates an important health problem by increasing health costs and mortality rates. Recently, important works have been carried out to control the spread of resistant pathogens and plants are investigated as new antibiotic resources [19].

Inhibition zones which were created by test extracts were demonstrated in Table 1. Both acetone and ethyl acetate extracts of *P. armeniaca* exhibited higher activity than tested other plant extracts. The weakest antibacterial activity generally was found in *M. oleifera*. DMSO which was used as negative control didn't show any activity against test bacteria. Gentamycine which was used as positive control showed higher activity than tested plant extracts except for acetone extract of *P. armeniaca* against *E. faecalis*. The weakest antibacterial effect was found in acetone extracts of *E. tenuifolia* subsp. *sibthorpiana* against *S. enterica* (6.5±0.70) and acetone extract of *M. oleifera* against *P. vulgaris* (6.5±0.70). The highest antibacterial effect was detected in acetone extract of *P. armeniaca* against *E. faecalis* (21±1.41).

MIC is defined as the lowest concentration which inhibits microorganisms growth [20]. In MIC assay, extracts which showed inhibition zones ≥10 mm were studied [21]. MIC values of the extracts were given in Table 2.

Table 1. Inhibition zones which was created by plant extracts and gentamycine (mm).

Bacteria	EAE	PAE	MAE	EEE	PEE	MEE	DMSO	CN
S. enterica serovar *typhimirium*	6.5±0.70	14.5±0.70	6.5±0.70	-	8±1.41	8±0.00	-	17.5±0.70
P. vulgaris	10±0.00	12±1.41	6.5±0.70	6.5±0.70	7±0.00	-	-	16±1.41
Y. pseudotuberculosis	7.5±0.70	11±1.41	11±1.41	9±0.00	8.5±0.70	9.5±0.70	-	18±0.00
B. subtilis	8.5±0.70	13.5±0.70	8±1.41	-	-	-	-	15.5±0.70
B. cereus	7±1.41	8±1.41	-	11±1.41	12±1.41	7±1.41	-	20±1.41
E. aerogenes	11±1.41	12±1.41	-	12.5±0.70	11±1.41	9±0.00	-	18.5±0.70
S. aureus subsp. *aureus*	11.5±0.70	12.5±0.70	11.5±0.70	14±0.00	20.5±0.70	11±1.41	-	19.5±0.70
G. rubripertincta	11.5±0.70	12.5±0.70	-	16±0.00	10.5±0.70	9±0.00	-	22±1.41
L. monocytogenes	9±1.41	8.5±0.70	7.5±0.70	13.5±0.70	11.5±0.70	11.5±0.70	-	21.5±0.70
E. faecalis	17±1.41	21±1.41	-	8±1.41	8.5±0.70	8.5±0.70	-	20±0.00

EAE: Acetone extract of *E. tenuifolia* subsp. *sibthorpiana*; PAE: Acetone extract of *P. armeniaca*; MAE: Acetone extract of *M. oleifera*; EEE: Ethyl acetate extract of *E. tenuifolia* subsp. *sibthorpiana*; PEE: Ethyl acetate extract of *P. armeniaca*; MEE: Ethyl acetate extract of *M. oleifera*; CN 10: Gentamycine 10 µg/ml.

Table 2. MIC values of the extracts (µg/ml).

Bacteria	EAE	PAE	MAE	EEE	PEE	MEE
S. enterica serovar *typhimirium*	-	93.75	-	-		-
P. vulgaris	187.5	375	-	-	-	-
Y. pseudotuberculosis	-	46.88	375	-	-	-
B. subtilis	-	187.5	-	-	-	-
B. cereus	-	-	-	187.5	93.75	-
E. aerogenes	187.5	187.5	-	187.5	93.75	-
S. aureus subsp. *aureus*	187.5	375	375	375	187.5	187.5
G. rubripertincta	93.75	23.44	-	375	93.75	-
L. monocytogenes	-	-	-	375	187.5	187.5
E. faecalis	93.75	375	-	-	-	-

EAE: Acetone extract of *E. tenuifolia* subsp. *sibthorpiana*; PAE: Acetone extract of *P. armeniaca*; MAE: Acetone extract of *M. oleifera*; EEE: Ethyl acetate extract of *E. tenuifolia* subsp. *sibthorpiana*; PEE: Ethyl acetate extract of *P. armeniaca*; MEE: Ethyl acetate extract of *M. oleifera*.

While acetone extracts of the plants are ranges from 23.44 µg/ml to 375 µg/ml; ethyl acetate extracts of the plants are ranges from 93.75 µg/ml to 375 µg/ml. Lower MIC values show higher antibacterial activity. The lowest MIC value was exhibited by acetone extract of *P. armeniaca* against *G. rubripertincta* as 23.44 µg/ml.

There are studies about the antibacterial activities of *P. armeniaca*. *E. tenuifolia* subsp. *sibthorpiana* and *M. oleifera*. For example, Aybey investigated antibacterial activity of ethyl acetate extract of *P. armeniaca* and it was found that this extract created 12 mm and 14 mm inhibition zones on *Bacillus subtilis* and *Salmonella typhimurium*, respectively. Also, MIC values were found as 0.625 mg/ml and 1.25 mg/ml against these bacteria [22]. In our study, it was found ethyl acetate extract of *P. armeniaca* showed no activity against *B. subtilis* and created 8 mm inhibition zone against *S. typhimurium*. These differences arised from collecting samples from different locations and using different extraction methods.

In literatures, there are studies generally about antibacterial activity of essential oils of *E. tenuifolia* subsp. *sibthorpiana.* Gökbulut et al. found MIC values of essential oil of *E. tenuifolia* subsp. *sibthorpiana* as 125 µg/ml. 62.5 µg/ml and 500 µg/ml against *Staphylococcus aureus. Bacillus cereus* and *Enterococcus faecalis*, respectively [23]. In our current study, MIC values of extract of *E. tenuifolia* subsp. *sibthorpiana* found at 187.5 µg/ml and 93.75 µg/ml against *S. aureus* and *E. faecalis*, respectively. These different results might be arised from different secondary metabolites which in our extract and essential oil. Fouad et al. searched antibacterial effect of *M. oleifera* leaf extract against pyogenic bacteria isolated from camel abscess [24].

3.2. Antioxidant activity

3.2.1. Total phenolic content

The total phenolic content of the extracts was presented in Table 3. While the highest total phenolic content was found in acetone extract of *P. armeniaca* (434.21±0.011 µg GAE/ml), the lowest total phenolic content was found in ethyl acetate extract of *M. oleifera* (25.66±0.003 µg GAE/ml).

In a study which was carried out by Yumrutaş and Saygıdeğer, total phenolic content of methanol and hexane extracts of *P. armeniaca* was found as 320.37±6.97 mg GAE/g and 55.90±1.01 mg GAE/g, respectively [25]. Using different extraction methods and solvents cause different results between our study and Yumrutaş and Saygıdeğer's study.

Table 3. Total phenolic content of the tested plant extracts (µg GAE/ml).

Plant Extract	Total Phenolic Content (µg GAE/ml)
Acetone extract of *P. armeniaca*	434.21±0.011
Acetone extract of *E. tenuifolia* subsp. *sibthorpiana*	46.97±0.001
Acetone extract of *M. oleifera*	27.48±0.003
Ethyl acetate extract of *P. armeniaca*	120.06±0.009
Ethyl acetate extract of *E. tenuifolia* subsp. *sibthorpiana*	141.6±0.006
Ethyl acetate extract of *M. oleifera*	25.66±0.003

3.2.2. Total flavonoid content

Total flavonoid content of the extracts were given in Table 4. Total flavonoid content of ethyl acetate extracts of the plants were found higher than acetone extracts of the plants except for ethyl acetate extract of *M. oleifera*. The highest and the lowest total flavonoid content was found in ethyl acetate extract of *E. tenuifolia*

subsp. *sibthorpiana* (739.17±0.010 µg QE/ml) and acetone extract of *P. armeniaca* (177.49±0.011 µg QE/ml), respectively.

Table 4. Total flavonoid content of the tested plant extracts (µg QE/ml).

Plant Extract	Total Flavonoid Content (µg QE/ml)
Acetone extract of *P. armeniaca*	177.49±0.011
Acetone extract of *E. tenuifolia* subsp. *sibthorpiana*	344.64±0.044
Acetone extract of *M. oleifera*	354.27±0.037
Ethyl acetate extract of *P. armeniaca*	228.95±0.049
Ethyl acetate extract of *E. tenuifolia* subsp. *sibthorpiana*	739.17±0.010
Ethyl acetate extract of *M. oleifera*	320.92±0.089

3.2.3. Total antioxidant capacity

Total antioxidant capacity of the tested plant extracts were presented in Table 5. Total antioxidant capacity of ethyl acetate extracts of the plants were detected higher than acetone extracts of the plants except for ethyl acetate extract of *P. armeniaca*. The highest total antioxidant capacity was found in ethyl acetate extract of *E. tenuifolia* subsp. *sibthorpiana* (373.50±0.033 µg AAE/ml) and the lowest total antioxidant capacity was found in acetone extract of *M. oleifera* (85.23±0.010 µg AAE/ml).

Table 5. Total antioxidant capacity of the tested plant extracts (µg AAE/ml).

Plant Extract	Total Antioxidant Capacity (µg AAE/ml)
Acetone extract of *P. armeniaca*	162.26±0.014
Acetone extract of *E. tenuifolia* subsp. *sibthorpiana*	100.32±0.020
Acetone extract of *M. oleifera*	85.23±0.010
Ethyl acetate extract of *P. armeniaca*	119.56±0.025
Ethyl acetate extract of *E. tenuifolia* subsp. *sibthorpiana*	373.50±0.033
Ethyl acetate extract of *M. oleifera*	169.41±0.023

3.2.4. DPPH radical scavenging activity

Figure 1 shows DPPH radical scavenging activity of extracts and standards. DPPH radical scavenging activity of acetone extract of *M. oleifera* is higher than BHT and Rutin which were synthetic antioxidants at 1000 µg/ml concentration. When the activities of extracts, BHT and Rutin are compared at 1000 µg/ml concentration, we can make a ranking as follows: Acetone extract of *M. oleifera* > BHT > Rutin > Acetone extract of *E. tenuifolia* subsp. *sibthorpiana* > Ethyl acetate extract of *P. armeniaca* > Ethyl acetate extract of *E. tenuifolia* subsp. *sibthorpiana* > Ethyl acetate extract of *M. oleifera* >Acetone extract of *P. armeniaca.*

Abdulkadir et al. found DPPH radical scavenging activity (% inhibition) of methanol extract of *M. oleifera* ranges from 58.62±1.13 and 83.62±1.32. Moreover, DPPH radical scavenging activity (% inhibition) of hexane extract ranges from 15.98±1.24 and 32.91±1.63 [26].

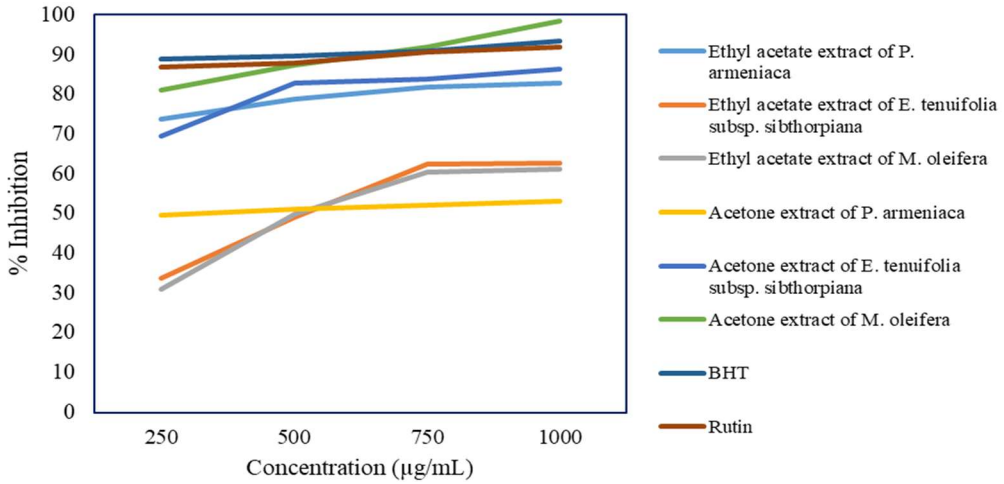

Figure 1. DPPH scavenging activity extracts and standards.

Table 6. CUPRAC activity of extracts and BHT.

Plant Extract	Concentration (µg/ml)	Absorbance (nm)
PEE	250	0.6856±0.024
	500	1.4135±0.021
	750	1.8411±0.018
	1000	2.048±0.026
EEE	250	0.8741±0.018
	500	1.4926±0.023
	750	1.970±0.016
	1000	2.127±0.030
MEE	250	0.3248±0.028
	500	0.4933±0.016
	750	0.7494±0.024
	1000	1.0246±0.038
PAE	250	0.6213±0.007
	500	0.8782±0.003
	750	0.8842±0.004
	1000	0.9676±0.019
EAE	250	0.068±0.011
	500	0.2171±0.002
	750	0.4048±0.012
	1000	0.5634±0.027
MAE	250	0.074±0.006
	500	0.2245±0.018
	750	0.4269±0.031
	1000	0.5799±0.0001
BHT	250	0.6635±0.023
	500	0.7016±0.021
	750	0.8283±0.024
	1000	0.9716±0.014

PEE: Ethyl acetate extract of *P. armeniaca*; EEE: Ethyl acetate extract of *E. tenuifolia* subsp. *sibthorpiana*; MEE: Ethyl acetate extract of *M. oleifera*; PAE: Acetone extract of *P. armeniaca*; EAE: Acetone extract of *E. tenuifolia* subsp. *sibthorpiana*; MAE: Acetone extract of *M. oleifera.*

3.2.5. CUPRAC activity

CUPRAC activity of the extracts was demonstrated in Table 6. When CUPRAC activity of extracts are compared at 1000 µg/ml concentration, the highest activity was detected in ethyl acetate extract of *E. tenuifolia* subsp. *sibthorpiana* and the lowest activity was detected in acetone extract of *E. tenuifolia* subsp. *sibthorpiana.* Ethyl acetate extracts exhibited higher activity than acetone extracts of extracts. Moreover. ethyl acetate extracts of the plants showed higher CUPRAC activity than BHT which was used standard antioxidant agent.

Sarıkürkçü et al. found CUPRAC activity of ethyl acetate extract of *P. armeniaca* was higher than methanol and water extracts [27]. In our study, we also found CUPRAC activity of ethyl acetate extract of *P. armeniaca* higher than acetone extract.

4. CONCLUSION

P. armeniaca, *E. tenuifolia* subsp. *sibthorpiana* and *M. oleifera* can be seen as an alternative to synthetic antioxidants and antibacterial agents. Therefore, researches about the isolation and identification of substances with antibacterial and antioxidant properties in these plants should be increased.

Authors' contributions: SA performed antioxidant activity. SA made figures and tables in antioxidant part. SA also wrote and revised the manuscript. MS performed antibacterial activity. MS made figures and tables in antibacterial part. All authors read and approved the final version of the manuscript.

Conflict of interest: The authors declare no potential conflict of interest.

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