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ANTIMICROBIAL ACTIVITIES OF SOME *EUPHORBIA* SPECIES

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Abstract

In this study, the antimicrobial activities of methanolic extracts and latex of some *Euphorbia* species used for medical purposes in Turkey were investigated. The extracts of *Euphorbia aleppica* L., *Euphorbia szovitsii* Fisch.&Mey. var. *harputensis* Aznav. ex M. S. Khan, *Euphorbia falcata* L. sub. *falcata* var. *falcata*, *Euphorbia denticulata* Lam., *Euphorbia macroclada* Boiss., *Euphorbia cheiradenia* Boiss.&Hohen, Euphorbia virgata Waldst.&Kit., *Euphorbia petiolata* Banks&Sol. were prepared with methanol. The antimicrobial activities of these extracts were examined on test microorganisms as follows: *Staphylococcus aureus* COWAN 1, *Bacillus megaterium* DSM 32, *Proteus vulgaris* FMC 1, *Klebsiella pneumonia* FMC 5, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* DSM 50071, *Candida albicans* FMC 17, *Candida glabrata* ATCC 66032, *Epidermophyton* sp. and *Trichophyton* sp. by the disc diffusion methods and well agar method. The MIC values of extracts were determined according to the broth microdulitions method. Results indicated that extracts of *Euphorbia* species inhibited the growth of tested microorganisms in the different ratio. Also, the MIC values of extracts were determined as 31,2-1000 µg.

Keywords: Antimicrobial activity, Pathogen microorganisms, Medicinal plants

Introduction

Various plants, which serve as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health. Since ancient times medicinal plants have continued to be an important therapeutic aid for alleviating the ailments of humankind (Nair and Chanda, 2007). These medicinal herbs constitute indispensable components of the traditional medicine practiced worldwide due to their low cost, easy accessbility and ancestral experience (Natarajan et al., 2005).

Euphorbia is a genus of flowering plants belonging to the family Euphorbiaceae. *Euphorbia* is the richest genus represented by 80 species in Turkey (Baytop, 1999).The effects of different *Euphorbia* species on pathogens microorganism have been studied by researchers in different parts of the world (Oksüz et al., 1994; Ferreira et al., 1996; Öksüz et al., 2002; Al-Mughrabi, 2003; Cateni et al., 2003; Madureira et al., 2003; Annapurna et al., 2004; Papp, 2004; Sudhakar et al., 2006; Ogbulie et al., 2007; Barla et al., 2007; Kamba and Hassan, 2010).

The latex of *Euphorbia* species used in this study is used widely because of this plant species' different substantial features. For instance, these plants are used for the treatment of hypertension, destruction of warts and skin diseases. Despite these attributes, no study is found in the literature about the antimicrobial activities of these *Euphorbia* species. Our purpose is to evaluate the potential antimicrobial activities of *E. aleppica*, *E. szovitsii* var. *harputensis*, *E. falcata* sub. *falcata* var. *falcata*, *E. denticulata*, *E. macroclada*, *E. cheiradenia*, *E.virgata*, *E. petiolata* on some bacteria, yeasts and dermatophyta fungi. The results suggest that *Euphorbia* species possesses compounds with antimicrobial properties that might be utilised for developing new drugs.

Materials and Methods

Plant materials and extraction procedure

E. aleppica, *E. szovitsii* var. *harputensis*, *E. falcata* sub. *falcata* var. *falcata*, *E. denticulata*, *E. macroclada*, *E. cheiradenia*, *E. virgata*, *E. petiolata* were collected from Elazig Province in the Eastern Anatolia of Turkey. They were collected from the following locations:*E. aleppica*, B7 Elazig: Harput, inceler village, field 1400m, 26.06.2011, SK.1001*E. szovitsii* var. *harputensis*, B7 Elazig: Harput, inceler village, field 1400m 26.06.2011.SK,1002*; E. falcata* sub. *falcata* var. *falcata*, B7 Elazig: Harput, Buzluk cave, road sides 1450m 26.06.2011, SK.1003; *E. denticulata*, B7 Elazig: Sugözu village, field 1400m, 27.06.2011, SK.1004 *;E. macroclada*, B7 Elazig: Firat university campus 1020m 26.06.2011,SK.1005*; E. cheiradenia*, B7 Elazig: Ulukent, field 1400m 27.06.2011, SK.1006*; E. virgata*, B7 Elazig: Elazig: Firat University campus 1020m 26.06.2011, SK.1007 and *E. petiolata*, B7 Elazig: Harput, around castle, field 1200m 26.06.2011, SK.1008 The voucher numbers were Voucher No: 1001,1002,1003,1004,1005,1006,1006,1007,1008 respectively.

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The taxonomic identification of plant materials was determined using Flora of Turkey (Davis, 1970-1984-1985). The collected flowering branches were powdered under steril conditions. Each of the powdered plant materials (5 g) was extracted in 25 mL methanol (98.1) solvent by keeping on a rotary shaker (100 rpm) for 24h. The extracts were filtered using Whatman filter paper and then concentrated in vacuum at 37°C using a Rotary evaporator. The extracts were dissolved in DMSO and stored at 4°C for further studies. Then, 500 µg extracts were injected into empty antibiotic paper discs having a diameter of 6 mm (Schleicher&Shüll No: 2668, Germany). The Standard antibiotics nystatin and streptomycin were used as a positive control (respectively for yeasts and bacteria). The experimental studies were repeated three times.

Test Microorganisms

A total of 6 bacteria (*Staphylococcus aureus* COWAN 1, *Bacillus megaterium* DSM 32, *Proteus vulgaris* FMC 1, *Klebsiella pneumoniae* FMC 66032, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* DMS 50071 SCOTTA), 3 yeasts (*Candida albicans* FMC 17, *Candida glabrata* ATCC 66032, *Candida tropicalis* ATCC 13803), and 2 dermatophyte species (*Trichophyton* sp., *Epidermophyton* sp.) were used in the present investigation. Microorganisms were provided by the Department of Biology, Faculty of Science and Arts, Firat University, Microbiology Laboratory, Elazig-Turkey.

Antimicrobial activity

Antimicrobial tests were carried out by disc diffusion and well agar method using 100 µl of suspension containing 10^6 cells / mL of bacteria, 10^4 cells / mL yeast per McFarland standard, inoculated into Mueller Hinton Agar (Difco), Malt Extract Agar (Difco) and Sabouroud Dextrose Agar (Oxoid), respectively. Agar disc diffusion method was prepared using the discs (6 mm in diameter), containing 25 µl (500 µg) of the extracts (20 mg/ml), placed on the inoculated Mueller Hinton Agar (Difco), Malt Extract Agar (Difco) and Glukoz Sabouroud Agar (Oxoid), respectively. For agar well diffusion method, wells were prepared in the plates with the help cork-borer (0. 85 cm). 25 µl of the latex of plants was introduced directly into the well. Petri dishes were placed at 4 °C for 2h. Then, the inoculated plates were incubated at 37±0.1°C, at 24 h for bacterial strains and also at 25±0.1°C at 72 h for yeast and dermatophyta fungi. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms (Collins and Lyne, 1987).

Microdilution assays

The MIC values of plant extracts were determined acording to the method of microdulitions (NCCLS, 2000). But MIC could not be made in latex, as there was no sufficient amount of latex. The inocula of microorganisms were prepared from 12 h broth cultures, and suspensions were adjusted to 0.5 McFarland standard turbidity. The *Euphorbia* species were first diluted to the highest concentration 100 µl to be tested, and then serial 2-fold dilutions were made in a concentration range from 3.12 to 100 µl (31,2-1000 µg) in 10 ml sterile test tubes containing nutrient broth and sabouraud dextrose broth. MIC values of these plant latexes against bacteria, yeasts and dermatophyta were determined based on a micro-well dilution method (Güllüce et al., 2004). Microbial growth was determined by absorbance values at 600 nm using an ELx 800 universal microtiter plate reader. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of microorganisms.

Statistical analysis

SPSS 15.0 software was used for statistical analysis of the data. Analysis of variance (ANOVA) and least significant difference (LSD) tests were also used for comparisons of groups and the control group.

Results and Discussion

The antimicrobial activities of *Euphorbia* species, negative control group, and standard antibiotics are given in tables 1, 2 and 3. It has been found that the extracts of *Euphorbia* species have antibacterial and antifungal activity to the microorganisms tested, and it seems that the antimicrobial activity of those plants extract are changeable as seen in Tables 1-2 .

Extracts of plants obtained from *E. falcata*, followed by *E. aleppica*, *E. macroclada* and *E. virgata,* have the highest antimicrobial efficiency as seen in tables 1- 2. The activity of the plants against test bacteria, yeasts and dermatophyte fungi may be indicative of the presence of broad spectrum antibiotic compounds in the plant. The antimicrobial effects of extract and latex of *E. falcata* were observed to be very high as seen in Table 1 and Table 2 respectively; 8-23 mm, 10-21 mm and the lowest MIC value were determined in *C tropicalis, P. vulgaris.* (Table 3).

Table 1 and table 2 show that extract and latex of *E. aleppica* have antibacterial and antifungal activity as 8-25 and 10-21 mm zone of inhibition to the microorganisms tested, and are also observed to be very high to *Epidermophyton*. MIC value of *E. aleppica* was determined 31,2-1000µg against the tested microorganisms. The lowest MIC value was determined in *Epidermophyton* sp.

As seen in Tables 1-2, the latex and extract of *E. szovitsii* have the low antimicrobial activity against to *E. coli* and *P. aeruginosa*. Extract of *E. szovitsii* has antimicrobial activity on the tested microorganisms from high to low respectively; *Epidermophyton* sp, *C. tropicalis, Trichophyton* sp., *K. pneumoniae, P. vulgaris, B. megaterium, C. albicans, S. aureus* and *C. glabrata*. However, the latex of *E. szovitsii* showed inhibitory effect at different ratio against some bacteria, yeasts and

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dermatophyta tested (8-15 mm as showed in table 2). MIC value of *E. szovitsii* was determined as 31,2-1000µg and the lowest MIC value was determined in *Epidermophyton* sp. (table 3).

Table 1. Antimicrobial activity of some *Euphorbia* species according to the disc diffusion method

	Inhibition zone in diameter (mm)									
Microorganisms	**E.A**	**E.S**	**E.F**	**E.D**	**E.M**	**E.C**	**E.V**	**E.P**	**Control**	**Standart**
S. aureus	14.66±0.33	10±0.57	20±1.15 ^{d}	17±1.15 ^{cd}	11±0.88	8.33±0.33	11±0.57	13±0.57		13**±0.33
B. megaterium	20±0.57 ^{cd}	11±0.33	15±0.57 ^{cd}	-	13±0.57 ^{d}	9±0.57	12±0.57	8.33±0.33	-	9**±0.33
P. vulgaris	14±0.33 ^{d}	12±0.33	23±1.15 ^{cd}	11±0.57	11±0.57	13±0.88 ^{d}	8.66±0.33	9±0.33	-	11**±0.33
K. pneumoniae	11±0.33	12±1.15	15±1.15 ^{cd}	8.33±0.33	9±0.33	8.33±0.33	11±0.57	11±1.15	-	9**±0.33
E. coli	8.33±0.33	9±0.57	8.33±0.33	-	8.33±0.33	12±0.33	8.33±0.33	11±1.15	-	11**±0.57
P. aeruginosa	10±0.88	9±0.57	15±0.57	13±0.57 ^{d}	13±0.57	9±0.33	9±0.33	9±0.33	-	11**±0.57
C. albicans	15±0.33	11±0.57	17±1.15	9±0.33	12±0.33	13±1.15	9±0.57	8.33±0.33	-	18.3*±0.33
C. glabrata	13±0.33	9.33±0.33	12±0.57	11±0.57	11±1.15	8.66±0.33	15±0.57 ^{cd}	8±0.33	-	12*±0.33
C. tropicalis	20.33±0.33 ^{cd}	15±0.57 ^{cd}	23±1.15 ^{cd}	20±0.57 ^{cd}	13±0.33 ^{d}	9±0.33	13±0.57 ^{d}	17±0.57 ^{cd}	-	13*±0.57
Trichophyton sp.	18±0.57 ^{cd}	13±1.15 ^{d}	17±0.57 ^{cd}	13±1.15 ^{d}	23±0.57 ^{cd}	11±0.57	17±0.57 ^{cd}	8.33±0.33	-	NT
Epidermophyton sp.	25±0.57 ^{cd}	23±1.15 ^{cd}	15±0.57 ^{d}	12±0.57	23±0.57 ^{cd}	11±0.57	21±0.57 ^{cd}	12±0.57	-	NT

E.A. : *E. aleppica*, **E.S.**: *E. szovitsii*, **E.F**.: *E. falcata*, **E.D**. :*E. denticulata*, **E.M**.: *E. macroclada*, **E.C**.: *E. cheiradenia*, **E.V**.: *E. virgata*, **E.P.:** *E. petiolata*, Extract*:* 500 µg/disc, *:Nystatin (30 µg/disc), **: Streptomycin sulphate (10 µg /disc), Control, NT: not tested cd: p<0.0001, d: p<0.001, c: p<0.01, b: p<0.05

Table 2. Antimicrobial activity of the latex of some *Euphorbia* species

E.A. : *E. aleppica*, E.S.: *E. szovitsii*, E.F.: *E. falcata*, E.D. :*E. denticulata*, E.M.: *E. macroclada*, E.C.: *E. cheiradenia*, E.V.: *E. virgata*, E.P.: *E. petiolata* Latex:500µg/disc, p<0.0001, d: p<0.001, c: p<0.01, b: p<0.05

Microorganisms	Inhibition zone in diameter (mm)									
	E.A	**E.S**	**E.F**	**E.D**	**E.M**	**E.C**	**E.V**	**E.P**	**Control**	**Standart**
S. aureus	17±1.15 ^{d}	12±1.15	15±1.15	15±1.15	10±1.15	10±1.15	13±1.15	13±1.15	-	13**±0.33
B. megaterium	13±1.15 ^{d}	11±0.33	10±1.15	-	8.33±0.33	9±0.57	9±0.33	10±1.15	-	9**±0.33
P. vulgaris	10±1.15	12±1.15	9±0.57	12±1.15	9±0.57	11±1.15	9±0.57	15±1.15	-	11**±0.33
K. pneumoniae	15±1.15 ^{d}	12±1.15	20±1.15 ^{cd}	20±1.15 ^{cd}	23±1.15 ^{cd}	11±1.15	18±1.15 ^{cd}	12±1.15	-	9**±0.33
E. coli	10±1.15	8.33±0.33	8.33±0.33	-	8.33±0.33	12±1.15	8.33±0.33	10±1.15	-	11**±0.57
P. aeruginosa	10±1.15	8.33±0.33	10±1.15	8.33±0.33	9±0.57	9±0.33	11±1.15	9±0.33	-	11**±0.57
C. albicans	15±1.15	15±1.15	20±1.15	14±1.15	21±1.15 ^{d}	13±1.15	11±1.15	111.15	-	18.3*±0.33
C. glabrata	10±1.15	11±1.15	12±1.15	11±1.15	15±1.15	13±1.15	10±1.15	11±1.15	-	12*±0.33
C. tropicalis	21±1.15	15±1.15	19±1.15	19±1.15	15±1.15	12±1.15	18±1.15	13±1.15	-	13*±0.57
Trichophyton sp.	15±1.15	14±1.15 ^{d}	13±1.15	11±1.15	15±1.15 ^{cd}	14±1.15 ^{d}	13±1.15 ^{d}	8.33±0.33	-	NT
Epidermophyton sp.	11±1.15	13±1.15 ^{d}	15±1.15 ^{cd}	11±1.15	8±0.33	13±1.15 ^{d}	13±1.15 ^{d}	13±1.15	-	NT

Table 3. The MIC values of *Euphorbia* extracts to microdilution assay

Microorganisms	E.A	E.S	E.F	E.D	E.M	E.C	E.V	E.P
S. aureus	25	50	6.25	12.5	50	100	50	25
B. megaterium	6.25	50	12.5	-	25	100	25	100
P. vulgaris	25	25	3.12	50	50	25	100	100
K. pneumoniae	25	25	12.5	100	100	100	50	50
E. coli	100	100	100	-	100	25	100	50
P. eruginosa	50	100	12.5	25	25	100	100	100
C.albicans	12.5	50	12.5	100	25	25	100	100
C. glabrata	25	100	25	50	50	50	100	12.5
C. tropicalis	6.25	12.5	3.12	6.25	25	100	25	12.5
Trichophyton sp.	6.25	25	12.5	25	3.12	50	12.5	100
Epidermophyton sp.	3.12	3.12	12.5	25	3.12	50	3.12	25

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E.A.: *E. aleppica*, E.S.: *E. szovitsii*, E.F.: *E. falcata*, E.D. :*E. denticulata*, E.M.: *E. macroclada*, E.C.: *E. cheiradenia*, E.V.: *E. virgata*, E.P.: *E. petiolata* MIC: Values given as µl (10 mg/ml)

The extracts of *E. macroclada* have antibacterial and antifungal activity as 8-23 mm zone of inhibition against the tested microorganisms as seen in tables 1-2. MIC value of *E. macroclada* was determined at 31,2-1000µg (table 3). The lowest MIC value was determined in yeast. It seems that the antimicrobial activity of the extract of *E. macroclada* is compatible with those reported by other researchers. This extract demonstrated the highest flavonoid content and all tested showed a very high antioxidant activity (Barla et al., 2007).

As shown in Table 1 and 2, the extract of *E. denticulata* did not show any activity against *E. coli* and *B. megaterium.* The extract was shown however to be more active against *S. aureus* (17 mm) *and C. tropicalis* (20 mm inhibition zone .

As seen in table 1 and table 2, extract and latex of *E. cheiradenia* showed inhibitory effect against the test microorganisms (8-13 and 9-14 mm inhibition zone). MIC value of *E. cheiradenia* was determined at 31,2-1000µg (table 3).

The latex of *E. virgata* showed a maximum activity against *K. pneumoniae* (24 mm) and *C. albicans* (21 mm inhibition zone). MIC value of *E. virgata* was determined as seen in table 3. The effects of many medicinal plant extracts may be used as response to spesific health problems.

As can be seen from tables 1 and 2, the extract of *E. petiolata* showed activity against *E. coli* (11 mm inhibition zone). MIC value of *E. petiolata* was determined at 31,2-1000µg as seen in table 3. Results of these kinds herald an interesting promise of constructing a potentially active antimicrobial additive agent of plant origin (Adwan et al., 2006).

As shown in Table 1, the control disc showed no inhibitory effect against the test microorganisms. The antimicrobial activities of different *Euphorbia* species are changeable according to other research findings (Öksüz et al., 1994; Ferreira et al., 1996; Öksüz et al., 2002; Al-Mughrabi, 2003; Cateni et al., 2003; Madureira et al., 2003; Annapurna et al., 2004; Papp, 2004; Sudhakar et al., 2006; Ogbulie et al., 2007; Barla et al., 2007). These characteristics may arise from the genetic structure of plant species and physical, bioactive-biochemical constituents and chemical differences of plant extract, solvent and test microorganisms. This study indicated that there are differences in the antimicrobial effect of plant species due to phytochemical differences among species. The mentioned researchers claimed that sensitivity of microorganism to chemoterapeutic compounds can change even against different strains. In similar studies, the extract of various plants inhibited the growth of some microorganisms at different rations. Different plants possess different constituents in different concentrations, which account for differential antimicrobial effect as also suggested.

The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potentials as they can serve this purpose with lesser side effects that are often associated with synthetic antimicrobials. Further exploration of plant-derived antimicrobials is needed today. Further research is necessary to determine the identitity of the antibacterial compounds isolated from these plants and also to determine their full spectrum of efficacy (Parekh and Chanda, 2007).

Although studies have been conducted about bioactive compounds and antimicrobial activity in some *Euphorbia* species, no study is found in the literature about the antimicrobial activity of *E. falcata*, *E. aleppica*, *E. macroclada* and *E. virgata*, *E. szovitsii*, *E. denticulata*, *E. petiolata E.cheiradenia*. In the end, we have found that the extracts of *E. falcata* followed by *E. aleppica*, *E. macroclada* and *E. virgata* revealed antimicrobial activities against to most bacteria, yeasts and dermatophyta. And equally the extracts of *E. szovitsii*, *E. denticulata*, *E. cheiradenia*, *E. petiolata* revealed antimicrobial activities against bacteria, yeasts and dermatophyta. However, *E. denticulata* did not reveal any effect on *B. megaterium* and *E. coli.*

The results in the study suggest that those extracts may possess the compounds with antibacterial and antifungal properties that can be used as antimicrobial agents in the development of new drugs for the treatment of infectious disease.

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