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Antimicrobial activity of some plant species used for the medical purpose in Turkey

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ABSTRACT

İn this study, the antimicrobial activities of eight species plants, used for treatment of various diseases, were investigated. The extracts of *Rhus coriaria* L., *Pistacia terebinthus* L. subsp. palestina, *Centaurea virgata* Lav., *Euphorbia macroclada* Boiss., *Ceterach officinarum* DC., *Echinophora tenuifolia* L. subsp. sibthorpianal (Guss) Tutin, *Equisetum romasissimum* Desf., *Umbilicus erectus* DC. have been prepared with methanol and the antimicrobial activities of these extracts have been examined on test microorganisms as follows: Staphylococcus aureus COWAN 1, *Bacillus megaterium* DSM 32, *Klebsiella pneumoniae* FMC 5, *Escherichia coli* ATCC 25922, *Candida albicans* FMC 17, *Candida glabrata* ATCC 66032, *C. tropicalis* ATCC 13803, *Trichophyton* sp. and *Epidermophyton* sp. by disc diffusion methods. The MIC values of plant extracts were determined acording to the microdilutions assays. Results from obtained experimental studies showed that the extracts of *R. coriaria*, *P. terebinthus* sub. sp. *palestina*, *U. erectus* and *C. virgata* have been inhibited the growth of all over the microorganisms used in the test at different ratio. But the extracts of *E. macroclada*, *C. officinarum*, *E. tenuifolia*, *L. sibthorpianal*, *E. romasissimum* had no effect against some bacteria, yeasts and dermatophyta used in study. Also, The MIC values of real extracts have been determined as 20 - 0.3125 mg/ml.

Keywords: Antimicrobial activity, Pathogen microorganisms, Medicinal plants.

INTRODUCTION

A various spesific plants, as sources of medicinal compounds have continued to play a dominant role in the maintenance of human healt since ancient times and medicinal plants continued to be an important therapeutic aid for alleviating the ailments of humankind. Today, there is a renewed interest in traditional medicine and increasing demand for more drugs from plant source. This revival of interest in plant derived drug is mainly due to the current widespread belief that "green medicine" is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects [1]. This situation provided the impetus to the search for new antimicrobial substances from various sources like medicinal plants [2]. Synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Therefore, there is a need to search for new infection fighting strategies to control microbial infections [3-4]. The world health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80 % of the worlds population [5], over 50 % of all modern clinical drugs are of natural product origin [6].

These plants used in this study, are used widely because of different substantial features. For instance; an infusion of the dried fruits of *R. coriaria* are used for antiseptic, sore throat and skin disease. *P. terebintus* subsp. *palestina* is used for diuretic, bracing, restorative and lesion regenerative. *C. virgata* is used for the therapeutic of allergy. *E. macroclada* is used for defuse, wart and skin disease. *C. officinarum* is used for diuretic and skin disease. *E. tennuifolia* subsp. s*ibthorpianal* is used for vulnerary and paregoric and also nervine. *E. romasissimum* is used for treatment of nephritic disease, oedema and eczema. *U. erectus* is used for strangury, diuretic and lesion regenerative [7, 8].

The purpose of this study is to evaluate the potential antimicrobial activities of *R. coriaria*, *P. terebinthus* subsp. *palestina*, *C. virgata*, *E. macroclada*, *C. officinarum*, *E. tenuifolia* subsp. *sibthorpianal*, *E. romasissimum*, *U. erectus* on the some bacteria, yeasts and dermatophyta. Thus, the results suggest that eight species plants possess compounds with antimicrobial properties that might be utilized for developing new drugs.

MATERIALS AND METHODS

Plant Materials and extraction procedure

R. coriaria, *P. terebinthus* subsp. *palestina*, *C. virgata*, *E. macroclada*, *C. officinarum*, *E. tenuifolia* subsp. *sibthorpianal*, *E. romasissimum*, *U. erectus* were collected from were collected from Elazig province in the Eastern Anatolia of Turkey. The taxonomic identification of plant materials was determined using by Flora of Turkey [9]. The collected plant materials were dried and powdered. The dried and powdered plant materials (20g) were extracted in 400 mL methanol (% 98.1) solvent by keeping on a rotary shaker (100 rpm) for 24 h. The extracts were filtered using Whatman filter paper (No1) and then consantrated in vacuom at 37 °C using a Rotary evaporator. They were dissolved in dimethylsulfoxide and stored at 4 °C for further studied. Then, 100 µl extracts were injected into empty antibiotic paper discs having a diameter of 6 mm (Schleicher&Shüll No: 2668, Germany). Discs injected with methanol served as negative controls.

Test Microorganisms

A total 4 bacteria (*S. aureus* COWAN 1, *B. megaterium* DSM 32, *K. pneumoniae* FMC 5, *E. coli* ATCC 25922), 3 yeasts (*C. albicans* FMC 17, *C. glabrata* ATCC 66032, *C. tropicalis* ATCC 13803) and 2 dermatophyta species (*Trichophyton* sp., *Epidermophyton* sp.) were used in the present investigetion. Microorganisms were provided by the Department of Biology, Faculty of Science, Firat University, Microbiology Laboratory, Elazig-Turkey.

Antimicrobial activity

Antimicrobial tests were carried out by disc diffusion method using 100 µL of suspension containing 10^6 cells / mL of bacteria, 10^4 cells / mL yeast and cells / mL dermatophyta fungi as per McFarland standard, inoculated into Mueller Hinton Agar (Difco), Malt Extract Agar (Difco) and Sabouroud Dextrose Agar (Oxoid), respectively. The discs (6 mm diameter) were impregnated with 100 µL of the extracts, placed on the inoculated Mueller Hinton Agar (Difco), Malt Extract Agar (Difco) and Sabouroud Dextrose Agar (Oxoid), respectively. Steril petri dishes (9cm diameter) 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 yeasts and dermatophyta fungi. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms [10]. The MIC values of active extracts were determined acording to the microdilutions assays [11]. Each assay in this experiment was replicated in three times.

RESULTS AND DISCUSSION

The antimicrobial activities acccording to disc diffusion and microdilution assays of plant extracs, negative control group and standart antibiotics are showed in table 1-2. It has been found that the extracts of plants 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 Table 1-2 and also some plants extracts have a higher activity usually as to comparison antibiotics.

The extracts of plant were obtaining from *R. coriaria*, followed by *P. terebinthus* subsp. *palestina*, *C. virgata* and *U. erecetus* have the highest antimicrobial effeciency (showed in table 1). The antimicrobial activity of plants against to test bacteria, yeasts and dermatophyta fungi may be indicative of the presence of broad spectrum antibiotic compounds in the plant.

Result from obtained study indicated that the extract of *R. coriaria* has antimicrobial activity as 20-33 mm zone of inhibition to the tested microorganisms and the MIC values of this extract were determined as 0.3125-2.5 mg/ml against the tested microorganisms (table 1-2). In addition, many reports demonstrated the extract of *R. coriaria* has the antibacterial activity against to *B. subtilis*, *P. aeuroginosa* and *S. aureus* [12, 13]. *R. coriaria*, followed by *P. terebinthus* subsp. *palestina* and *C. virgata* have great potential as antimicrobial compounds against microorganisms and can be used in the treatment of infectious disease caused by resistant microorganisms.

The extract of *P. terebinthus* subsp. *palestina* showed that the maximum activity against to *C. tropicalis* (24 mm), followed by *K. pneumoniae*, *Trichophyton* sp., *B. megaterium*, *S. aureus*, *E. coli*, *C. albicans* and *C. glabrata* (22-10 mm zone of inhibition). The extract showed low inhibitory effect against to *Epidermophyton* sp. (showed in table 1) and also except for *Epidermophyton* sp., the MIC values of *P. terebinthus* subsp. *palestina* extract were determined as 2.5-5 mg/ml as seen in table 2. But, the ethanol extract of *P. terebinthus* subsp. *palestina* did not show any activity against to *E. coli*, *K. pneumoniae*, *P. aeruginosa* ve *S. agalactea* as reported by Keleş et al. [14]. Additionally, the essential oil from the leaves of *P. terebinthus* showed antibacterial activities as reported by Ulukanlı et al. [15]. Its effect on both bacteria and yeast, dermatophyta have not been reported in the literature. The activity of the plant extract against to test microorganisms may be indicator of the presence of the broad spectrum antibiotic compounds in the plant.

It can be seen in the table 1, the extract of *C. virgata* showed inhibitory effect at different ratio against to test bacteria, yeasts and dermatophyta as 15-31 mm zone of inhibition zone and observed to be very high activity in *B. megaterium* (31 mm). Smilarly, the MIC values of *C. virgata* were determined as 0.3125-5 mg/ml against all of the tested microorganisms and also the antibacterial activity of this extract observed to be very high in *B. megaterium* as 0.3125 mg/ml (showed in table 2). The broad spectrum of antimicrobial activity may be attributed to the presence of bioactive metabolities of various chemical types of plant compounds. The antibacterial effects of flavonoids which were identified from *C. virgata* were found to be active against Gram-negative bacteria such as *K. pneumoniae*, *P. vulgaris*, *P. aeruginosa*, and *E.coli*, which are similar with our study [16]. Besides, no any study was found about antibacterial, antifungal and antidermatophyte of *C.* virgata.

The extract of *E. macroclada* did not show any activity against to *C. glabrata* and *Epidermophyton* sp., where as it has antibacterial and antifungal effect on the other tested microorganisms: *S. aureus*, *B. megaterium*, *K. pneumoniae*, *E. coli*, *C. albicans*, *C. tropicalis* and *Trichophyton* sp. (8-20 mm) as seen in table 1. Further more; the MIC values for this extract were determined as 2.5- 10 mg/ml (showed in table 2). It seems that the antimicrobial activity of *E. macroclada* extract is compatible to those reported by other researchers and also the alcohol, ethanol and chloroform extract of *Euphorbia fusiformis* showed that the activity against to *E. coli*, *Staphylococcus* sp., *Proteus* sp. and *Pseudomonas* sp. as reported by Mathabe et al., [17]. While the all extracts of *Spirotachys africana* Sond. showed that the activity against to *S. aureus*, *V. colera*, *S. dysentry*, *S. flexner* ve *S. boydi*

methanol and aceton extract of this plant indicated activity against to *E. coli* ve *S. typhi* as reported earlier [18].

Table 1-2 shows that, the extract of *C. officinarum* has not any activity against to *B. megaterium* and *C. albicans*, while this extract showed the maximum activity against *K. pneumoniae* (29 mm zone of inhibition and 1.25 mg/ml MIC value). The extract of *C. officinarum* has antimicrobial effect as reported by other researchers [19,20].

The extract of *E. tenuifolia* subsp. s*ibthorpianal* has activity on *K. pneumoniae*, *E. coli*, *C. albicans*, *C. glabrata*, *C. tropicalis*, *Trichophyton* sp. and *Epidermophyton* sp. (21 mm, 15 mm, 22 mm, 9 mm, 15 mm, 24 mm and 9 mm zone of inhibition respectivelly) while it has not antimicrobial activity on *S. aureus*, *B. megaterium*, *C. glabrata* (table 1). Furthermore; Table 2 shows that, the MIC values results of *E. tenuifolia* were determined as 2.5- 10 mg/ml. Previous study, has been demonstrated that the essential oil of *E. sibthorpiana* has essential oil antimicrobial effective in the inhibition of different pathogens [21].

The extract of *E. romassisimum* did not any activity against to *S. aureus*, *E. coli*, *C. glabrata* and *Epidermophyton* sp., while observed antibacterial and antifungal activity to the other tested microorganisms in table 1-2 (8-24 mm, 2.5-5 mg/ml MIC value). The extract of *Umbelliferae* family did not indicate any inhibitory effect against to tested microorganisms as reported by İşçan et al., [22]. The effects of many medicinal plants extracts may be used as response to spesific healt problems.

It can be seen in the table 1-2, the extract of *U. erectus* has antibacterial and antifungal activity on the tested microorganisms from high to low respectivelly; *C. tropicalis* (27 mm), *K. pneumoniae* (24 mm), *Epidermophyton* sp (23 mm), *E. coli* (18 mm), *Trichophyton* sp. (17 mm), *S. aureus* (13 mm), *B. megaterium* (11 mm), *C. albicans* (11 mm) and *C. glabrata* (10 mm). The MIC values of *U. erectus* were determined as 1.25-5 mg/ml against the tested microorganisms and also as more active against *C. tropicalis* (1.25 mg/ml) as seen in table 2. These values were supported by previous case reports. The antifungal activity of *U. erectus* was observed to the against to test fungi as reported by Gürhan & Ezer [23]. The extract of *C. martini* showed the activity against to test bacteria as reported earlier [24]. Results of these kind hearald an interesting promise of consructing a potentially active antimicrobial additive agent of plant origin [12].

As shown in Table 1, the control disks injected with 10 µL of methanol showed no inhibitory effect against the test microorganisms.

Table 1: Antimicrobial activity of methanol extracts of some plant species (100 µl) acording to the disc diffusion method

Materials	**R.C**	**P.T**	**C.V**	**E.M**	**C.O**	**E.T**	**E.R**	**U.E**	**Negative Control**	**Standart**
Microorganisms	**Inhibition zone in diameter (mm)**									
S. aureus	30	17	23	13	24	-	-	13	-	13**
B.megaterium	20	20	31	8	-	-	9	11	-	9**
K. pneumoniae	24	22	15	19	29	21	17	24	-	9**
E. coli	27	16	28	11	15	15	-	18	-	13**
C.albicans	33	13	21	15	-	22	20	11	-	18*
C.glabrata	27	10	23	-	13	9	-	10	-	12*
C. tropicalis	31	24	28	18	19	15	24	27	-	11*
Trichophyton sp.	30	21	27	20	23	24	8	18	-	NT
Epidermophyton sp.	27	9	15	-	9	9	-	24	-	NT

R.C.: *R. coriaria*, **PT.:** *P. terebinthus*, **C.V.:** *C. virgata*, **E.M.:** *E. macroclada*, **C.O.:** *C. officinarum*, **E.T.:** *E. tenuifolia*, **E.R.:** *E. romasissimum*, **U.E.:** *U. Erectus*, (-): No inhibiton zone, Control: methanol, *:Nystatin (30 µg/disc), **: Streptomycin sulphate (10 µg/disc), NT: Not tested

The antimicrobial activity of different plant extracts are changeable acording to the other researchers findings [25-30], which may arise from the genetic structure of plant species and physical, bioactive-biochemical constituents and chemical differences of plant extract, solvent and test microorganisms the other research shows clearly at when compareted the other plant species. However, the use of medicinal plants as herbal remedies to prevent and cure several ailments differs from community to community [31].

This study indicated that there are differences in the antimicrobial effect of plant group, due to phyto chemical differences among species. The mentioned researchers claimed that sensitivity of microorganisms to chemoterapeutic compounds can change even against different strain. In similar studies, the extract of various plants inhibited the growth of some microorganisms at different ration. Different plants possess different constituents in different concentration, which account for differantial antimicrobial effect, as also suggested.

The results in the study suggest that those extracts may be possess some of 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 diseases.

Table 2: The MIC values of methanol extracts of some plant species against the microorganisms to microdilution assay (MIC in 20 mg/mL)

Microorganisms	MIC values (mg/ml)							
	R.C	**P.T**	**C.V**	**E.M**	**C.O**	**E.T**	**E.R**	**U.E**
S. aureus	0.625	2.5	2.5	5	2.5	-	-	5
B. megaterium	2.5	2.5	0.3125	-	-	-	-	5
K. pneumoniae	2.5	2.5	5	2.5	1.25	2.5	5	2.5
E. coli	0.625	2.5	1.25	10	5	5	-	2.5
C. albicans	0.3125	5	2.5	5	-	2.5	2.5	5
C.glabrata	0.625	5	1.25	-	5	-	-	5
C. tropicalis	0.3125	2.5	1.25	2.5	2.5	5	2.5	1.25
Trichophyton sp.	0.625	2.5	1.25	2.5	2.5	2.5	-	2.5
Epidermophyton sp.	0.625	-	5	-	-	10	-	2.5

R.C.: *R. coriaria*, **PT.:** *P. terebinthus*, **C.V.:** *C. virgata*, **E.M.:** *E. macroclada*, **C.O.:** *C. officinarum*, **E.T.:** *E. tenuifolia*, **E.R.:** *E. romasissimum*, **U.E.:** *U. Erectus*, (-): No inhibiton zone

CONCLUSION

In the end of studies, we have found that the extracts of *R. coriaria*, *P. terebinthus* subsp. *palestina*, *U. erectus* and *C. virgata* revealed antimicrobial activities against to bacteria, yeasts and dermatophyta, but the extracts of *E. macroclada*, *C. officinarum*, *E. tenuifolia* subsp. *sibthorpianal*, *E. romasisimum* revealed antimicrobial activitiesagainst to bacteria, yeasts and dermatophyta. However; there was no effect of this extracts on the some bacteria, yeasts and dermatophyta. In this study, unlike other studies dermatophytes and fungi were used as test microorganisms.

Conflict of interest

Authors declare that there is no conflict of interest to reveal.

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