Antimicrobial screening of some selected Turkish medicinal plants

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Abstract: For many years, plants are used for treatment of various diseases. In general, plants have rather more therapeutic benefits and fewer adverse effects compared with the synthetic drugs. The purpose of this study was to determine the *in vitro* antimicrobial potentials of fifteen plant species from Anatolia region of Turkey against some selected bacteria and a yeast strain. The extracts from belong to nine families were examined against *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 43300 (MRSA), *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 10231 using disc diffusion and micro dilution methods. According to the obtained results, all plant extracts showed different ranges of antibacterial activity against *S. aureus* ATCC 43300 and *S. aureus* ATCC 25923 strains. Flower and leaves extracts of *R. lutea*, leaves extract of *E. ritro*, flower and leaves extracts of *H. europaeum*, leaves extract of *E. macroclada*, fruit and leaves extracts of *Z. fabago*, flower extract of *C.crupinastrum*, leaves extract of *D. tenuifolia* showed different ranges of antifungal activity against *C. albicans*.

Keywords: Antimicrobial activity, medicinal plants, microdilution, disc diffusion.

INTRODUCTION

Herbs produce a diversified range of bioactive components, which make them as a plentiful source of several kinds of medicines. In pharmaceutical studies plants are usually used for the extensive range of components present in herbs which have been used to remedy chronic infectious ailments. Throughout the world more frequent occurrence of the infectious diseases are the major problem and another problem associated with these diseases is the antibiotic resistance which develops with intense use of antibiotics. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune suppression and allergic reactions. Because of these problems, alternative evaluation of natural ingredients which can be obtained from natural sources such as medicinal herbs can be made. These drugs from plants are less toxic and side effects are limited. They are impressive in the remedy of infectious disorders while simultaneously reducing many of the side effects that are often associated with synthetic antimicrobials (Rios and Recio, 2005; Hemalatha *et al*., 2013; Kapoor *et al*., 2015).

In this study fifteen medicinal herbs species which belong to nine families were screened. Some of these plants are used by local people as traditional medicines. *Echinophora tenuifolia* (Apiaceae) is used as antispasmodic and digestive. *Centaurea virgata* (Asteraceae) has antiallergic effect (Çakılcıoglu and Turkoglu, 2010). *Euphorbia macroclada* (Euphorbiaceae) is good for arthritis (Ozbilgin and Saltan Çitoglu, 2012). Flowers of *Melilotus officinalis* (Fabaceae) display remarkable wound healing activity (Dweck, 2009). *Reseda lutea* (Resedaceae) extracts are good for allergy and eczema. Phytochemical analysis of aerial parts of *R. lutea* has shown the presence of flavonoids, anthocyanins and glucosides (Radulovic *et al*., 2014). *Cichorium intybus* (Asteraceae) is used for digestion and has diuretic properties. Moreover, it was reported to have diuretic and tranquillizer effects (Nandagopal and Kumari, 2007). *Zygophyllum fabago* (Zygophyllaceae) is used as a drug for treatment of rheumatism and gout disorders (Mudassir and Sidney, 2005). In the folk medicine, the extract of the roots of *Eryngium campestre* (Apiaceae) is used as diuretic, appetizer, stimulant and aphrodisiac (Duke *et al*., 2002). Phytochemical studies demonstrate that roots and aerial parts of *E. campestre* contain flavonoids, monoterpene glycosides, coumarin derivatives and saponins (Kartal *et al*., 2005, 2006). The same studies also displayed that the aerial parts of *Diplotaxis tenuifolia* (Brassicaceae) possess significantly high concentration of flavonoids, tannins, glucosinolates, sterols and vitamin C (Martínez-Sánchez *et al*., 2008). The purpose of this study was to evaluate the *in vitro* antimicrobial activities of fifteen plant species from Anatolia region of Turkey.

MATERIALS AND METHODS

Plant materials

Plants samples were harvested in July 2010 from Ankara-Turkey and were authenticated by Prof. Dr. Fatmagül Geven, in Department of Biology, Faculty of Science, Ankara University. The plant specimens with their localities and the necessary field records were written and numerated as voucher specimen number. They were deposited in Herbarium Department at Ankara University.

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Table 1: Scientific names, studied organs and voucher specimen numbers of the studied plants

Family	Species	Part of Plant	Voucher Specimen (AEF) No
Resedaceae	*Reseda lutea*	Flowers- leaves	FG 2010-13
Apiaceae	*Eriyngium campestre*	Flowers- leaves	FG 2010-18
	Echinophora tenuifolia	Leaves	FG 2010-24
Asteraceae	*Carlina oligocephala*	Flowers	FG 2010-17
	Cichorium intybus	Flowers	FG 2010-27
	Echinops ritro	Flower- leaves	FG 2010-19
	Centaurea virgata	Flowers	FG 2010-11
	Crupina crupinastrum	Flowers	FG 2010-22
Fabaceae	*Melilotus alba*	Flowers	FG 2010-26
	Melilotus officinalis	Dlowers	FG 2010-21
Boraginaceae	*Heliotrpoium europaeum*	Flowers, leaves	FG 2010-20
Euphorbiaceae	*Euphorbia macroclada*	Flowers, leaves	FG 2010-25
Zygophyllaceae	*Zygophyllum fabago*	Fruit, leaves	FG 2010-16
Brassicaceae	*Diplotaxis tenuifolia*	Leaves	FG 2010-10
Apocynaceae	*Cynanchum acutum*	Leaves	FG 2010-23

The plant specimens used in this study were listed in table 1.

Extraction of plant samples

Different parts of plant samples such as flowers and leaves were washed with tap water and dried at room temperature. For methanol extraction, 2 gr of dried samples were grounded into a fine powder with liquid nitrogen, then mixed with 20 mL methyl alcohol at room temprature in 160 rpm for 24 hr. The extracts obtained was filtered by use Whatman No. 1 paper. The methanol was removed by using a rotary evaporator at 40°C to obtain dry extracts of each plants. The obtained extracts was then dissolved in DMSO and kept at 4°C until used (Shomali Moghaddam *et al*., 2015).

Antibacterial and antifungal activities of plant specimens

Disc diffusion method

The antibacterial activities of plant extracts were investigated against *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and antifungal activity against *Candida albicans* ATCC 10231 by disc diffusion method (CLSI, 2006). Tested bacteria were incubated at 37°C into the Nutrient Broth (Difco, Difco Laboratories, Detroit, MI, USA) for 24 h and *C. albicans* at 27°C into the Malt Extract Broth (Difco, Difco Laboratories, Detroit, MI, USA) for 48 hr. For inoculation, colonies of each organism were transfered into 0.9% sterile saline solution until the visible turbidity was equal to 0.5 McFarland standard having approximately 10^8 cfu/mL for bacterial and 10^7cfu/mL for *C. albicans* (Silici and Koç, 2006). Nutrient Agar was used for antibacterial and Malt Extract Agar was used for antifungal activity testing. 0.02mL of each plant extracts were exerted on sterile paper discs of 6mm diameter, then discs were left to dry over night at room temperature. The surface of the plates were inoculated with the microbial suspensions. Discs were placed on the agar surface of each petri plate. The diameters of the inhibition zones were determined in millimeters after incubating petri plates at 30°C for 24-48h. DMSO and methanol were used as negative control. Gentamicin (10mg), ampicillin (10mg), streptomycin (10mg) and tetracycline (30mg) were used as positive control.

Minimum inhibitory concentrations

Microbroth dilution method was used to determine the minimum inhibitory concentrations (MIC) (CLSI, 2009; EUCAST, 2013). *B. subtilis* ATCC 6633, *S. aureus* ATCC 43300 (MRSA), *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *C. albicans* ATCC 10231 were used as test microorganisms. Mueller Hinton Broth (Difco, Difco Laboratories, Detroit, MI, USA) was used for testing. Serial two-fold dilutions between 250 to 1.95µg/mL were prepared in medium. A set of wells which contain inoculated broth were used as control. At the end of the incubation period (18-24 h at 35±1°C for bacteria-48h for fungi), the last well with no microbial growth was recorded to represent MIC value (mg/mL).

RESULTS

Results of disc diffusion assay of plant extracts were presented in table 2. According to obtained results from disc diffusion method, flowers and leaves extracts *R. lutea* and flower extract of *C. virgata* showed different ranges of antibacterial potential against all tested bacteria strains. However, flowers and leaves extracts of *R. lutea*, leaves extract of *E. ritro*, flowers and leaves extracts of *H. europaeum*, leaves extract of *E. macroclada*, fruit and leaves extracts of *Z. fabago* showed antifungal activity against *C. albicans*.

Table 2: Disc diffusion results of the tested plant extracts (mm)

Species	Part of plant	*S. aureus* ATCC 25923	*B. subtilis* ATCC 6633	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	*C. albicans* ATCC 10231
Reseda lutea	Flowers	10	20	10	13	9
	Leaves	13	20	10	13	9
Eriyngium campestre	Flowers	10	7	-	-	-
	Leaves	8	-	-	8	-
Echinophora tenuifolia	Leaves	9	-	-	13	-
Carlina oligocephala	Flowers	9	13	9	-	-
Cichorium intybus	Flowers	8	10	11	-	-
Echinops ritro	Flowers	9	7	9	-	-
	Leaves	10	16	9	-	9
Centaurea virgata	Flowers	16	18	18	15	-
Crupina crupinastrum	Flowers	9	-	-	-	-
Melilotus alba	Flowers	10	-	10	7	-
Melilotus officinalis	Flowers	10	-	9	-	-
Heliotrpoium europaeum	Flowers	11	16	11	-	16
	Leaves	12	8	11	-	11
Euphorbia macroclada	Flowers	9	11	10	-	-
	Leaves	13	12	9	-	12
Zygophyllum fabago	Fruit	11	16	16	-	16
	Leaves	12	14	16	7	16
Diplotaxis tenuifolia	Leaves	12	10	13	-	-
Cynanchum acutum	Leaves	13	-	-	10	-
Ampicillin		20	30	20	-	-
Gentamicin		30	18	-	15	-
Streptomicin		20	25	23	15	-
Tetracycline		13	13	35	20	-

Table 3: The MIC results of the plant extracts (µg/ml)

Species	Part of plant	*S. aureus* ATCC 25923	*S. aureus* ATCC 43300	*E. coli* ATCC 25922	*B. subtilis* ATCC 6633	*C. albicans* ATCC 10231
Reseda lutea	Flowers	31.25	62.5	62.5	62.5	125
	Leaves	125	62.5	62.5	62.5	125
Eriyngium campestre	Flowers	125	125	-	125	-
	Leaves	250	62.5	-	-	-
Echinophora tenuifolia	Leaves	125	250	125	-	-
Carlina oligocephala	Flowers	125	250	125	125	-
Cichorium intybus	Flowers	62.5	250	125	125	-
Echinops ritro	Flowers	125	125	125	125	-
	Leaves	62.5	250	125	31.25	250
Centaurea virgata	Flowers	125	250	62.5	62.5	-
Crupina crupinastrum	Flowers	125	250	62.5	-	31.25
Melilotus alba	Flowers	250	250	125	-	-
Melilotus officinalis	Flowers	125	250	62.5	-	-
Heliotrpoium europaeum	Flowers	125	250	125	31.25	31.25
	Leaves	250	250	125	250	125
Euphorbia macroclada	Flowers	125	250	125	125	-
	Leaves	62.5	31.25	250	31.25	125
Zygophyllum fabago	Fruit	125	125	62.5	62.5	62.5
	Leaves	125	125	62.5	125	62.5
Diplotaxis tenuifolia	Leaves	250	250	125	250	250
Cynanchum acutum	Leaves	250	250	250	-	-

MIC results of the tested extracts were presented in table 3. All plant extracts showed different ranges (31.25-250 µg/mL) of antibacterial activity against *S. aureus* ATCC 43300 (MRSA) and *S. aureus* ATCC 25923 strains. At the same time, flowers and leaves extracts of *R. lutea*, leaves extract of *E. ritro*, flowers and leaves extracts of *H. europaeum*, leaves extract of *E. macroclada*, fruit and leaves extracts of *Z. fabago*, flowers extract of *C. crupinastrum*, leaves extract of *D. tenuifolia* showed different ranges (31.25-250µg/mL) of antifungal activity against *C. albicans*.

DISCUSSION

For a long time, medicinal plants have been considered a healthy source of life for all people. The tendency in modern medicine is to use synthetic drugs that eventually were modelled on compounds obtained mainly from plants. Many of these plants were screened for various biological activities including antibacterial, antiviral and antifungal. Therefore, whether the plants are used as a whole, or extracts or their synthetics, their discovery originated from the long term practice of medical herbalism by man (Rates, 2001).

In the previous studies, Fazly Bazzaz and Harirzadeh (2003) were tested methanolic extracts from different parts of the *C. virgata*, *C.intybus*, *R. lutea*, *M. officinalis* and *Z. fabago* against *B. subtilis, S. aureus, E. coli, P. aeruginosa* and *C. albicans*. They offered that flowering stem part extracts of *C. virgata* showed a good effect against *B. subtilis*, *P. aeruginosa* and *E. coli.* Aerial parts extract of *C. intybus* had evident antimicrobial activity against *B. subtilis*, *S. aureus* and *P. aeruginosa*. Aerial parts extract of *R. lutea* showed remarkable activity against *P. aeruginosa, E. coli* and *S. aureus* strains. In this study we observed that the extracts of different parts of *M. officinalis* and *Z. fabago* didn't show any antimicrobial activity against *S. aureus, B. subtilis, E. coli, P. aeruginosa* and *C. albicans*. In our study, *C. virgata* showed antibacterial activity against *B. subtilis*, *S. aureus*, MRSA, *P. aeruginosa, E. coli*. *C. intybus* displayed antibacterial activity against *B. subtilis, E. coli, S. aureus,* MRSA however no antibacterial activity was observed against *P. aeruginosa*. *R. lutea* showed antimicrobial activity against *B. subtilis,* MRSA, *S. aureus, E. coli, P. aeruginosa* and *C. albicans.* Most of our findings comply with the results of this study.

In another study, antibacterial activity of the ethyl acetate, water and ethanol extracts of *C. intybus* were studied. According to obtained results, all the tested extract samples displayed antibacterial activity, the ethyl acetate extract was found the best active one (Petrovic *et al*., 2004). In a study, Nandagopal and Kumari (2007) used hexane, ethyl acetate, petroleum ether and water for extraction of active components from the root of *C. intybus*. They found that hexane and ethyl acetate root extracts had evident inhibition activity against *B. subtilis, E. coli* and *S. aureus*. Liu *et al*., (2013) demonstrated that root extract of *C. intybus* showed antibacterial activity against *B. subtilis*, *S. aureus, E. coli, B. thuringiensis* and *Salmonella typhi*, while *Aspergillus sp.*and *Penicillium sp.*resisted against the extract.

Tekeli *et al*., (2011) screened the methanol extracts of twelve *Centaurea* species for antibacterial activity against *E. coli*, *B. cereus*, *Salmonella enteritidis*, *S. aureus* strains. *C. cariensis* showed an antimicrobial effect against all of tested microorganisms. Also the results exhibited the extracts from eight *Centaurea* species (*C. balsamita, C. calolepis, C. cariensis* subsp. *maculiceps*, *C. cariensis* subsp. *microlepis*, *C. kotschyi* var. *kotschyi, C. solstitialis* subsp. *solstitialis*, *C. urvillei* subsp. *urvillei* and *C. virgata*) contained antibacterial potential against several of the tested microorganisms.

Mudassir and Sidney (2004). reported that methanolic extract of aerial parts of *Z. fabago* had a strong effect against *C. albicans* and good effect against *E. coli* and *B. subtilis*. In our study, *Z. fabago* showed significant antimicrobial activity against *B. subtilis*, MRSA, *S. aureus*, *E. coli* and *C. albicans.*

In a study, the antimicrobial activity of ethanolic extracts from leaves and roots of *E. planum, E. campestre, E. maritimum* were investigated against *B. subtilis* ATCC 6633, *S. aureus* ATCC 25923, *C. albicans* ATCC 10231, *C. glabrata* clinical strain, *Cryptococcus neoformans* clinical strain, molds *Aspergillus niger* ATCC 16404 and dermatophyte *Trichophyton mentagrophytes*. The results have shown that the ethanolic extracts inhibit the growth of *S. aureus* and all tested fungi (Thiem *et al*., 2010). In our study, *E. campestre* showed good antibacterial activity against Gram positive bacteria. Bu it didn't show any antifungal activity against *C. albicans.*

In present study, we investigated the *in vitro* antimicrobial activities of fifteen plant species from Anatolia region of Turkey against *B. subtilis* ATCC 6633, *S. aureus* ATCC 43300 (MRSA), *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922 and *C. albicans* ATCC 10231. According to the results, most of the tested species showed antimicrobial activity in different ranges against various test microorganisms. These medicinal plants are commonly used in traditional medicine to treat some of diseases and as a conclusion it can be associated with their antimicrobial activity.

CONCLUSION

In this research, all plant extracts showed antibacterial activity against *S. aureus* ATCC 43300 and *S. aureus* ATCC 25923 strains. Besides, flower and leaves extracts

of *R. lutea*, leaves extract of *E. ritro*, flower and leaves extracts of *H. europaeum*, leaves extract of *E. macroclada*, fruit and leaves extracts of *Z. fabago*, flower extract of *C. crupinastrum*, leaves extract of *D. tenuifolia* showed antifungal activity against *C. albicans*. Therefore, the methanol extracts from plant samples can be suggested as a new potential source of anti-microbial agents.

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