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Antimicrobial potential in *Artemisia scoparia* and *Echinacea purpurea*

Rabia[1], Khush Bakht Sultan[1], Muhammad Zakir[1], Hina Fazal[2], Haroon Khan[3] Murad Ali Khan*[1].

1.Department of Chemistry, Kohat University of Science and Technology, Kohat, Pakistan
2.Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex, Peshawar, Pakistan.
3.Gandhara College of Pharmacy, Gandhara University, Peshawar

ABSTRACT

In the present work the antimicrobial activity of leaves of *Echinacea purpurea* and whole plant of *Artemisia scoparia* were tested against *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Bacillus atrophoeous* (human pathogenic bacteria) *Ervinia carotovora*, *Agrobaterium tumefactions* (plant pathogenic bacteria) and a fungus, *Candida albicans* by using agar disc diffusion method. Azithromycin, Ciprofloxacin, Clotrimazole were used as standard antibiotics. The extracts of *E. purpurea* showed marked susceptibility against the microbes when tested in two different concentrations (1 mg/6 µl and 2 mg/12 µl). Similarly the whole plant of *Artemisia scoparia* produced significant activity against tested pathogens but was comparatively less pronounced. Based on the results, it is concluded that the extract of both plant could be effective natural healing agents against infections caused by the test organisms.

Key words: *Artemisia scoparia*, *Echinacea purpurea*, antimicrobial

*Corresponding Author Email: drmalikhan@yahoo.com
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INTRODUCTION

Plants contain a large number of bioactive molecules which are the source of different types of medicines[1]. Medicines obtained from plants have made great contributions to human health and well being. They play a key role in the development of new medicines either by: (1) become a precursor for the development of a new drug, or: (2) used as a phytomedicine for curing of various diseases[2]. A large number of traditional drugs derived from the medicinal herbs and plants are used by more than 70% people in advanced countries. In recent years such efficacy is proved that many plants contain antimicrobial potentials which are synthesized during secondary metabolism of plant[3]. Many works have been done which aim at investigating the different antimicrobial properties of plant extracts as the demand for safe medicine due to the misuse of commercially available antibiotics and an increase in Immuno-deficiency[4]. In the present study the crude extract and different solvent soluble fractions of the whole plant of *Artemisia scoparia* and leaves of *Echinacea purpurea* were used.

The genus *Artemisia* L. consists of a variable number of species (from 200 to more than 400, depending on the authors) found throughout northern half of the world[5]. The aerial parts of the plant have been widely used for their hypoglycemic, hypolipidaemic, diuretic and anti-inflammatory activities[6].

The genus *Echinacea* of family Asteracea has nine species common in North America[7]. Stem and root extracts of Echinacea have been used as painkiller, cold remedy and for curing of snakebite by Native Americans for centuries[8]. In European community Echinacea has been used for its antimicrobial and immunostimulatory activities in recent years[9].

MATERIALS AND METHODS

Plant material

Fresh plant of *Artemisia scoparia* was collected from Parachinar Valley, Pakistan and *Echinacea purpurea,* a cultivated herb obtained from PCSIR Peshawar during 2011. The taxonomic identities of these plants were determined by qualified plant taxonomist at department of Botany Kohat University of Science and Technology, Pakistan. These plants were washed 2-3 times with running tap water followed by shade-drying. They were then powdered and used for extraction.

Test microorganisms

Five gram negative bacterial strains such as *Escherichia coli (*ATCC# 25922*), Pseudomonas aeruginosa (*ATCC# 9721*), Salmonella typhi* (clinical isolate), *Erwinia carotovora* (clinical isolate*), Agrobaterium tumefactions* (clinical isolate), one gram positive bacterial strain such as

Bacillus atrophoeous (clinical isolate), and a fungal strain such as *Candida albicans* (clinical isolate) were used.

Preparation of solvent extraction

2 kg of the shade dried, powder of plant materials were soaked separately in methanol for 10 days, extracted three times at room temperature in the same solvent and then filtered. The diluted extracts were concentrated on the vacuumed rotary evaporator under reduced pressure at a temperature of 46˚C to give a residue (extract), which was further suspended in water and partitioned successively with *n*-hexane, chloroform and ethyl acetate to *n*-hexane-soluble, chloroform-soluble and ethyl acetate-soluble fractions, respectively. All the extracts were concentrated using rotary flash evaporator. After complete solvent evaporation, each of these extract was weighed and stored in airtight bottles for further use. 400mg of each extract was dissolved in 2.4 ml of DMSO as a solvent and were used as the test extracts for antimicrobial assay.

Antimicrobial assay

Antimicrobial activities of solvent extracts; methanol, *n*-hexane, chloroform, ethyl acetate and aqueous were determined by Disc-diffusion method on nutrient agar medium[10]. Bacterial cultures were adjusted to 0.5 McFarland turbidity standards and inoculated onto nutrient agar plates of 15cm diameter. Sterile filter paper (wattman-1) discs of 6mm diameter impregnated with plant extracts in concentration of 1mg and 2 mg $disc^{-1}$ in 6µl and 12µl volume were applied on the discs containing 0.5 McFarland and 10^6cfu/ml cultures of bacteria and fungi respectively. Antibiotics (Azithromycin, Ciprofloxacin, Clotrimazole) were applied (6µl $disc^{-1}$) on separate plates as positive control for gram positive bacteria, gram negative bacteria and *Candida albicans* respectively. DMSO (6µl $disc^{-1}$) was also applied as negative control. These plates were then incubated at 36˚C for 24 h and zone of inhibition if any around the discs were measured in mm.

Data analysis

Data are mean of three different experiments.

RESULTS AND DISCUSSION

To search for traditionally used medicinal plants with antimicrobial properties, two important plants viz., *A. scoparia* and *E. purpurea* were screened against different microorganisms. In assay, two concentrations of each extract 1 mg/6 µl and 2 mg/12 µl were used against six strains of bacteria including both human and plant pathogenic strains, and one fungal strain.

The extracts of *A. scoparia* displayed outstanding activity against human infectious bacteria (Table 1). Overall, the extracts demonstrated zone of inhibition in a dose dependent manner. of the fractions, ethyl acetate exhibited maximum zone of inhibition (25 mm) against *E. coli*, crude extract (24 mm) against *P. aeruginosa*, crude extract (30 mm) against *S. typhi*, chloroform fraction (30 mm) against *B. atrophoeous*.

Table 1: Antimicrobial activities crude and different solvent soluble extracts from leaves of *Echinacea purpurea* against various microorganisms.

Microorganism	**Diameter of zone of inhibition (mm)**										
	Methanolic		**n-hexane**		**chloroform**		**Ethyl acetate**		**Aqueous**		**standard**
	C-1	**C-2**	**C-1**	**C-2**	**C-1**	**C-2**	**C-1**	**C-2**	**C-1**	**C-2**	**C-3**
Human pathogens											
E. coli	13	15	22.5	23.5	19.5	25	12	13.5	10	15	28
P. aeruginosa	20.5	24	15	16	18	19.5	11	15	12	17	30
S. typhi	26	30	15.5	17	14	19	10.5	11.5	12.5	15	26
B. atrophoeous	17.5	19	20	25	29	30	12	15.5	12	16	25*
Plant pathogens											
E. carotovora	10	12	12	19	11	15	11	20	--	--	24
A. tumificiens	12	14.5	15	16.5	20	25	13	14	13.5	17	33

Data are mean of three readings. C-1= 1st concentration (1 mg/6 µl), C-2= 2nd concentration (2 mg/12 µl), C-3=3rd concentration (30 (50 µg/6 µl), (--)=Not effective

When tested against plants pathogenic bacteria, the extracts of *A. scoparia* showed reasonable activity. The effect was in a concentration dependent manner. Ethyl acetate fraction showed maximum activity (20 mm) against *E. carotovora* while chloroform fraction (25 mm) against *A. tumefaciens.*

Table 2: Antimicrobial activities crude and different solvent soluble extracts from whole plant of *Artemisia scoparia* against various microorganisms

Microorganism	**Diameter of zone of inhibition (mm)**										
	Methanolic		***n*-hexane**		**Chloroform**		**Ethyl acetate**		**Aqueous**		**Standard**
	C-1	**C-2**	**C-1**	**C-2**	**C-1**	**C-2**	**C-1**	**C-2**	**C-1**	**C-2**	**C-3**
Human pathogens											
E. coli	--	--	14.5	15	--	--	11.5	14.5	8.5	11.5	28
P. aeruginosa	14.5	15	13.5	18.5	--	--	11	19.5	10	12.5	30
S. typhi	--	--	--	--	--	--	11.5	13.5	9	11.5	26
B. atrophoeous	14	15	13.5	18.5	--	--	15	19.5	8	12	25*
Plant pathogens											
E. carotovora	11.5	15	14.5	19.5	--	--	13	17.5	9.5	15	24
A. tumefaciens	12	15	14	15.5	--	--	12	18.5	9.5	11.5	33

Keys: C-1= 1st concentration (1 mg/6 µl), C-2= 2nd concentration (2 mg/12 µl), C-3=3rd concentration (30 µg/6 µl), (*) = 4th concentration (50 µg/6 µl), (--)=Not effective.

Similarly, the results of extracts of leaves of *E. purpurea* are presented in table 2. Hexane fraction was most susceptible against *E. coli* with 15 mm zone of inhibition, while ethyl acetate most active against rest of human pathogenic bacteria in the order of 19.5 mm against *P. aeruginosa*, 13.5 mm against *S. typhi* and 19.5 mm against *B. atrophoeous*. In case of plant pathogenic strains, hexane fraction showed maximum activity (19.5 mm zone of inhibition) against *E. carotovora* and ethyl acetate fraction (18.5 mm) against *A. tumefaciens*.

Both the plant had promising activity against the only fungus tested as shown in table 3. The hexane fraction of *A. scoparia* caused outstanding activity (32 mm zone of inhibition) against *C. albicans* while the remaining was also very profound. The sensitivity of extracts of *E. purpurea* was also prominent and maximum inhibition (17.5 mm) was shown by the crude extract.

Table 3: Antifungal activities of crude extract and different solvent fractions from leaves of *Echinacea purpurea* and whole plant of *Artemisia scoparia* and against *Candida albicans*

	Plants	Diameter of zone of inhibition (mm)										
		Methanolic		*n*-Hexane		Chloroform		Ethyl acetate		Aqueous	Standard	
		C-1	C-2	C-1	C-2	C-1	C-2	C-1	C-2	C-1	C-2	C-3
C. albicans	*Echinacea purpurea*	15.5	19	25	32	26.5	30	12	15	13	16	24*
	Artemisia scoparia	17.5	22	12.5	16	--	--	12	17	9	11.5	24*

The test pathogens have been causative agents in several human infections[11, 12]. However, the current therapeutic options available for the treatment of resulting infections are facing problem of resistant[13-15]. In this scenario, these two plants could be ideal natural healing agents for the effective management of such disorders.

CONCLUSIONS

In conclusions, the extracts of tested plants, *A. scoparia* and *E. purpurea* illustrated marked antimicrobial activity. The study validated folk uses of the plant as antimicrobial. Moreover, further detail studies could led to the isolation of clinical useful antimicrobial.

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