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Short Communication

Antibacterial and antifungal activities of the crude extracts from the stem of *Chenopodium ambrosioides* Linn., an indiginous medicinal plant

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The crude methanolic extract along with the n-hexane, ethyl acetate, dichloromethane, n-butanol and aqueous fractions from the stem of *Chenopodium ambrosioides* Linn was studied against human pathogenic bacterial strains of *Escherichia coli, Klebsiella pneumoniae* (Gram negative bacteria), *Staphylococcus aureus, Bacilus subtilis* and *Staphylococcus epidermidis* (Gram positive bacteria) while against the fungal strains of *Aspergillus niger, Aspergillus parasiticus, Trycophyton horzianum, Rhizopus tolenapur* and *Aspergillus flavus.* The tested bacterial strains were taken from Center for Phytomedicine and Medicinal Organic Chemistry (CPMMOC) University of Peshawar, Pakistan which were previously collected from hospital patients of Khyber Teaching Hospital, University road Peshawar, Pakistan while the antifungal strains were collected from Center for Biotechnology and microbiology (CBM) University of Peshawar, Pakistan which were also in advance collected from hospital patients of Khyber Teaching Hospital, University road Peshawar. The selected strains were tested against crude extract and its fractions. Zones of inhibition were measured by using National Committee for Clinical Lab Standards (NCCLS) method in which for antibacterial activities, streptomycine while meconozole was used as standard drugs for antifungal activities. Dimethyl sulphoxide (DMSO) was used as negative control in both cases. The n-hexane, ethyl acetate, dichloromethane as well as n-butanol fractions showed moderate to significant activities against all bacterial strains especially against *B. subtilus, K. pneumoniae* and *S. epidermidis.* The fractions showed low antifungal activities against these strains.

Key words: Antibacterial, antifungal, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Staphylococcus epidermidis*, *Klebsiella pneumonia*, *Aspergillus niger, Aspergillus Parasiticus, Trycophyton horzianum, Rhizopus tolenapur* , *Aspergillus flavus,* moderate, significant.

INTRODUCTION

Chenopodium ambrosioides Linn. is used traditionally as antihelmintic and antiparasitic agent in America, Asia and Europe (Monzote et al., 2009; Gadano et al., 2006). The roadsides and river banks in Pakistan, at Peshawar, Baluchistan, Dir, Swat, Kohala, Kashmir and Rawalpindi are widely covered by this plant (Stewart, 1972; Nisar et al., 2010). The important medicinal plant family of Chenopodiaceae, consisting of 102 genera and 1400

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Table 1. Criteria for determination of antibacterial activity.

Entry	Diameter (mm)	Activity
1	Below 9	No activity
2	9-12	Non-significant
3	13-15	Low
4	16-18	Good
5	Above 18	Significant

species (Marie, 1965), having an elevated importance for phytochemical investigation and medicinal evaluation, also provide space to this specie and thus increases the curiosity of researcher for antimicrobial potential and phytochemical studies of this plant. Due to a characteristic smell of the plant, the essential oil from this plant, known as 'Baltimore oil', has remained a main focus for the researchers and from the beginning of 19th century, was used for curing the patients with worms (Monzote et al., 2009).

The essential oil obtained from this plant has been reported for antifungal (Jardim et al., 2008; Kumar et al., 2007) and insecticidal (Cloyd and Chiasson, 2007) activities. The main component of the whole plant is ascaridol (45 to 70%). Its content is very variable and depends on the environment and the time of harvesting. Because of its toxicity, the content should not exceed 62 to 65% for pharmaceutical preparations. Ascaridole is the main constituent of the essential oil of this plant along with carvacrole and cryophyllene oxide and the toxic effects of these compounds on mitochondria has been reported (Monzote et al., 2009). Other components isolated from the essential oil of this plant are limonene, transpinocarveol, ascaridole-glycol, aritasone, β-pynene, myrcene, phelandrene, alcanphor and α-terpineol (De Pascual et al., 1980; Sagrero-Nieves and Bartley, 1995).

However, after a thorough literature survey it was found that no medicinal or phytochemical evaluations were carried out using whole plant or parts of the plant. Major studies are carried out on the essential oil of this plant through steam distillation along with the leaves extracts. Due to this very reason the plant stem methanolic extract was used to study antimicrobial potential of this medicinal plant.

MATERIALS AND METHODS

Plant collection

The plant was collected from Peshawar, Pakistan in June and the plant stem was separated from other parts. The well known Botanist of Peshawar University, Dr. Abdur Rashid, identified the plant. After getting shade dried in a closed room, the plant stem was grinded to powder form. The powdered plant stem was extracted with methanol three times by maceration for one week each time and thus methanolic crude extract was obtained. The crude methanolic extract was concentrated at 40°C through vacuum distillation by using rotary evaporator. This methanolic extract was further concentrated till complete dryness in water bath at 25°C temperature. The dried methanolic crude extract was further dissolved in distilled water and was further fractionated using n-hexane, ethyl acetate, dichloromethane, and n-butanol solvent systems leaving at the end the aqueous fractions. All these fractions along with crude methanolic extract were tested for antibacterial and antifungal potential.

Antibacterial bioassay

The antibacterial activity was checked by the agar well diffusion method (Nisar et al., 2010, 2013). The solid nutrient agar was prepared by dissolving 28 g nutrient agar in distilled water and making the solution up to 1 ml. The bacterial culture was kept on stock nutrient cultural agar. A bacterial colony was inoculated in broth and incubated at 37 ± 1°C for 24 h. After a day, soft agar was melted and cooled up to 40°C and was added by 100 μl bacterial culture, shaken and poured in plates containing nutrient agar. After rotating to cause even distribution of culture, the plates were further allowed for solidification. Wells were dug in the medium with the help of sterile metallic cork borer (6 mm diameter) in each plate at least 24 mm apart with their centers. Stock solution of the test samples in the concentration of 22 mg/ml were prepared in the dimethyl sulphoxide (DMSO) and 150 micro liter dilutions were added in their respective wells. The antibacterial activity of samples was compared with standard drug, streptomycin. The concentration of streptomycin was 2 mg/ml in DMSO. The standard drug streptomycin and DMSO were used as positive and negative control. Sample solutions (22 mg/ml in DMSO) were added in their respective wells by sterilized dropping pipettes. Two wells were filled with DMSO and streptomycin as negative and positive control. Plates were incubated at 37 ± 1°C for 24 h. The inoculums with two to eight hours bacterial strains containing approximately 10^4 to 10^6 colony forming units/ml (CFU) was spread on the surface of Mueller-Hinton agar plates with the help of a sterile cotton swab. All the agar surface of each plate was streaked for three times each with swabbed cotton turning the plate 60° between each streaking. All the solutions along with positive and negative control were added in their respective wells. The plates were again incubated for 14 to 19 h or more at 37°C. The antibacterial activity of samples was compared with standard drug, streptomycin showing inhibition in mm. Antibacterial potential of samples was then determined as per criteria mentioned in Table 1. Growth inhibition was calculated with reference to positive control.

Antifungal bioassay

The antifungal activity was determined by the agar well diffusion method (Nisar et al., 2010, 2013). In this method, Sabouraud dextrose agar (SDA) was prepared by mixing and dissolving mycological peptone, 10 g, glucose, 40 g, agar 15 g in approximately 900 ml of deionized water while the pH was maintained at 5.6 with hydrochloric acid and the total solution was made up to 1 L. The mixture was heated for at least 10 min and was then sterilized in autoclave at 121°C and 15 lb/in^2 pressure for a total of 15 min. The culture of organisms was maintained at SDA. Miconazole was used as the standard drug while DMSO was used as negative control. The extracts were dissolved in DMSO (24 mg/ml). Sterile Sabouraud dextrose agar medium (7 ml) was placed in a test tube and inoculated in a sample solution (40 μg/ml) kept in slanting position at room temperature overnight. Each tube was inoculated with a piece of inoculums of 4 mm diameter. The fungal culture was then inoculated on the slant. The samples were incubated for 7 days at 30°C and growth inhibition was observed. The percent growth inhibition was calculated with reference to the negative control by applying the formula:

Table 2. Criteria for determination of antifungal activity.

Entry	Percent inhibition	Activity
1	30-40	Low
2	50-60	Moderate
3	61-70	Good
4	Above 70	Significant

Table 3. Diameter of zone of inhibition (mm) of samples against bacterial strains.

Sample	*Escherichia coli*	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Bacilus subtilis*	*Staphylococcus epidermidis*
Crude methanolic extract	10	12	10	08	12
n-hexane fraction	14	18	13	14	15
Ethyl acetate fraction	13	20	12	14	11
Dichloromethane fraction	00	13	12	00	12
n-butanol fraction	16	12	14	12	10
Aqueous fraction	00	00	00	00	11
DMSO	00	00	00	00	00
Streptomycin	26	28	26	30	30

% Inhibition = (Linear growth of the negative control - Linear growth of sample) × 100

Growth in medium containing crude extract and fractions was determined. The results were evaluated by comparing with Table 2.

RESULTS AND DISCUSSIONS

Antibacterial bioassay

The antibacterial activity was studied against various human pathogens including *Escherichia coli, Klebsiella pneumonia* (Gram negative bacteria), *Staphylococcus aureus, Bacilus subtilis* and *Staphylococcus epidermidis*. The tested bacterial strains were taken from Center for Phytomedicine and Medicinal Organic Chemistry (CPMMOC) University of Peshawar, Pakistan which were previously collected from hospital patients of Khyber Teaching Hospital, University road Peshawar, Pakistan. The diameter of zone of inhibition (mm) samples against the bacteria is given in Table 3.

The crude extract showed non significant activity against bacterial strains. The n-hexane fraction showed low to good activities against all bacterial strains. It showed good activity against *K. pneumoniae* and low against four other bacterial strains. The ethyl acetate fraction showed non significant, low and significant activities. This fraction showed significant activity against *K. pneumoniae,* low against *E. coli* and *B. subtilis* while non significant against *S. aureus* and *S. epidermidis.* Dichloromethane fraction showed no activity against *E. coli* and *B. subtilis,* non significant against *S. aureus* and *S. epidermidis* while low activity against *K. pneumoniae*. n-Butanol fraction showed non significant activities against *K. pneumoniae, B. subtilis* and *S. epidermidis*, low activity against *S. aureus* and good activity against *E. coli* while the aqueous fraction remained non significant against *S. epidermidis* and non active against all other bacterial strains. Streptomycin used as a standard drug showed zone of inhibition (mm) 26, 28, 26, 30 and 30 against *E. coli, K. pneumoniae, S. aureus, B. subtilis* and *S. epidermidis,* respectively.

Antifungal bioassay

Antifungal activities were performed against the five fungal strains including *Aspergillus niger, Aspergillus parasiticus, Trycophyton horzianum, Rhizopus tolenapur* and *Aspergillus flavus* which were collected from Center for Biotechnology and microbiology (CBM) University of Peshawar, Pakistan and were also in advance collected from hospital patients of Khyber Teaching Hospital, University road Peshawar. The % inhibition of samples against fungal strains is shown in the Table 4. The crude as well as all the fractions showed from none to low activities against all fungal strains. The best activities are of ethyl acetate fraction against *A. niger* and *R. tolenapur* with % inhibition of 40 and 35, respectively.

Conclusion

The results confirm the antimicrobial strength of the crude extracts of the stem of this plant which supports the traditional medicinal use of this plant extracts. The results

Table 4. Percentage inhibition of samples against fungal strains.

Sample	*Aspergillus niger*	*Aspergillus parasiticus*	*Trycophyton horzianum*	*Rhizopus tolenapur*	*Aspergillus flavus*
DMSO	100	100	100	100	100
Crude methanolic extract	10	08	12	00	00
n-hexane fraction	25	20	21	18	19
Ethyl acetate fraction	40	09	27	35	00
Dichloromethane fraction	11	18	24	00	00
n-butanol fraction	00	28	22	10	27
Aqueous fraction	24	13	00	18	22

also go in favor of the importance of screening plants as a potential source of bioactive compounds. However further studies are required to investigate this important medicinal plant for isolation of novel compounds.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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