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Full Length Research Paper

Antimicrobial studies of the crude extracts from the roots of Chenopodium ambrosioides Linn.

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Antibacterial and antifungal activities of *Chenopodium ambrosioides* Linn have been studied for their medicinal potential by agar well diffusion method. Five bacterial strains, *Escherichia coli, Klebsiella pneumoniae* (Gram negative bacteria), *Staphylococcus aureus, Bacillus subtilis* and *Staphylococcus epidermidis* (Gram positive bacteria) as well as five fungal strains, *Aspergillus niger, Aspergillus parasiticus, Trycophyton horzianum, Rhizopus tolenapur* and *Aspergillus flavus* , were used to study the antimicrobial potential of the crude methanolic extract along with n-hexane, ethyl acetate, dichloromethane, n-butanol and aqueous fractions from the roots of *Chenopodium ambrosioides* Linn. The tested bacterial strains were taken from Center for Phytomedicine and Medicinal Organic Chemistry (CPMMOC) University of Peshawar, Pakistan; they were previously collected from hospital patients while the antifungal strains were collected from Center for Biotechnology and Microbiology (CBM) University of Peshawar, Pakistan which were also in advance collected from hostel patients of Khyber Teaching Hospital, University road Peshawar. The selected strains were tested against crude extract and its fractions. Zone of inhibition were measured by using National Committee for Clinical Lab Standards (NCCLS) method in which for antibacterial activities, Streptomycine while for antifungal activities, Miconazole were used as standard drugs. Dimethyl sulphoxide (DMSO) was used as negative control in both cases. All fractions remained inactive against *K. pneumonia* while other fractions showed good to non-significant activities against other bacterial strains. The n-hexane fraction showed moderate activity against *A. niger* while all other fractions showed low activity against antifungal strains. The Statistical Package for the Social Sciences (SPSS) calculations by using test statistics "t" shows the 'p' value of lower than α=0.05, while the confidence interval (CI), 95% is also significant.

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

INTRODUCTION

Chenopodium Ambrosioides Linn, being a therapeutic plant, is widely used in the traditional medicinal system in Asia, Europe and America especially as an antihelmintic agent and as a remedy for parasitic disorders (Monzote et al., 2009; Gadano et al., 2002). In Pakistan, the plant is widely distributed in Peshawar, Baluchistan, Dir, Swat, Kohala, Kashmir and Rawalpindi (Nasir et al., 1972; Nisar et al., 2013). This plant is a member of an important plant

family, *Chenopodiaceae,* which have an elevated importance for phytochemical investigation and medicinal evaluation. The family *Chenopodiaceae* consists of 102 genera and 1400 species (Manske et al., 1965). At the beginning of the 19th century, the essential oil from this plant, known as 'Baltimore oil' was used for treating patients with worms (Monzote et al., 2009). The essential oil obtained from this plant has been reported to have antifungal (Kumar et al., 2007; Jardim et al., 2008) and insecticidal activities. Ascaridole was the first compound isolated from this plant in 1895 by a German Pharmacist, named Hüthig. Ascaridole is the main constituent of the essential oil of this plant together with carvacrole and cryophyllene oxide, and the toxic effects of these compounds on mitochondria have 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., 1981).

However, literature does not provide any information regarding the use of the whole plant or different parts of the plant for phytochemical studies or medicinal evaluation. Major studies were carried out on the essential oil of this plant together with the leaves extract. Due to this reason, we studied the antimicrobial potential of the extracts from the roots of this medicinal plant.

MATERIALS AND METHODS

Plant collection

The plant was collected from Peshawar, Pakistan in June and its different parts were separated. The plant was identified by Dr. Abdur Rashid, Department of Botany, University of Peshawar, having assigned voucher number BOT20056 (PUP). The plant roots were shade dried. After shade dryness, the plant roots were grinded and converted to powdered form. The powdered plant roots 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 were extracted with methanol for three times by maceration for four nights each and thus methanolic crude extract was obtained. The at same 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 at the end aqueous fractions. All the five fractions together with crude extract were tested for antibacterial and antifungal activities.

Antibacterial bioassay

The antibacterial activity was checked by the agar well diffusion method (Nisar et al., 2010; Shah, 2014). In this method, one loop full of 24 h old culture containing approximately 10^4-10^6 CFU (colony forming units/ml) was spread on the surface of Mueller-Hinton agar plates. Wells were dug in the medium with the help of sterile metallic cork borer. Stock solution of the test samples in the concentration of 22 mg/ml was prepared in the dimethyl sulphoxide (DMSO) and 150 µl dilutions were added in their respective wells. The antibacterial activity of samples ware compared with standard drug, streptomycin. The concentration of streptomycin was 2 mg/ml. The standard drug streptomycin and DMSO were used as positive and negative control. The amount of growth in each well was determined visually by comparing with the growth in the controlled wells. Antibacterial potential of sample was then determined as per criteria mentioned in Table 1. Percent growth inhibition was calculated with reference to positive control.

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

Antifungal bioassay

The antifungal activity was determined by the Agar Well Diffusion Method (Nisar et al., 2011; Nisar et al., 2013). In this method, Miconazole was used as the standard drug. The samples 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. 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:

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

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

RESULTS AND DISCUSSION

Antibacterial bioassay

Antibacterial activity was studied against various human pathogens including *Escherichia coli, Klebsiella pneumoniae* (Gram negative bacteria), *Staphylococcus aureus, Bacilus subtilis* and *Staphylococcus epidermidis* (Gram positive bacteria) as shown in Figure 1. The tested bacterial strains were taken from the culture of Microbiology Laboratory, PNRL (Pakistan Nuclear

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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

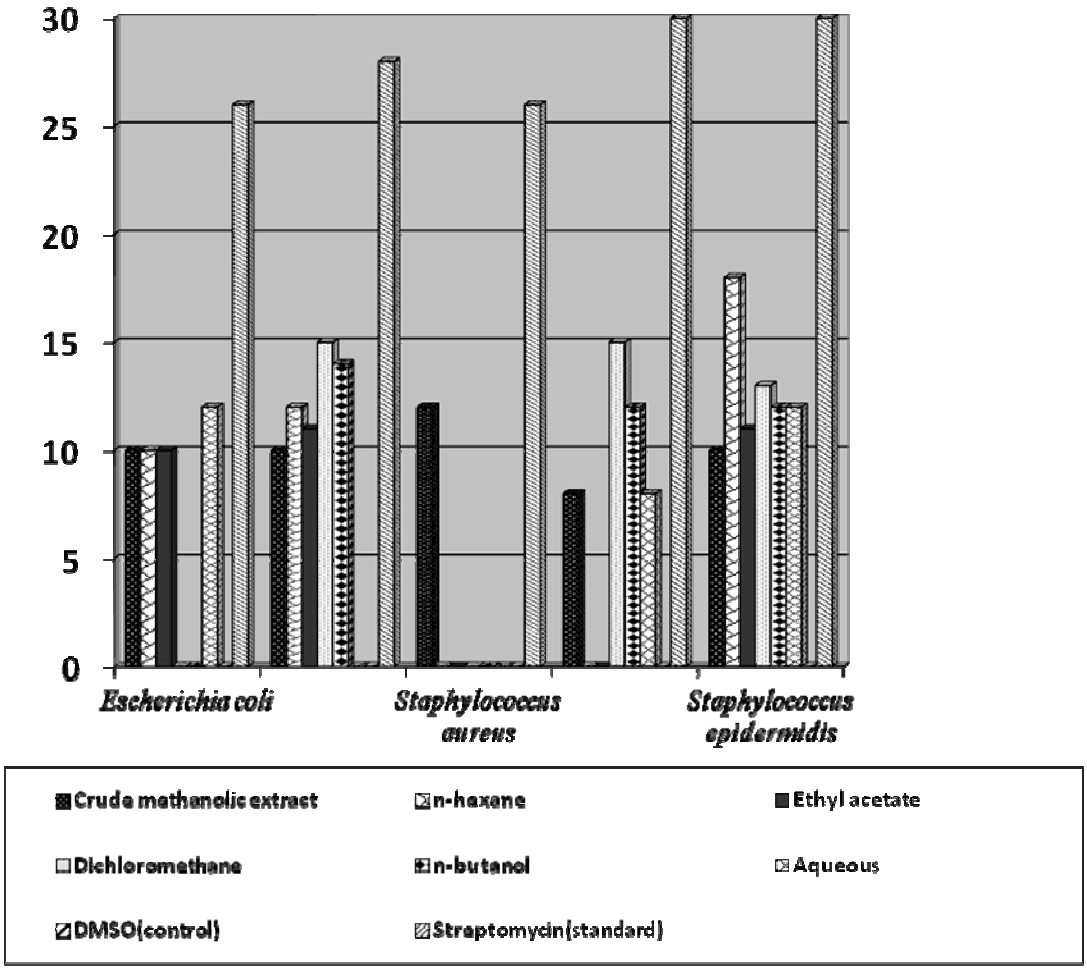

Figure 1. The graphical representation of antibacterial activities (mean only).

Research Laboratories, Peshawar, Pakistan); they were previously isolated from hospital patients. The diameter of zone of inhibition (mm) of samples against the bacteria is given in the Table 3.

The crude extract showed non significant activity against bacterial strains. The n-hexane fraction showed good to low activities against all bacterial strains. It showed good activity against *S. epidermidis* and low to non significant against four other bacterial strains. The ethyl acetate fraction showed low and non significant activities. This fraction showed non significant activity against *K. pneumoniae,* low against *E. coli* and *B. subtilis* while no activity against *S. aureus* and non significant against *S. epidermidis.* Dichloromethane fraction showed no activity against *E. coli* and *S. aureus,* while low activity against *B. subtilis, S. epidermidis* and *K. pneumonia.* The n-butanol fraction showed no activities against *S. aureus and E. coli*, non significant activities against *S. epidermidis and*

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

Sample/fraction	*Escherichia coli*	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Bacilus subtilis*	*Staphylococcus epidermidis*
Crude methanolic extract	10 ± 1.03	10 ± 0.23	12 ± 0.35	08 ± 0.32	10 ± 1.06
n-hexane	10 ± 1.54	12 ± 0.70	00 ± 0.11	00 ± 00	18 ± 0.85
Ethyl acetate	10 ± 0.28	11 ± 0.39	00 ± 00	00 ± 00	11 ± 0.32
Dichloromethane	00 ± 00	15 ± 1.23	00 ± 0.89	15 ± 0.81	13 ± 0.65
n-butanol	00 ± 00	14 ± 0.85	00 ± 00	12 ± 1.32	12 ± 0.62
Aqueous	12 ± 1.50	00 ± 00	00 ± 1.23	08 ± 0.54	12 ± 1.35
DMSO(control)	00	00	00	00	00
Streptomycin(standard)	26 ± 0.11	28 ± 0.13	26 ± 0.56	30 ± 0.61	30 ± 0.98

The data is represented as triplicate with ±SD.

Table 4. One-sample statistics of antibacterial activities.

	N	Mean	Std. deviation	Std. error mean
Sample mean	40	9.38	9.189	1.453

Table 5. One-sample test of antibacterial activities.

Sample mean	Test value = 0					
	t	df	Sig. (2-tailed)	Mean difference	95% Confidence interval of the difference	
					Lower	Upper
Sample mean	6.452	39	.000	9.375	6.44	12.31

B. subtilis while low activities against *K. pneumoniae* and *S. aureus* while the aqueous fraction remained non significant against *S. epidermidis* and *E. coli* and non activities against all other bacterial strains. Streptomycin was used as a standard drug, it showed zone of inhibition (mm) 26, 28, 26, 30 and 30 against *E. coli, K. pneumoniae, S. aureus, B. subtilis* and *S. epidermidis*, respectively.

The good activities of n-hexane fraction against *S. epidermidis* shows the presence of some good antibacterial novel drugs in this fraction which may lead to isolation of many good antibiotics.

SPSS antibacterial interpretation

The SPSS antibacterial calculations are listed below (Tables 4 and 5).

From SPSS output, the total size is 40, mean is 9.38, standard deviation is 9.189 (measure of dispersion), the 'P' value is 0.000 which is less than the level of significance, α=0.05. Therefore on the basis of sufficient evidence, it is concluded that antibacterial activities are significant, whereas the 95% confidence interval (CI) is 6.44, 12.31.

Antifungal bioassay

Antifungal activities were performed against the five fungal strains including *A. niger, Aspergillus parasiticus, Trycophyton horzianum, Rhizopus tolenapur* and *Aspergillus flavus* (Figure 2). The inhibition (%) of samples against fungal strains is shown in the Table 6.

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, which on further chemical investigations will lead to isolation of antifungal chemicals.

SPSS antibacterial interpretation

SPSS interpretation of antifungal activities is given in Tables 7 and 8.

The SPSS output of the antifungal activities of the plant

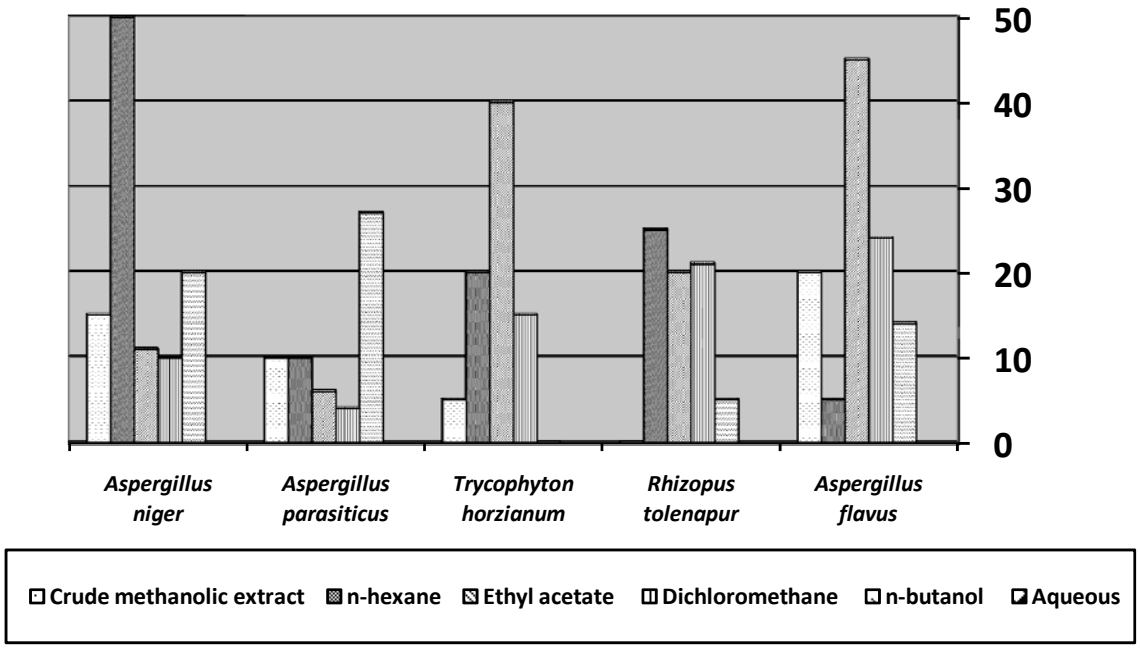

Figure 2. The graphical representation of antifungal activities (mean only).

Table 6. Percent inhibition (mm) of samples against fungal strains.

Sample/fraction	*Aspergillus niger*	*Aspergillus parasiticus*	*Trycophyton horzianum*	*Rhizopus tolenapur*	*Aspergillus flavus*
Crude methanolic extract	15 ± 0.23	10 ± 0.58	05 ± 0.55	00 ± 00	20 ± 0.21
n-hexane	50 ± 0.51	10 ± 0.29	20 ± 0.65	25 ± 0.65	05 ± 0.19
Ethyl acetate	11 ± 0.20	06 ± 0.12	40 ± 1.13	20 ± 0.63	45 ± 0.12
Dichloromethane	10 ± 0.32	04 ± 0.82	15 ± 0.24	21 ± 0.52	24 ± 0.51
n-butanol	20 ± 0.22	27 ± 0.54	00 ± 00	05 ± 0.12	14 ± 0.58
Aqueous	10 ± 0.17	15 ± 0.54	21 ± 0.21	18 ± 1.58	20 ± 0.59
DMSO	00	00	00	00	00
Miconazole	100	100	100	100	100

The data is represented as triplicate with SD±.

Table 7. One-sample statistics of antifungal activities.

N	Mean	Std. deviation	Std. error mean
30	16.87	12.099	2.209

Table 8. One-sample test of antifungal activities.

Data	Test value = 0					
	t	df	Sig. (2-tailed)	Mean difference	95% confidence interval of the difference	
					Lower	Upper
Data	7.635	29	.000	16.867	12.35	21.38

samples of total size is 30, mean is 16.87, standard deviation is 16.87 (measure of dispersion), the 'P' value by test statistics 't' is 0.000 , in this case is lower than the level of significance, α = 0.05. Considering these values, the antifungal activities of the plant are significant. The CI 95% is 12.35 and 21.38.

Conclusion

The above results confirm the antimicrobial strength of the crude extracts of the roots of this plant, which is also supported by SPSS calculations, and supporting the traditional medicinal use of this plant extracts. The results also show 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.

REFERENCES

de Pascual JT, Bellido I, Gonzalez M, Muriel M, Hernandez J(1981). Aromatic compounds from Artemisia campestris subsp. Glutinosa. Phytochemistry 20(10):2417-2420.

Gadano A, Gurni A, López P, Ferraro G, Carballo M (2002). *In vitro* genotoxic evaluation of the medicinal plant Chenopodium ambrosioides L. J. Ethnopharmacol. 81(1):11-16.

Jardim CM, Jham GN, Dhingra OD, Freire MM (2008). Composition and antifungal activity of the essential oil of the Brazilian Chenopodium ambrosioides L. J. Chemical Ecol. 34(9):1213-1218.

Kumar R, Mishra AK, Dubey N, Tripathi Y (2007). Evaluation of Chenopodium ambrosioides oil as a potential source of antifungal, antiaflatoxigenic and antioxidant activity. Int. J. Food Microbial. 115 (2):159-164.

Manske RH, Holmes HL, Rodrigo R (1965). The alkaloids: chemistry and physiology. Academic Press. Vol. 18.

Monzote L, Stamberg W, Staniek K, Gille L (2009). Toxic effects of carvacrol, caryophyllene oxide, and ascaridole from essential oil of Chenopodium ambrosioides on mitochondria. Toxicol. Appl. Pharmacol. 240(3):337-347.

Nasir E, Ali S, Stewart RR (1972). Flora of West Pakistan: an annotated catalogue of the vascular plants of West Pakistan and Kashmir. Fakhri.

Nisar M, Kaleem WA, Qayum M, Hussain A, Zia-Ul-Haq M, Ali I, Choudhary M (2011). Biological screening of Zizyphus oxyphylla edgew stem. Pak. J. Bot. 43(1):311-317.

Nisar M, Qayum M, Shah MR, Kaleem W, Ali I, Zia-Ul-Haq M (2010). Biological screening of Impatiens bicolor royle. Pak. J. Bot. 42(1): 523-526.

Nisar M, Shah H, Khan I, Rehman F, Khan SM, Marwat KS, Niazi RZ, Ullah A (2013). Antimicrobial potential and phytochemical investigation of Fixed oil of plant *Chenopodium ambrosioides* Linn. Asian J. Chem. 25(2):1069-1072.

Shah H (2014). Antibacterial and antifungal activities of the crude extracts from the stem of *Chenopodium ambrosioides* Linn, an indiginous medicinal plant. Afr. J. Pharm. Pharmacol. 8(8):231-234.