WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 7, 1030-1036. Research Article ISSN 2277– 7105

ANTIMICROBIAL ACTIVITY OF *CHENOPODIUM ALBUM* L. FROM NORTHERN INDIA

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Article Received on
01 May 2016,
Revised on 21 May 2016,
Accepted on 12 June 2016
DOI: 10.20959/wjpr20167-6510

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ABSTRACT

Extract of organic solvent (Aqueous, methanol and acetone) of medicinal plant *Chenopodium album* L. were evaluated for their antimicrobial activity against Gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram negative bacteria (*Escheichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*). These were carried out by taking organic extract of leaves of plants at concentration of 5mg/ml and their activities were recorded by estimating zones of inhibition as produced by Disc Diffusion method on nutrient broth. The acetone and aqueous extracts of leaves of various tetraploid plants also revealed no toxic or antibacterial activity. The methanol extract of leaves collected from the tetraploid plants of the species showed antibacterial activity against only two bacterial strains *Klebsiella pneumoneae* and *Pseudomonas aeruginosa*. For these Zone of Inhibition was estimated as 9.66-14.00 for *Klebsiella pneumoneae* and 10.66-13.66 for *Pseudomonas aeruginosa.*

KEYWORDS: Antimicrobial, Northern India, *Chenopodium album*, Disc diffusion method.

INTRODUCTION

Plants are part and parcel of human society to combat diseases, from the dawn of civilization.[1] The history of medicinal plants is connected with history of civilization. A number of reports have been published showing development of microbial resistance in numerous pathogens to the available antibiotics. This has led authors to investigate the antimicrobial activity of medicinal plants.[2-4] The antimicrobial activities of plant extracts may reside in a variety of different components including aldehyde and phenolic compounds. Naturally occurring combinations of these compounds can be synergistic and often result in crude extracts have antimicrobial activity than purified individual constituents. In this work I

selected *Chenopodium album* (Family-Chenopodiaceae, common name- lamb's quarters, goosefoot) from Northern India. This study was conducted to address their antimicrobial activities against some pathogenic bacteria causing acute diarrhea, food poisoning and other diseases when extracted their leaves in different organic solvents.

MATERIALS AND METHODS

(i) Bacteria and growth conditions

Five bacterial species were employed as test organisms which include *Staphylococcus aureus* (MTCC ACC NO 96), *Bacillus subtilis* (MTCC ACC NO 2757), *Escheichia coli* (MTCC ACC NO 3261),*Klebsiella pneumonia* (MTCC ACC NO 3384), *Pseudomonas aeruginosa* (MTCC ACC NO 1035). Inocula were prepared by adding an overnight culture of organism in nutrient broth gm/L (Peptone digest of animal tissue: 5.00,Beef extract: 1.50, Yeast extract: 1.50, NaCl : 5.00, pH (at 25˚C): 7.4±0.2). cells were allowed to grow and then suspension was diluted.

(ii) Test plants and their extraction

Chenopodium album plants were collected from ten districts of Punjab. Plants were washed under running tap water. Mature healthy leaves were separated from the plants, cut into small pieces and were air dried for several days. Leaves were then ground into coarse powder.

The 5gm of dried and ground plant powder was extracted in 100ml distilled water for 6 hrs at 60˚C , in air tight , clean flat bottomed container with occasional stirring and shaking. Extract was then filtered with Whatman filter paper (No.1) and then centrifuged at 6000rpm for 25min.The filtrate was concentrated using a rotary evaporator at low temperature (40-45˚C) and pressure. Dried extract was dissolved in DMSO (1:1w/v) and stored at 4˚C in airtight bottles. Similarly methanol and acetone extracts were prepared by applying same procedure. Then it was tested for antimicrobial activity.

(iii)Determination of antibacterial activity

The antimicrobial activity of aqueous, acetone and methanol extracts of leaves was studied by Disc diffusion method[5] using *B.subtilis, E.coli, P.aeruginosa, S.aureus, K.pneumonia*. All tests were performed in triplicate.

Petri plates and nutrient Agar[6] were autoclaved at 121˚C. 30ml of growth media was poured to the petriplates. It was allowed to solidify for 15min. Then the 0.5ml of inoculation

(inoculums' size 10^4cells/ml) was spread on the agar plates. Sterile paper discs measured 6mm diameter that absorbed 20µl of test sample were placed on the solidified plates under aseptic conditions. Each disc must be pressed down to ensure complete contact with agar surface. The inoculated plates were made to stand for 1hr and then plates were inverted and placed in an incubator at 37˚±1˚C for 24hr.[7-8] After 24hr of incubation, each plate was examined. The diameter of zones of complete inhibition was measured, including the diameter of the disc. Zones were measured the nearest whole millimeter using a scale, which was held on the back of inverted petriplate.

Chloramphenicol (20mg/ml) was used as positive control and DMSO as negative control as standard references. The lowest concentration that killed 100% of inoculum bacteria was recorded as minimum bactericidal concentrations (MBC).

(iv) Calculation

Observations were made in triplicate and mean values were taken for further use. Activity index was calculated by comparing the zone of inhibition by plant extract with that of chloramphenicol.

$$\text{Activity index} = \frac{\text{Inhibition zone of test sample (extract)}}{\text{Inhibition zone of standard antibiotic}}$$

RESULTS

The results of antimicrobial determinations for all organic extracts of leaf of *Chenopodium album* L. against five bacterial species were investigated in disc diffusion method. Fig. 1 illustrates representative plate showing antibacterial activity of extracts of *Chenopodium album* that produced zones of inhibition against *Klebsiella pneumoneae* and *Pseudomonas aeruginosa*.

Table 1: Zone of inhibition recorded (mm in diameter) for varius extracts of *Chenopodium album* L.

Sr. No.	Species	Chloramphenicol	DMSO	Methanol	Aqueous	Acetone
BARNALA						
1.	*Bacillus subtilis*	30	NIL	NIL	NIL	NIL
2.	*Escherichia coli*	33	NIL	NIL	NIL	NIL
3.	*Klebsiella pneumoniae*	36	NIL	9.66	NIL	NIL
4.	*Pseudomona saeruginosa*	32	NIL	NIL	NIL	NIL
5.	*Staphylococcus aureus*	32	NIL	NIL	NIL	NIL
BATHINDA						
1.	*Bacillus subtilis*	30	00	NIL	NIL	NIL
2.	*Escherichia coli*	33	00	NIL	NIL	NIL
3.	*Klebsiella pneumoniae*	36	00	11.33	NIL	NIL
4.	*Pseudomonas aeruginosa*	32	00	10.66	NIL	NIL
5.	*Staphylococcus aureus*	32	00	NIL	NIL	NIL
JALANDHAR						
1.	*Bacillus subtilis*	30	00	NIL	NIL	NIL
2.	*Escherichia coli*	33	00	NIL	NIL	NIL
3.	*Klebsiella pneumoniae*	36	00	10.66	NIL	NIL
4.	*Pseudomonas aeruginosa*	32	00	11.66	NIL	NIL
5.	*Staphylococcus aureus*	32	00	NIL	NIL	NIL
SANGRUR						
1.	*Bacillus subtilis*	30	00	NIL	NIL	NIL
2.	*Escherichia coli*	33	00	NIL	NIL	NIL
3.	*Klebsiella pneumoniae*	36	00	14.00	NIL	NIL
4.	*Pseudomonas aeruginosa*	32	00	13.66	NIL	NIL
5.	*Staphylococcus aureus*	32	00	NIL	NIL	NIL

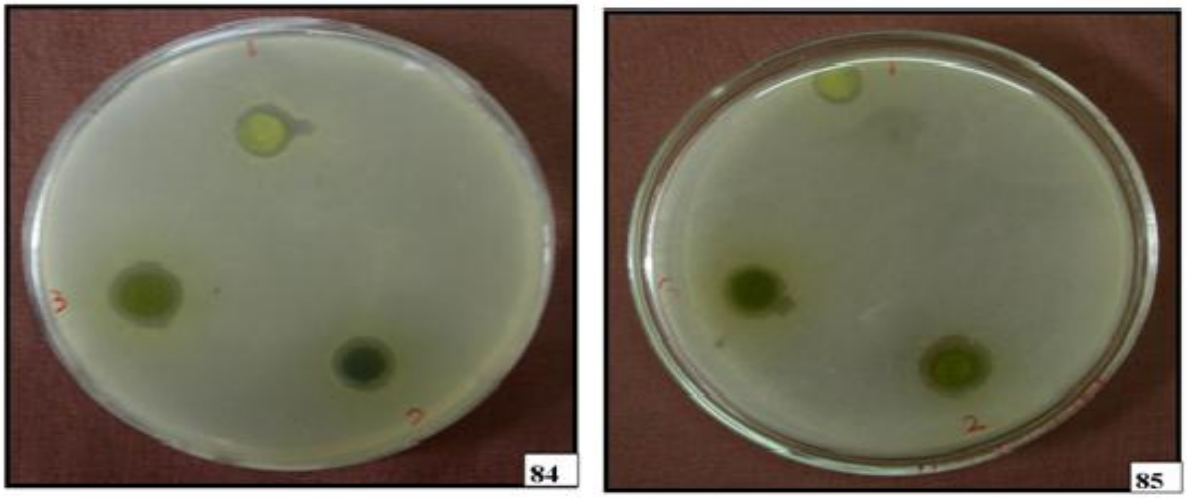

Figure 1 Antibacterial activity. Effect of plant extract on *Klebsiella pneumoneae* and *Pseudomonas aeruginosa* .

Table 2: Activity index for different extracts of *Chenopodium album* L. collected from different populations.

Sr. No.	Species	Aqueous	Acetone	Methanol
BARNALA				
1.	*Bacillus subtilis*	NIL	NIL	NIL
2.	*Escherichia coli*	NIL	NIL	NIL
3.	*Klebsiella pneumoniae*	NIL	NIL	**0 .26**
4.	*Pseudomonas aeruginosa*	NIL	NIL	NIL
5.	*Staphylococcus aureus*	NIL	NIL	NIL
BATHINDA				
1.	*Bacillus subtilis*	NIL	NIL	NIL
2.	*Escherichia coli*	NIL	NIL	NIL
3.	*Klebsiella pneumonia*	NIL	NIL	**0.31**
4.	*Pseudomonas aeruginosa*	NIL	NIL	**0.33**
5.	*Staphylococcus aureus*	NIL	NIL	NIL
JALANDHAR				
1.	*Bacillus subtilis*	NIL	NIL	NIL
2.	*Escherichia coli*	NIL	NIL	NIL
3.	*Klebsiella pneumonia*	NIL	NIL	**0.29**
4.	*Pseudomonas aeruginosa*	NIL	NIL	**0.36**
5.	*Staphylococcus aureus*	NIL	NIL	NIL
SANGRUR				
1.	*Bacillus subtilis*	NIL	NIL	NIL
2.	*Escherichia coli*	NIL	NIL	NIL
3.	*Klebsiella pneumonia*	NIL	NIL	**0.38**
4.	*Pseudomonas aeruginosa*	NIL	NIL	**0.42**
5.	*Staphylococcus aureus*	NIL	NIL	NIL

The acetone and aqueous extracts of leaves of various tetraploid plants also revealed no toxic or antibacterial activity. The methanol extract of leaves collected from the tetraploid plants of the species showed antibacterial activity against only two bacterial strains *Klebsiella pneumoneae* and *Pseudomonas aeruginosa*. For these Zone of Inhibition was estimated as 9.66-14.00 for *Klebsiella pneumoneae* and 10.66-13.66 for *Pseudomonas aeruginosa*. Zone of inhibition and activity index for each extract are presented in table 1 and 2.

DISCUSSION

Plant and plant products have been used extensively throughout history to treat medical problems. The traditional medical methods play a vital role to cover basic health needs in developing countries in the last decades. In this connection plants continue to be a rich source of therapeutic agents. These defense chemicals are mostly secondary metabolites like alkaloids, steroids, tannins, flavanoids, phenols, etc. Plant originated antimicrobial drugs are of interest because many human and animal pathogens show multidrug resistance and certain

antibiotics have undesirable side effects.[9] All the differences in antimicrobial activity of extracts might be due to chemical composition of plants, the species of microorganisms used and method of extraction. Different solvents have been reported to have capacity to extract different phytoconstituents depending upon their solubility and polarity of the solvent.[10]

Present study revealed that only methanol extract of leaves tetraploid of *C.album* L. was effective against *Klebsiella pneumonia* and *Pseudomonas aeruginosa* . The methanol extract of leaves obtained from hexaploid plants of *C.album* was not effective against any organism. Similar results were obtained by Nayak.[11] The incidence of antimicrobial activity in the tetraploid and not in hexaploids might be due to genetic differences in the two cytotypes. However, ethanol extract of leaves was not tested in this study and therefore demands to be investigated that might yield any antibacterial effect.

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