In Vitro Study of Antibacterial Activity of *Chenopodium Album* against Certain Bacterial Pathogens

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Abstract

The multidrug resistance of pathogenic bacteria is very big challenge in drug discovery at present time in allover world. This research work was designed to examine the in vitro antimicrobial activities of leaves aqueous, ethanolic amd chloroform extracts of *Chenopodium album* L. *Chenopodium album* leaves were collected from Mahendergarh district, Haryana and Jhunjhunu district, Rajasthan. The effects of aqueous, ethanolic amd chloroform extracts were tested against 6 bacterial strains by using disc,well-diffusion method. Results showed that leaves aqueous, ethanolic amd chloroform extracts of *C.album* has activity against the selected bacterial strains.

Introduction

Human beings have used plants for the treatment of diverse ailments for thousands of years [1, 2]. According to the World Health Organization, most populations still rely on traditional medicines for their psychological and physical health requirements [3], since they cannot afford the products of Western pharmaceutical industries[4], together with their side effects and lack of healthcare facilities [5]. Rural areas of many developing countries still rely on traditional medicine for their primary health care needs and have found a place in day-to-day life.

Chenopodium album commonly named as Chilva. It is herbaceous, 0.3-3.5m high, erect or ascending, mealy or green or reddish, inodorous. Bakshi *and* his friends (1999) reported that the plants and their parts are useful in curing anorexia, cough, dysentry, diarrhoea, piles and kills small worms [6]. K. P. Singh *et al*. (2011) evaluated the antibacterial activities of *C. album* L. against human pathogenic bacteria[7].

Thus the principal objective of present study was to screen antibacterial activity of *Chenopodium album* against gram negative and grma positive bacteria.

MATERIAL AND METHODS

Bacterial strains

The bacteria used in this study are Escherichia *coli, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus vulgaris, Klebsiella pneumoniae, Shigella dysentriae* and all were collected from Singhania University hospital.

Preparation of crude extracts

The leaves of *Chenopodium album* (Chilva) were collected locally from Mahendergarh district, Haryana and Jhunjhunu district, Rajasthan, India. The shade dried leaves were grinded into fine powder and the total mass was subjected to extraction by a hot percolation method with aqueous solution, Methanol and Chloroform in Soxhlet apparatus (fig.2).

Antibacterial activity

Antibacterial activity by disc diffusion method and agar well diffusion method[8] .The microorganism was activated by inoculating a loopful of the strain in the nutrient broth (30ml) and incubated on a rotary shaker. Then 0.2 ml of inoculum (inoculum size was 10^8 cells/ml as per McFarland standard) was inoculated into the molten Muller Hinton agar media and after proper homogenization it was poured into the Petri plate. For agar disc diffusion method, the test compound (0.1 ml) at four different concentrations i.e. 15 µg/ml, 20 µg/ml 25 µg/ml 30 µg/ml, was introduced on the disc (0.7 cm) and then allowed to dry. Then the disc was impregnated on the seeded agar plate. For agar well diffusion method (Klastrup, 1975), a well was made in the seeded plates with the help of a cup-borer. The test compound at four different concentrations i.e. 15, 20, 25, 30µg/ml, was introduced into the well and the plates were incubated at 37ºC for 24 h. Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain, controls were maintained in which pure solvents were used instead of the extract. The control zones were subtracted from the test zones and the resulting zone

diameter is shown in the graph. The experiment was done three times and the mean values are presented.

Results and Discussion

The antibacterial activity of **aqueous extract** of *Chenopodium album* was tested against six different isolated pathogens mentioned above at different concentrations. The various concentrations used were 15, 20, 25, 30µg/ml. The mean value of the diameter of inhibition zone (in mm) was observed. It was observed that the aqueous extract of *Chenopodium album* is most effective against *Staphylococcus aureus* with a zone of inhibition 17.6 ± 0.2mm at a minimum concentration of 15µg/ml, which is slightly less than the used standard antibiotic Chloramphenicol (17.8 ± 0.3mm) at same concentration (fig.3).After *Staphylococcus aureus,* this extract was found to be effective against *Shigella dysentriae* (13.4 ± 0.2mm) (Table-1).

The antibacterial activity of **ethanol extract** of *Chenopodium album* was tested against six different isolated pathogens mentioned above at different concentrations. The various concentrations used were 15, 20, 25, 30µg/ml. The mean value of the diameter of inhibition zone (in mm) was observed. It was observed that the ethanol extract of *Chenopodium album* is most effective against *Staphylococcus aureus* with a zone of inhibition 17.3 ± 0.3mm at a minimum concentration of 15µg/ml, which is slightly less than the used standard antibiotic Chloramphenicol (17.8 ± 0.3mm) at same concentration(fig.4). After *Staphylococcus aureus*, this extract was found to be effective against *Shigella dysentriae* (13.0 ± 0.3mm) (Table-1).

The antibacterial activity of **chloroform extract** of *Chenopodium album* was tested against six different isolated pathogens mentioned above at different concentrations. The various concentrations used were 15, 20, 25, 30µg/ml. The mean value of the diameter of inhibition zone (in mm) was observed. It was observed that the chloroform extract of *Chenopodium album* is most effective against *Staphylococcus aureus* with a zone of inhibition 17.1 ± 0.2mm at a minimum concentration of 15µg/ml, which is less

than the used standard antibiotic Chloramphenicol (17.8 ± 0.3mm) at same concentration(fig.5). After *Staphylococcus aureus*, this extract was found to be effective against *Shigella dysentriae* (12.8 ± 0.3mm) followed by *Pseudomonas aeruginosa* (12.4 ±0.2mm) (Table-1).

Plant extracts have been used for many thousands of years in food preservation and pharmaceuticals. It is necessary to survey those plants theoretically which have been used in traditional medicine to modify the quality of healthcare. The plant extracts are potential sources of modern antimicrobial compounds especially against bacterial pathogens [9].In vitro studies in this work showed that the aqueous and ethanol extracts of *Chenopodium album* leaves have significant role against growth of *Staphylococcus aureus and Shigella dysentriae* but Chloroform extract of *Chenopodium album* leaves very effective against *Staphylococcus aureus, Shigella dysentriae* and *Pseudomonas aeruginosa.* We found dissimilar results from study of Amjad,L. & Alizad,Z.(2012)[9] where it is reported that flowers and leaves methanolic and ethanolic extracts of *Chenopodium album* L. do not have any activity against the selected bacterial strains like *Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus* and *Staphylococcus aureus* whereas previous study in China supported our study and reported that leaves 95% ethanolic extract of *Chenopodium album* have inhibition effect on the *Escherichia coli* and *Staphylococcus aureus*[10].

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Fig. 1:- ***Chenopodium album***

Fig.2:- Soxhlet apparatus

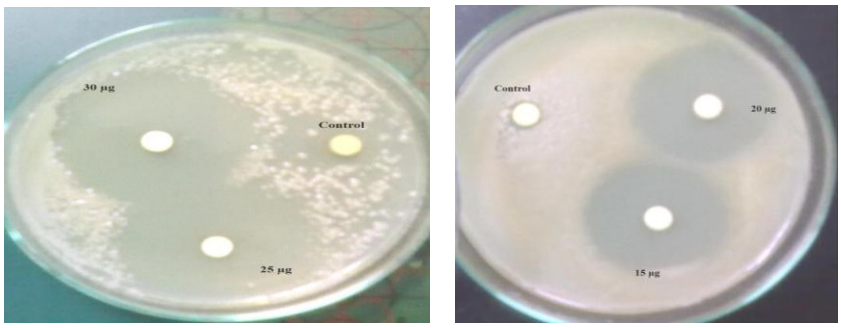

Fig.-3:- Effect of aqueous extract of leaves of *Chenopodium album* against *Staphylococcus aureus*

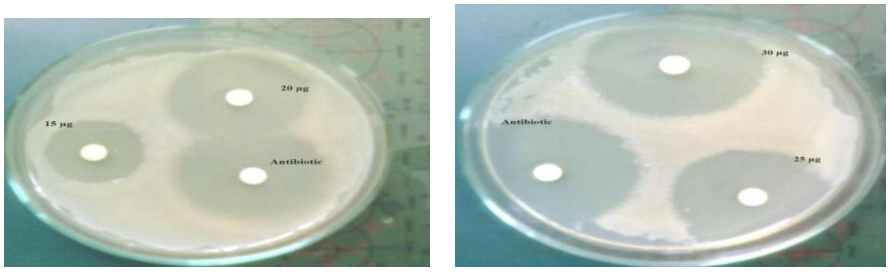

Fig.-4:- Effect of ethanol extract of leaves of *Chenopodium album* against *Staphylococcus aureus*

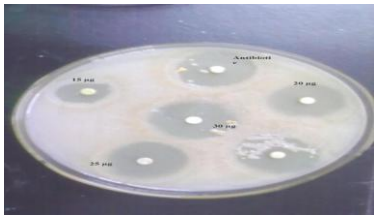

Fig.5:-Effect of Chloroform extract of leaves of *Chenopodium album* against *Staphylococcus aureus*

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Table 1: Antimicrobial activity of aqueous, Ethanol, Chloroform extracts of *Chenopodium album* against certain pathogens (Mean ± SEM)

Pathoge	*Chenopodium album*												Chloramphenicol
	15 µg/ml			20 µg/ml			25 µg/ml			30 µg/ml			15 µg/ml
	Aqueous	Ethanol	Chloroform	Aqueous	Ethanol	Chloroform	Aqueous	Ethanol	Chloroform	Aqueous	Ethanol	Chloroform	
Escherichia coli	12.2 ±0.2	12.0 ±0.1	11.9 ±0.4	14.7 ±0.2	14.3 ±0.1	14.0 ±0.1	18.1 ±0.3	17.5 ±0.2	17.3 ±0.2	22.6 ±0.4	22.2 ±0.3	22.0 ±0.2	12.7 ±0.2
Pseudomonas aeruginosa	13.1 ±0.1	12.6 ±0.2	12.4 ±0.2	14.5 ±0.1	14.2 ±0.2	14.1 ±0.3	18.6 ±0.2	18.1 ±0.2	17.9 ±0.3	22.6 ±0.2	22.1 ±0.3	21.8 ±0.4	13.2 ±0.3
Staphylococcus aureus	17.6 ±0.2	17.3 ±0.3	17.1 ±0.2	20.2 ±0.2	19.8±0.2	19.6 ±0.2	23.7±0.1	23.3±0.1	23.1 ±0.4	25.9*±0.3	25.3±0.3	25.0 ±0.4	17.8±0.3
Proteus vulgaris	12.2 ±0.2	11.6 ±0.2	11.3 ±0.2	15.0 ±0.4	14.7 ±0.3	14.5 ±0.3	18.4 ±0.2	18.0 ±0.3	17.9 ±0.3	23.1 ±0.3	22.7 ±0.4	22.5 ±0.5	13.9 ±0.4
Klebsiella pneumoniae	13.2 ±0.3	12.6 ±0.1	12.4 ±0.3	15.3 ±0.2	15.0 ±0.3	14.7 ±0.1	18.6 ±0.2	18.3 ±0.2	18.1 ±0.2	23.2 ±0.2	22.7 ±0.2	22.6 ±0.3	13.8 ±0.3
Shigella dysentriae	13.4±0.2	13.0±0.3	12.8 ± 0.3	16.6±0.3	16.2±0.2	16.0±0.1	18.9±0.1	18.6±0.1	18.4±0.2	22.6±0.2	22.2±0.1	22.0±0.2	13.9 ±0.4