

Study of Antibacterial Activity of *Chenopodium album* Leaves Extract

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ABSTRACT

This study describes the antibacterial activities of three different solvent extracts of leaves of *Chenopodium album*. Methanol, acetone and chloroform extracts of *C. album* were prepared. The antibacterial activity was assessed using well plate method and were examined for the size of zone of inhibition. Different extracts were investigated against the test organisms namely *Lactobacillus*, *Bacillus subtilis* and *Escherichia coli*. The maximum activity was observed at 100% concentration of different extracts of leaves. The maximum zone of inhibition for 100% concentration were observed as *E. coli* (19 mm) and *Lactobacillus* (19 mm) in diameter respectively. *C. album* did not show any antibacterial activity against *B. subtilis*. Antibacterial activity was compared with standard Amoxicillin and it was found to be 23 mm diameter for *Lactobacillus* and 25 mm for both *E. coli* and *B. subtilis* in terms of zone of inhibition.

Keywords: Antibacterial activity, *C. album*, *Lactobacillus*, *B. subtilis* and *E. coli*.

INTRODUCTION

Human beings have been influenced in various ways by plants and their products. Ever the dawn of civilization, man has used plants for shelter, food and some plants are used to cure innumerable ailments which disturb his physical beings¹. Medicinal plants are used by the people in the ancient times also without the knowledge of their active ingredients. In modern times, the active ingredients and curative actions of medicinal plants were investigated through the use of various scientific methods^{2,3,4}.

It has been pointed out that more than 80% of the world's population depends on plants to meet their primary health care needs (WHO 2005). Among the estimated 2,50,000-5,00,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller. Over 2000 plants and 8000 different species are found to have medicinal purpose in different forms⁵. Even after the discovery of large number of antibiotics severity of bacterial infections has risen up, because of escalating levels of antibiotic resistant strains and increase in population with decreased immunity⁶. As per the WHO report on antimicrobial resistance in 2014, overcoming the antibiotic resistance is the major issue for next millennium. Screening of plants for antimicrobial activities has gained much importance as WHO is encouraging and promoting the development and utilization of medicinal plant resources for traditional system of medicine⁷.

Methanol extract of stem of *Salicornia herbacea*, annual herb of *Chenopodiaceae* family has been found to exhibit antioxidant, antibacterial effect against several pathogenic

microorganisms. It has also been reported as potent inhibitor of cytochrome P450 activity against three CYP isozymes⁸. The methanolic extracts from roots of *Caltha palustris* var. *alba* has been evaluated for anthelmintic, antimicrobial, antioxidant and cytotoxic activity⁹. The nutraceutical potential of *Chenopodium quinoa* leaves was assessed through analysis of their phenolic content, effect of phenolic compounds on cancer cell properties and estimation of their antioxidant activity. The observations indicated the chemo preventive and anticarcinogenic effect of phenolic compounds¹⁰. Essential oil from *Chenopodium ambrosioides* var. *ambrosioides* in combination with conventional antimicrobials showed synergistic antimicrobial action against tested strains of bacteria. It also showed considerable antioxidant activities¹¹. *Kaempferia pandurata* and *Sennaalata* has also been evaluated for antibacterial activity against antibiotic resistant strains of bacteria based on the minimum inhibitory concentration using Mueller-Hinton broth in a Micro dilution method¹².

C. album represents a rich source of anthelmintic agents, plant is used medicinally in different countries as a source of many potent and powerful drugs. Ethanol leaf extract of *C. album* has been found to show antibacterial activity on all Gram (+) and Gram (-) microorganisms and strongest activity was recorded on *B. subtilis* with 13 mm zone of inhibition at 1000 µg/ml concentration¹³. *C. album* is a fast growing "weedy annual plant" with about 150 species occurring almost everywhere in the world¹⁴.

C. album is cultivated and consumed as a food crop in Northern India and is also given to the animals as their

Table 1: Phytochemical Testing of *C. album*.

Test Parameters	Methanol extract	Acetone extract	Chloroform extract
Terpenoids	+	+	+
Reducing sugars	+	-	-
Saponins	+	+	+
Tannins	+	-	+
Flavonoids	-	-	-
Phylobatanin	+	-	+
Alkaloids	+	-	-

+ represent the presence of constituent

- represent the absence of constituent

necessary feed. In the English texts it may be called by its Hindi name Bathua¹⁵. The leaves generally contain about 3.9% proteins, 0.76% fats, 8.93% carbohydrates and 3% ash, calcium, phosphorus, vitamin A and some others¹⁶.

Iron and fibre are also usually present in too small quantities to do any harm¹⁷. The literature survey reveals that antibacterial activities of *C. album* were observed with streptomycin, ampicillin, chloramphenicol, ciprofloxacin, gentamycin, tetracycline, ofloxacin^{18,19,20,21,13}. During present investigations, it was planned to study the antibacterial activities against *Lactobacillus*, *B. subtilis* and *E.coli*. with amoxicillin as standard as methanol, acetone and chloroform extracts of the *C. album*. Specific plant compounds such as vitamins, minerals and saponins have been proposed to have direct antimicrobial activity.

MATERIALS AND METHOD

Collection of the plant material

The leaves of *C. album* were collected from the fields of village Jai Singh Wala (Moga district of Punjab, India). The leaves were dried in the absence of direct sunlight. After drying, the leaves were crushed in a mixer grinder.

Preparation of the plant extracts

50 gms of the powder of the plant material was taken in a Soxhlet's apparatus on a heating mantle with 150-200 ml of various solvents (methanol, chloroform and acetone) according to the boiling points of different solvents for 48 hours. After the completion of extraction, distillation of solvent-plant mixture was done. The semi-solid extract was collected and was placed in desiccator for drying and preserved for further studies.

Phytochemical Testing

The extracts of *C. album* were analyzed for the presence of terpenoids, reducing sugars, saponins, tannins, flavonoids, alkaloids, Phylobatanin and some others by standard methods.^{22,23}

Study of the Antibacterial Activity

Collection of microorganisms

Lactobacillus, *E.coli* and *B. subtilis* species were obtained from the Department of Biotechnology of M. M. Modi College Patiala (District in Punjab, India) and the pure cultures of bacteria were maintained on nutrient agar stands for their vegetative growth. The cultures were maintained in a refrigerator for use and were regularly checked for contamination, periodic transfers were also made aseptically.

Preparation of the dilutions of extract

10 mg of crude plant extract was taken in a test tube and dissolved in 10 ml of Dimethyl Sulphoxide (DMSO) which acts as an inert solvent. From this 100%, 75%, 50% and 25% dilutions were made by adding DMSO. Same procedure was followed for different solvent extracts and these dilutions were preserved in a refrigerator for future use.

Preparation of culture media for the study

Composition of nutrient agar medium.

Agar powder - 7 gm

Distilled water - 250 ml

Nutrient broth - 1.3 gm

The above ingredients were carefully weighed and dissolved in measured amount of distilled water in properly sterilized containers.

Preparations of bacterial inoculums

The agar was poured in the petriplates and was left for 25-30 minutes in laminar flow to solidify the agar on plates. Then 2-3 drops of micro-organisms were poured on the plates and were spread equally with the help of a spreader. These agar plates were left for 1.5- 2 hours for proper growth of the micro-organisms at room temperature. The growth obtained on the agar plates were transferred to test the antibacterial activity of the herbal extract.

Well plate method

The antibacterial activity was assessed using well plate method and was examined for the size of zone of inhibition. In this method, wells or holes were prepared on the agar plates and filled with one or two drops of extract dilutions. The same procedure was repeated for the four different concentrations of solvent extracts i.e. 25%, 50%, 75% and 100% respectively. In the centre of the agar plate, control solvent was also added. The zones of inhibition were measured on the underside of the plate using Vernier calipers in mm.

RESULTS AND DISCUSSION

According to the literature survey and preliminary analysis, solvent extracts of the leaves of *C. album* have shown the presence of terpenoids, saponins, tannins, Phylobatanin and to less extent of reducing sugars and alkaloids (Table 1).

The antibacterial activity in terms of zone of inhibition is shown in Table 2. Different extracts of leaves of *C. album* (chloroform, acetone and methanol) were studied for zones of inhibition against the test organisms *Lactobacillus*, *B. subtilis* and *E.coli*. The Disc Diffusion Method was used for the study. The maximum activity was observed at 100% concentration of different extracts of leaves. The zones of inhibition for the test organism *Lactobacillus* were maximum as 13 mm, 19 mm and 12 mm and for *E.coli* were 19 mm, 16 mm and 16 mm in diameter for 100% concentration of chloroform, acetone and methanol extracts respectively. The zone of inhibition for different solvent extracts for *B. subtilis* were observed as nil.

The zones of inhibition of solvent control (chloroform, acetone and methanol) were nil and of standard (Amoxicillin), the zone of inhibition for *Lactobacillus* was

Table 2: Antibacterial activity of *C. album*.

Concentration of Extract	Test Organism	Zone of inhibition		
		Chloroform Extract	Acetone Extract	Methanol Extract
25%	<i>Lactobacillus</i>	8 mm	9 mm	6 mm
	<i>E.coli.</i>	10 mm	11 mm	9 mm
	<i>B. subtilis</i>	Nil	Nil	Nil
50%	<i>Lactobacillus</i>	11mm	13 mm	10 mm
	<i>E.coli.</i>	13mm	15 mm	11 mm
	<i>B. subtilis</i>	Nil	Nil	Nil
75%	<i>Lactobacillus</i>	12mm	14 mm	13 mm
	<i>E.coli.</i>	14mm	18 mm	16 mm
	<i>B. subtilis</i>	Nil	Nil	Nil
100%	<i>Lactobacillus</i>	13mm	19 mm	12 mm
	<i>E.coli.</i>	19mm	16 mm	16 mm
	<i>B. subtilis</i>	Nil	Nil	Nil
Control	<i>Lactobacillus</i>	Nil	Nil	Nil
	<i>E.coli.</i>	Nil	Nil	Nil
	<i>B. subtilis</i>	Nil	Nil	Nil
Standard (Amoxicillin)	<i>E.coli.</i>	25mm	25 mm	25 mm
	<i>B. subtilis</i>	25mm	25 mm	25 mm
	<i>Lactobacillus</i>	23mm	23mm	23mm

23 mm and for both *B. Subtilis* and *E.coli* were 25 mm in diameter.

CONCLUSION

Methanol, chloroform and acetone extracts of leaves of *C. album* have been found to be quite effective against bacteria namely *Lactobacillus* and *E.coli*. *C. album* did not show any antibacterial activity against *B. subtilis*. The maximum zone of inhibition for 100% concentration were observed as *E. coli* (19 mm) and *Lactobacillus* (19 mm) in diameter respectively. Comparison of antibacterial activity against standard Amoxicillin has been found as 23 mm for *Lactobacillus* and 25 mm for both *E.coli* and *B. subtilis* in terms of zone of inhibition.

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