



## Antibacterial activity of important medicinal plants on human pathogenic bacteria

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Article published on June 23, 2015

**Key words:** Antibacterial Activity, Medicinal Plants, Agar Well Diffusion Method.

### Abstract

The resistance of pathogenic bacterial strains to antibiotics is the major burning issue around the world. To minimize the strain resistance to antibiotic local medicinal plant were selected. The leaves of medicinal plants (*Ajuga bracteosa* Benth, *Calotropis procera* and *Zizyphus sativa* Geartn) were collected and antibacterial activity was examined through agar well diffusion method. The crude extracts were obtained by using methanol and n-hexane as the extraction solvent. Three concentrations (100 mg/ml, 50 mg/ml, and 25 mg/ml) were used to check the antibacterial activity of plant extracts. Each plant sample was tested against two Gram-positive (*Bacillus cereus*, *Bacillus subtilis*) and three Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*) bacteria. The methanol extracts showed better activity against all the tested bacterial strains, except *Zizyphus sativa* Geartn showed inactive against *Pseudomonas aeruginosa* and *Salmonella typhi*. The n-hexane extracts showed moderate activity against all the bacterial strains, whereas the n-hexane extract of *Zizyphus sativa* Geartn were inactive against *Salmonella typhi* and *Bacillus cereus*. It is concluded that methanol extract showed good antibacterial activity as compared to n-hexane extract and further study is needed to check the activity of the tested plants against the other bacterial strains.

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## Introduction

Pakistan has a rich flora, including medicinal plants having good potential for therapeutic uses (Walter *et al.*, 2011). Medicinal plants can provide a wealth of antimicrobial agents, which can be used as an alternate source of antibiotics (Malik *et al.*, 2011; Walter *et al.*, 2011). The plants antibacterial agent is the notable source against bacterial infections, but in the last three decades, rapid increases in antibiotic resistant bacteria threaten human health, especially the immune suppressed patients (Nasrullah *et al.*, 2012).

In the last few decades, most of pathogenic bacteria developed resistance to many antibiotics and this is a major threat to human health. Medicinal plants are sources of diverse molecules, many of which display antimicrobial and antioxidant properties which protect human body from both pathogens and cellular oxidation. Thus, it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential (Bajpai *et al.*, 2005; Wojdylo *et al.*, 2007). A large number of antimicrobial agents derived from traditional medicinal plants are available for treating various diseases caused by micro-organisms (Jain, 1994). Although hundreds of plant species have been tested for antimicrobial properties, the vast majority have not been adequately evaluated (Balandrin, 1985).

*Ajuga bracteosa* Benth belongs to the family Lamiaceae (Ali and Nasir, 1990). Various species of this genus are used as analgesics, to dissolve blood clots, and to relieve fever, diarrhea, eye problems, and diseases of the bladder. The leaves, stems, and seeds are officinal; the juice of fresh leaves is plastered on the bites of insects, burns, cuts, and tumors, and applied as hot fomentations against carcinomas. The seeds are used to relieve stomach ache and diarrhea (Perry and Metzger, 1980).

*Calotropis procera* R.Br. belongs to the family Asclepiadaceae (Klein and Johnson, 1987). *C. procera* R. Br. had been widely used in traditional medicine due to its pharmacological active compounds found in all plant parts; barks, roots, leaves and especially its

milky latex which exudates from damaged and broken leaves and stems. *C. procera* R.Br. has been used for treatment of a number of diseases, such as ulcers, tumors, leprosy, piles and diseases of the spleen, liver and abdomen (Kumar and Arya, 2006).

The genus *Zizyphus* belongs to the family Rhamnaceae (Cherry, 1985). The fruits are applied on cuts and ulcers and also used to treat pulmonary ailments and fevers and to promote the healing of fresh wounds, for dysentery. The leaves are applied locally to sores, and the roots are used to cure and prevent skin diseases (Adzu *et al.*, 2001).

The aim of the present study, to evaluate the potential of antibacterial activity of methanol and n-hexane crude extracts of *Ajuga bracteosa* Benth, *Calotropis procera* R.Br and *Zizyphus sativa* Gaertn leaves against clinically isolated pathogenic bacterial strains.

## Materials and methods

### *Plant materials collection and identification*

Disease free fresh leaves of *Ajuga bracteosa* (Benth.), *Calotropis procera* (R.Br.) and *Zizyphus sativa* (Gaertn.) were collected from the Wari Valley, District Dir upper Khyber Pukhtunkhawa Pakistan. The plant specimens were identified with the help of taxonomists, previous available literature (Ali and Qaiser, 2004) and flora of Pakistan (Stewart, 1967).

### *Ethno botanical study*

A questionnaire was designed to interview the local inhabitants during field plant collection trips and ethnobotanical information were gathered. Generally, the aged, people including men and women, who were familiar with traditional uses of local plants, were quizzed for the extraction of folk knowledge.

### *Extraction of plant materials*

Three hundred grams of each powdered air-dried plant material was soaked in 70% methanol and other three hundred grams of each powdered air-dried plant material was soaked in 90% n-hexane. They were regularly shaken for maximum extraction at 80rpm for 7 days. After 7 days, the extract was filtered

using Whatman filter paper (No. 1). The extracts solutions were evaporated to dryness under reduced pressure at a temperature of 45 °C using a vacuum pump with the rotary evaporator. The paste obtained after rotary evaporation contained some water contents which was further dried in water bath at 60 °C for one hour. The thick pastes obtained are known as the crude extract. The extracts were kept in sterile bottles at 4°C until use.

#### *Test microorganisms and microbial culture*

Five bacterial strains were used in this study in which two were Gram positive: *Bacillus cereus* and *Bacillus subtilis* and three Gram negative: *E. coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*. All the bacterial strains were clinical isolates obtained from the Department of Pharmacy, University of Malakand, Chakdara, Khyber Pakhtunkhwa, Pakistan. All the bacterial strains were cultivated in nutrient agar medium and incubated at 37°C for 24hrs.

#### *Growth media*

Nutrient agar media are best growth media for bacteria. The Media was composed of Beef extract 3.0g, Agar 15.0g and Peptone 5.0g. One liter media were prepared by dissolving 40g of nutrient agar in 700ml of distilled water. After complete dissolution, the final volume of the media was raised to 1000ml by adding more distilled water. The media were boiled using a hot plate. The PH was adjusted to 7.0 at 25°C, using 0.1M NaOH and 0.1M HCl. The needed media and all glassware were sterilized through autoclaving at 15psi at 121 for 20 minutes.

#### *Antibacterial activity*

The about 20ml nutrient agar was plated in Petri dishes and allowed to solidify for 30 minutes<sup>3</sup>. Wells of 6mm in diameter and about 2 cm apart were punctured in the culture media using sterile cork borers to make three to five uniform wells in each Petri dish. A drop of molten nutrient agar was used to seal the base of each well. The wells were filled with 50µl (use micropipette) of the extract concentration of 25mg/ml, 50mg/ml and 100mg/ml and allow diffusing for 45 minutes. In the central well 50µl of

90% n-hexane and 70% methanol was used as negative control for respective extracts. The antibacterial activities were determined after 24 hours at 37°C incubation in the incubator. The diameter of zone of inhibition produced by the extract was measured and compared with the standard. Each sample was used in triplicate for the determination of antibacterial activity. The work was carried out in laminar flow.

#### *Statistical Analysis*

All the tests were conducted in triplicates. The data were statistically analyzed and expressed as mean ± S.D.

### **Results and discussion**

The present study revealed that the tested medicinal plants; *Calotropis procera* R.Br. *Zizyphus sativa* Geartn and *Ajuga bracteosa* Benth leaves extracts possess potent antibacterial activity against five bacterial strains, including two Gram-positive bacterial strains (*Bacillus subtilis*, *Bacillus cereus*) and three Gram-negative bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*) Table (1). The preliminary ethnobotany study showed that these medicinal plants are extensively used in local pharmacies for different treatments (Table 2).

Methanol extracts of *Calotropis procera* R.Br. Showed activity at all tested concentration against all bacterial strains except *Pseudomonas aeruginosa* and *Salmonella type* in 50, 100 mg/ml. The highest activity was observed against *Escherichia coli* 21 mm and lower against *Salmonella type* of 10 mm. While n-hexane extract of *Calotropis procera* R.Br. Showed activity at all higher concentrations against all bacterial strains. The maximum activity was noted against *E. coli* 19 mm and least activity against *Salmonella typhi* 10.3 mm. The finding also correlated with Kareem *et al.*, 2008 that the ethanolic extracts of *Calotropis procera* R.Br. Latex give the widest zone of inhibition 14.1mm against *E. coli* and 10.66mm against *Pseudomonas aeruginosa* using agar well diffusion method.

The result showed that *Ajuga bracteosa* Benth. methanolic extracts showed activity against *B. subtilis*, *Bacillus cereus*, *Salmonella typhi* and *E. coli* at all concentrations except *Pseudomonas aeruginosa* at high concentrations. The highest activity was recorded against *B. subtilis* 20.6 mm while lowest against *E. coli* 10.6 mm. The n-hexane extract showed activity against *Bacillus cereus* and *E. coli* at all concentrations. Although *B. subtilis*, *Pseudomonas aeruginosa* and *Salmonella typhi* showed activity at

high concentrations. The maximum activity was observed against *E. coli* 16.3 mm and least against *Bacillus cereus* and *Pseudomonas aeruginosa* 10 mm. By using the broth dilution method Cyrus *et al.* (2008) reported that the methanolic extract of *Ajuga bracteosa* Benth showed activity against *Bacillus cereus* and *E. coli*, while no activity against *Pseudomonas aeruginosa*. The findings suggest that the Well diffusion method shows high activity as compared to the broth dilution method.

**Table 1.** Antibacterial activity of selected Medicinal Plants.

Plants	Solvents	Concentrations (mg/ml)	Diameter of zone of inhibition (mm)				
			<i>B.s</i>	<i>B. c</i>	<i>E.c</i>	<i>P. a</i>	<i>S.t</i>
<i>Calotropis procera</i> R.Br.	Methanol	25	10.2±0.1	12.5±0.1	10.0±0.5	-	-
		50	13.3±0.2	17.0±0.1	14.0±0.3	12.0±0.1	10.0±0.1
		100	15.0±0.2	19.0±0.3	21.0±0.2	12.5±0.3	12.1±0.3
	n-hexane	25	-	10.6±0.5	10.6±0.1	-	-
		50	10.7±0.5	14.2±0.8	14.6±0.5	11.6±0.5	-
		100	14.4±0.5	18.3±0.5	19.0±0.3	13.3±0.3	10.3±0.1
<i>Ajuga bracteosa</i> Benth.	Methanol	25	11.6±0.5	11.0±0.1	10.6±0.5	-	11.3±0.5
		50	15.3±0.4	13.2±0.5	12.5±0.2	15.0±0.1	14.0±0.5
		100	20.6±0.4	17.3±0.1	17.5±0.2	19.3±0.5	14.6±0.4
	n-hexane	25	-	10.0±0.1	11.0±0.1	-	-
		50	12.3±0.3	12.6±0.4	13.0±0.1	10.0±0.3	11.3±0.5
		100	15.0±0.1	14.0±0.2	16.3±0.5	14.6±0.5	12.7±0.5
<i>Zizyphus sativa</i> Geartn.	Methanol	25	11.8±0.5	10.1±0.5	10.3±0.1	-	-
		50	15.3±0.5	12.5±0.2	15.0±0.1	-	-
		100	18.0±0.2	16.4±0.4	18.0±0.2	-	-
	n-hexane	25	10.5±0.1	-	-	-	-
		50	13.0±0.1	-	11.3±0.5	10.0±0.1	-
		100	14.8±0.1	-	15.0±0.1	13.7±0.3	-
Ciprofloxacin	100µg/ml	31.1±0.1	26.9±0.4	30.2±0.1	25.1±0.1	28.4±0.2	

The significant activity of Methanol extract was against *E. coli* followed by *B. subtilis* and *B. cereus* at all used concentrations. Although zero activity against *Pseudomonas aeruginosa* and *Salmonella typhi*. The highest activity was noted against *E. coli* and *B. subtilis* 18 mm and lowest against *B. cereus* 10.1 mm. The n-hexane extract of *Zizyphus sativa* Geartn leaves showed activity against *B. subtilis* at all concentrations, while showing activity against

*Pseudomonas aeruginosa* and *E. coli* at high concentrations (50, 100 mg/ml). While no activity was observed against *B. cereus* and *Salmonella typhi*. Our findings correlate with Mahesh and Satish, (2008) stated that the Methanol leaf extracts of *Zizyphus Mauritania* showed significant antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, (15,18 mm) zones of inhibition.

**Table 2.** Ethno botanical study of selected medicinal plants.

S/No	Botanical Name	Family	Local name	Habit	Altitude/m	Part used	Medicinal Uses
1.	<i>Calotropis procera</i> R.Br.	Asclepiadaceae	Spalmay	Herb	1300-2000	Leaves & Stem	Grinded leaves are used in different formulation as pain killer and for Scabies and leaves milk are highly used for Asthma treatments.
2.	<i>Ajuga bracteosa</i> Benth.	Lamiaceae	Buty	Herb	2000-4000	Leaves	As a stimulant and Leaves decoction is used for jaundice, pain killer and blood purifier.
3.	<i>Zizyphus sativa</i> Geartn.	Rhamnaceae	Markhany	Tree	1500-2300	Leaves & Fruit	Fruits are edible having market value. Leaves decoction is used for fever, fruit as blood purifier.

## Conclusion

The present study concluded that the selected medicinal plants have good activity against the clinically isolated pathogenic bacterial strains; further study is needed on the selected medicinal plants.

## Acknowledgment

The authors owe a depth of gratitude to the Department of Pharmacy, University of Malakand, Chakdara, for facilitating the research and also to taxonomists Mr Mehboob-ur-Rehman for assistances in plant identification.

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