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## ***In-vitro Antimicrobial Activity of Bark Extracts of an Ethnic Plant *Zanthoxylum armatum* DC. Against Selected Human Pathogens in Uttarakhand Himalaya***

**Nidhi Srivastava, Anup Kainthola and A.B. Bhatt**

### **ABSTRACT**

The present study is focused to evaluate the antimicrobial efficacy of *Zanthoxylum armatum* DC. (Rutaceae) against four different microorganisms viz *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa*. *Z. armatum* is extensively used in indigenous system of medicine as a tonic, carminative, stomachic and antihelminthic. Present investigation is an attempt to reveal the antibacterial activity of the chloroform, methanol and acetone extracts of *Z. armatum* bark using the well diffusion method against four different bacterial strains. Highest ZOI was observed in acetone extract against *S. aureus* (42.3 mm) followed by methanolic extract against *S. aureus* (28.7mm) while highest chloroform extract against were found *P. vulgaris* (28.3 mm). Overall the methanol and acetone extract of bark was found to be more effective for *S. aureus* and chloroform extract for *P. vulgaris*. The results of the extracts were compared with the standard antibiotics.

**Key words:** *Zanthoxylum armatum* DC, Antimicrobial, Well Diffusion, Zone of Inhibition.

### **1. Introduction**

The Garhwal Himalaya is known for its rich bio-resources and ethnoculture diversity. The use of plants and plant products as medicines could be traced far back as the beginning of human civilization. The earliest mention of medicinal use of plants in Hindu culture is found in *Rigveda*, which is said to have been written between 4500-1600 B.C. and is supposed to be the oldest repository of human knowledge. It is *Ayurveda*, the foundation of medicinal science of Hindu culture, where in eighth division deals with specific properties of drugs and various aspects of science of life and the art of healing [1]. Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants growing in different parts of the country. Following the advent of modern medicine, herbal medicine suffered a setback; however, during last two to three decades advances in plant chemistry in the identification of effective plant compounds against certain diseases have renewed the interest in herbal medicines [2]. Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants [3]. Antimicrobial drugs of plant origin have enormous therapeutic potential. These are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. The extract of *Camellia sinensis* and *Acalypha indica* is most effective against *Staphylococcus aureus* [4]. Compounds extracted from different parts of the plants can be used to cure diarrhea, dysentery, cough, cold, cholera, fever bronchitis, etc. In Pakistan, China, India, Japan, Sri Lanka and Thailand the practice of traditional medicine is widespread [5]. Increasing bacterial resistance is prompting resurgence in research of the antimicrobial role of herbs against resistant strains [6, 7]. The seed powder of *Zanthoxylum armatum* DC. (Rutaceae) can be taken orally with warm water to treat constipation, stomach pain, toothache and cold [8, 9]. Traditionally, leaves and fruits are used as mouth freshener and

in tooth care while bark is used as ischiotoxic or piscicidal [10]. Leaves, fruits and barks are used as spice [11]. The present study aims to screen and evaluate antibacterial activity of crude acetone, chloroform and methanol extracts of *Z. armatum* bark against both gram positive and gram negative bacteria.

## 2. Materials and methods:

### 2.1 Collection of samples

The medicinal plant *Zanthoxylum armatum* DC used for the present study has been found growing abundantly in lesser and higher Himalaya at an altitudinal range of 700- 2000m amsl. The plant samples collected for the present investigation have been matched with the specimens of Garhwal University Herbarium (acronym GUH), placed in the Department of Botany and Microbiology, Srinagar, Uttarakhand.

### 2.2 Preparation of extracts

Clean dry (dried under shade) plant sample were collected in cotton bags. The material was ground using a coffee grinder. Two grams of grounded material was soaked in each 25 ml of chloroform, acetone and methanol in different flask for 24 hours and filtered using standard filter paper. The material was again mixed with fresh 25 ml of each of three solvents and filtered after 24 hours. Same process was repeated once again. The extracts after treating with 75 ml (25 ml × 3 times) chloroform, acetone and methanol were then filtered, all three filtrates were transferred into vials and allowed to evaporate until completely dry. Once dry, the extracts were re-suspended in 2 ml of chloroform, acetone and methanol. Thus the concentration of the final extracts was 1g dry material/ ml [12, 13].

### 2.3 Collection of test organism and preparation of stock culture

Four species of bacteria, one gram-positive (*Staphylococcus aureus*) and three gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*) were isolated from infected sites

of patients attending OPD of V.C.S.G Base Hospital (a tertiary healthcare centre) Srinagar for testing. These were cultured in nutrient broth for 24 hours and the fresh inoculums were taken for the test and reconfirmed by gram staining and sub culturing in appropriate selective media.

### 2.4 Preparation of standard culture inoculums of test organism

Three to four isolated colonies were inoculated in 2 ml nutrient broth and incubated till the growth in the broth was equivalent with Mac-Farland standard (0.5%) as recommended by WHO at which the number of cells was assumed to be  $1.5 \times 10^8$  cfu/ml.

### 2.5 Determination of Zone of Inhibition (ZOI)

The antibacterial activity was assessed by agar well diffusion method. Muller Hinton agar medium was prepared by using 15g agar dissolved in 1L distilled water. Muller Hinton agar medium was poured into each Petri plate of 20 x 90mm and allowed to cool to 45°C to solidify. The freshly prepared inoculums were swabbed all over the surface of the MHA plate using sterile cotton swab. Wells of 8 mm diameter were made in the agar with a sterile cork borer. Hundred micro-liters of the working suspension/solution of different plant extracts were loaded in each well and same volume of extraction solvent for control was filled in the wells with the help of micropipette. Plates were left for some time till the extracts diffused in the medium with the lid closed and incubated at 37°C for 24 hour. The tests were performed three times and the zones of inhibition were measured for each extract using a ruler and the results were recorded.

### 3. Results and Discussion:

Among all the pathogens, all gram negative and positive bacteria were inhibited by all type of plant extracts. The plant extracts shown inhibitory action against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*. The results are summarized in table I.

**Table: 1** Antimicrobial activity of bark extracts against different pathogenic microorganisms.

| Test organisms       | Plant Extracts |          |         |
|----------------------|----------------|----------|---------|
|                      | Chloroform     | Methanol | Acetone |
| <i>P. aeruginosa</i> | 14.3           | 23.7     | -       |
| <i>S. aureus</i>     | 24.7           | 28.7     | 42.3    |
| <i>E.coli</i>        | 25.7           | -        | 31.7    |
| <i>P. vulgaris</i>   | 28.3           | 25.0     | 21.7    |

No zone of inhibitions

**Table 2:** Antimicrobial activity of standard antibiotics

| Test organisms       | Tetracycline | Streptomycin | Ampicillin |
|----------------------|--------------|--------------|------------|
| <i>P. aeruginosa</i> | -*           | 10.0         | -          |
| <i>S. aureus</i>     | 24.6         | 10.1         | 8.1        |
| <i>E.coli</i>        | 20.3         | 17.2         | 10.2       |
| <i>P. vulgaris</i>   | -            | 9.1          | 20.2       |

When the three extracts of bark were tested against the test microorganisms, acetone and methanol bark extracts exhibited greater inhibitory effects against *S. aureus*, than the chloroform extracts showing an inhibitory action against *P. vulgaris*.

In a control experiment the antimicrobial activity of three standard antibiotics against test organisms were also observed and are summarized in Table II. The results of both tables when compared confirm the potential antibacterial activity of the *Z. armatum* bark. Activity of bark extract were compared with the standard antibiotics, all antibiotics shown antibacterial activity but *P. vulgaris* could not be inhibited and showed negative results against Tetracycline. All extracts have exhibited greater inhibitory effect against all pathogen than the antibiotics.

Results of assessment of antibacterial activity revealed variability in different extracts against the test bacterial pathogen, In case of *Z.*

*armatum* bark extracts were found to have maximum inhibitory potential against all bacterial pathogens. Infectious diseases account for high proportion of health problems in the developing countries like India. Microorganism has developed resistance to many antibiotics and this has created immense clinical problem in the treatment of infectious diseases. The resistance of the organism increased due to the indiscriminate use of commercial antimicrobial drugs commonly used for the treatment of infectious diseases. This situation forced the scientist to search for new antimicrobial substances from various sources including medicinal plants [14]. Many of the plants used today were known to the people of ancient culture throughout the world for their preservative and medicinal powers [15].



**Plate 1:** Zone of inhibition of *Zanthoxylum armatum*

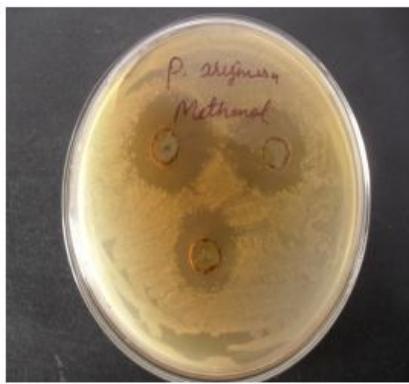
*P. aeruginosa* when tested against standard antibiotics, no zone of inhibition was observed for ampicillin and tetracycline and 10.0 mm for streptomycin, whereas the methanol bark extract showed 23.7 mm zone of inhibition, chloroform showed 14.3 mm which is more active than streptomycin and acetone was showed negative result.

When *P. vulgaris* tested against the standard antibiotics, no zone of inhibition was observed for tetracycline, 20.2 and 9.1 mm for ampicillin and streptomycin respectively, but the chloroform extract exhibited 28.3mm, methanol 25.0mm and acetone displayed 21.7 mm zone of inhibition which was found to be more active than the antibiotic. The growth of *P. vulgaris* was controlled by the bark extracts of *Z. armatum* indicating that the latter enhances immunity against the pathogen which can cause urinary tract infections.

When *S. aureus* tested against standard antibiotics, zone of inhibition was observed for tetracycline 24.6mm and 10.1 mm, 8.1 mm for ampicillin and streptomycin. Acetone bark extract was shown exhibited greater inhibitory effect against *S. aureus* and the zone of inhibition was found 42.3mm, methanol showed 28.7 mm zone of inhibition, and chloroform showed 24.7 mm zone of inhibition, which is more active than standard.

The test organisms *S. aureus* and *P. aeruginosa*, included in the study were isolated from patients and their growth was strongly inhibited by bark extracts of *Z. armatum*. The other organisms included in the study were isolated from clinical specimens and this clearly indicates that the bark could be attributed to the treatment of diseases caused by the test microorganisms.

Extracts of bark were tested against *E.coli*. Chloroform and acetone



were shown inhibitory action which is more effective than antibiotics. Tetracycline has shown 20.3 and 17.2mm and 10.2mm for ampicillin and streptomycin. Whereas the acetone bark extract showed 31.7 mm, chloroform displayed 25.7 mm and methanol extract was showed negative result.

The growth of *E.coli* was controlled by extract of *Z. armatum*, which indicates that the plant could inhibit the activity of bacteria that can cause diarrhea and dysentery. *Z. armatum* is most common and one of the widely used plants of the area, its bark was used traditionally in stomach problem and as popular household spices.

#### 4. Conclusion:

The findings of the present study support the conventional usage of the plant under consideration and suggest that some of the plant extracts possess compounds with antimicrobial properties that can be further explored for antimicrobial activity. According to WHO [16], medicinal plants are the best source to obtain a variety of drugs. Therefore, such plants need further investigations to better understand their properties, safety and efficacy. The study has revealed that many secondary metabolites are present in the bark of the study plant owing to its high antimicrobial activity. This antibacterial activity of the plant extracts demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases and this plant as a remedy for dental carries, skin infection, and urinary tract infection caused by the pathogens studied, and this plant can be exploited for new potent antimicrobial agent.

These findings support the traditional knowledge of local users and

it is a preliminary, scientific, validation for the use of this plant for antibacterial activity to promote proper conservation and sustainable use of such plant resources. Awareness of local community should be enhanced incorporating the traditional knowledge with scientific findings.

### **5. Acknowledgements:**

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