

# Antibacterial Activity of *Zanthoxylum Armatum* (Timur) on Gram Positive Bacteria and Gram Negative Bacteria

Sunita Shrestha (Singh)\*

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## Abstract

*Zanthoxylum armatum* is commonly known as Timur which is a major indigenous spice of Nepal. The objective of this research work is to determine the antibacterial activity of *Z. armatum*. The fresh fruits seeds of *Z. armatum* were collected from Pyuthan District of Nepal. About 25 gram of seeds were grinded to make powder and mixed with 500 ml ethanol for ethanol extraction. Then, extraction was separated by filtration and left for time until residue obtained. The residue obtained was mixed with DMSO (10%) and diluted to 1% and 10% dilution. Agar well diffusion method was done for studying antimicrobial activity for the Gram positive bacteria (*Staphylococcus aureus* and *Bacillus* spp.) and Gram negative bacteria (*Escherichia coli*, *Klebsiella pneumonia*, *Salmonella Typhi* and *Shigella dysenteriae*). For the quality control of test, antimicrobial activity of *Z. armatum* was also done for *Staphylococcus aureus* (ATCC: 25923) and *Escherichia coli* (ATCC: 25922) along with positive control (Ofloxacin) and negative control (10% DMSO). Among different concentration of 1%, 10%, and 100% of ethanol extract of *Z. armatum*, 10% and 100% of ethanol extract were found to be effective for both Gram positive bacteria and Gram negative bacteria. From this study, it was concluded that antibacterial activities of *Z. armatum* was found to be effective.

**Keywords:** Agar Well diffusion method, Bacteria, Ethanol Extraction, *Zanthoxylum armatum*,

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## Introduction

Among different species of family Rutaceae, *Zanthoxylum armatum* is commonly known as Timur which is a major indigenous spice of Nepal (Nair and Nayar, 1997). In Nepal, it is found from west to east in open places or in forest under grow

\* Department of Science, Padmakanya Multiple College, Bagbazar, Kathmandu, E-mail: shresthasunitasingh@gmail.com

that an altitude of 1000m to 2500m (DPR, 2016). The dried fruit of *Z. armatum* contain an aroma that is present in brown fruit wall (pericarp-shell). It may be able to develop numbing or anesthetic feeling on the tongue. Seeds are solitary, globose, shining and have bitter taste (Brijwal et al., 2013). Herbal medicinal practice, which uses plant sources to cure various infectious diseases, is also getting popularity these days. Among different herbs, *Z. armatum* contain chemical compounds such as alkaloids, flavonoids, glycosides, terpenoids, steroids, phenols, lignins, coumarins, and benzoids (Li, 2006; Phuyal et al., 2020a) have been reported from different parts of the plant like leaves, fruits, seeds, and bark which are responsible for several biological activities including antimicrobial, antioxidant, antipyretic, larvicidal, and anti-inflammatory properties. The fruits and seeds are employed as an aromatic tonic in fever, dyspepsia, and expelling roundworms (DPR., 2016; Kala, 2005). The fruits are used as condiments and spices. *Z. armatum* is not only used as flavouring in cooking but also its seed oil and crushed seeds are added to cereal seeds and legumes to protect against damages caused by stored grain pests (Tiwary et al., 2007). In this today's world, a wide range of antimicrobials are used for the treatment of several contagious diseases, which may result in undesirable side effects and serious medical problems resulted in the development of multiple drug resistance (Marchese and Schito, 2000; Portillo et al., 2001). So, it is urged for researchers to identify or extract natural antimicrobials from natural sources with less health severity (Agrawal et al., 1996).

Medicinal plants have been used for curing diseases for many centuries in different indigenous systems of medicine as well as folk medicines. Unfortunately, no much research has been done on seeds of *Z. armatum* to explore its antimicrobial properties against bacteria. Hence, this study was done to evaluate the antimicrobial activity of ethanol extracts of seeds of *Z. armatum* against the human pathogenic bacteria.

## **Materials and Methods**

### **Sample collection and transportation**

The fresh fruits seeds of *Z. armatum* were collected from Pyuthan District of Nepal. Samples were transported to Laboratory then cleaned with distill water and shade dried for a week. This study was done in Microbiology laboratory of Padmakanya Multiple, Campus from April 2022 to July 2022.

### **Alcoholic extraction**

Dry seed of *Z. armatum* was granded to form powder. Finely form of *Z. armatum* (25gm) was treated with 100ml of Ethanol and kept for 24hrs at ambient conditions.

Then the mixtures were filtered using cheese cloth and extracts were obtained. Extract was left until residues obtained from extraction then labeled it. The obtained extracts were then used for antibacterial activity assay. Residue of extracts was used as 100% as crude extraction. Then 100% extraction was diluted to 1% and 10% with 10% DMSO (Agarwal et al., 2010).

### **Preparation of microbial cultures**

For study of antibacterial activity of *Z. armatum*, pure cultures of bacteria were collected from Med-Micro Research laboratory, Kathmandu. All Gram positive bacteria (*Staphylococcus aureus* and *Bacillus* spp.) and Gram negative bacteria (*Escherichia coli*, *Klebsiella pneumonia*, *Salmonella* Typhi and *Shigella dysenteriae*) were subcultured on Nutrient Agar (NA) and stored at 4°C before use in experiments. ATCC culture of *Staphylococcus aureus* (ATCC: 25923) and *Escherichia coli* (ATCC:25922) were used for quality control.

### **Antibacterial Activity**

Agar well diffusion method was used to determine the antibacterial activity of extract of *Z. armatum* (Perez et al., 1990). Colonies of bacteria from Nutrient Agar were transferred to the Nutrient broth, and their turbidity was visually adjusted with the broth to equal 0.5 McFarland turbidity standard. A sterile cotton swab was dipped into the inoculum and rotated against the wall of the tube above the liquid to remove excess inoculum then lawn culture was done. The overall procedure of inoculum preparation and inoculation of culture media remained the same for all bacteria.

The inoculated plate was allowed to stand for 15min, before making wells for different compounds to be tested. A cork borer of 7mm diameter was sterilized and pressed above the inoculated agar plates. It was removed immediately by making five well in the plate. In each well of each plate, 70µl of extracts (1%, 10% and 100%), positive control (Ofloxacin) and negative control (10% DMSO) were poured. Then, plates were incubated at 37°C in an incubator for 24hrs to 48hrs in aerobic condition. After incubation, clear zone of inhibition was measured around the wells.

### **Data entry**

All data were entered in MS Excel and analysis was done.

## Result

Table 1. Antibacterial activity of *Z. armatum* (Timur) extracts against Gram positive bacteria

Among Gram positive bacteria, both 10% and 100% were found to be effective in which *S. aureus* showed higher zone of inhibition (26mm) followed by ATCC:25923 *S. aureus* (18mm) and *B. cereus* (13mm).

Extraction	Zone of inhibition (mm)			
	Gram Positive Bacteria	1%	10%	100%
Ethanol extraction	<i>S. aureus</i> (ATCC:25923)	0	10	18
	<i>S. aureus</i>	0	14	26
	<i>B. cereus</i>	0	10	13

Table 2. Antibacterial activity of *Z. armatum* (Timur) against Gram negative bacteria

Among Gram negative bacteria, only 100% were found to be effective in which *E. coli* showed higher zone of inhibition which was 22mm followed by *S. Typhi* (20mm) and other Gram negative bacteria.

Extraction	Zone of inhibition (mm)			
	Gram Negative Bacteria	1%	10%	100%
Ethanol extraction	<i>E. coli</i> (ATCC:25922)	0	0	16
	<i>E. coli</i>	0	0	22
	<i>K. pneumoniae</i>	0	0	15
	<i>S. Typhi</i>	0	0	20
	<i>S. dysenteriae</i>	0	0	10



A

B

C

A. Dry seed of *Timur* B. Powder of *Timur* C. Antibacterial activity of *Bacillus* spp.

## Discussion

In this study, the antibacterial activity of ethanol extracts of seed of *Timur* was determined against Gram positive bacteria and Gram negative bacteria by the agar well diffusion method. Several experiments have demonstrated considerable amount of antibacterial activities of *Z. armatum* of leaf, fruit, seed, and bark extracts against different bacterial strains (Joshi and Gyawali, 2012; Joshi and Joshi, 2000). Antibacterial activity of extracts was done for 100%, 10% and 1% dilution. Extracts was not found to be effective at low concentration (1%) for both Gram positive bacteria and Gram negative bacteria. This may be due to low concentration unable to inhibit the bacteria. On the other hand 10% extracts of *Z. armatum* was found to be highly effective for only Gram positive bacteria in which *S. aureus* showed higher zone of inhibition (14mm) followed by ATCC:25923 *S. aureus* (10mm) and *Bacillus cereus* (10mm). Due to high resistance capacity of Gram negative bacteria as compare to Gram positive bacteria, low concentration inhibited Gram positive bacteria but didnot inhibit Gram negative bacteria.

Similarly, Joshi and Gyawali (2012) studied on antibacterial activity of *Z. armatum*, zone of inhibition produced by the ethanolic of *Z. armatum* showed antibacterial activities against different bacteria. In the study of Barkatullah et al., (2013), zone of inhibition produced by the extract against *B. subtilis* and *E. coli* both were 11.67 mm and against *S. aureus* was 17.33 mm for ethanolic extract. However, in another study (Joshi and Joshi, 2000), instead of ethanolic extracts, methanolic extracts of the fruits was done and showed 7mm zone of inhibition against *S. aureus*.

Extracts was found to be highly effective at 100% for both Gram positive bacteria and Gram negative bacteria. At this higher concentration, *S. aureus* showed higher zone of inhibition (26mm) among Gram positive bacteria and *E. coli* showed higher zone of inhibition (22mm) among Gram negative bacteria. Among Gram positive bacteria, *S. aureus* (26mm) showed higher zone of inhibition at 100% followed by *B. cereus* (18mm) and ATCC:25923 *S. aureus* (13mm). Among Gram negative bacteria, *E. coli* (22mm) showed higher zone of inhibition followed by *S. Typhi* (20mm), ATCC:25922 *E. coli* (16mm), *K. pneumonia* (15mm) and *S. dysenteriae* (10mm). All Gram negative bacteria used in this study was highly pathogenic as well as resistance bacteria as compare Gram positive bacteria. Among all tested bacteria, Gram positive bacteria *S. aureus* showed higher zone of inhibition at 100% dilution as well as 10% dilution of extracts. So, *Z. armatum* can be used for treatments of diseases caused by the pathogenic bacteria. From this study, antibacterial activity of fruit extracts of *Z. armatum* was found to be effective. This

might to be due to the higher phenolic and flavonoid contents in fruits. *Z. armatum* has been reported to produce structurally diverse chemicals including terpenoids, flavonoids, coumarins, sterols, and alkaloids that show antibacterial activity. Many active components have been identified from the plant that might be developed into novel drugs (Phuyal et al., 2020b).

## Conclusion

From this research work, it can be concluded that ethanol extraction of *Z. armatum* was found to be highly effective for Gram positive bacteria and Gram negative bacteria. So, ethanol extraction of *Z. armatum* showed antibacterial activity for bacteria and suggested that use of *Z. armatum* would be helpful in the treatment of infections caused by different bacteria.

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