

## A Comparative Study of In Vitro Antibacterial Activity Capacity of Leaf Extract of *Zanthoxylum armatum* (Timur)

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### ABSTRACT

*Zanthoxylum armatum* also known as Timru and toothache tree. This is a sub deciduous aromatic, branched, scandent, or erect shrub or a small tree that grows to 6–7 meter in height and belongs to the Rutaceous family. This plant is common in the hot valleys of the Himalayas from Jammu to Bhutan, Nepal, and Pakistan. It covers a considerable portion of Orissa and Andhra Pradesh in India This plant is considered as a divine plant in contiguous areas. The seeds contain many important phytoconstituents and essential oils which contribute to its biological activities including larvicidal, anti-inflammatory, analgesic, antinociceptive, antioxidant, antibiotic, hepatoprotective, antiplasmodial, cytotoxic, antiproliferative, anthelmintic, antiviral and antifungal. It contains alkaloids, essential oil, resins, sterols, triterpenes, glycosides, saponins, tannins and flavonoids. Because of its Significant medicinal properties and excessive increase in demand has put this plant in the category of endangered plants. This research paper focuses on the Antibacterial activity of the *zanthoxylum armatum* plant.

**Keywords-** *Zanthoxylum armatum*, qualitative investigation and antibacterial activity.

### I. INTRODUCTION

*Zanthoxylum armatum* is also known as the toothache tree, Nepal Piper and Indian prickly bush it is common in the north east India and also known as Tejpal in Hindi, Tejowati in Sanskrit, Timur in Nepali and Mukthrub in Manipuri, it is prevalent in north east India. Different parts of the *Z. armatum* plant, including the fruits, stem, leaves, and bark, have been used in various indigenous medical systems to treat gastric problems, fever, and appetizers. It works well to treat toothaches, irritation, and stomach aches. Due to its pharmacological benefits, *Zanthoxylum armatum* is in high demand on both the domestic and global markets. Around 1,748 distinct types of medicinal plants can be found in the Indian Himalayan region. It is a big spiky shrub or small tree.[1]

Due to its exceptional therapeutic efficacy, *Z. armatum* (family: Rutaceae) is regarded as a significant medicinal plant. Many *Z.* species are traditionally used as medicinal plants in managing various health conditions. Secondary metabolites isolated from parts of plant species in this genus have demonstrated several pharmacological activities, such as antioxidant, analgesic, anti-inflammatory activities, and modulatory effects against obesity, dementia, and diabetes [2,3] The fruit part of the plant may use to purify the water. Also used as insect repellent. The wood of this plant may be very heavier and stronger than it is used for walking sticks. *Z. armatum* also gives and showed work against antioxidants [4] antinociceptive, antifungal [5]] anti-inflammatory, hepatoprotective [6] pesticides, anthelmintic, antiproliferative [7] etc



Figure: Parts of *Z. armatum*

## II. PLANT PROFILE

*Zanthoxylum armatum* trees are widely dispersed around the world. *Zanthoxylum armatum* is also grown in nations including China, Japan, Korea, Taiwan, Bangladesh, Bhutan, Nepal, Pakistan, Laos, Myanmar, Thailand, Vietnam, and Indonesia. There are numerous kinds of the tree in the Indian states of Andhra Pradesh, Jammu & Kashmir, Assam, Manipur, Meghalaya, Nagaland, Orissa, and Uttar Pradesh[9]. *Zanthoxylum armatum* is mostly found in North-East India and is distributed throughout India from Jammu and Kashmir to Bhutan at an altitude of about 2500 m. The species' primary habitats include wastelands, mountains, valleys, and forests(10)*Zanthoxylum armatum* is a small tree having large spiny shrubs. The Leaves are distinctively trifoliolate with the leaf-stalk winged. Leaflets are stalk less, 2.0-7.5 × 1.0-1.7 cm, elliptic to ovate-lance like, entirely to slightly toothed, sharp tipped, base sometimes oblique. Minute yellow flowers arise in leaf axils. Flowers have 6-8 acute sepals. Petals are absent. Male flowers have 68 stamens and large anthers because of which the flowers look yellow. Female flowers have 1-3 celled ovaries, pale red, splitting into two when ripe. Seeds are round 3 mm in diameter shining black colour(11)Fruits have purplish red colour and have a round 4 mm diameter. Flowers having growth April to May and fruits may appears in tree all over the year (12) The traditional uses of *Zanthoxylum* species as food and in medicinal practices are highlighted in this section. Pastes prepared from *Z. species* are used in South Africa and Kenya to relieve wound pain and speed up the healing process. (13,14) *Z. species* like *Z. zanthoxyloides* are used to treat sickle cell anaemia, rheumatism, urinary tract infection, and venereal diseases. (15)

Fruits and seeds are usually used as a condiment and spice in momos, chowmien, biscuits, thukpa, sweetened cakes etc. The fruit part of the plant may be used to purify the water. Also used as insect repellent. The wood of this plant may be much heavier and stronger than it is used for walking sticks. *Z. armatum* also contains essential oils and is also known as an ornamental plant (16).

Table 1: Uses of *Z. armatum* in various disease

S.no	Country	Part of plant	Traditional uses	References
1	India	Seeds and bark Aerial parts	fever, dyspepsia, cholera. insecticidal	18
2	Japan	Seed	Git problem and depression	19
3	Nepal	Fruit Berries Bark Fruits	Decoction is used for abdominal pain Carminative, antispasmodic, anti-rheumatic cholera, diabetes and asthma diarrhoea, dysentery and stomach-ache	20
4	Pakistan	Fruit	Spice and condiment Powder of its dried fruit along with <i>Mentha longifolia</i> Twigs are used as toothbrush during gum problems and toothache	21
5	China	Aerial part	As infusion in vinegar is used to expel bugs or worms infecting ear. Scabies is treated by the plant using a lotion applied to the skin	22

The alkaloids found in the plant *Zanthoxylum armatum* include g-fagarine, b-fagarine, magnoflorine, nitidine, chelerythrine, and tambatarine. It also contains linalool, beta-sitosterol, tamblin, tambulating, aramatamide, lignans, asarinin and fragesin Bark of plant contains yellow crystalline compounds named as berberine (23) Many chemical studies are done which introduced the isolation of further two new phenolic compounds 3-3',4'-dimethoxyflavone-5-β-d-xylopyranoside along with the five known compounds, 1-methoxy- 1,6,3-anthraquinone, 1-hydroxy- 6,13-anthraquinone, 2-hydroxybenzoic acid, 2-hydroxy-4-methoxybenzoic acid and stigmasta-5-en-3β-deglucopyranoside, on the basis of spectral data and chemical analyses(24) Two new phenolic glycoside, 2-methoxy-4-hydroxyphenyl-1-O-α-L-rhamnopyranosyl-(1"→ 6')-β- D- glucopyranoside and threo-3-methoxy-5-hydroxy-phenylpropanetriol-8-O-β-Dglucopyranoside were isolated from the stems of *Zanthoxylum armatum*(25)

Along with other ingredients including arginine, aspartic and glutamic acid, glycine, histidine, threonine, tyrosine, and others, it contains alkaloids, flavonoids, saponin, steroids, terpenes, phenols, carbohydrates, proteins, and essential oil. (26) From the bark of this plant armatamide which is a amide is identified. Seed part of this plant consist hydroxylicenolic acid and other volatile compound (27). Stem and root part of plant consists beta-amyrin, beta-sitosterol, lasarinin and L-plananin and zanthobungeneanine. Mythylcinnamate, limonine, carvone, linalool, palmitic acid, p-cymene are some other compounds present in *Zanthoxylum armatum* (28) On the other hand, the aerial section of the plant contains sitosterol and pinoresinol diethyl ether. Linalyl acetate, phenols, methyl-n-nonyl ketone, sesquiterpenes hydrocarbon, tricosane, citronellal, 1,8-lineole, and limonene are among the compounds found in the oil extracted from *Zanthoxylum armatum* (29) Important active ingredients found in the plant *Zanthoxylum armatum* include lignin, alkaloids, coumarin, benzenoids, amino acids, phenols, and flavonoids (30,31) And many more derivatives are found in the research (32)

### III. MATERIAL AND METHOD

Collection and processing of samples. The fresh leaves *Z. armatum* were collected from wild and cultivated populations from Pithoragarh District of Uttarakhand during (august 2023) samples were cleaned and shade dried for a week before the extraction procedure.) e herbarium of the voucher specimens was prepared, which are deposited at the Forest research institute Dehradun

#### **Extraction of the Samples**

The dried samples were then powdered separately in a grinder. 35gm weight of the powdered samples was loaded in a thimble and put inside the Soxhlet apparatus the apparatus was run for 72 hours till the colored solvent appeared in the siphon for obtaining the crude extracts of the samples. After complete extraction, the solvents were evaporated in a rotary vacuum evaporator at 65°C under reduced pressure

#### **Analysis of bioactive component**

Leaves extracts prepared in three different solvents (water, petroleum ether, chloroform) were used for experimental purposes.

#### **Test for flavonoids:**

When 2-3 drops of sodium hydroxide are added to 2 milliliters of petroleum ether extract, the mixture turns a rich yellow color, suggesting the presence of flavonoids. Add two to three drops of sodium hydroxide to two milliliters of chloroform extract, and the mixture turns colorless. Add a few drops of diluted HCL, and the mixture turns yellow, indicating the presence of flavonoids. When 2-3 drops of NaOH are added to 2

milliliters of water in an aqueous solution, a yellow color appears, indicating the presence of flavonoids.

#### **Test for alkaloids:**

One millilitre of chloroform extract was mixed with a few drops of Mayer's reagent. The lack of a yellowish precipitate produced suggests that there were no alkaloids present. A precipitate was generated in an aqueous solution containing 1 millilitre of water extract and a few drops of Mayer's reagent, suggesting the presence of alkaloids in the solution. When a few drops of Meyer's reagent are added to one millilitre of petroleum ether, a yellow precipitate is produced, signifying the presence of alkaloids.

#### **Test for phenol:**

In aqueous solution few drop of ferric chloride solution were added to 1ml of water extract. A dark green precipitate was formed, indicating presence of phenol. In pet. ether 1 ml of pet. ether extract add few drop of ferric chloride solution give greenish color which indicate there is presence of phenol compound. Similarly in chloroform extract it give greenish color was formed which indicate there is presence of phenol.

#### **Test for anthraquinone**

In the anthraquinone test, the Bromine test is used. In pet. ether 2 ml of bromine to add equal volume of pet ether extract does not give pink precipitate which indicate absence of anthraquinone. In water and chloroform extract which do not give pink precipitate which mean there is no presence of anthraquinone

#### **Test for terpenoids:**

5ml of pet. ether, an aqueous solution was mixed with 2ml of chloroform and 3ml of concentrated sulphuric acid were carefully added. A reddish brown coloration of the interface was formed to show the presence of terpenoids.

#### **Test for tannins:**

In the tannins test ferric reagent is use in this test 3 drop of ferric reagent were added to a 2ml of sample (chloroform, pet. ether, water) extract the chloroform extract and pet. extract does not give gray color which indicate the absence of tannin water give gray color which indicate there is presence of tannin.

#### **Test for steroids**

This test is given by Liebermann Burchard reaction to the chloroform solution in a test tube few drop of acetic anhydride was added 1ml of concentrated sulphuric acid allowed to stand a reddish ring was formed which mean there is presence of steroids in the pet. ether and aqueous solution doesn't give positive reaction

#### **Test for glycosides:**

Phytochemical in glucosides test at first 0.5ml of glacial acetic acid and 2-3 drop of ferric chloride was mixed with Chloroform, pet. ether, water then 1 ml of concentrated H<sub>2</sub>SO<sub>4</sub> then water and chloroform gives deep blue color which indicate presence of glycosides.

**Table 2: Phytochemical Screening on plant sample in different solvents**

S. No	Test	Pet. ether	Chloroform	Water
1	Flavonoids	+	+	+
2	Alkaloids	+	-	+
3	Phenol	+	+	+
4	Anthraquinone	-	-	-
5	Terpenoids	+	-	+
6	Tannins	-	-	+
7	Steroids	-	+	-
8	Glycosides	-	+	+

**Preparation of agar media**

In a beaker, 28 grams of the dehydrated powder or lab-prepared media is added to 1000 milliliters of distilled water. The suspension is then heated to boiling to dissolve the medium completely. The dissolved medium is then autoclaved at 15 lbs pressure (121°C) for 15 minutes. Once the autoclaving process is complete, the beaker is taken out and cooled to a temperature of about 40-45°C. The media is then poured into sterile Petri plates under sterile conditions. Once the media solidifies, the plates can be placed in the hot air oven at a lower heat setting for a few minutes to remove any moisture present on the plates before use.



**Agar well diffusion**

Agar well diffusion method is widely used to evaluate the antibacterial activity of plants. Similarly to the procedure used in the disk-diffusion method, the agar plate surface is inoculated by spreading a volume of the

bacterial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100 µL) of the antibacterial agent or extract solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antibacterial agent diffuses in the agar medium and inhibits the growth of the bacterial strain tested.

**Antibacterial Activity**

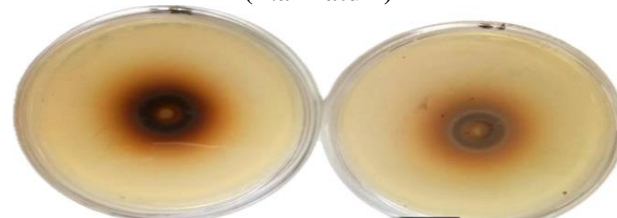
The chloroform, pet.ether, and water extract was prepared by using the Soxhlet apparatus of zanthoxylum armatum to study its antibacterial potential. Antibacterial analysis of extract was carried out against lacto-bacillus organisms. The zone of inhibition in mm for the tested organism with the Chloroform, pet.ether and water extract of Zanthoxylum armatum and by agar well diffusion method. In the present study, Chloroform, pet.ether, and water, stem extract Zanthoxylum armatum obtained by Soxhlet extraction was screened to detect the presence or absence of several bioactive compounds which are reported to cure different diseases. Anti-microbial analysis of stem extract was carried out against lacto-bacillus organisms by agar well diffusion method. It was observed that the zone of inhibition was recorded against lacto-bacillus organisms. The results indicate that the chloroform, pet.ether and water extract of Zanthoxylum armatum is having anti-bacterial efficiency in controlling the bacteria.



**Zone of inhibition of lactobacillus from potassium ether extract (Z. armatum)**



**Zone of inhibition from chloroform extract (Z. armatum)**



**Zone of inhibition from water extract (Z. armatum)**

**Fig: 2 Minimum inhibitory concentration of Zanthoxylum Armatum plant**



**Table 2: Antibacterial activity inhibitory concentration of *Zanthoxylum Armatum* plant**

Extract	Standard(am oxicillin) in mm	25mc g	50mc g	100m cg	MI C
Pet.ether	24	20	22	24	22
Chloroform	21	19	21	25	21
Water	25	19	18	26	23

#### IV. CONCLUSION

This paper's phytochemical analysis identified a wide range of phytochemicals, including as alkaloids, flavonoids, amino acids, steroids, saponins, and tannins. The use of leaves as a medicinal remedy was confirmed by the phytochemical component. This research can assist identify zanthoxylum armatum, which is used widely by Indians today as therapeutic herbs and phytochemicals. Research has been done on the antibacterial activity of water, pet ether, and chloroform extract from *Zanthoxylum armatum infortunatum* against organisms that are resistant to a variety of medications. The investigation's conclusions show that zanthoxylum armatum powdered leaves extract has potent antibacterial activity against drug-resistant bacteria, such as lactobacillus, in a variety of solvents.

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