# Evaluation of Antimicrobial Studies of Traditional Medicine

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**Abstract**

Synthetic drugs, which are having serious side effects, are cytotoxic to human beings. Moreover, bacteria slowly become resistant against antibiotics. In order to overcome these problems, scientists are continuing to search for antibacterial and novel principles from plants. *Zanthoxylum limonella* has been popularly used in traditional systems of medicine for the treatment of dental caries, cardiac, respiratory diseases and stomach problems. The present study was therefore focused to find out the medicinal properties of the bark of *Z. limonella* tested against some selected bacteria using disc-diffusion method at the concentration of 100, 200 and 300 mg/ml. The methanol and water extracts of the bark exhibited effective antibacterial activity against *S. aureus* at a concentration of 300 mg/ml than the other extracts. Phytochemcial screening results also support the present findings and proved the traditional usage of this plant by tribal healers in Kolli hills of Tamil Nadu.

**Key words:** *Zanthoxylum limonella*, medicinal plant, tribal healers, antibacterial

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## 1. INTRODUCTION

*Zanthoxylum limonella* L. (Dennst.) Alston belongs to the family Rutaceae. It is a climbing shrub, capable of growing upto 50 meter height and the cylindrical stem has small spikes on the surface. *Zanthoxyllum limonella* commonly inhabits temperate regions of Assam, Bhutan, Himalaya, Tamil Nadu (Kolli hills) and Sri Lanka. *Zanthoxylum limonella* is called "Veerasingam pattai" in vernacular language. It is popularly used for the treatment of cardiac, respiratory diseases, tooth infection as well as against stomach affection[1]. The various parts of *Z. limonella* are used as traditional medicine in India. The bark is bitter and is used as a tonic and to cure rheumatism[2]. Essential oil extracted from the fruit is commonly known as 'Mullilam oil'; it is frequently used as anti-inflammatory, antiseptic, anticholera, diarrhea and hypocholesterlamic; fruits are used as pickle[3]. Several authors have reported on the isolated classes of constituents obtained from stem bark of *Zanthoxylum sprucei* such as lepeol, β-sitosterol, (+)-sesamin, trans-dimethylmataairresinol, hesperidin, (-)-cis-N-methylcanadine, sucrose[4], liriodenine, sesamin, lichexanthone, piperitol, savinin, lirioodenine and decrine from *Z. tetraspermum* and *Z. caudatum*[5]. *Zanthoxylum limonella* has not been studied in detail. The present investigation was aimed at studying the antibacterial properties; phytochemical screening was also carried out.

## 2. MATERIALS AND METHODS

### 2.1. Collection of plant bark

The fresh stem bark was collected based on the medicinal information obtained from the Natives (Malaiali tribes) in Kolli hills, Salem district, Tamil Nadu, India, in August 1995. The plant was identified with the help of Flora of Tamil Nadu Carnatic[6]. Voucher specimen (PBB 002/95) is maintained in our department.

### 2.2. Extraction

The bark was separated, dried and ground into powder. 50 g of bark powder was extracted successively in soxhlet apparatus using 300 ml of solvents such as hexane, diethyl ether, dichloromethane, ethyl acetate and methanol; 50 g of bark powder was suspended with 300 ml of distilled water over night in the room temperature. The extracts were filtered and filtrates were evaporated using vacuum rotary evaporator at 50°C[7]. Crude extracts yielded hexane 1 g, ethyl acetate 1.2 g, diethyl ether 0.8 g, dichloromethane 1.8 g and methanol 2.3 g; from water 1.5 g of air dried (1:6 w/v) bark.

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### 2.3. Bacterial culture

*Escherichia coli, Klebsiella aerogenus, Proteus vulgaris, Pseudomonas aeruginosa* and *Staphylococcus aureus* were obtained from the Department of Medical Microbiology, Dr. ALM Post Graduate Institute of Basic Medical Sciences, Taramani, Chennai - 600 113, Tamil Nadu, India. The pure cultures of bacteria were maintained by frequent subculturing on Muller Hinton agar slants (pH 7.4) and stored at 4°C.

### 2.4. Standard drugs

The following chemotherapeutic agents were included in the test as references: Chloramphenicol (30 µg/disc) and Streptomycin (30 µg/disc) were supplied by HiMedia private Ltd, Mumbai, India.

### 2.5. Antibacterial activity

Sterile Muller Hinton agar medium 10 ml was poured into the petridishes and left to set for solidification. 100-300 mg/ml crude extracts were weighed from the stock and dissolved in 1% of the acetone. Filter paper discs (9 mm in diameter) were suspended in the extract separately and introduced on the medium. Acetone served as a negative control; Chloramphenicol (30 µg/disc) and Streptomycin (30 µg/disc) were used as drug control (references). Three replicates were used for each concentration of the extract. Plates were incubated at 37°C for 24 h; after the incubation, the inhibition zones were measured around the disc[8].

### 2.6. Phytochemical screening

Phytochemical analysis of various extracts of *Z. limonella* was performed[9].

#### 2.6.1. Alkaloids

Five grams of powdered material was extracted with 15 ml of 0.1 N aqueous sulphuric acid. The filtrate was treated with 1.5 ml of concentrated ammonia and extracted with diethyl ether. The organic phase was divided into two parts. One part was evaporated to dryness and the residue was redissolved in 1 ml 3% HCl and assayed by Meyer's test. A white precipitate indicated the presence of alkaloids. Other part was concentrated under reduced pressure and developed on TLC using two standard solvents (Acetone-$H_2O$-conce.Ammonia 90:7:3 and acetone-conce. Ammonia 97:3) for alkaloids. Alkaloids were detected by spraying the chromatograms with Dragendorff's reagent[10].

### 2.6.2. Flavonoids

The powdered bark material (2g/20ml) was boiled for 3 minutes in a water bath. 2 ml of filtrate was added to 2 ml of acetic acid-alcohol (EtoAc:$H_2O$:HCl), solid magnesium and about 1 ml of t-amyl alcohol. The appearance of rose-orange indicated the presence of flavonoids.

### 2.6.3. Tannins

The decoction was treated with 1% FeCl, solution. The appearance of precipitate indicated the presence of tannins.

### 2.6.4. Triterpene

Wet powder 1 gm was extracted with 10 ml of ether for 24 h. 1 ml of this extract was evaporated to dryness and the residue was redissolved in several drops of acetyl anhyride; then a drop of $H_2SO_4$ was added. The presence of triterpene and steroids was indicated by a green coloration. A complete assay was performed with $H_2SO_4$.

### 2.6.5. Saponins

The bark decoction of 1 ml was shaken vigorously for 10 seconds in a test tube. It was allowed to settle for 10 minutes and the height of foam was measured in mm[11].

## 3. RESULTS AND DISCUSSION

The bark of *Z. limonella* extract was tested against diverse bacteria and the results revealed that all the extracts were found to inhibit the bacterial growth with varying degree within the concentrations tested. Methanol and water extract showed the maximum antibacterial effect on *S. aureus* at 300 mg/ml concentration (Table 2). The results correlate with that of Moura et al[1], who reported that the methanol, aqueous crude extract and basic fraction of zanthoxylin from the bark of *Zanthoxylum rhoifolium* had antibacterial properties. Among them, the crude methanol extract and its basic fraction showed potential antibacterial activity against all the tested bacteria. Zanthoxylin did not exhibit bacterial inhibition. The crude ethanolic extract from the leaves of *Zanthoxylum liebmannianum* exhibited inhibitory effect on the reproduction of *Entamoeba histolytica* ($IC_{50}$ = 3.48 µg/ml); asarinin was the most active ($IC_{50}$ = 19.86 µg/ml) on *E. histolytica*[11]. The ethyl acetate and diethyl ether extracts were found to control the growth of bacteria only moderately. Moreover, hexane and dichloromethane extracts did not show any inhibitory effect in all the tested organisms. However, water extracts showed broad spectrum of activity. Potential bacterial inhibition was observed at the highest concentration of the extract. This may be due to the chemical compounds such as alkaloid, terpenes and tannin etc. Four different organic and water

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extracts of the bark were tested for the screening of chemical components. Terpenoid was present in all the extracts whereas alkaloids were recorded in the methanol and water extracts. Binutu and Cordell[4], also reported that the known compounds such as lepeol, β-sitosterol, (+)-sesamine, trans-dimethyl mataairesinol, hesperidin, (-)-cis-N-methylcanadine and sucrose were isolated from the *Zanthoxylum spruce.* The different solvent extracts of this bark proved the presence of alkaloid component (Table 1). The present findings corroborate with the findings of Nissanka et al[5], who reported that the alkaloids such as liriodenine, sesamin, lichexanthone and piperitol gamma-gamma-diethyl ether from the *Zanthoxylum tetraspermum* and compounds such as sesamin, savinin, lirioodenine, decrine isolated from *Z. caudatum* showed significant antibacterial and antifungal activity. *Zanthoxylum limonella* bark extracts showed a wide spectrum of antibacterial activity at various concentrations in methanol and water extracts. Water extract was the most effective against *S. aureus;* this may be due to the water soluble chemical compounds that are present in the stem bark.

**4. Conclusion**

From the above findings it can be concluded that *Z. limonella* possesses some properties related to the traditional claims of this plant.

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**Table 1**: Phytochemical screening of different solvent extract of *Zanthoxylum limonella* bark.

| | Chemical components | | | |
|---|---|---|---|---|
| Bark extracts | Alkaloid | Steroid | Terpenoid | Tannin |
| Hexane | + | - | + | - |
| Ethyl acetate | - | + | + | - |
| Diethyl ether | - | - | + | + |
| Dichloromethane | - | + | + | - |
| Methanol | + | + | - | - |
| Water | + | - | + | + |

(+) Indicates the presence of the respective compound

(-) Indicates the absence of the respective compound

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**Table 2:** Antibacterial activity of different extracts of bark of *Zanthoxylum limonella* by disc-diffusion method.

| | | Size of the inhibition zone (9 mm) | | | | |
|---|---|---|---|---|---|---|
| Tested extract | Conc. mg/disc | *E. coli* | *K. aerogenus* | *P. vulgaris* | *P. aeruginosa* | *S. aureus* |
| Hexane | 100-300 | - | - | - | - | - |
| Ethyl acetate | 100 | 2.2 | 1.0 | 3.1 | 1.1 | 3.3 |
| | 200 | 2.4 | 2.6 | 3.4 | 2.0 | 4.1 |
| | 300 | 3.5 | 3.1 | 4.1 | 3.0 | 5.1 |
| Diethyl ether | 100 | 1.0 | 1.1 | 1.1 | 1.3 | 1.1 |
| | 200 | 2.1 | 2.3 | 2.3 | 2.4 | 2.2 |
| | 300 | 2.3 | 3.0 | 2.4 | 3.2 | 3.2 |
| Dichloromethane | 100-300 | - | - | - | - | - |
| Methanol | 100 | 3.2 | 3.3 | 4.4 | 2.0 | 6.2 |
| | 200 | 4.2 | 4.2 | 4.9 | 2.2 | 7.1 |
| | 300 | 6.2 | 4.0 | 6.4 | 6.1 | 8.8 |
| Water | 100 | 3.4 | 3.0 | 6.2 | 6.4 | 7.1 |
| | 200 | 5.1 | 3.9 | 7.8 | 6.0 | 8.0 |
| | 300 | 5.1 | 4.9 | 6.7 | 3.1 | 9.1 |
| Chloramphenicol 30µg/disc | | 9.2 | 9.3 | 8.8 | 9.0 | 10.2 |
| Streptomycin 30 µg/disc | | 9.0 | 7.0 | 8.0 | 6.0 | 10.0 |

Bacteria: *Escherichia coli, Klebsiella aerogenus, Proteus vulgaris, Pseudomonas aeruginosa* and *Staphylococcus aureus*

Inhibition zone diameters less than the original disc (9 mm in diameter)

(-) Absence of activity

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