Journal of Advanced Medical and Dental Sciences Research

@Society of Scientific Research and Studies

Journal home page: www.jamdsr.com

doi: 10.21276/jamdsr

Index Copernicus value = 82.06

(e) ISSN Online: 2321-9599;

(p) ISSN Print: 2348-6805

Original Research

In vitro antimicrobial activity of fruit extract of *Zanthoxylum armatum* against important bacterial and fungal strains

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ABSTRACT:

Background: Since numerous years antimicrobials are utilized to treat fungal and bacterial infections however their unfavourable impacts have lead researchers to think about their substitutes. Extremely minute examination is done on the antimycotic and antibacterial activity of certain plants is fairly specultative. In villages of Himachal Pradesh *Zanthoxylum armatum* leaves, fruits and stem are utilized to keep up oral hygiene by individuals and their oral hygiene was found comparable to those using other means to clean oral cavity. Aim of the present investigation was to check the antimicrobial activity of *Z.armatum* and to compare it with chlorhexidine and clotrimazole. **Material and methods**: The aqueous and ethanolic concentrates of dried fruits of *Z.armatum* were made and their antimicrobial impacts were analyzed. **Results**: Ethanolic extract was found to have progressive antimicrobial activity against tested bacterial and fungal aqueous extracts. **Conclusion**: Thus it can be concluded that *Z.armatum* has good antibacterial properties so it can be used as an active ingredient in plaque control measures.

Key words: Antibacterial, Antifungal, Medicinal plants, Zarmatum

Received: 10 January, 2020

Accepted: 22 January, 2020

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This article may be cited as: Mahajan A, Aneja T, Sharma A, Swati. In vitro antimicrobial activity of fruit extract of *Zanthoxylum armatum* against important bacterial and fungal strains. J Adv Med Dent Scie Res 2020;8(2):104-106.

INTRODUCTION:

Plants that have natural potentially active ingredients are used to cure disease or reduce pain are known as medicinal plants.¹ Plants have a therapeutic and restorative function in shielding human beings from the adverse impacts of diseases and other complications, thus considered to have a beneficial role in healthcare system. Due to this large percentage of inhabitants of the developing countries still rely on herbal medicines. In spite of their significance, therapeutic plants are only sometimes taken care within an organized way and many of them are exploited with practically no regard for the future.² Noteworthy boost in usage of medicinal plants is seen constantly for both traditional users and pharmaceutical industry. Medicinal plants give

chances for biological screening, techniques valuable for industry and trends in the pharmacological examination of natural products. Plants have been examined broadly yet at the same time vast number of them have not touched base to the traditional health care system.^{3,4} In recent times because of advancement of modern and new complex techniques, plant researchers are keen in doing new investigations and checking out new medications from natural and biologically active compounds of the plants, which could serve as an inexhaustible resources for pharmaceutical industries.⁵

Zanthoxylum armatum is a standout amongst the most important therapeutic plants in Indian medicinal Literature. Practically all parts of this plant are utilized in Indian traditional framework for the treatment of different diseases.⁶

Zanthoxylum armatum belongs to family Rutaceace. In common language it is called as Indian Prickly Ash, Toothache tree, Nepal Pepper. Traditional names of this plant are: Tejphal (Hindi), Mukthrubi (Manipuri) Tejowati (Sanskrit) and Timur (Nepal). It is widely distributed in India. It is also found in most places of Bhutan, China, Japan, Taiwan, Nepal, Philippines, Pakistan and Malaysia at altitudes of 1,300-1,500 m. Valleys and thickets in the mountains, wasteland and the under-storey of mixed forest are customary locations of the species. The locals of North America smash the bark and apply on their gums for subsequent relief therefore it is known as the toothache tree.⁷ On reviewing its antimicrobial activity present research was done to evaluate the antimicrobial activity of Z.armatum fruit extract against important bacterial and fungal strains.

MATERIAL AND METHODS:

The fresh and healthy fruits of *Z.armatum* were collected from nearby areas. Plants were collected on the basis of the information provided in the ethnobotanical survey of India. Each specimen was labelled, numbered and noted with date of collection.

Table1: showing strain, culture media and agar used.

In laboratory the fruits were washed with distilled water to remove soil or dust particles from them. Further cleaning of fruits was done with ethanol. The fruits were shade dried for ten days and grinded in electric grinder. 20mg of powder was mixed in 100ml of distilled water and ethanol separately with sonicator. Extracts were kept for 48 hours and then filtered using whatman filter paper No. 4. Both the extracts were heated over water bath for 5-6 hours till they become viscous. Antimicrobial activity was performed by disc diffusion method. All the microbial strains used in the study were procured from Institute of microbial technology (IMTECH), Chandigarh, India. Table 1 shows the details of strain, culture media and agar used. 20 petriplates were taken for each microorganism. 20ml of agar was poured into sterile petriplate. After solidification 100 µl of microbial inoculum was swabbed on respective plates. Five wells were punched over the agar plates using sterile well puncher. The punched wells were filled with 100 µl of plant extracts. 0.2% chlorhexidine, clotrimazole, ethanol and distilled water taken as controls. Plates were incubated at 37°C for 24 hours. After incubation zones of inhibition for extracts were measured using vernier caliper.

Microorganism	Microbial type culture collection	Culture media	Agar used
Streptococcus mutans	MTCC 890	Brain heart infusion broth	Brain heart infusion agar
Lactobacillus acidophilus	MTCC 10307	Lactobacillus infusion broth	Lactobacillus MRS agar
Candida albicans	MTCC 854	Sabouraud dextrose broth	Sabouraud's dextrose agar
Candida tropicalis	MTCC 184	Sabouraud dextrose broth	Sabouraud's dextrose agar

RESULTS:

Table 2: mean zone of inhibition (mm)

Extract	Streptococcus mutans	Lactobacillus acidophilus	Candida albicans	Candida tropicalis
	Mean ±S.D	Mean ±S.D	Mean ±S.D	Mean ±S.D
Zanthoxylum ethanol	15.2 ±1.17	12.8 ±1.1	21.8 ±2.2	24.8 ±2.11
Zanthoxylum water	11.05 ±2.26	8.4 ±2.1	8.4 ±1.1	10 ±1.98
Ethanol	10.5 ±1.17	6.2 ±0.18	5.5 ± 1.18	5 ±0.88
Chlorhexidine	17.9 ±2.38	17.4 ±1.1	NA	NA
Clotrimazole	NA	NA	30 ±1.12	30 ±1.27
Distilled water	-	-	-	-
P value	0.00	0.00	0.00	0.00

DISCUSSION:

The unsystematic use of commercial antimicrobial drugs utilized for management of infectious diseases has prompted development of multiple drug resistance so there is a requirement for more up to date and powerful therapeutic agents. Medicinal plants not only have the enormous therapeutic potential but are also free from the side effects caused by synthetic antimicrobials.8 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.⁹ Many of the plants used today were known to the people of ancient culture throughout the world for their preservative and medicinal powers.⁸

Tiwary M et al¹⁰, found linalool (57%) and limonene (19.8%) as major component of essential oil of *Zanthoxylum armatum*. Monoterpenes like linalool and limonene are responsible for the antimicrobial property of this plant. The various alkaloids, flavonoids, flavonal glycosides, lignins, phenolics, sterols, terpenoids, fatty acids, alkenicacids, amino acids, various aromatic and volatile and number of other compounds have also been identified and isolated from Z. armatum essential oil in good quantity.¹¹ The antifungal activity of essential oil is generally due to linalool content.¹²

In present study highest zone of inhibition was observed in ethanol extract against *Candida tropicalis* (24.8mm) followed by *Candida albicans* (21.8mm), *Streptococcus mutans* (15.2mm) and *Lactobacillus acidophilus* (12.8mm). Ethanolic (Z.armatum) extract was found to have more antimicrobial activity against tested bacteria and fungi than aqueous extract (Z.alatum). Antibacterial activity of ethanolic extract was found to be even more than the positive control (chlorhexidine) whereas, antifungal activity of ethanolic extract was less than the positive control (clotrimazole).

Srivastava N et al⁸ in their study used the Z. armatum bark against four different bacterial strains. Highest zone of inhibition 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.

CONCLUSION:

Thus, it can be concluded that Z.armatum has good antibacterial properties so it can be used as an active ingredient in plaque control measures. The present study is a scientific validation of the traditional use of this plant. After further studies it can be incorporated as a constituent in mouthwashes and toothpastes.

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