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EVOLUTION OF ANTI-BACTERIAL ACTIVITY OF *ZANTHOXYLUM OVALIFOLIUM* WIGHT (RUTACEAE). AGAINST SELECTED PATHOGENIC BACTERIA

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Abstract

The present study explores antibacterial study of fruit extracts of *Zanthoxylum ovalifolium Wight.* The plant *Zanthoxylum ovalifolium* belongs to Rutaceae family. The antibacterial activity of the extracts was screened against five bacterial strains viz., *E. coli, P. syringae, Klebsiella pneumoniae, S. aureus and P. aeruginosa*. Antibacterial activity was conducted by the agar well diffusion method. The extract showed varies levels of antibacterial activity on different test bacteria. The zone of inhibition was determined against the microorganisms and the effects of these extracts were compared to standard drug amoxicillin.

Key words: Antimicrobial activity, *Zanthoxylum ovalifolium* Wight, Bacterial strains.

Introduction

The existence of livelihood is very difficult on earth without plants. Plants and plant products are very essential and plays valuable role in human world and helps to maintain human health. Throughout the life man was completely depending on plants for his basic needs. Medicinal plants produce hundred to thousand varieties of chemical compounds which help in the healing of many disorders and disease which in term shows their medicinal action toward the patient. Hence, the herbal and aromatic plants have been used for the purpose of medicine long before prehistoric period and attainting significant role in health care. Whole population of the world depends on the plant-based medicine due to their therapeutically activity against ailments. Plants have their own diverse number of bioactive compounds, which find the wealthy solution for many countries to treat many diseases like diarrhea, cancer, inflammation, pain, rheumatism, cold, cough, jaundice etc. (Gangola *et al*., 2017). According to the report on hand by World Health Organization (WHO), that about 80% of people from developing countries have been using medicinal plants to meet their primary health care requirements.

Zanthoxylum ovalifolium belongs to family Rutaceace. The genus *Zanthoxylum* is the largest genus consisting of about 250 species (Arun and Paridhavi. 2012). Many species of this genus have been largely studied and used in the traditional systems of medicine to cure many diseases such as cholera, colic asthma, cancer, snakebite, cold, microbial infections, diabetes, cough, fever, headache, and toothache (Medhi *et al.,* 2013) On study, the species of *Zanthoxylum* have studied antimicrobial, anti-inflammatory, analgesic, antimalarial, and cytotoxic properties (Poornima *et al*., 2018; Nidhi *et al.,* 2013; Marquez *et al.,* 2005; Alam *et al.,* 2017; Islam *et al.,* (2014).

The plant *Zanthoxylum ovalifolium* commonly called as thorny yellow wood. aromatic shrub or small tree, about 1- 6m tall, it grows in dense dry deciduous to ever green forests throughout Western Ghats, India, in Karnataka distributed in Chikmagalur, Dakshina and Uttara Kannad, Kodugu, Shimoga. Leaves of plant simple, 3- foliolate, alternate, gland dotted, Spines present on the lower surface of leaf, dense paniculate cyme inflorescence and Fruits capsule with number of oil dots.

The survey of literature showed that, no antibacterial work has been done on plant *Zanthoxylum ovalifolium* fruit. The present study evaluated the antibacterial activity of *Zanthoxylum ovalifolium* fruit extract.

Materials and Methods

Collection and identification plant material

The fresh sample of *Zanthoxylum ovalifolium* Fruit were collected in the month of July-August 2018 in the field of different regions of Agumbe and Sringeri belongs to Shimoga and Chikkamangaluru district, Karnataka. The plant were authenticated, prepared with voucher specimen KU/AB/RN/PP/002/2017 and deposited in the department of Botany Kuvempu University, Shanakaragatta.

The collected plant material were washed thoroughly with water to take away of extraneous waste and was shade dried about two weeks and mashed with the help of electric blender.

Processing and extraction

About 250gms of powdered plant fruit material was extracted by hot extraction method using soxhlet apparatus. The extraction method was carried out using different solvent based on increasing polarity viz., hexane, ethyl acetate and methanol solvents. Solvents were

Fig. 1: fruits of *Zanthoxylum ovalifolium*.

Table 1: Percentage yield of different solvent extract from 250gm of powdered *Zanthoxylum ovalifolium* fruit in 800ml of solvent.

Solvent name	% Yield of extract in grams
Hexane	2%
Ethyle acetate	1.2%
Methanol	1.6%

Fig. 2: Shows extract obtained from different solvent of fruit.

Fig. 3: Extraction process using soxhlet apparatus.

Table 2: Antibacterial activity of different solvent extract of *Zanthoxylum ovalifolium* fruit against two pathogenic bacteria.

S. No.	*Zanthoxylum ovalifolium*	Bacterial strains	Inhibition zone in mm			Ciprofloxacin (standard)
			25(µg/ml)	50(µg/ml)	100(µg/ml)	1(µg/ml)
1	Hexane	*Klebsiella pneumoniae*	1.33±2.3	10.66±2.08	14±1.73	17.66±1.52
		S. aureus	0.33±0.57	1.66±2.88	10±0	19±1
2	Ethyl acetate	*Klebsiella pneumoniae*	10.33±0.57	14±0	15±1	17.66±1.52
		S. aureus	8.66±2.3	13.66±4.04	16±1.73	19.33±0.5
3	Methanol	*Klebsiella pneumoniae*	6.33±1.15	12±0	13±1	17.6±1.52
		S. aureus	6.66±1.15	13.33±0.57	14.66±2.51	19.66±0.57

evaporated under reduced pressure and stored in a glass bottle at °C for use (Dabur *et al.,* 2004). (R. Bhatia *et al., 2010*).

$$Percentage\ yield = \frac{Dry\ weight\ of\ extract}{Dry\ weight\ of\ plant\ material} \times 100$$

(R. Bhatia *et al., 2010*)

Screening for antibacterial activity:

Selection of Bacterial Strains:

Antibacterial activities of the fruit extracts of *Zanthoxylum ovalifolium* was screened against clinical isolated Gram positive and Gram negative pathogen bacterial strains *viz., Staphylococcus aureus* and *Klebsiella pneumonia* were stored in department of Applied Botany, Kuvempu University, shankaraghatta.

Inoculum Preparation:

Liquid culture medium was prepared by adding different volume of (0.6%) peptone, (0.15%) yeast extract and Sodium hydroxide (0.36%) solvents in the distilled water and this solution sterilized in an autoclave at 151Lbs and 120^{0}C of temperature. The loopful bacterial strain form slant culture was inoculated into 10ml of sterile nutrient broth and the cultured was maintained at 28^{0}C ± 2^{0}C. The culture was swabbed over the Muller Hinton Agar (MHA) plates. Determination of susceptibility by using medicinal plant extracts. (Divya *et al.,* 2018: H.S. Shubha *et al.,* 2010).

Agar well radical diffusion assay:

The antibacterial activity of *Z. ovalifolium* fruits extract was evaluated by using standard agar well radial diffusion method (Bauer AW, *et al.,* 1966) against one positive (*Staphylococcus aureus*) and one negative (*Klebsiella pneumonia)* pathogenic bacteria. The sterilized nutrient agar medium was poured into sterilized glass petri plates. Liquid broth containing 100 µl of 24 h previous bacterial cultures was spread separately over the solid nutrient agar media plates and it was punched by using sterilized cork borer of size about 6mm diameter. Each well was loaded with 25 µL leaf extract of different solvent (Hexane, Ethyl acetate and Methanol) and concentration like, 25, 50, 100 µg/ml and negative control (DMSO) and standard drug 1µg/ml ().The culture plates were incubated at 37°C for 24 h. the experiment was triplicate for each extract (Thippeswamy *et al.,* 2011)

Statistical analysis:

Agar well diffusion data of three replicate of each extraction, positive and negative control were analyzed by using statistical analysis and result expressed as mean ± SD.

Results and Discussion

In the present study, dried fruit powder was extracted by soxhlet method using 800ml different solvent such as hexane, ethyl acetate and methanol solvent. Each and solvent showed good value yield extraction whereas, hexane solvent produce highest percentage of yield extract about 2% when compared to the rest of solvent such as, Methanol solvent gives 1.6% of extract and ethyl acetate solvent was 1.2% of extract respectively (Table 1).

Nowadays treatment of disease with synthetic drug shows several problems which intern leads to many problems such as side effects, very expansive in its price and causing resistance to antibiotics. Hence naturally available active compounds from herbs or medicinal plants

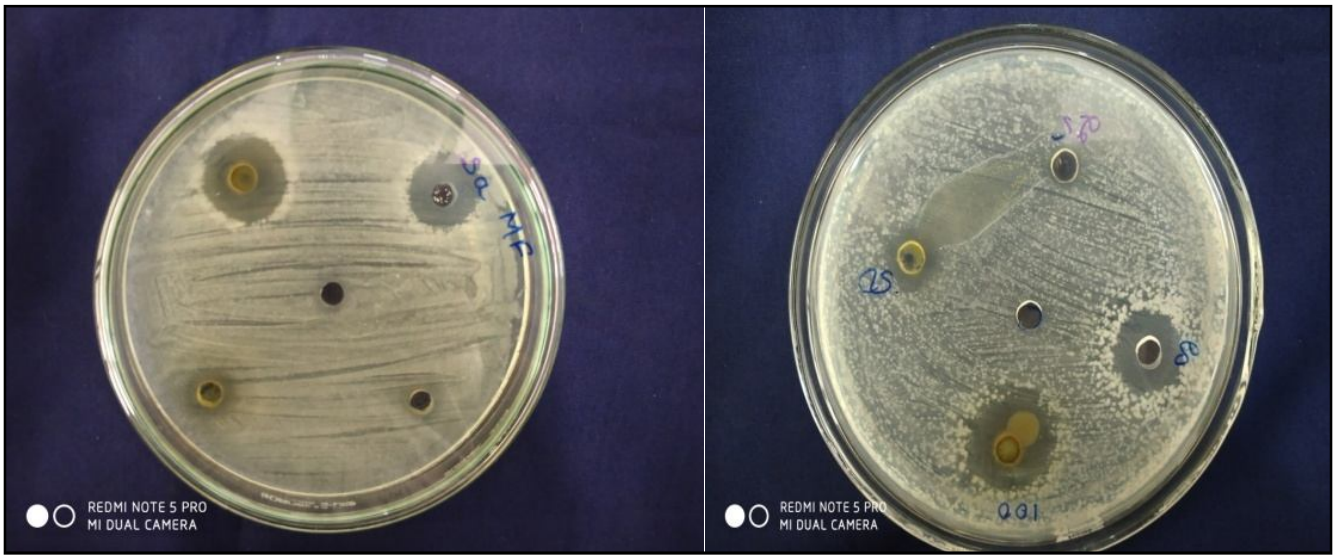

Fig. 4: Showed antibacterial activity against selected pathogenic bacteria.

help to cure many ailments. for that reason, there is a need of quality research to discover plant based medicine to treat many diseases.(YAVUZ1. C *et al.,* 2017). The plant *Z. ovalifolium* fruit of different solvent extract were evaluated for antibacterial activity against *K. pneumoniae* and *S. aureus* pathogenic bacteria. All tested solvent extract exhibits antibacterial activity the zone of inhibition range from 0.33 to 16mm of diameter (Table 2).

Among the three solvent extracts *viz.,* hexane, ethyl acetate and methanol fruit extract of *Z. ovalifolium, the* methanolic extract showed highest growth inhibition against *K. pneumonia and followed by S. aureus* bacterial was observed at highest concentration(100mg/mL) *i.e.,* 16 and 15mm. Moderate zone of inhibition was observed at 100mg/mL of methanol extract of fruit against S. aureus and K. pneumonia *i.e.* 14.66 and 13mm. Similarly hexane exhibits varied zone of inhibition against K. pneumonia and S. aureus about 14 and 10 mm at higher concentration 100mg/mL. likewise potent antibacterial activity was observed in some other Zanthoxylum species such as, the significant antibacterial activity of different solvent extract was observed in plant Zanthoxylum alatum against pathogenic bacteria such as Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus, and Escherichia coli. (Guleria. S *et al.,* 2013**)** and Zanthoxylum tingoassuiba (Costa S.R *et al.,* 2017) showed significant antimicrobial activity when compared with standard drug.

Conclusion

The present showed that, the plant *Zanthoxylum ovalifolium* is important medicinal plants which showed considerable antibacterial activity of different solvent extract against gram positive and gram negative bacteria. Therefore, from the present study, it is confirmed that the fruit extract of *Z. ovalifolium* has antibacterial property and further work is need to identify bioactive compounds which responsible for pharmacological action towards microbial disease.

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