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### ***In vitro* Antibacterial Activity of Tuber Extracts of *Zehneria scabra***

<sup>1</sup>M. Kandhasamy and <sup>2</sup>K.D. Arunachalam

<sup>1</sup>Department of Microbiology, PGP College of Arts and Sciences,  
Namakkal, Tamil Nadu, India

<sup>2</sup>School of Biotechnology, SRM University, Chennai, Tamil Nadu, India

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**Abstract:** In the present study, the antibacterial activity of six different extracts of tuber of *Zehneria scabra* was evaluated by agar diffusion method in Muller-Hinton agar medium. The crude ethanolic and methanolic extracts inhibited the growth of all the pathogenic bacteria used in the present study. Benzene and chloroform extracts of *Z. scabra* exhibited moderate antibacterial activity, where as petroleum ether and hexane extracts showed the least antibacterial activity. Petroleum ether and hexane extracts inhibited the growth of *Bacillus subtilis* and *Staphylococcus aureus*, respectively. The gram positive bacteria were more susceptible to all the crude extracts screened for its antibacterial activity. *Pseudomonas aeruginosa*, gram negative and multidrug resistant bacteria was sensitive to almost all the extracts, but *E. coli* was more susceptible to the crude extracts and showed higher antibacterial activity by producing the higher diameter of inhibitory zone. The inhibitory zone diameter produced by *E. coli* was ranged from 10 to 14 mm. The crude extracts have greater potential and can be used for controlling the growth of methicilin resistant bacteria *Staphylococcus aureus* and multi drug resistant nosocomial pathogen *Pseudomonas aeruginosa*.

**Key words:** Medicinal plants, *Zehneria scabra*, tuber extracts, pathogenic bacteria, antibacterial activity

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### **INTRODUCTION**

India has long tradition of using the herbal medicine for management and treatment of diseases both in humans and livestock. The traditional medicine is mainly based on the information and knowledge of the plants used, part of plants used and doses, which are passed on from generation to generation.

Higher plants play an important role in providing new remedies and in some cases, they are important sources for old remedies. According to Van *et al.* (1997), 50% of the drugs used in the clinical treatment are derived from the plant sources.

More recently antitumor and anticancer drugs are derived from leaves of *Pterocarpus indicus*, species of *Taxus* (Yao and Hu, 2001) and rhizome of *Dioscorea colletti* (Van *et al.*, 1997). These discoveries indicated the values of higher plants and its importance in the field of medicine. Higher plants produce a variety of substances like small peptides, unsaturated long chain of aldehyde, alkaloids, essential oils and phenol as the secondary metabolites.

These secondary metabolites act as chemotherapeutic, bactericidal, bacteriostatic agents (Evans *et al.*, 1986; Purohit and Bohra, 1998) and they provide important sources for new molecules and provide basic structure for synthesizing new drugs (Tomoko *et al.*, 2002; Nair *et al.*, 2005).

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**Corresponding Author:** Dr. M. Kandhasamy, Centre for Marine and Coastal Studies, School of Energy Sciences, Madurai Kamaraj University, Madurai, Tamil Nadu, India Tel: +91-9865518261

Infectious disease causing pathogenic bacteria are now becoming resistant to drugs, due to indiscriminate use of antimicrobial drugs. It becomes a greater problem of giving treatment against the pathogenic bacteria (Sieradzki *et al.*, 1999). More over the cost of the drugs are high and also produce adverse effect on the host, that include hypersensitivity, depletion of beneficial microbes in the gut (Idose *et al.*, 1968).

Due to these reasons plants herbal medicines are reexamined for their potential resources of new drugs (Cowan, 1999) and the plants, which are used in the traditional medicines reexamined with hope of findings, new or improved medication (Grierson and Afolayan, 1999).

Tubers, corm and rhizome are the modification of stem. The main function of the modified stem is storage of food materials, especially carbohydrates. The modified stems play important role in folk and traditional medicine, from the ancient days. Corms of *Hypoxis hemerocallidae* (Hypoxidaceae) used to treat the urinary bladder disorders, including Benign Prostatic Hyperplasia (Van *et al.*, 1997) and also in rheumatoid arthritis, immune system disorders and tuberculosis (Watt and Breyer-Brandwijk, 1962). The yacon tubers contain fructan. The microbes present in the intestine have the ability to ferment fructan and converted it into  $\beta$  glucon and it acts as non-specific immune stimulators and also influences the intestinal microflora and modifies the hyperlipidemias.

Tubers are also rich in phenolic compounds such as catechins, epicatechins, chlorogenic acid, leucoanthocyanins and anthocyanins (Imbert and Seaforth, 1998) and flavonoids compounds that act as antimicrobial compounds. The phenolic compounds undergone biotransformation through oxidation reaction. The biotransferred compounds act as antioxidants (Farombi *et al.*, 2000).

Tuber used in the present study was used by the tribal of Holli malai, for different purpose. They are using infusion of tuber for jaundice, decoction for piles and fever, juice for cough and fever and dry powder paste for skin afflictions like sores, burns, cut etc. Based on the information, the present work was undertaken to find out the antibacterial activity of the crude extracts, obtained from the tuber of *Zehmaria scabra*, using six different organic solvents.

## MATERIALS AND METHODS

### Plant Collection and Processing

The material used in the present study was a tuber. The tubers were collected from the Kolli malai, Namakkal district, Tamil Nadu, India. The plants were collected during October, 2006 and identified, using the herbarium specimens available at Rapinat Herbarium (RPT), St. Joseph College, Trichirapalli. Tubers were washed with sterile-distilled water and cut into small pieces and air-dried at room temperature (28°C). After complete drying the pieces of tubers was ground to powder using an electric blender.

### Solvents

The organic solvents such as ethyl alcohol, methanol, petroleum ether, hexane, benzene and chloroform were used for extraction of the bioactive compounds. They were purchased from Merck Company. Nutrient agar, Nutrient broth were used to culture the pathogenic bacteria and Muller-Hinton agar medium was used to assess the antibacterial activity. They were purchased from Hi-Media Company, Chennai.

### Microorganisms

Bacteria causing infectious diseases both in animals and humans were used in the present study. They were clinical isolates and brought from Apollo Hospital, Madurai. They were both gram negative and gram positive. Eight gram negative bacteria were *Escherichia coli*, *Klebsiella pneumonia*,

*Salmonella typhi*, *Salmonella paratyphi A*, *Salmonella paratyphi B*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Serratia* sp. and two gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* were used in the present study.

#### **Extraction Procedure**

Twenty five gram of powder of *Zehmaria scabra* was weighed and mixed with respective solvent individually in the ratio of 1:6. They were macerated and kept in the temperature (28°C) for 72 h. The mixture was stirred at every 24 h using sterile glass rod separately. Then each mixture was filtered through the Whatmann No. 1 filter paper and the filtrate were concentrated in vacuum rotary evaporator to reduce the volume. The residue was dissolved (3 mg/10 mL) in respective solvents and it was used for assessing the antibacterial activity.

#### **Preparation of Antibiotic Discs**

Sterile empty antibiotic discs (6 mm in diameter) were purchased from Hi-Media Company, Chennai. Ten microliter (containing 30 µg of the extract) of respective solvent extract were added to the discs individually and aseptically and allowed to dry. After drying discs were used to assess the antibacterial activity.

#### **Preparation of Inoculum**

Pure culture of each bacterial pathogen was taken from the nutrient agar medium and transferred to the nutrient broth and incubated at 37°C for 24 h. An inoculum size of  $10^8$  cfu mL<sup>-1</sup> was prepared. 0.1 mL culture corresponding to  $10^5$  cfu mL<sup>-1</sup> was used to assess the antibacterial activity.

#### **Assay of Antibacterial Activity**

Antibacterial activity was assessed by agar diffusion method (Bauer *et al.*, 1966). For assessing the antibacterial activity of each crude extract (10 µL/disc-containing 30 µg extract) containing disc, chloramphenicol disc (30 mcg/disc) as positive control and disc-impregnated with respective solvent (10 µL/disc), as negative control were used for assessing antibacterial activity.

## **RESULTS**

The antimicrobial screening of different extracts of tuber *Z. scabra* on *E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, *S. typhi*, *S. paratyphi A*, *S. paratyphi B*, *S. marcescens*, *S. aureus* and *B. subtilis*, shown in the Table 1. It was observed that almost all extracts inhibited the growth of any one of the pathogenic bacteria tested in the present study and significant variation in their antibacterial activity was also observed. Among the pathogenic bacteria gram positive bacteria were more sensitive against all extracts used in the present study (Table 1).

The growth of the all the pathogenic bacteria used in the present study was inhibited by the crude ethanolic and methanolic extracts of the tubers of *Z. scabra*. Even though ethanolic and methanolic extract showed higher antibacterial activity and variation in the activity between the solvent extracts were also observed. The crude ethanolic extract of *Z. scabra* showed higher antibacterial activity than methanolic extracts (Table 1). It inhibited all pathogenic bacterial growth and produced zone of inhibition and its diameter ranged from 7-13 mm.

The extracts of benzene and chloroform showed moderate antibacterial activity, variation in antibacterial activity between the two extracts were observed. Chloroform extract inhibited the growth of all the pathogenic bacteria except *P. mirabilis*, *S. typhi*, *S. paratyphi B* and *S. marcescens*. Benzene extracts did not inhibited the growth of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *S. typhi* and *S. paratyphi B*. Both extracts were active on inhibiting the growth of *S. aureus* and *B. subtilis* (Table 1).

Table 1: Antibacterial activity of crude extracts of tuber of *Zehmaria scabra* against pathogenic bacteria

Species	Inhibition zone diameter (mm) solvents used for extraction						
	Positive control chloramphenicol (30 mcg/disc)	Ethanol	Methanol	Pet. ether (30 µg/disc)	Hexane	Benzene	Chloroform
<i>E. coli</i>	14±0.0	14±0.5	13±0.6	ND*	ND	ND	10±0.2
<i>K. pneumoniae</i>	9±0.0	10±0.3	9±0.3	ND	ND	ND	7±0.4
<i>P. mirabilis</i>	15±0.0	10±0.2	8±0.2	ND	ND	ND	ND
<i>P. aeruginosa</i>	11±0.0	9±0.3	9±0.4	ND	ND	8±0.4	9±0.3
<i>S. typhi</i>	11±0.0	7±0.4	7±0.6	ND	ND	ND	ND
<i>S. paratyphi A</i>	10±0.0	8±0.1	8±0.3	ND	ND	10±0.2	10±0.2
<i>S. paratyphi B</i>	10±0.0	9±0.2	7±0.2	ND	ND	ND	ND
<i>S. marcescense</i>	12±0.0	8±0.4	7±0.1	ND	ND	10±0.2	ND
<i>S. aureus</i>	10±0.0	14±0.4	12±0.5	ND	11±0.6	7±0.4	9±0.3
<i>B. subtilis</i>	12±0.0	14±0.5	11±0.2	7±0.2	ND	9±0.5	10±0.4

ND\* : Antibacterial activity not detected

The crude petroleum ether and hexane extracts showed the least antibacterial activity, which were active only against the gram positive bacteria *S. aureus* and *B. subtilis*. Petroleum ether extract inhibited the growth of *B. subtilis*, whereas *S. aureus* growth was inhibited by hexane extract. The crude hexane extract was more active on inhibiting *S. aureus* and produced zone of inhibition and its diameter was 12 mm (Table 1).

The gram-positive bacteria were sensitive against all the crude extract. Among the gram-positive bacteria *S. aureus* was more sensitive against all the extracts and the diameter of the inhibition zone ranged from 7-14 mm. Among gram-negative bacteria *P. aeruginosa* was more susceptible to ethanolic, methanolic, benzene, chloroform extracts. But *E. coli* was more susceptible and showed higher antibacterial activity by producing the inhibitory zone of diameter, ranged from 10 to 14 mm (Table 1).

## DISCUSSION

In India large section of people, especially in villages using the herbal medicine to combat the infectious diseases and disorders. Gradually people move towards the traditional medicine. The reason for that is, trusts on herbal medicine, which improve the diseases conditions, after the herbal medicine treatment. No side effect or fewer side effects is observed due to herbal medicine. Another reason is the cost of the drugs and cost of the treatment is low. People in developing countries now prefer the herbal medicine.

The present study revealed that ethanolic and methanolic extract tuber of *Z. scabra* inhibited the growth of both gram positive and gram-negative bacteria (Table 1). It may be due to the reason that tuber contains different 197 types of polyphenolic compounds and flavanoids, that are pharmacologically important (Coursey, 1967) and act as antimicrobial agents (Farombi, 2003).

From the study it was observed that gram-positive bacteria were more sensitive against all extracts used in the present study. Gram positive bacteria such as *S. aureus* (except pet. ether extract) *B. subtilis* (except hexane extract) growth was inhibited by all the extracts (Table 1). The present study was well corroborated with the studies of Rabe and Van Staden (1997), Kokoska *et al.* (2002) and Kelmanson *et al.* (2000). According to their studies medicinal plants that were used to screen the antibacterial activity were more active against the gram-positive bacteria than gram negative. The crude extract of tuber of *Dioscorea dregeana* and the tuber bark of *D. sylvatica* inhibited the growth of the gram-positive bacteria (Kelmanson *et al.*, 2000). The general observation was that gram-negative bacteria were more resistant to antibiotics than gram-positive bacteria (Paz *et al.*, 1995). The resistance is due to the different in their cell wall structure and their composition. In gram-negative

bacteria the outer membrane acts as a barrier to many environmental substances including antibiotics (Tortora *et al.*, 2001). Presence of thick urine layer in the cell wall prevents the entry of the inhibitors (Martin, 1995).

It was also observed from the study that all the crude extracts of the tuber *Zehmaria scabra* inhibited the growth of the any one of the bacterial pathogens tested (Table 1). Kelmanson *et al.* (2000) also observed the similar results. They explained reasons for inhibition of growth of any one of the bacteria used in their study. The reason is that the tubers have constant contact with soil. The tuber produces different types of antimicrobial substances in response to the infection of the soil pathogens that infect the tubers. Those substances are used for self-defense and inhibited the growth of the soil pathogens.

In the present study, two important findings were observed. First the gram negative bacteria *P. aeruginosa* was inhibited by all the solvents extract, except petroleum ether and hexane extracts. Generally *P. aeruginosa* was said to be multi drug resistant, nosocomial pathogen. Secondly the growth of *K. pneumoniae* was inhibited by the ethanolic, methanolic and chloroform extracts of the tuber of *Z. scabra*. Such observations are important and deserve further study in order to isolate and purify the bioactive compounds responsible for inhibiting the growth of multi drug resistant bacteria *P. aeruginosa*.

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