Research Article
www.ijrap.net

ANTAGONISTIC MICROBIAL SCREENING OF SHOOT EXTRACTS OF *ZEHNERIA SCABRA* (L.F.) SONDER

S.P. Anand[1]*, A. Doss[1] and R. Jeyachandran[2]
[1]PG & Research Department of Botany, National College (Autonomous), Tiruchirappalli, Tamil Nadu, India
[2]Department of Plant Biology and Plant Biotechnology, St. Joseph's College (Autonomous), Tiruchirappalli, Tamil Nadu, India

Received on: 22/10/2011 Revised on: 09/01/2012 Accepted on: 20/01/2012

***Corresponding author**
Dr. S.P. Anand, Assistant Professor, PG & Research Department of Botany, National College (Autonomous), Tiruchirappalli - 620 001, Tamil Nadu, India. E-mail: dranandsp@gmail.com

ABSTRACT
Zehneria scabra is a climber belongs to the family Cucurbitaceae, which has a wide traditional uses. The plant powder is used as curative agent of headache and stomachache when applied externally. Shoot paste also cures skin rashes and itches. Fruits are reported to cure stomachache. Ethanol, methanol, ethyl acetate, chloroform and aqueous extracts of the shoot were tested against various pathogenic bacteria by the disc diffusion and streak plate methods. The shoot extracts displayed higher activity against the Gram-positive *Staphylococcus aureus* rather than the other bacteria tested. *Zehneria scabra* shoot parts elevated range of inhibition capability against *Staphylococcus aureus* being one of the casual organisms for skin diseases.
Key Words: Microorganisms, Medicinal plant, Cucurbitaceae, antimicrobial, disc diffusion,

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world. They have been used as a source of medicine. The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas and the Bible, has been traced to the occurrence of natural products with medicinal properties. In fact plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines.

Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. The effects of plant extracts on bacteria have been studied by a number of researchers in different parts of the world[1]. It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumour and antimicrobial agents[2]. The selection of crude plant extracts for screening programmes has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural product[3].

Such one of the important medicinal *Zehneria scabra* (L.f.) Sonder attracts greater attention because it's high medicinal value both in herbal folklore practices and also the lack of adequate information on the nature of bioactive principle and its therapeutic action. The plant has enormous ethnobotanical value, as used by tribes for various treatments such as stomach pain, fever and skin diseases etc. It acts as an important medicine for livestock in various ailments. *Zehneria scabra* acts as an important medicinal plant as illustrated by the tribes of Bodamalai and foot hills people. Fruits are reported to cure stomachache. Tribal people used the root of *Zehneria scabra* to hang in front of their house believing that it will prevent the entry of disease causing pathogens. Root of the plant is used with milk in fever and diarrhoea[4]. The antibacterial activities in root extract of *Zehneria scabra* have been studied by Anand *et al*.,[5]. Present antibacterial research work focus on the above medicinal plant using various bacterial pathogens by different methods especially in the shoot samples.

MATERIALS AND METHODS

Plant materials

The healthy shoot portions of *Zehneria scabra* were collected and spread out shade dried in the laboratory at room temperature for seven days. Once completely dry, these were ground to a fine powder using an electronic blender and it was stored in a closed container at room temperature.

Tested bacteria

Eight bacterial species were tested. These bacterial strains were collected from microbial type culture collection, Institute of Microbial Technology, Chandigarh, Punjab, India. The bacteria such as *Staphylococcus aureus, Shigella dysenteriae, Bacillus subtilis* (Gram positive Bacteria); *Escherichia coli, Salmonella typhi*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Enterobacter faecalis* (Gram negative bacteria).

Preparation of extracts

Fifty grams of the dried shoot powder were soaked separately with 300 ml of each of the solvents viz. ethanol, methanol, ethyl acetate, chloroform and hexane in a Soxhlet apparatus for 48 hr until complete extraction of the materials. At the end of 48 hr, each extract was filtered through Whatman No.1 filter paper and filtrates were concentrated at room temperature in order to reduce the volume. The paste like extracts was stored in pre-weighed screw caped bottles and the yield of extract has been weighed. These screw cap bottles were kept in refrigerator at 4° C. Each of the extract was individually reconstituted and the required extract was diluted for use. Aqueous extracts were prepared by using 50 grams of dried powdered plant materials were extracted with 300 ml of sterile distilled water (1:6 w/v). The aqueous extract

was maintained in a Soxhlet apparatus over 48 hr, filtered and concentrated.

Media preparation

The readymade Nutrient agar and Nutrient broth medium were used for these studies. Required amount of Nutrient agar and Nutrient broth were weighed and dissolved in distilled water made into 1000 ml and autoclaved at 121° C for 15 minutes.

Methods of antibacterial screening

The antibacterial screening of the aqueous and other organic solvent shoot extracts of *Zehneria scabra* were investigated through different methods such as disc diffusion method[6] and streak plate method[7].

RESULTS AND DISCUSSION

Disc diffusion method

The antibacterial sensitivity of the plant extract was observed using the disc diffusion method by measuring the diameter of the growth inhibition zone. For disc diffusion method, high range of inhibition zone was exhibited against ethanolic and methanolic shoot extracts. The rate of inhibition zones moderate in ethyl acetate and chloroform extracts were followed by hexane and aqueous extracts. In the case of both ethanolic and methanolic shoot extracts of *Zehneria scabra* they revealed similar kind of results, which affected the activity of *Staphylococcus aureus* to a great extent followed by *Salmonella typhi.* Other bacterial strains induced negligible amount of inhibition zones. Streptomycin (antibiotic disc) was maintained as a control (Table 1).

Zehneria scabra ethyl acetate shoot extracts showed high degree of inhibition against *Staphylococcus aureus, Escherichia coli* and moderate inhibition was observed in *Salmonella typhi* and *Enterobacter faecalis.* There was no inhibition against other microorganisms (Table 1). Both chloroform and hexane shoot extracts exhibited less activity against *Staphylococcus aureus* and no satisfactory results against other tested bacteria (Table 1). Aqueous extracts of the same part were found to possess less inhibition against *Staphylococcus aureus* and there was no inhibition against other pathogenic bacteria (Table 1). The concordant result was reported in *Wrightia tinctoria*[8].

Streak plate method

The *in vitro* antibacterial sensitivity of shoot extracts of *Zehneria scabra* against eight pathogenic bacteria by streak plate method is depicted in Table 2 & 3. The data showed that there is a strong inhibition with the increasing percentage of concentration of the shoot extracts of *Zehneria scabra.* All the Gram-positive and Gram-negative organisms tested were sensitive to the crude extracts at the level of 100% concentration. The antibacterial sensitivity was determined by the magnitude of presence or absence of growth. In control plates of each solvent without plant extracts, the growth rate of bacteria was noted to be excessive whereas in experimental plates, the degree of growth inhibition increased with the gradual increase in the concentration of shoot extracts.

Table 1: Antibacterial activity of shoot extract of *Zehneria scabra* against various bacteria [Disc diffusion method]

Microorganisms	Ethanol	Methanol	Ethyl acetate	Chloroform	Hexane	Aqueous
B. subtilis	0.10 ± 0.00	0.10 ± 0.00	–	–	–	–
E. faecalis	–	–	0.10 ± 0.04	–	0.10 ± 0.00	–
E. coli	0.10 ± 0.00	0.10 ± 0.00	0.20 ± 0.04	0.10 ± 0.02	0.10 ± 0.00	0.01 ± 0.00
K. pneumoniae	0.14 ± 0.04	0.10 ± 0.04	–	–	–	–
P.aeruginosa	0.12 ± 0.02	0.10 ± 0.01	–	–	–	–
S. typhi	0.27 ± 0.06	0.21 ± 0.02	0.12 ± 0.02	0.10 ± 0.04	0.10 ± 0.02	0.10 ± 0.00
S. dysenteriae	0.17 ± 0.04	0.10 ± 0.09	–	–	–	–
S. aureus	0.50 ± 0.11	0.45 ± 0.04	0.30 ± 0.02	0.21 ± 0.09	0.15 ± 0.02	0.10 ± 0.02

Values represent diameter of inhibition zone in cm (Mean ± SD)

'–' Represents absence of measurable inhibitory action

Table 2: Growth rate of different bacterial strains against different concentrations of Ethanol and Methanol shoot extracts of *Zehneria scabra* [Streak plate method]

Microorganisms	Concentration (%)									
	Ethanol					Methanol				
	C	25	50	75	100	C	25	50	75	100
B. subtilis	3+	3+	3+	2+	2+	3+	3+	2+	2+	2+
E. faecalis	3+	3+	3+	2+	2+	3+	3+	2+	2+	1+
E. coli	3+	2+	2+	2+	1+	3+	2+	2+	2+	1+
K. pneumoniae	3+	3+	2+	2+	2+	3+	3+	2+	2+	2+
P.aeruginosa	3+	3+	3+	2+	2+	3+	3+	3+	2+	2+
S. typhi	3+	3+	2+	2+	1+	3+	3+	2+	2+	1+
S. dysenteriae	3+	3+	3+	2+	2+	3+	3+	3+	2+	2+
S. aureus	3+	2+	1+	1+	–	3+	2+	2+	1+	–

Table 3: Growth rate of different bacterial strains against different concentrations of Ethyl acetate and Chloroform shoot extracts of *Zehneria scabra* [Streak plate method]

Microorganisms	Concentration (%)									
	Ethyl acetate					Chloroform				
	C	25	50	75	100	C	25	50	75	100
B. subtilis	3+	3+	2+	2+	2+	3+	3+	3+	3+	2+
E. faecalis	3+	3+	3+	2+	2+	3+	3+	3+	2+	2+
E. coli	3+	2+	3+	2+	2+	3+	3+	3+	2+	1+
K. pneumoniae	3+	3+	3+	2+	1+	3+	3+	3+	2+	2+
P.aeruginosa	3+	2+	2+	1+	1+	3+	3+	3+	2+	2+
S. typhi	3+	2+	2+	2+	1+	3+	3+	3+	2+	1+
S. dysenteriae	3+	3+	2+	2+	2+	3+	3+	3+	2+	1+
S. aureus	3+	1+	1+	–	–	3+	3+	2+	1+	1+

Note: Hexane and aqueous extracts showing excessive growth in all concentrations.
C : Control, 3+ : Excessive growth (No inhibition), 2+ : Moderate growth (Partial inhibition), 1+ : Poor growth (Strong inhibition), - : No growth (Complete inhibition)

Hence the higher concentrations *viz.* 75% and 100% strongly or completely inhibit the growth of test bacteria. *Staphylococcus aureus* were susceptible to the shoot extracts (ethanol, methanol, ethyl acetate, chloroform and hexane) at higher concentration *viz.* 75% and 100% where complete inhibition (absence of bacterial growth) was observed. Ethanolic and ethyl acetate shoot extracts were more inhibitory on *Staphylococcus aureus* than the other solvents used (Table 2,3). The growth rate of *Staphylococcus aureus* was controlled by plant species *Hypericum*[9] in earlier report.

The ethanol extract of *Zehneria scabra* effectively inhibited the growth of both the Gram-positive and Gram-negative bacteria. The activity of the aqueous extracts of the plants showed nil activity. There is a general opinion that aqueous extracts do not possess and antibacterial activity against various pathogens as reported earlier by Aburjai *et al*[10].

On the basis of results obtained, it is suggested that the antibacterial principles present in *Zehneria scabra* interact with microbes of both polar and non-polar compounds. These observations suggest that the organic solvent extraction method is better for the isolation of antibacterial compounds. Similar findings and conclusions were drawn by Singh and Singh[11] in their experiments which represent a very good mechanism of biological control of microorganisms. In addition, the antibacterial nature of plant can be attributed not only to a particular compound but also to the cumulative effect of various bioactive substances. Some examples include alkaloids, flavonoids, triterpenoids, thymol and other compounds of phenolic nature which are classified as antimicrobial compounds. The present study showed the efficacy of antibacterial activity exclusively for bacterial pathogens which really shows the presence of biological principles. It leads to the isolation and characterization of the active principles present in the experimental plant.

The results showed the presence of antimicrobial activities agreeing with comparable results of previous researches using extracts of other plant species like *Plumbago zeylanica, Boerhavia diffusa, Tinospora cordifolia, Berberis aristata, Terminalia chebula, Zingiber officinale, Azadirachta indica, Trichosanthes dioica* and *Picrorrhiza kurrooa*[12,13].

These results suggested that the ethanol, methanol and ethyl acetate produced comparatively higher activity than the other solvents. *Zehneria scabra* shoot parts possess the curative property against the skin diseases, which was conformed by the existence of elevated range of inhibition capability against *Staphylococcus aureus* being one of the casual organisms for skin diseases.

REFERENCES

1. Erdogrul OT. Antibacterial activities of some plant extracts used in folk medicine. Pharmaceutical Biol. 2002; 40: 269-273.
2. Vlietinck AJ, Van Hoof L and J. Totte. Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. J. Ethnopharmacol. 1995; 46: 31- 47.
3. Kusumoto IT, Nakabayashi T and Kida H. Screening of various plant extracts used in *Ayurvedic* medicine for inhibitory effects on human immunodeficiency virus type 1 (HIV-l) protease. Phytotherapy Res. 1995; 9: 180-184.
4. Kirtikar KR. and Basu BD. Indian medicinal plants. Vol II. Jayyed press. New Delhi. pp. 1161-1162, 1975.
5. Anand SP, Jeyachandran R and Francis Xavier T. Antibacterial activities of *Zehneria scabra* root extracts. J. Tropical Med. Pl. 2003; 4 (2): 173-179.
6. Maruzella JC and Henry PA. The antimicrobial activity of perfume oils. Journal of American Pharmaceutical Association 28: 471, 1958.
7. Orzechowski G. Antibiotika in hoheren pflanzen. Pharmazie zn unserer zeit. 1: 42-53, 1972.
8. Waghmare BM, Shinde SR and Sumanth GT. Studies of antimicrobial activity of some medicinal plant extracts against pathogenic bacteria and isolated seed borne fungi. J. Microbial World, 2009; 11 (2): 173 -178.
9. Mu Q, Gibbons S, Stavri M, Smith E, Zhou FS and Hu CQ. Antibacterial effects of *Hypericum ascyron* and *Hypericum japonicum* against multidrug-resistant *Staphylococcus aureus*. Pharmaceutical Biol. 2006; 44: 157-159.
10. Aburjai T, Darwish M, AI-Khalil and AI-Abbadi. Screening of antibiotic resistant inhibitors from local plant materials against two different strains of *Pseudomonas aeruginosa*. J. Ethnopharmacol. 2001; 79: 39-44.
11. Singh I. and Singh VP. Antifungal properties of aqueous and organic solution extracts of seed plants against *Aspergillus flavus* and *A. niger*. Phytomorphol. 2000; 50: 151-157.
12. Sohni YR, Padmaja K, Bhatt RM and Kaimal P. Prophylactic therapy of *Salmonella typhi*. Septicemia in mice with a traditionally prescribed drug formulation. J. Ethnopharmacol.1995; 45: 141-147.
13. Prakash P, Kulkarni PH and Paranjpe P. Comparative efficacy of four Ayurvedic formulation in the treatment of *Acne vulgaris*. J. Ethnopharmacol. 1995; 49: 127-132 .

Source of support: Nil, Conflict of interest: None Declared