International Journal of Herbal Medicine
Available online at www.florajournal.com

E-ISSN: 2321-2187
P-ISSN: 2394-0514
IJHM 2017; 5(5): 192-195
Received: 14-07-2017
Accepted: 15-08-2017

Prashith Kekuda TR
Department of Microbiology, S.R.N.M.N College of Applied Sciences, N.E.S Campus, Balraj Urs Road, Shivamogga, Karnataka, India

Vinayaka KS
Department of Botany, Kumadvathi First Grade College, Shimoga Road, Shikaripura, Karnataka, India.

Praveen Kumar SK
Department of Studies in Biochemistry, Karnatak University, Dharwad, Karnataka, India

Correspondence
Prashith Kekuda TR
Department of Microbiology, S.R.N.M.N College of Applied Sciences, N.E.S Campus, Balraj Urs Road, Shivamogga, Karnataka, India

Antimicrobial activity of *Salix tetrasperma* Roxb. (Salicaceae)

Prashith Kekuda TR, Vinayaka KS and Praveen Kumar SK

Abstract
Salix tetrasperma Roxb. belongs to the family Salicaceae. The plant is traditionally used and is reported to exhibit several bioactivities. The present study was carried out to investigate antimicrobial activity of leaf extract of *S. tetrasperma*. Extraction of shade dried and powdered leaf material was carried out by maceration process. Antibacterial activity was evaluated by agar well diffusion method against 4 Gram positive and 4 Gram negative bacteria. Antifungal activity was determined against 4 seed-borne fungi by poisoned food technique. The extract exhibited concentration dependent activity against test bacteria. Among Gram positive and Gram negative bacteria, *S. epidermidis* and *P. aeruginosa* displayed highest susceptibility to extract respectively. Inhibition of test fungi by extract was concentration dependent. Among fungi, marked and least susceptibility to extract was shown by *Rhizopus* sp. and *Cladosporium* sp. respectively. In suitable form, the leaf of *S. tetrasperma* can be used to treat bacterial infections and to manage seed-borne fungal diseases of plants.

Keywords: *Salix tetrasperma*, Maceration, Agar well diffusion, Poisoned food technique

1. Introduction
Antibiotic resistance is a global problem and is a serious and widespread problem in developing countries. Emergence of resistant pathogens is mainly due to indiscriminate use of antibiotics and the resistant pathogens are of serious concern in both hospitals and the community. Bacteria such as *Staphylococcus aureus*, *Mycobacterium tuberculosis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are few among the antibiotic resistant bacteria. Antibiotic resistant bacteria are known limit the efficacy of current drugs and cause high mortality each year due to failure of treatment. This situation triggered immense research on searching for alternatives for disease therapy. Natural products, in particular botanicals, offer as an important alternative strategy for therapy against infectious diseases. Extracts and purified compounds from higher plants have shown to exhibit potent inhibitory activity against a wide range of pathogenic bacteria including antibiotic resistant bacteria [1-7].

Seed is an important input in the production of several crops. Seeds are considered as passive carriers of several pathogenic microorganisms. Fungi such as species of *Aspergillus*, *Helminthosporium*, *Cladosporium*, *Penicillium*, *Rhizopus*, *Fusarium*, *Curvularia*, *Alternaria* and *Cercospora* are associated with seeds. Many seed-borne fungi are known to cause reduction in seed quality, seed abortion, seed rot, reduction of germination ability and seedling damage. Besides, some seed-borne fungi such as *Aspergillus* and *Penicillium* are known to elaborate certain toxins (mycotoxins) which cause severe health hazards on consumption. Fungal diseases of plants are one of the major constraints in successful crop production and are known to cause severe yield loss. The unrestrained use of synthetic fungicides for the control of phytopathogenic fungi and diseases of crop plants resulted in serious threat to human health as well as environment. Extensive use of fungicide is leading to disturbed biodiversity (due to effects on non-target organisms) and development of resistance in the plant pathogens. Hence there is immense scope for searching alternative strategies for diseases control. The use of natural products such as plants and biological control agents appears to be the best alternatives for chemical agents [8-11].

The genus *Salix* comprises about several species mainly distributed in the temperate regions of the world and in higher altitudes of the tropics. *Salix tetrasperma* Roxb., belonging to the family Salicaceae, is a small sized tree characterized by glabrous to silky pubescent twigs and simple, shiny, elliptic-lanceolate leaves with acute apex and characteristic margin. Bark is steel grey in color, farrowed and scaly in appearance. Flowers are unisexual, sessile and are in catkin. Fruit is a 2-valved capsule, dry and dehiscent. The plant is commonly known as Indian willow and often grows along water bodies. The plant is found distributed in various parts of

Karnataka such as Bangalore, Belgaum, Chikamagalur, Shimoga, Mysore, Kodagu, Hassan and Uttara Kannada [12, 13]. The plant is used as fuel wood, and in making furniture, roof, fence and agricultural tools. The twigs are used to make baskets [14-16]. Various parts of *S. tetrasperma* are used traditionally for the treatment of ailments or diseases such as pain, fever, rheumatism, epilepsy, venereal diseases, swellings, piles and bladder stone [17-21]. Several bioactivities such as analgesic [13], anti-inflammatory [13], antipyretic [22], antioxidant [13, 23], antimicrobial [24, 25], hypoglycaemic [26], laxative [27], diuretic [27], cytotoxic [28], insecticidal [24] and CNS activity [29]. The present study was carried out to investigate antibacterial and antifungal activity of solvent extract obtained from leaves of *S. tetrasperma*.

2. Materials and Methods

2.1. Media and chemicals

Three culture media viz. Nutrient agar, Nutrient broth and Potato dextrose agar and chemicals viz. methanol, Streptomycin and Dimethyl sulfoxide (DMSO) were purchased from HiMedia laboratories, Mumbai, India.

2.2. Plant material and extraction

The plant *S. tetrasperma* was collected at Siddarahalli, near Matturu, Shivamogga, Karnataka during January 2017. The plant was authenticated by Prof. D. Rudrappa, Department of Botany, S.R.N.M.N College of Applied Sciences, Shivamogga. The leaves were separated, washed, dried under shade and powdered. Extraction of leaf powder (10g) was carried out by maceration technique using methanol (100ml). The leaf material was left in methanol for two days in stoppered container and later filtered [30]. The leaf extract (LE) obtained was used for antimicrobial studies.

2.3. Test bacteria and inoculum preparation

Four Gram positive bacteria (*Staphylococcus aureus* NCIM 5345, *Staphylococcus epidermidis* NCIM 2493, *Bacillus subtilis* NCIM 2063 and *Bacillus cereus* NCIM 2016) and four Gram negative bacteria (*Klebsiella pneumoniae* NCIM 2957, *Escherichia coli* NCIM 2065, *Pseudomonas aeruginosa* NCIM 2200 and *Salmonella typhimurium* NCIM 2501) were used. The test bacteria were maintained on in nutrient agar slants under refrigeration condition. The test bacteria were seeded into sterile nutrient broth tubes and incubated overnight at 37 ºC.

2.4. Antibacterial activity of LE of *S. tetrasperma*

Agar well diffusion method as employed by Raghavendra *et al*.[31] was used to evaluate antibacterial activity of two concentrations of LE (10mg/ml and 20mg/ml) of *S. tetrasperma*. The method involved swabbing of overnight growth broth cultures of test bacteria on the surface of sterile nutrient agar plates aseptically followed by punching wells of 8mm diameter using a sterile cork-borer. The wells were labeled and filled with 100µl of LE, reference antibiotic (Streptomycin, 1mg/ml of sterile distilled water) and DMSO. The plates were left undisturbed for 24 hours at 37ºC followed by measuring the diameter of inhibition zones formed around the wells.

2.5. Test fungi

Four seed-borne fungi viz. *Cladosporium* sp., *Helminthosporium* sp., *Rhizopus* sp. and *Penicillium* sp. isolated previously from paddy seeds were used. The test fungi were maintained on potato dextrose agar slants in refrigerator.

2.6. Antifungal activity of LE of *S. tetrasperma*

Poisoned food technique was employed to evaluate antifungal potential of LE of *S. tetrasperma*. Potato dextrose agar was prepared, autoclaved, poisoned with LE (0.5 and 1.0mg/ml of medium) and poured into sterile plates. The control (without LE) and poisoned potato dextrose agar plates were inoculated aseptically with well sporulated (7 days old) cultures of test fungi and the plates were incubated at room temperature for 5 days. Later, the diameter of fungal colonies was measured using a ruler. Extent of inhibition of mycelial growth of fungi (%) was calculated using the formula:

Mycelial growth inhibition (%) = (Dc – Dt / Dc) x 100, where Dc refers to colony diameter of fungi in control plates and Dt denotes diameter of fungal colonies in poisoned plates[11].

2.7. Statistical analysis

Antibacterial and antifungal experiments were carried out in triplicate. The results were presented as Mean ± Standard deviation (S.D).

3. Results and Discussion

3.1. Antibacterial activity of LE of *S. tetrasperma*

In the present study, the LE of *S. tetrasperma* was found to exhibit concentration dependent inhibitory activity against test bacteria as noticed by an increase in the diameter of zones of inhibition around wells on increasing in the concentration of extract. LE exhibited varied inhibitory activity against test bacteria. Marked and least inhibitory activity was observed against *S. epidermidis* and *E. coli* respectively. At 10mg/ml concentration, LE was not effective in inhibiting *E. coli*. Among Gram positive and Gram negative bacteria, *S. epidermidis* and *P. aeruginosa* displayed highest susceptibility to extract respectively. Reference antibiotic displayed higher inhibition of test bacteria when compared to LE. Overall, LE as well as reference antibiotic were more active against Gram positive bacteria when compared to Gram negative bacteria. In an earlier study, Islam *et al*.[24] also observed the inhibition of leaf extract of *S. tetrasperma* against Gram positive bacteria while most of Gram negative bacteria remain unaffected. In another study, a herbal formulation containing *S. tetrasperma* was shown to inhibit acne causing *Propionibacterium acnes* and *Staphylococcus epidermidis*[32]. Abdel-Hameed *et al*.[33] evaluated antibacterial activity of leaves of *S. tetrasperma* and observed that the plant was not effective in exhibiting antibacterial activity at 1mg/ml concentration tested. The lower susceptibility of Gram negative bacteria to LE of *S. tetrasperma* and antibiotic could be attributed to the presence of an outer membrane which might have acted as an additional barrier for the entry of LE and antibiotic.

Table 1: Antibacterial activity of leaf extract of *S. tetrasperma*

Test bacteria	Zone of inhibition in cm (Mean± S.D)			
	LE 10mg/ml	LE 20mg/ml	Antibiotic	DMSO
S. aureus	1.03±0.00	1.30±0.00	2.86±0.05	0.00±0.00
S. epidermidis	2.10±0.10	2.66±0.05	3.30±0.00	0.00±0.00
B. cereus	1.73±0.05	2.10±0.00	3.10±0.10	0.00±0.00
B. subtilis	1.60±0.00	1.80±0.10	3.20±0.00	0.00±0.00
K. pneumoniae	1.33±0.05	1.60±0.00	2.53±0.05	0.00±0.00
P. aeruginosa	1.50±0.10	1.70±0.00	2.60±0.00	0.00±0.00
S. typhimurium	1.30±0.00	1.53±0.05	2.50±0.00	0.00±0.00
E. coli	0.00±0.00	1.10±0.00	2.20±0.10	0.00±0.00

3.2. Antifungal activity of LE of *S. tetrasperma*

A considerable reduction in mycelial growth of test fungi was

observed in plates poisoned with LE of *S. tetrasperma*. The LE displayed concentration dependent inhibition of test fungi (Table 2). Among fungi, marked and least susceptibility to extract was shown by *Rhizopus* sp. and *Cladosporium* sp. respectively. At extract concentration of 1mg/ml, an inhibition of >60% of all fungi was observed. At 0.5mg/ml concentration, *Cladosporium* sp., *Rhizopus* sp., *Helminthosporium* sp. and *Penicillium* sp. were inhibited to 25.79%, 39.70%, 27.20% and 33.33% respectively. At 1.0mg/ml concentration, *Cladosporium* sp., *Rhizopus* sp., *Helminthosporium* sp. and *Penicillium* sp. were inhibited to 61.13%, 70.14%, 62.79% and 63.63% respectively. In a similar study, Pushpavathi *et al*.[25] observed marked inhibitory activity of leaf extract of *S. tetrasperma* against seed-borne fungi isolated from sorghum seeds. The study of Abdel-Hameed *et al*.[33] showed no inhibitory activity of leaf extract of *S. tetrasperma* against *Candida albicans* and *Aspergillus niger*. In a study by Deepak *et al*.[34], *S. tetrasperma* failed to show inhibition of zoosporangium formation of *Sclerospora graminicola*, causal agent of downy mildew disease of pearl millet.

Table 2: Colony diameter of test fungi in control and poisoned plates

Test fungi	Colony diameter in cm		
	Control	LE 0.5mg/ml	LE 1.0mg/ml
Cladosporium sp.	2.83±0.05	2.10±0.10	1.10±0.10
Rhizopus sp.	6.80±0.00	4.10±0.00	2.03±0.05
Penicillium sp.	3.30±0.00	2.20±0.10	1.20±0.00
Helminthosporium sp.	4.30±0.10	3.13±0.05	1.60±0.00

4. Conclusion

In the present study, the LE of *S. tetrasperma* exhibited concentration dependent inhibitory activity against bacteria and fungi. The plant appears to be a suitable candidate for developing herbal formulation and lead drugs for the treatment of infectious bacterial diseases and management of plant diseases caused by seed mycoflora.

5. Acknowledgements

Author is thankful to head of the department of Microbiology and Principal, S.RN.M.N College of Applied Sciences and N.E.S, Shivamogga for support. Author also thanks Prof. D. Rudrappa for assisting in the collection and identification of plant material.

6. References

1. Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Reviews 1999; 12(4):564-582.
2. Nascimento GGF, Locatelli J, Freitas PC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Brazilian Journal of Microbiology. 2000; 31:247-256.
3. Srivastava J, Chandra H, Nautiyal AR, Karla SJS. Antimicrobial resistance (AMR) and plant-derived antimicrobials (PDAms) as an alternative drug line to control infections. 3 Biotech. 2014; 4:451-460.
4. Compean KL, Ynalvez RA. Antimicrobial activity of plant secondary metabolites: A review. Research Journal of Medicinal Plants. 2014; 8(5):204-213.
5. Al Laham SA, Al Fadel FM. Antibacterial activity of various plants extracts against antibiotic-resistant *Aeromonas hydrophila*. Jundishapur Journal of Microbiology. 2014; 7(7):e11370.
6. Nagalingam M, Arumugam G, Panneerselvam A. Antimicrobial activity of some Indian folklore medicinal plants against drug resistant bacteria and fungi isolated from clinical samples. Asian Journal of Plant Science and Research. 2015; 5(6):49-56.
7. Wikaningtyas P, Sukandar EY. The antibacterial activity of selected plants towards resistant bacteria isolated from clinical specimens. Asian Pacific Journal of Tropical Biomedicine. 2016; 6(1):16-19.
8. Hasan MM, Chowdhury SP, Alam S, Hossain B, Alam MS. Antifungal effects of plant extracts on seed-borne fungi of wheat seed regarding seed germination, seedling health and vigour index. Pakistan Journal of Biological Sciences. 2005; 8(9):1284-1289.
9. Kumari A, Kumar R, Maurya S, Choudhary JS, Kumar S. Antifungal efficacy of aqueous extracts of neem cake, karanj cake and vermicompost against some phytopathogenic fungi. The Bioscan. 2013; 8(2):671-674.
10. Baka ZAM. Plant extract control of the fungi associated with different Egyptian wheat cultivars grains. Journal of Plant Protection Research. 2014; 54(3):231-237.
11. Kekuda PTR, Raghavendra HL. Antifungal activity of *Helichrysum buddleioides* DC. against seed borne fungi. EC Microbiology. 2017; 6(2):54-59.
12. Manjunatha BK, Krishna V, Pullaiah T. Flora of Davanagere district, Karnataka, India. Regency Publications, New Delhi, India. 2004.
13. El-Shazly A, El-Sayed A, Fikrey E. Bioactive secondary metabolites from *Salix tetrasperma* Roxb. Zeitschrift für Naturforschung. 2012; 67c:353-359.
14. Ahmad H, Khan SM, Ghafoor S, Ali N. Ethnobotanical study of Upper Siran. Journal of Herbs, Spices and Medicinal Plants. 2009; 15:86-97.
15. Narayanan RMK, Mithunlal S, Sujanapal P, Kumar AN, Sivadasan M, Alfarhan AH *et al*. Ethnobotanically important trees and their uses by Kattunaikka tribe in Wayanad Wildlife Sanctuary, Kerala, India. Journal of Medicinal Plants Research. 2011; 5(4):604-612.
16. Ajaib M, Haider SK, Zikrea A, Siddiqui MF. Ethnobotanical studies of shrubs and trees of Agra valley Parachinar, upper Kurram agency, Pakistan. FUUAST Journal of Biology. 2014; 4(1):73-81.
17. Ahmad KS, Qureshi R, Hameed M, Ahmad F, Nawaz T. Conservation assessment and medicinal importance of some plants resources from Sharda, Neelum valley, Azad Jammu and Kashmir, Pakistan. International Journal of Agriculture and Biology. 2012; 14(6):997-1000.
18. Sarwar GAKM. Medicinal plant genetic resources of Bangladesh – genera represented by single species and their conservation needs. Journal of Medicinal Plants Studies. 2015; 3(2):65-74.
19. Zhasa NN, Hazarika P, Tripathi YC. Indigenous knowledge on utilization of plant biodiversity for treatment and cure of diseases of human beings in Nagaland, India: A case study. International Research Journal of Biological Sciences. 2015; 4(4):89-106.
20. Bajpai O, Pandey J, Chaudhary LB. Ethnomedicinal uses of tree species by Tharu Tribes in the Himalayan Terai region of India. Research Journal of Medicinal Plant. 2016; 10(1):19-41.
21. Nitheesh A, Paul A, Fathima KM, Aravind R, Nair SC. Study on medicinal plants in Kozhenchery Taluk, Pathanamthitta district, Kerala. International Journal of Pharmaceutical Sciences Review and Research. 2017; 42(1):274-299.
22. Virupaksha JH, Nadendla RR, Satishkumar M. Evaluation of antinociceptive and antipyretic activities of *Salix tetrasperma* Roxburg. International Journal of

Pharma and Bio Sciences. 2016; 7(3):89-94.
23. Kishore NR, Mangilal T, Anjaneyulu N, Abhinayani G, Sravya N. Investigation of anti-inflammatory and *in vitro* antioxidant activities of hydroalcoholic extract of bark of *Salix tetrasperma* Roxb. International Journal of Pharmaceutics and Drug Analysis. 2014; 2(5):506-509.
24. Islam SM, Zahan R, Nahar L, Alam BM, Naznin M, Sarkar GC *et al*. Antibacterial, insecticidal and *in vivo* cytotoxicity activities of *Salix tetrasperma*. International Journal of Pharmaceutical Sciences and Research. 2011; 2(8):2103-2108.
25. Pushpavathi D, Shilpa M, Petkar T, Siddiqha A, Kekuda PTR. Evaluation of antifungal activity of some plants against seed-borne fungi. Scholars Journal of Agriculture and Veterinary Sciences. 2017; 4(4):155-159.
26. Chhetree RR, Dash GK, Mondal S, Parhi R. Studies on the hypoglycaemic activity of the bark of *Salix tetrasperma* Roxburgh. International Journal of Drug Development and Research. 2010; 2(4):799-805.
27. Sumanta M, Ramana H, Suresh P, Raj CR. Studies on diuretic and laxative activity of the bark of *Salix tetrasperma* Roxburgh. International Research Journal of Pharmacy. 2010; 1(1):145-149.
28. Moustafa SMA, Menshawi BM, Wassel GM, Mahmoud K, Mounier MM. Screening of some plants in Egypt for their cytotoxicity against four human cancer cell lines. International Journal of PharmTech Research. 2014; 6(3):1074-1084.
29. Virupaksha JH, Nadendla RR, Kumar SM, Kavya S. Effect of *Salix tetrasperma* Roxburgh leaf extracts on central nervous system activities. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2017; 7(4):2060-2064.
30. Agbor AM. Methanol extracts of medicinal plants used for oral healthcare in Cameroon. Biochemistry and Pharmacology. 2015; 4:164.
31. Raghavendra HL, Kekuda PTR, Akarsh S, Ranjitha MC, Ashwini HS. Phytochemical analysis, antimicrobial and antioxidant activities of different parts of *Pleocaulus sessilis* (Nees) Bremek (Acanthaceae). International Journal of Green Pharmacy. 2017; 11(2):98-107.
32. Bhatt PC, Sharma S. Anti-Microbial effects of Purodil Gel on acne causing *Propionibacterium acnes* and *Staphylococcus epidermidis*. International Journal of Research in Cosmetic Science. 2014; 4(1):7-9.
33. Abdel-Hameed ES, El-Nahas HA, Abo-Sedera SA. Antischistosomal and antimicrobial activities of some Egyptian plant species. Pharmaceutical Biology. 2008; 46(9):626-633.
34. Deepak SA, Oros G, Sathyanarayana SG, Shetty SH, Sashikanth S. Antisporulant activity of watery extracts of plants against *Sclerospora graminicola* causing downy mildew disease of pearl millet. American Journal of Agricultural and Biological Sciences. 2007; 2(1):36-42.