In vitro Antimicrobial Screening of the Fruit Extracts of Two Syzygium Species (Myrtaceae)

K. Venkata Ratnam and R.R. Venkata Raju

Department of Botany, Sri Krishnadevaraya University, Anantapur 515 003, India

Abstract: The present study carried out to evaluate the antimicrobial properties of Syzygium alternifolium and S. samarangense fruits, against certain bacterial and fungal strains using disc diffusion method. All the test extracts exhibited significant antimicrobial activity on certain pathogens. The petroleum ether extract of S. alternifolium, petroleum ether and methanol extracts of S. samarangense exhibited significant inhibition. The minimum inhibition and minimum bacterial/fungal concentrations were determined by microdilution method using 96-well microtitre plate method. As the disc dosage level increases the inhibitory effect also increased. The extracts were proved as strong inhibitors against Gram negative bacteria than Gram positive bacteria. Among the test extracts S. samarangense found to be effective drug on both Gram positive and Gram negative bacteria.

Key words: Antimicrobial activity • Syzygium alternifolium • Syzygium samarangense

INTRODUCTION

Herbal medicine represents one of the most important fields of traditional medicine. WHO recognized that medicinal plants played an important role in the health care of about 80% of the world population in developing countries and depend largely on traditional medicine [1]. Plant derived products are present in 14 of the 15 therapeutic categories of pharmaceutical preparations that are currently recommended by medicinal practitioners and they form an important part of the health care system in the western world [2]. It is estimated that about 75% of the 120 biologically active plant derived compounds, presently in use world wide, have been derived through follow up researchers to verify the authenticity of data from folk and ethnomedical uses. So, there is a great scope for new drug discoveries based on traditional plant uses [3]. There is a need to establish the pharmacological activities for identifying and comparing the various crude drugs for potency.

Syzygium alternifolium (Wt.) Walp. (Myrtaceae) is an endemic aromatic tree, distributed in Assam and Andhra Pradesh states of India. Locally it is known as mogi/ movi. The plant parts were used in traditional medicine to cure various diseases viz., tender shoots and fruits for dysentery, seeds for diabetes and stem bark was used to treat gastric ulcers [4].

S. samarangense (Blume) Merrill is a deciduous tree, commonly known as samarang apple. It was introduced from Malacca, which is under cultivation in different states of India for their edible fruits. The fruits also used in traditional medicine to cure diabetes.

Several Syzygium species were reported to possess antibacterial [5-7], antifungal [8] and anti inflammatory [9] activities. S. alternifolium was reported to possess hypoglycemic and antihyperglycemic activity [10] and flavonoid constituents [11]. The flavonoids, isolated from S. samarangense, were reported to possess antihyperglycemic activity [12] spasmylytic [13] and immunomodulatory activity [14]. Based on review of literature, no reports are available regarding to antimicrobial properties of S. alternifolium and S. samarangense. Hence, the present study gains importance to screen the antimicrobial properties of such fruits.

MATERIALS AND METHODS

Plant material: The fruits of S. alternifolium and S. samarangense were collected from different localities of forests of Eastern Ghats, India (June 2004). The specimens were identified with the help of regional and local floras [15,16] and deposited at Sri Krishnadevaraya University Herbarium (SKU), Anantapur.

Preparation of extracts and paper discs: The collected fruits were shade dried, powdered and extracted with petroleum ether, ethyl acetate and methanol using Soxhlet
apparatus for 6 hours. The extracts were filtered and the filtrates were concentrated under reduced pressure at 40°C using a rotoflash evaporator. The crude samples were subjected to antimicrobial screening against the bacteria and fungi.

Known weights of crude extracts (25 and 50 mg/mL) were dissolved in dimethyl sulfoxide (DMSO). Sterilized Whatmann No. 1 filter paper discs of 6 mm diameter were saturated with 20 µL of the extract and allowed to dry at room temperature in laminar air flow bench.

Microorganisms used: The microbial strains viz., *Bacillus cereus* MTCC 1429, *Staphylococcus aureus* MTCC 737, *Escherichia coli* MTCC 1687, *Pseudomonas aeruginosa* MTTC1688, *Klebsiella pneumoniae* MTCC 109 and yeast, *Candida albicans* MTTC 183, were used to test the extracts. The organisms were obtained from the Microbial Type Culture Collection Centre, Institute of Microbial Technology (IMTECH), Chandigarh, India, were used in the study.

Antimicrobial activity: The antimicrobial activity of the extracts was evaluated by disc diffusion method [17]. Previously prepared paper discs containing different concentrations of extracts were placed on the surface of the petriplates, containing 20 mL of respective media seeded with 0.1 ml of previously prepared microbial suspensions (10^5 CFU/mL). Standard antibiotics viz., ampicillin, kanamycin, tetracycline and vancomycin (30 µg/disc) obtained from Hi-media, Mumbai, were used as positive controls. The discs containing petroleum ether, ethyl acetate and methanol served as negative controls. The assessment of antimicrobial activity was based on measurement of inhibition zones formed around the discs. The plates were incubated for 24 h at 37°C and the diameter of the inhibition zones was recorded. Three independent trials were conducted for each concentration.

The minimum inhibitory concentration (MIC) was determined, using a common broth micro dilution method in 96-well microtiter plates [18, 19]. Two fold dilutions of each extract were carried out, starting from 16 to 0.062 mg/mL. 10 µL of the previously prepared different microbial suspensions (10^5 CFU/mL) were added to each well. Plates were incubated for 18 h at 37°C and then were examined with Elisa reader (TECAN, Sunrise, China) at 620 nm and the lowest concentration of each extract showing no growth was taken as its minimum inhibition concentration (MIC). The solution DMSO (100 µL/mL) served as the negative control. All the samples were tested in triplicates to confirm the activity.

The minimum bacterial/ fungal concentration (MBC/MFC) was determined by adopting standard methods [20,21]. To determine MBC, 10 µL of broth medium from each well of MIC tested plate was taken and incubated in Nutrient agar at 37°C, for 24 h for bacteria or in Sabouraud Dextrose agar at 30°C, for 48 h for the yeasts. The least concentration showing no visible (except one or two colonies) growth on agar sub culture was taken as MBC/MFC value. This is the lowest concentration, expressed in mg/mL. Each test was performed in triplicates and repeated twice and results were tabulated.

RESULTS AND DISCUSSION

Data on *in vitro* antimicrobial properties of two *Syzygium* species were depicted in Table 1, 2, 3 and 4. Present observations revealed that the test extracts of *S. alternifolium* and *S. samarangense* possessed good antimicrobial activity against both bacteria and fungi.

Among the test extracts of *S. alternifolium*, petroleum ether extract exhibited maximum inhibition zones (9-13 mm) against the pathogens, except *Staphylococcus aureus* and *Candida albicans*, while the ethyl acetate and methanol extracts showed moderate inhibition zones (6-10 mm) (Table 1). In the present investigation, Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*) were more susceptible than Gram positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*). As the disc dosage level increases the inhibitory effect also increased. Pulla Reddy et al., (2005) isolated two flavonoids viz, eucalyptin and tephrowatsin and a terpenoid, friedelin from *S. alternifolium*. Eucalyptin was reported to possess antimicrobial activity [22], while friedelin reported to possess antileishmanial activity [23]. So, the broad spectrum of antimicrobial activity observed in present study appeared to be due to the individual or combined effect of the above mentioned chemical constituents.

Minimum inhibitory concentration values of 250 to 500 µg/mL were obtained for petroleum ether and methanol extracts, while 250 µg/mL for bacteria and 1000µg/mL for yeast, was recorded for ethyl acetate extract (Table 2). A Gram negative bacterium, *Pseudomonas aeruginosa* showed complete inhibition at 4-mg/ml concentration as minimum bacterial concentration (MBC) to petroleum ether and ethyl acetate extracts. *Klebsiella pneumoniae* was completely inhibited by ethyl acetate extract (4 mg/mL). The methanol extract expressed MBC/MFC values, 8 mg/mL for all tested strains.
All the test extracts of *S. samarangense* possess significant antimicrobial activity against the pathogenic microbial strains. Interestingly both Gram positive and Gram negative bacteria were sensitive to *S. samarangense* fruit extracts. Among the three extracts, the methanol extract showed a higher activity than other extracts. This may be due to the solvent extract containing different constituents having antimicrobial activity. Methanol was proved as the most effective solvent for extracting broad spectrum of antimicrobial compounds from plants [24].

The petroleum ether extract of *S. samarangense* showed maximum inhibition (MIC 125 µg/mL) against Gram negative bacterium *Klebsiella pneumoniae*, but *Bacillus cereus* (Gram positive) and *Klebsiella pneumoniae* were completely inhibited by the same extract (MBC 4 mg/mL). The methanol extract exhibited similar results on tested pathogens, where as the ethyl acetate extract exhibited strongest inhibition against Gram negative bacterial strains viz., *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (MIC 125 µg/mL; MBC 4 mg/mL for each).

In conclusion, the present observations confirm the presence of antimicrobial principles in all examined crude drugs. Among the test extracts *S. samarangense* found to be effective drug on both Gram positive and Gram negative bacteria, while *S. alternifolium* showed good antimicrobial activity on bacteria. The observations confirm the folk uses of these crude drugs and justify the ethnobotanical approach in the search for novel bioactive compounds.

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REFERENCES


