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Short Communication

ANTIMICROBIAL ACTIVITY OF *SYZYGIUM CALOPHYLLIFOLIUM* WALP LEAF EXTRACT

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ABSTRACT

Objective: To evaluate the antimicrobial activity of methanol extract of *Syzygium calophyllifolium* Walp. leaf against the gram-negative bacteria, namely, *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella typhi*, *Proteus miriabilis* and *Pseudomonas aeruginosa,* the gram-positive bacteria *Bacillus cereus*, *Bacillus subtilis*, *Sarcina lutea*, *Staphylococcus aureus* and *Bacillus megaterium,* three fungal species strains, commonly causing systemic infections in immune compromised patients such as *Candida albicans*, *Aspergillus niger* and *Saccharomyces cerevisiae*.

Methods: The dried leaves were ground finely and extracted in methanol for 48 h at room temperature (26 °C-28 °C). This was then filtered using Whatman No.1 filter paper. The different concentration of (5 µl, 10 µl, 15 µl) methanol extract of leaves of *S. calophyllifolium* was investigated *in vitro* by an agar diffusion method and the MIC by macro-broth dilution method in the present study. The antibacterial antibiotics Ampicillin (10µ/ml), Ofloxin (1 mg/ml) and the antifungal antibiotics Nyastatin (20µg/ml), Tobramycin (10µ/ml) were used as positive controls.

Results: The largest zone of inhibition was noted against *E. coli* (25±mm in diameter) and *S. cerevisiae* (25±mm in diameter) at the highest concentration (15 µl). *B. cereus, K. pneumoniae, P. aeruginosa* and *E. coli* showed the highest MIC (10±mg/ml) and MBC (20±mg/ml).

Conclusion: The result revealed that the methanol extract of *S. calophyllifolium leaf* has a broad spectrum of antimicrobial activity.

Keywords: *Syzygium calophyllifolium*, *Eugenia calophyllifolia,* Myrtaceae, Antimicrobial activity, Minimum inhibitory concentration

For a long period of time, plants have been a valuable source of natural products for maintaining human health. The use of plant extracts and phytochemicals, both of known antimicrobial properties, can be of great significance in therapeutic treatments [1]. Nowadays, an increasing number of infectious agents are becoming more resistant to commercial antimicrobial compounds [2]. The antimicrobial activity of plant extracts has formed the basis of many applications. These include their raw and processed potential as natural agents in food preservation, pharmaceuticals, alternative medicine and natural therapies [3, 4]

The necessity to develop new drugs requires varied strategies, among them, the bio prospection of secondary metabolites produced by medicinal plants [5, 6]. Among the different plant derivatives, secondary metabolites proved to be the most important group of compounds that showed a wide range of antibacterial and antifungal activity [7, 8]. The resistance that pathogens build against antibiotics and the oxidative stress caused by free radicals has sparked interest in the search for new antibacterial and antioxidant compounds also from nature [9-12].

Many plants from the family Myrtaceae have been investigated for their antimicrobial activity. But there is no much investigation made on the methanol extract of leaves of *S. calophyllifolium.* In this present study the antimicrobial activity of methanol extract of *S. calophyllifolium* Walp. (*Eugenia calophyllifolia* Wt) leaf against the gram-negative bacteria, *E. coli*, *K. pneumoniae*, *S. typhi*, *P. miriabilis* and *P. aeruginosa*, the gram-positive bacteria, *B. cereus*, *B. subtilis*, *S. lutea*, *S. aureus* and *B. megaterium,* three fungal species strains such as *C. albicans*, *A. niger,* and *S. cerevisiae* was investigated by an agar diffusion method.

The sample leaves of *S. calophyllifolium* were collected from Doddabetta, The Nilgiris, Tamilnadu. The leaves were washed with tap water and dried under shade to a constant weight. The dried leaves were ground finely with the help of mixer grinder. From which varying amounts were taken and extracted in methanol for 48 h at room temperature (26 °C-28 °C). This was then filtered using Whatman No.1 filter paper.

Bacterial strains, representing common human bacterial pathogens were used for the antibacterial screenings. The gram-negative bacteria *E. coli*, *K. pneumoniae*, *S. typhi*, *P. miriabilis* and *P. aeruginosa* and the gram-positive bacteria *B. cereus*, *B. megaterium*, *B. subtilis*, *S. lutea*, *S. aureus*. Three species of fungal strains such as *C. albicans*, *A. niger,* and *S. cerevisiae* (Department of Microbiology, RVS Arts and science, Coimbatore) were also chosen since it causes severe meningoencephalitis in immune compromised patients.

An agar diffusion method [13-15] was used to screen the plant for antibacterial, and antifungal activities of the selected plant extract and the antibacterial and antifungal activity were followed based up on the work of [16, 17]. In brief, in the beginning of the experiments, the bacterial or fungal strains to be used were inoculated on Nutrient agar and Saboraud agar respectively, and grown for 24 h. A small amount of the culture was transferred to sterile isotonic sodium chloride, and the turbidity was measured spectrophotometrically at 625 nm. The suspensions were diluted in 0.9 % (w/v) NaCl to an absorbance of 0.1 at 625 nm. 240 µl of a diluted suspension of bacteria/fungi was inoculated on agar dishes containing Isosensitest and Saboraud agar for the bacteria and fungi respectively. Sterile Whatman filter paper discs (Ø = 12.7 mm) containing different concentration (5µl, 10µl, 15µl) of extracts were applied to the dishess. Alternatively, steel cylinders (Ø = 12.7 mm) or holes (Ø = 12.7 mm) board in the agar loaded with different concentration (5µl, 10µl, 15µl) of extracts were used. The petri dishes were kept in room temperature for one hour prior to incubation in the dark at+35 °C for 24 h. The diameter of the inhibition zones was measured after incubation and the results were expressed as the mean of 5-25 diameters. Ampicillin (10µ/ml) and Ofloxin (1 mg/ml) (Sigma-Aldrich Chemicals, USA) were used as positive controls for the bacteria. For the fungal species Nyastatin (20 µg/ml) and Tobramycin (10 µ/ml) (Sigma-Aldrich Chemicals, USA) were used as positive controls.

The minimum inhibitory concentration (MIC) was determined by macro-broth dilution techniques as specified by National Committee for Clinical Laboratory Standards [18]. A twofold serial dilution of the reconstituted extract was prepared in Mueller-Hinton Broth. Each dilution was seeded with 100 µl of the standardized suspension of the test organism (1 × 106 cfu/ml) for Gram-positive bacteria and (5 × 105 cfu/ml) for Gram-negative bacteria and

incubated for 24 h at 37 °C. MIC was determined as the highest dilution (i.e. lowest concentration) of the extract that showed no visible growth.

MBC was determined by selecting tubes that show no growth during MIC determination, and a loop full from each of the tubes was subcultured on the Mueller-Hinton Agar. The plates were incubated for 24 h at 37 °C. The MBC was determined as the least concentration that showed no visible growth [18].

Data were presented as the mean±SD (n = 5). Statistical analyses used one-way analysis of variance (ANOVA) to account for the different treatments and were complemented with unpaired t-test. Differences were considered statistically significant at P<0.05 [19].

In this present investigation, the anti-microbial activity of methanol leaf extract of *S. calophyllifolium* was evaluated using disc diffusion method. The test extract possesses a significant antibacterial activity against some gram-positive and gram negative of human pathogens. The extract of different concentrations showed moderate to good antibacterial activity, and the largest zone of inhibition (25±0.08 mm in diameter) was recorded at the concentration of 15 µl against *E. coli* followed by *P. aeruginosa* (22±0.07 mm in diameter). Antibacterial antibiotics Ampicillin (10 µ/ml) and Ofloxin (1 mg/ml) were also found to be active against all the bacterial tested in the present study (table 1).

The antifungal activity was tested against some fungal species, and the higher zone of inhibition was noted at the concentration of 15 µl against *S. cerevisiae* (25±0.24 mm in diameter) followed by *C. albicans* (22±0.38 mm in diameter). The lower zone was recorded in *A. niger* (10 mm in diameter). The activity of standard Antifungal antibiotics Nyastatin (20 µg/ml) and Tobramycin (10µ/ml) were also recorded (table 2).

The result of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was recorded (table 3). The result showed that *B. cereus, K. pneumoniae, P. aeruginosa* and *E. coli* had the highest MIC (10 mg/ml) and MBC (20 mg/ml) while *S. lutea* showed the lowest MIC (4 mg/ml) and MBC (8 mg/ml). *B. megaterium, B. subtilis* and *P. miriabilis* had the MIC (5 mg/ml) and MBC (10 mg/ml). *S. typhi* showed the MIC (8 mg/ml) and MBC (10 mg/ml).

Table 1: Antibacterial activity of *S. calophyllifolium* extract

S. No.	Bacterial species	Zone of inhibition (mm)				
		***S. calophyllifolium* extract concentration**			**Known drug concentration**	
		5 µl	**10 µl**	**15 µl**	**Ofloxin (1 mg/ml)**	**Ampicillin (10µ/ml)**
1.	*Staphylococcus aureus*	8±0.08	10±0.20	15±0.08	15±0.22	20±0.11
2.	*Sarcina lutea*	5±0.10	8±0.07	10±0.14	10±0.10	15±0.28
3.	*Bacillus megaterium*	9±0.06	11±0.25	15±0.13	15±0.22	20±0.37
4.	*Bacillus subtilis*	5±0.08	10±0.13	15±0.18	10±0.20	18±0.25
5.	*Bacillus cereus*	10±0.11	15±0.10	20±0.07	15±0.24	20±0.09
6.	*Pseudomonas aeruginosa*	8±0.05	16±0.13	22±0.07	10±0.39	15±0.11
7.	*Salmonella typhi*	10±0.10	10±0.20	20±0.40	15±0.21	20±0.20
8.	*Klebsiella pneumoniae*	10±0.20	15±0.10	15±0.06	10±0.14	20±0.24
9.	*Proteus miriabilis*	5±0.13	10±0.11	10±0.10	10±0.11	15±0.14
10.	*Eschrichia coli*	15±0.16	20±0.38	25±0.08	15±0.23	20±0.06

Each value is expressed as mean±SD (n=5)

Table 2: Antifungal activity of *S. calophyllifolium* extract

S. No.	Fungal species	Zone of inhibition (mm)				
		***S. calophyllifolium* extract concentration**			**Known drug concentration**	
		5 µl	**10 µl**	**15 µl**	**Nyastatin(20µg/ml)**	**Tobramycin(10µ/ml)**
1.	*Saccharomyces cerevisiae*	15±0.32	20±0.06	25±0.24	15±0.37	25±0.40
2.	*Aspergillus niger*	5±0.39	10±0.08	10±0.32	10±0.08	15±0.06
3.	*Candida albicans*	15±0.11	20±0.07	22±0.38	15±0.14	15±0.20

Each value is expressed as mean±SD (n=5)

Table 3: The MIC and MBC regimes of the *S. calophyllifolium* extract at the concentration of 15 µl (15 mg/ml)

S. No.	Bacterial species	MIC (mg)	MBC (mg)
1.	*Bacillus megaterium*	5±0.37	10±0.08
2.	*Sarcina lutea*	4±0.06	8±0.10
3.	*Bacillus cereus*	10±0.40	20±0.13
4.	*Bacillus subtilis*	5±0.06	10±0.23
5.	*Staphylococcus aureus*	ND	ND
6.	*Klebsiella pneumoniae*	10±0.07	20±0.25
7.	*Salmonella typhi*	8±0.08	10±0.11
8.	*Pseudomonas* aeruginosa	10±0.39	20±0.28
9.	*Proteus miriabilis*	5±0.24	10±0.13
10.	*Escherichia coli*	10±0.38	20±0.32

Each value is expressed as mean±SD (n=5), MIC-Minimum inhibitory concentration, MBC-Minimum bactericidal, concentration, ND-Not determined

The medicinal property of herbs is due to the presence of different complex chemical substance as secondary metabolites, which are exclusively accumulated in different parts of the plants [20]. Successful prediction of chemical compounds largely depends on the type of solvents used for extraction. The earlier study proved that the antimicrobial activity of methanol extract of *Ocimum americanum, Syzygium cumini, Murraya koeningii, Lawsonia inermis, Eucalyptus maculate, Azardirecta indica, Tridax procumbens* and *Adathoda vasica* against *Escherichia coli* and *Staphylocaoccus aureus* [21].

The methanolic extract of *Psidium guajava* (Myrtaceae) showed toxicity against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Vibrio cholera* [22]. Similarly, it was found that in the present study the methanol extract of provide more consistent antimicrobial activity. The aqueous and methanol extracts of the leaves of *Syzygium cumini* (Myrtaceae) exhibited the antibacterial activity against some gram-positive and gram-negative bacteria [23]. In their study, the methanol extract was more active than the aqueous extract and the highest zone of inhibition were noted in *Salmonella typhi*.

The antibacterial activity of *Eucalyptus camaldulensis* (Myrtaceae) was investigated against few gram positive and gram negative bacteria [24]. The methanol leaf extract of *E. camaldulensis* showed greater activity against *Salmonella typhi, Staphylococcus aureus, and Bacilllus subtilis.* In this present study, the methnol extract of *S. calophyllifolium* showed the largest zone of inhibition against *E. coli*. Therefore, many plants from the family Myrtaceae showed the effective antimicrobial activity and the plant *S. calophyllifolium* showed no exception. It may be concluded from the present study that the methanoloic leaves extract of *Syzygium calophyllifolium* revealed antimicrobial activity against some gram positive, gram negative and few fungal species pathogens.

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CONFLICT OF INTERESTS

Declared none

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