

Antibacterial Activity of Leaf Methanolic Extract of *S. Caryophyllatum* (L.) Alston against Human Pathogenic Microorganisms

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Abstract: *S. caryophyllatum* (L.) Alston belongs to the family Myrtaceae is an endangered species. It possesses traditional as well as pharmacological properties. The objective of the present investigation was to find out the antibacterial activity of *S. caryophyllatum* leaf methanolic extract against some human pathogenic bacteria. It was followed by Disc Diffusion method using gram positive and gram negative bacterial strains such as *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus megaterium*, *Sarcina lutea*, *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella* Spp., *Salmonella typhi* and *Proteus mirabilis*. The result showed that the inhibitory effect on *Bacillus subtilis* (24mm) was high when compared to *E. coli* (21mm) and *Bacillus cereus* (20mm). This effect on the bacterial strains may be due to the presence of secondary metabolites present in the leaf methanolic extract of *Syzygium caryophyllatum*.

Keywords: *S. caryophyllatum*, *Bacillus subtilis*, *E. coli*, *Bacillus cereus*.

I. Introduction

Prevention, protection and treatment of various diseases are the three major areas of concern in today's scenario. The microbial infection is one among them due to the production of multi-resistant bacteria in the clinical trials. Scientists are also focusing to treat such diseases without causing any side effects in the human body. WHO also recommended the plant based remedies to treat such diseases. The *Syzygium* species belongs to the family Myrtaceae which possesses various phytochemicals, antioxidant and pharmacological properties. Tsakala et al. (1996) investigated the antibacterial activity of *S. guineense* extract in order to assess its activity on some bacterial strains involved in diarrhoeal diseases [1]. Studies on *S. cumini* and *S. jambos* have shown considerable activity against gram-positive and gram-negative bacteria and fungi [2-3]. Nascimento et al., 2000 obtained a promising antibacterial activity of Jamun against *Klebsiella pneumonia* [4]. This photo activity observed in *S. cumini* is thought to be due to the presence of monoterpene aldehydes [5]. Ahmad and Beg (2001) reported that the gram-positive bacteria are considered to be more sensitive when compared to gram-negative bacteria because, of the differences in their cell wall structures [6]. Rajakaruna et al. (2002) have reported the antibacterial activity of *S. cumini* and indicated the large zones of inhibition against both *S. aureus* and *B. subtilis* [7]. Shafi (2002) reported the antibacterial activity of the leaf essential oils of *S. cumini* and *S. travancoricum* [8].

S. caryophyllatum is an endangered species (Myrtaceae) having very rich amount of phytochemicals antioxidant and hypoglycaemic activities. Not so much informations are presently available for this species in the context of antimicrobial activity. So the objective of the present study was focused on the antibacterial activity of the leaf extract against gram positive and gram negative bacteria.

II. Materials And Methods

2.1 Collection of the plant material

Syzygium caryophyllatum (L.) Alston (Myrtaceae) leaves were collected during the month of October 2009 from Palani Hills, Tamil Nadu, India. It was identified and authenticated by Dr. S. Padmavathy, Associate Professor, Department of Botany, Nirmala College for Women (Autonomous), Coimbatore, Tamil Nadu.

2.2 Preparation of extracts

Freshly collected samples of *S. caryophyllatum* leaves were washed 2-3 times in water followed by distilled water and shade dried. All the dried parts were pulverized by mechanical grinder (Willy mill) to get the powder through 100-mesh sieve and then stored in a refrigerator. The shade dried powdered plant material (250g) was extracted with methanol using soxhlet apparatus. Then the extract was concentrated in a rotary evaporator.

2.3 Antibacterial activity

2.3.1 Strains of bacteria used: The bacterial strains were procured from the Department of Microbiology, PSG institute of Medical Science, Coimbatore, Tamil Nadu, India. Bacteria were maintained on nutrient agar slants and fungi on Potato Dextrose agar slants at 4° C and subcultured monthly.

The following microbial strains were used in the present study. They are gram-positive bacteria such as, *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus megaterium*, *Sarcina lutea* and gram-negative bacteria such as, *Esherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella Spp.*, *Salmonella typhi* and *Proteus mirabilis*.

2.3.2 Determination of antibacterial activity

2.3.2.1 Disc Diffusion Assay [9]

Two colonies of a 24-hour plate culture of each organism were transferred aseptically into 10 ml nutrient and potato dextrose broth in a test tube and mixed thoroughly using an electric shaker for uniform distribution. Petridishes were plated with Nutrient agar and Potato Dextrose agar medium were prepared according to the manufacturer's manual and allowed for 30 minutes to solidify. The test organisms were then spread on the surface of the media using a sterile swap stick. The different concentrations of the plant extracts were (10, 20, 30 & 40mg/ml) placed on the disc (0.7 cm) and then allowed to dry. The control tube contained only test microorganisms not plant extracts. Then the disc was impregnated on the agar plates and chloramphenicol used as reference drug. The plates were then incubated at 28±1°C for 24 h. Microbial growth was determined by measuring the diameter of zone of inhibition.

2.3.1.2 Determination of Minimum Inhibitory Concentrations (MICs) of the extract of *S. caryophyllatum*

The Minimum Inhibitory Concentration (MIC) was determined by adopting the standard reference method NCCLS (2002) [10]. The extracts were dissolved in 2% dimethyl sulfoxide (DMSO). The stock solution of each extracts was serially diluted in 96-well microtiter plate with Mueller Hinton broth to obtain a concentration ranging from 8 mg/ml to 0.125 mg/ml. A standardized inoculum for each bacterial strain was prepared so as to give an inoculum size of 10⁵ CFU/ml in each well. Chloramphenicol was used as a standard antibiotic for comparative analysis with the effectiveness of various extracts against tested clinical isolate and drug resistant bacteria. Microtiter plate was kept at 37°C and incubated for 24 h. Following incubation, the MIC was calculated as lowest concentration of the extracts inhibiting the visual growth of the test cultures on the agar plate.

2.4 Statistical analysis

All the data were subjected to Duncan's Multiple Range Test (DMRT) was done by using the SPSS version 2007 WINSAT software.

III. Result

In the present study, the leaf methanolic extract of *S. caryophyllatum* were tested against gram-positive bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus megaterium*, *Sarcina lutea* and gram-negative bacteria such as *Esherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella spp.*, *Salmonella typhi* and *Proteus mirabilis*. The results were observed in the form of zone of inhibition, which is tabulated in the table 1. The present study shows a concentration dependent level of inhibition. The zone of inhibition was varied in between 7- 24 mm at 40mg concentration of the extract. The highest diameter zone of inhibition was noticed at 40mg concentration against *Bacillus subtilis* (24mm) followed by *E. coli* (21mm) *Bacillus cereus* (20mm). The other bacterial strains exhibits moderate activity against the extract in the following order *S. aureus* > *Salmonella typhi* and *Bacillus megaterium* at 40mg. At the lowest concentrations of the extracts (10 mg and 20 mg), the *Sarcina lutea*, *Klebsiella spp.* and *Proteus miriabilis* showed resistant to the extract. So there is no inhibition at the lowest concentration of the extract. On the other hand, a trace amount of inhibition was also noticed in the highest concentration of the same. All these results showed more activity when compared to the standard drug Chloramphenicol. It was also subjected to get the MIC against the tested organisms and it was found to be 0.125mg/ml for *Bacillus subtilis*. Thus, MIC assay are capable of verifying that the compound has antibacterial activity and that it gives reliable indication of the concentration of drug required to inhibit the growth of microorganisms.

From the above results, it is clear that the leaf extracts exhibits average antibacterial activity against the gram-negative bacteria when compared to the gram-positive bacteria. And also it is evident that the inhibition was concentration dependent. More susceptibility was noticed in the higher concentration of the tested organism of *B. subtilis* when compared to the other organisms and also standard drug.

Table 1. Antibacterial Activity Of Methanol Extract Of *S. Caryophyllatum* Against Clinically Important Microbial Pathogens

Microorganisms	Zone of inhibition (mm)				Synthetic drug Chloram- phenicol (10mg)	MIC mg/ml
	<i>S. caryophyllatum</i> leaf extract					
	Concentrations (mg/ml)					
	10	20	30	40		
Gram Positive Bacteria						
Control	-	-	-	-	-	-
<i>Staphylococcus aureus</i>	11±0.01	13± 0.01	17±0.001	19±0.002	14±0.4	1
<i>Bacillus cereus</i>	11±0.01	12±0.03	16±0.002	20±0.001	13±0.03	0.500
<i>Bacillus subtilis</i>	10±0.1	12±0.01	19±0.020	24±0.001	17±0.03	0.125
<i>Bacillus megaterium</i>	8±0.02	10±0.01	13±0.001	17±0.002	15±0.1	4
<i>Sarcina lutea</i>	R	R	R	7±0.01	14±0.4	-
Gram Negative Bacteria						
<i>Escherichia coli</i>	9±0.01	15±0.02	19±0.01	21±0.02	12±0.03	0.250
<i>Pseudomonas aeruginosa</i>	7±0.01	10±0.01	12±0.02	14±0.02	14±0.02	8
<i>Klebsiella Spp.</i>	R	6±0.0	9±0.04	10±0.05	11±0.01	-
<i>Salmonella typhi</i>	11±0.00	14±0.01	17±0.002	18±0.05	14±0.05	12
<i>Proteus mirabilis</i>	R	R	R	R	10±0.02	-

IV. Discussion

The present study reveals that the methanol leaf extract of *S. caryophyllatum* showed antibacterial activity against all tested microorganisms with MIC values. From the available literatures it should be understood that root, bark, seeds, fruits and leaves of *Syzygium* species possess antibacterial properties. Also the studies revealed that methanol was the most effective solvent for extracting antibacterial compounds from the selected seaweeds [11]. This may be due to the solvent to extract the different constituents having antimicrobial activity. Similar observations were noticed in the *Syzygium jambolanum* seeds [13]. Phytochemical constituents such as alkaloids, flavonoids, tannins, phenols, saponins, and several other aromatic compounds are secondary metabolites of plants that serve as a defense mechanism against many microorganisms, insects and other herbivores [13]. So it is confirmed that the observed antibacterial effects is believed to be due to the presence of secondary metabolites.

V. Conclusion

The *Syzygium* species possesses antimicrobial properties against tested human pathogenic bacteria due to the presence of secondary metabolites present in the extracts. Eventhough *S. caryophyllatum* showed antibacterial activity, further studies are needed to identify and isolate the compound irresponsible for such activity.

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