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Research Article

ANTIMICROBIAL ACTIVITY OF *SYZYGIUM JAMBOS* AGAINST SELECTED HUMAN PATHOGENS

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ABSTRACT

The aim of this study is to evaluate the antimicrobial efficacy of *Syzygium jambos* (L) Alston (Myrtaceae) against eight different microorganisms such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Vibrio cholera*. *S. jambos* is a widespread medicinal plant traditionally used in India to treat infectious diseases. Aqueous and acetone extracts of bark, leaves and seeds of *S. jambos* were tested for antimicrobial activity *in vitro* by the agar well diffusion method in petri dishes. Both extracts showed some activity against the tested microorganisms. Among the three different parts, aqueous extracts of bark have exhibited a minimum inhibitory effect against *S. aureus*, *E. coli* and *S. typhi*, whereas seeds inhibited the growth of *P. aeruginosa* and *V. cholerae*, and leaves exhibited inhibitory effect only against *S. typhi*. Among the acetone extracts, bark was found to be effective against all the test microorganisms, leaves inhibited only *S. aureus*, whereas seed extracts failed to exhibit any inhibitory effect against the test organisms. These properties seem to be related to the high tannin content of *S. jambos* in bark (2.5 mg/ml) than seeds (1.9 mg/ml) and leaves (1.4 mg/ml). Overall the acetone extract of bark was found to be more effective. The results of the extracts were compared with the standard antibiotics ampicillin, streptomycin and tetracycline.

Keywords: Syzygium jambos, *Staphylococcus aureus*, *Bacillus subtilis*, Antibiotics, Ampicillin, Streptomycin

INTRODUCTION

Infectious diseases account for high proportion of health problems in the developing countries like India. Microorganism has developed resistance to many antibiotics and this has created immense clinical problem in the treatment of infectious diseases. The resistance of the organism increased due to the indiscriminate use of commercial antimicrobial drugs commonly used for the treatment of infectious diseases. This situation forced the scientist to search for new antimicrobial substances from various sources including medicinal plants [1]. Many of the plants used today were known to the people of ancient culture throughout the world for their preservative and medicinal powers [2]. However several plants are used in India in the form of crude extracts, infusions or plaster to treat common infections without scientific evidence of efficacy [3].

Natural products of plant origin have played significant role in the search of therapeutic drugs such as quinine from cinchone [4]. Search for new antimicrobials is very important in recent time considering the escalating levels of antibiotic resistance among pathogenic bacteria [5, 6]. Many more herbal ingredients are present over the counter drugs such as laxatives. Medicines that come from plants include aspirin from willow bark. Tannin content present in some plants has the ability to act against the microorganisms such as *S. aureus, Salmonella species, E. coli, Pseudomonas species* and so on. Tannin is a general descriptive name for a group of polymeric phenolic substances capable of tanning leather, or precipitating gelatin from solution, a property known as astringency. According to Scalbert, tannin can be toxic to filamentous fungi, yeast and bacteria. Condensed tannins have been determined to bind cell walls of ruminal bacteria [7].

Syzygium jambos (L) Alston (*Eugenia jambos*) is widespread in sub-Saharan Africa (Benin, Democratic Republic of Congo and Cameroon) where its bark is traditionally used to treat infectious diseases [8, 9]. It is also distributed in Reunion Island, Central America (Guatemala) and Asia (Malaysia, Nepal) where fruits are eaten [10]. Besides studies on the fruit volatiles and sugars [11], the only part of the plant chemico-pharmacologically studied was the leaves. Aqueous, methanol, and ethyl acetate extracts of *S. jambos* leaves from Guatemala have been shown to posses anti-inflammatory activity in adjuvant carrageenan induced inflammation model in rats [12]. *S. jambos* may be merely a shrub but is generally a tree reaching 7.5-12 m in height and has a dense crown of slender, wide spreading branches. In India, the fruit is regarded as a tonic for the brain and liver. An infusion of the fruit acts as a diuretic and sweetened preparation of the flowers is believed to reduce fever. The seeds are employed against diarrhea, dysentery and catarrh. In Nicaragua, it has been claimed that an infusion of roasted, powdered seeds is beneficial to diabetics [13].

In spite of the use of bark of this plant to treat infectious diseases, no investigation was made on comparison of tannin content of the bark, leaves and seeds and their antibacterial properties. Thus we decided to investigate the antimicrobial properties of aqueous and acetone extracts of bark, leaves and seeds of *S. jambos*. Hence the present study was undertaken in the Post Graduate and Research Department of Microbiology, Dr.N.G.P Arts and Science College, Coimbatore, Tamil Nadu, India to evaluate the efficacy of antimicrobial activity of barks, leaves and seeds of *S. jambos*. The efficacy was compared with the standard antibiotics and the results were discussed.

MATERIALS AND METHODS

Plant materials

Plant parts such as barks, leaves and seeds of *S. jambos* were collected from Kannur (Panoor), Kerala, India. Plant materials were dried under the shadow. The dried materials were fine powdered and stored in polythene bags at room temperature (30±2ºC) until use.

Chemicals

All chemicals used were of analytical grade and purchased from typical chemical companies.

Extract preparations

Aqueous extract

To obtain the aqueous extracts, dried and finely powdered bark, leaves and seeds of *S. jambos* were weighed about 30 grams each and homogenized using 150ml of water. They were added to Soxhlet apparatus and the boiling point of water was set up at 100ºC. The water evaporates continuously and was recycled, thereby extracting the compounds present in the samples. They were continuously extracted until the solution looses the colour.

Acetone extract

To obtain the solvent extracts, dried and finely powdered bark, leaves and seeds of *S. jambos* were weighed about 30 grams each and homogenized using 150ml of 70% acetone. They were added to

Soxhlet apparatus and the boiling point of acetone was set up at 56.6ºC. The solvent was recycled, thereby extracting the compounds present in the samples. They were continuously extracted until the solvent looses its colour. The extract was then transferred to a sterile petridish and kept for evaporation of acetone at room temperature. Residues of extracts were collected and stored in the refrigerator.

Microorganisms used and their growth conditions

The test organisms included the bacteria *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi* and *Vibrio cholerae* were obtained from Microbiology divisions of Kovai Medical Center and Hospital (KMCH), a 500 bed multi-speciality hospital, Coimbatore, Tamil Nadu, India. The bacteria were grown in nutrient broth at 37°C and maintained on nutrient agar slants at 4°C.

Antibacterial activity of plant extracts: Well diffusion method

Antibacterial activity of the aqueous and acetone extracts of barks, leaves and seeds of *S. jambos* were tested using well diffusion method. A loop full of culture was inoculated into peptone broth and incubated for 2 to 6 hours at 35°C until it achieved the turbidity of 0.5 McFarland's standard. The test cultures were swabbed on nutrient agar plates, within 15 minutes after adjusting the turbidity of the inoculum suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This removed the excess inoculum from the swab. The dried surface of a nutrient agar plate was inoculated by streaking the swab and the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar was swabbed and wells were made using the sterile well puncture. Different concentrations (60µg to 100µg) of the sterile aqueous and acetone extracts were added to each well. The plates were incubated in an upright position at 37°C for 24 hours. The diameter of inhibition zones were measured in mm and the results are recorded.

RESULTS AND DISCUSSION

In traditional medicine, people usually use aqueous decoctions to treat patients. That is why we first prepared the extract from an aqueous decoction. Preliminary phytochemical analysis of these extract by calorimetric reaction showed that tannins were the major compounds. Because of the presence of tannins, an extract was prepared with acetone 70%, a better solvent for tannin [14]. These extracts were tested on a panel of Gram positive and negative bacteria. The antibacterial activity of aqueous and acetone extracts of different parts (bark, leaves and seeds) of *S. jambos* are presented in table 1 and 2. Similarly, the zones of inhibition exhibited by standard antibiotics (ampicillin, streptomycin and tetracycline) were depicted in table 3.

When the aqueous extracts of bark, leaf and seed were tested against the test microorganisms, bark extracts have exhibited greater inhibitory effect against *S. aureus*, and *E. coli* than the leaves and seeds, showing an inhibitory zone of 15mm. However, the aqueous extracts of bark, leaf and seed did not show any antibacterial activity against *B. subtilis, K. pneumoniae* and *P. vulgaris*. In the case of *P. aeruginosa*, seed extracts possessed anti-bacterial activity but barks and leaves did not show any activity. Both the bark and leaf extracts exhibited inhibitory effect against *S. typhi*, but no activity was observed in seed extracts. The aqueous seed extracts showed a stronger activity (17mm) against *V. cholerae*. When compared to the standard antibiotics such as streptomycin and tetracycline, seed extract had greater effect. Thus the aqueous extracts of bark, leaf and seed showed a varying degree of effectiveness against the tested microorganisms.

The acetone extracts of bark, leaf and seed of *S. jambos*, when tested have exhibited different degrees of antibacterial activity against test microorganisms. Among them, barks of *S. jambos* were found to be effective against all test microorganisms with inhibition zones ranging from 9 to 17 mm. When these results were compared with standard antibiotics, it was found that acetone extract is more effective than the streptomycin, ampicillin and tetracycline against *S. aureus*. When the acetone extract of leaves tested against *S. aureus*, 9 mm zone of inhibition was observed, but no zone of inhibition was observed in seed extracts.

It is also clear from the table- 2, that the bark extracts of *S. jambos* has exhibited antibacterial activity against *E. coli* showing 17 mm zone of inhibition. This extract is more effective when compared to standard antibiotics ampicillin and tetracycline and have equal effect to streptomycin. In the case of *B. subtilis*, the acetone bark extract have exhibited a zone inhibition of 16 mm were same when compared to the standard antibiotics and the extracts were found to be more effective. Similarly, the acetone bark extracts were highly effective against *K. pneumoniae*, when compared to standard antibiotics.

When *P. vulgaris* tested against the standard antibiotics, no zone of inhibition was observed for tetracycline, 20 and 9 mm for ampicillin and streptomycin respectively, but the extract showed 12 mm zone of inhibition which was found to be more active than streptomycin. *P. aeruginosa* when tested against standard antibiotics, no zone of inhibition was observed for ampicillin and tetracycline and 10 mm for streptomycin, whereas the bark extract showed a zone inhibition of 15 mm, which is more active than streptomycin.

When the acetone extracts of bark, leaf and seed of *S. jambos* tested against *S. typhi*, bark extracts were more effective when compared to leaves and seeds. 12 mm zone of inhibition was noticed for acetone bark extract, where as the leaves and seeds showed no inhibitory effect. When compared with the standard antibiotics, the bark extract is less active than the standard antibiotics. The acetone extract of bark exhibited a zone of inhibition of 15 mm against *V. cholerae*, which is more effective when compared to the standard antibiotic streptomycin.

The acetone extracts of leaves and seeds tested against *E. coli, B. subtilis, K. pneumoniae, P. vulgaris, P. aeruginosa* and *S. typhi*, showed no significant zone of inhibition. It is clearly evident that the bark extract had exhibited stronger antibacterial activity than seed and leaf extracts. This may be due to the higher tannin content (2.5 mg/ml) present in bark than seeds (1.9 mg/ml) and leaves (1.4 mg/ml). The bark of *Syzygium guinease* with which, it is often confused to treat pernicious attack, amenorrhea, abdominal pain and diarrhea [8, 9].

The acetone extracts of leaves failed to show any activity against the tested microorganisms, but the aqueous extract of leaves showed antibacterial activity only against *S. typhi*. An infusion of *S. jambos* leaves has been tested for anti-diabetic activity by glucose tolerance test in a randomized, parallel, double blind clinical trial in non-diabetic subjects, but no significant effect was detected [15].

Ethanol extracts of *S. jambos* leaves possessed antiviral activity on Herpes Simplex type I and inhibited the replication of vesicular stomatitis virus but had no effect on polio virus replication [16]. But in the present study, leaves showed an antibacterial activity only against *S. aureus* and *S. typhi.* Similarly seeds exhibited antibacterial activity only against *P. aeruginosa* and *V. cholerae*.

The test organisms *S. aureus* and *P. aeruginosa*, included in the study were isolated from diabetic foot ulcer patients and they were strongly inhibited by barks of *S. jambos*. So bark extracts/powders can be used to treat diabetes patients with foot ulcers to prevent amputation. The other organisms included in the study were isolated from clinical specimens and this clearly indicates that the bark, leaves and seeds could be attributed to the treatment of diseases caused by the test microorganisms.

On comparing the aqueous and acetone extracts of bark, seed and leaf of *S. jambos*, it is confirmed that the acetone extracts are more active than the aqueous. Djipa *et al.* also reported that small differences are observed with some strains where the acetone extract is often more active than the aqueous one [13]. The results of the present study are in accordance with earlier findings.

This limelight's that the organic solvent acetone has the ability to extract the active phytochemicals present in the plant parts. Among

the different parts studied, the effectiveness of these parts are in the ranking order; highest by bark, followed by seeds and leaves which are intermediate in their action because they could show inhibitory action only against few microorganisms like *S. typhi* and *V. cholerae*, where as the bark exhibited inhibitory effect against all the tested microorganisms.

Tannins are well known to possess general antimicrobial properties [6]. Tannins are quite resistant to microbial attack and are known to inhibit the growth of some microorganisms. It is this antimicrobial effect of tannins that slow down the rate of biodegradation of soil organic matter. However, Deschamps *et al.* have found a number of bacteria, fungi and yeast that are resistant to tannin [17].

The study indicates that even though both extracts do not possess the same antibacterial spectrum, the observed activities seem to be generally related to the total tannin content. This also shows that strains do not seem to have the same sensitivity to the tannins present in the bark, leaf and seed extracts and that the antibacterial properties of *S. jambos* extracts can probably be explained by the presence of high concentration of antimicrobial tannins. It is evident from the present study that the extracts of *S. jambos* were active against the test microorganisms. Further more phytochemical studies are required to determine the type of compounds responsible for the antimicrobial effect of the plant and toxicity tests can be carried out before the drug development process using barks of *S. jambos*. In addition, the results support the use of this plant in traditional medicine for the treatment of infections.

Table 1: Therapeutic use of the medicinal plant *Syzygium jambos*

Scientific name	Local name	Family	Parts used	Infection / Therapeutic use
Syzygium jambos	Rose apple	Myrtaceae	Bark	Epilepsy, asthma, bronchitis, hoarseness
			Leaves	Sore eyes, diuretic rheumatism, powdered leaves rubbed on bodies of small pox patients
			Seed	Diarrhea, dysentery, catarrh, anesthetic property, diabetic

Table 2: Antimicrobial activity of aqueous extracts of *S. jambos*

Microorganisms	Zone of inhibition (mm)														
	Bark (µg/ml)					Leaf (µg/ml)					Seed (µg/ml)				
	60	70	80	90	100	60	70	80	90	100	60	70	80	90	100
S. aureus	7	7	8	9	9	-	-	-	-	-	-	-	-	-	-
B. subtilis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. coli	6	6	9	9	11	-	-	-	-	-	-	-	-	-	-
K. pneumoniae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. vulgaris	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. aeruginosa	-	-	-	-	-	-	-	-	-	-	9	9	11	11	11
S. typhi	8	8	8	9	11	7	7	9	10	10	-	-	-	-	-
V. cholera	-	-	-	-	-	-	-	-	-	-	14	14	14	17	17

'-'– No significant zone of inhibition

Table 3: Antimicrobial activity of acetone extracts of *S. jambos*

Microorganisms	Zone of inhibition (mm)														
	Bark (µg/ml)					Leaf (µg/ml)					Seed (µg/ml)				
	60	70	80	90	100	60	70	80	90	100	60	70	80	90	100
S. aureus	12	12	12	13	13	7	7	9	9	9	-	-	-	-	-
B. subtilis	12	14	15	16	16	-	-	-	-	-	-	-	-	-	-
E. coli	14	14	16	17	17	-	-	-	-	-	-	-	-	-	-
K. pneumoniae	12	13	13	14	15	-	-	-	-	-	-	-	-	-	-
P. vulgaris	9	9	11	11	12	-	-	-	-	-	-	-	-	-	-
P. aeruginosa	12	12	13	14	15	-	-	-	-	-	-	-	-	-	-
S. typhi	9	9	11	12	12	-	-	-	-	-	-	-	-	-	-
V. cholera	12	13	14	14	15	-	-	-	-	-	-	-	-	-	-

'-'– No significant zone of inhibition

Table 4: Antimicrobial activity of standard antibiotics

Microorganisms	Antibiotics / Zone of inhibition (mm)		
	Streptomycin	Ampicillin	Tetracycline
S. aureus	10	8	12
B. subtilis	13	10	13
E. coli	17	10	15
K. pneumoniae	10	12	13
P. vulgaris	9	20	-
P. aeruginosa	10	-	-
S. typhi	13	13	17
V. cholera	10	20	16

'-' - No significant zone of inhibition

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