Research Article

STUDIES ON ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF *SAMADERA INDICA*

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ABSTRACT

Samadera indica a bitter plant widely distributed throughout India. The bitterness is due to the presence of triterpenoid such as quassinoids, this group responsible for wide variety biological activities. In the current world population, incidence of infection is increasing tremendously and hence the present study was aimed to carry out the antimicrobial activity and antioxidant activity of methanolic extract of *Samadera indica*, both the activities can be used to predict the wound healing activity of this extract. The methanolic extracts of *Samadera indica* obtained by soxhletation and was investigated for in vitro antimicrobial activity against microorganism including Gram-positive (*Staphylococcus aureus, Bacillus Subtilis*), Gram-negative bacteria (*Pseudomonas aerueginosa*, *Escherichia coli* and *Proteus Vulgaris*) and few strains of fungus such as *Candida albicans, Aspergillus niger and Aspergillus fumigates*. In addition, evaluated antioxidant activity and estimated total phenolic and flavanoids content. Antimicrobial studies revealed that it has significant activity against gram positive, gram negative bacteria and *Candida albicans*, but was resistant against *Aspergillus niger and Aspergillus fumigates*. All the methods of antioxidant showed a prominent antioxidant activity and were compared with Quercetin. Antioxidant activity of extracts produced increased scavenging activity in a dose dependent manner. The present study revealed the presence of antimicrobial and antioxidant activity, hence further studies could be carried out to find out the wound healing activity.

Keywords: *Samadera indica*, antimicrobial activity, antioxidant activity, Phenolic content, flavonoid content.

INTRODUCTION

The incidence of infection in human population is increasing at an alarming rate and literature shows that the prevalence of infection was 51%. Therefore, there has been a pressing need for the development of newer antibiotic from the natural source as they produce the secondary source[1], which can be used as antimicrobial, pharmaceutical drugs[2].

Samadera indica Gaetrn (Simaroubaceae) is a bitter plant[3], previous data shows that it has antitumor[4], antifeedant[5], phytotoxic[6], antiviral[7], and antihelmintic[8] etc. The *Samadera indica* also proved to posses' antioxidant activity[9].

Thus, the main objective of the present study was to study the antimicrobial activities and to screen antioxidant activities of methanolic extract of *Samadera indica*, hence both antimicrobial and antioxidant activity can be used to study the wound healing activity.

MATERIALS AND METHODS

Plant Material

The fresh leaves of *Samadera indica* was collected from the locally growing area mostly from Ernakulam district, Kerala during the month of February. It was then botanically authenticated by taxonomist and a voucher specimen is currently deposited in the Department of Pharmacognosy, Amrita School of Pharmacy, Kochi. The leaves were separated, dried, coarsely powdered passed through sieve no 40 and stored in a closed container for further use[10].

Preparation of Plant Extract

The coarse powder (50gm) was extracted by soxhlation process. The powder was first defatted with n-hexane and then allowed to dry. The marc thus obtained was extracted for 48 hrs with methanol. The resulting solvents were removed under reduced pressure and resulting semisolid, which was dried by vacuum using rotary flash evaporator to get a solid residue. The dried extract thus obtained was used for assessment of antimicrobial and antioxidant activities.

Phytochemical Screening

The dried methanolic extract was used to analyze qualitatively various phytoconstituents like alkaloids, proteins, steroids, saponins, flavonoids, phenolic compounds and tannins, Gums and mucilages[11].

Estimation of total phenol content

Plant polyphenols, a diverse group of phenolic compounds (flavanols, flavonols, anthocyanins, phenolic acids, etc.) possess an ideal structural chemistry for free radical scavenging activity. Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors from the ability of the polyphenol derived radical to stabilize and delocalize the unpaired electron (chain-breaking function) and from their

potential to chelate metal ions (termination of the Fenton reaction)[13]. The amount of total phenol content can be determined by Folin-Ciocateu reagent[12]. This test is based on the oxidation of Phenolic groups with phosphomolybdic and phosphotungstic acids. After oxidation, the green–blue complex formed was measured at 750 nm. The plant extract of (1mg/ml) and gallic acid monohydrate solution (1mg/ml) were prepared. 200 µl of the extract (1 mg/ml) was mixed with 1 ml of Folin-Ciocalteu reagent and 800 µl of sodium carbonate. After shaking, it was kept for 2 hrs for reaction. The absorbance was measured at 750 nm. Using gallic acid monohydrate, standard curve was prepared and linearity was obtained in the range of 10-50 µg/ml. The total phenol content of the extract was determined from standard graph and expressed as gallic acid equivalent in mg/g of the extract[12].

Estimation of flavonoid content

The plant extract of (1mg/ml) was prepared and 0.2 ml of the extract was taken in a test tube and the final volume was made up to 2 ml with distilled water and to this 4 ml of vanillin reagent was added rapidly. Exactly after 15 min. absorbance was recorded at 500 nm against blank. The standard graph was prepared using various dilution of Phloroglucinol solution (1mg/ml) and similar procedure was carried out[13].

HPTLC Analysis

Chromatography was performed on glass-backed silica gel $60GF_{254}$ HPTLC layers (20 cm x 20 cm; 0.30mm layer thickness) prepared using a camag TLC plate auto coater. The methanolic extract of *Samadera indica* was dissolved with HPLC grade methanol and about 5 µl and 10 µl of sample was applied on the plates. The sample loaded plate was kept in TLC twin tough developing chamber with the mobile phase (Toluene:Methanol:Diethylamine (8:1:1)) up to 87mm. The developed plate was dried using hot air for 5 minutes. The developed spots were viewed under UV 254nm, 366nm[14].

***In-vitro* Antioxidant activity**

Nitric oxide Scavenging Activity

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacted with oxygen to produce nitrite ions, which were measured using the Griess reaction. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. 1 ml of sodium nitroprusside (10 mM) in phosphate buffered saline (0.2 M, pH 7.4) was mixed with 100ml sample solution of various concentrations (10, 20, 30, 40, 50µg) and incubated at room temperature for 150 min. The same reaction mixture without the sample was used as the control after the incubation period, 0.5 ml of Griess reagent (1% sulfanilamide, 2% H3PO4 and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) was added. The absorbance of the chromophore (pink colour) formed was read at 546 nm[15].

The percentage of nitric oxide radical was calculated using the following equation.

Percentage Scavenging=[1-(Abs sample/Abs control)]X100

Where, $Abs_{control}$ is the absorbance of control at 546nm;

Abs_{sample} is the absorbance of sample at 546nm.

Test was performed in triplicate and the results were averaged.

Superoxide anion scavenging activity assay

The scavenging activity of extract towards superoxide anion radicals was measured by the method of Ni-shimiki *et al.*[16] About 1ml of nitro blue tetrazolium solution (156 µM in 100 mM phosphate buffer, pH 7.4), 1 ml nicotine amide adenine dinucleotide solution (468 µM in 100 mM phosphate buffer, pH 7.4) and 0.1ml of different concentrations (10, 20, 30, 40, 50µg) of extract and standard in solvent were mixed. The reaction was initiated by adding 100 µl of phenazine methosulphate (PMS) solution (60 µM) in 100 mM phosphate buffer, (pH 7.4) to the mixture. The reaction mixture was incubated at room temperature for 5 min and the absorbance at 560 nm was measured against reagent blank in spectrophotometer[17]. The superoxide anion scavenging activity was calculated according to the following equation.

Percentage inhibition = [1-(Abs sample/Abs control)]X100.

Where, Abs control was the absorbance of the control (without extract) at 560 nm; Abs sample was the absorbance in the presence of the extract at 560 nm.

The experiment was repeated in triplicate and was averaged.

***In-vitro* Antibacterial Assay**

Test Organism

Clinical microbial extracts of Gram negative (*Proteus Vulgaris*, *Pseudomonas aerueginosa*, *Escherichia coli*) and gram positive (*Staphylococcus aureus*, *Bacillus Subtilis*) were used for antibacterial agents.

Antimicrobial Assay

In-vitro antibacterial activity was evaluated using agar well diffusion technique. Muller-Hinton agar was as the medium[19]. The sterile agar was inoculated with bacteria culture (*Proteus Vulgaris*, *S.aureus*, *Bacillus Subtilis*, *Pseudomonas aerueginosa*, *E coli*) for 48 hours at 37°C. Well were bored by using a sterile borer and the standard, plant extracts of concentration 250, 500 and 1000µg/ml were poured into it. Plates were kept for 2 hours in refrigerator to enable pre diffusion of the extracts into the agar. Then the plates were incubated overnight (24 hours) at 37°C. The spectrums of activities of extracts were compared with standard Chloramphenicol (30 µg/ml)[18-20].

In-vitro Antifungal Activity

Test Organism

The test organism cultures, *Candida albicans, Aspergillus niger and Aspergillus fumigates* were procured from National Centre for Industrial Microorganisms (NCIM), Pune, India.

Determination of antifungal properties by MIC method

Cultures on receipt were sub cultured Saboraud Dextrose Agar medium (Fungi) plates and further stored in slants as stock cultures and incubated at 28°C for 48 h for fungi[21]. The Minimum Inhibitory Concentration (MIC) of the test substances against *Candida albicans, Aspergillus niger and Aspergillus fumigates* were determined by liquid broth method of two-fold serial dilution technique. In this assay, the minimum concentration of each test substance required to inhibit the growth of microorganism was determined by the production of turbidity. The standard used in the study was clotrimazole[23-24].

Statistical Analysis

All experimental data were carried out in triplicate and were expressed as average of three analyses ± standard deviation. Statistical analyzes was performed by t-test.

RESULTS AND DISCUSSION

Samadera indica obtained from Simaroubaceae, is a bitter plant due to the presence of Quassinoids. Quassinoids is a triterpenoid responsible for wide variety of biological activity. The activities like antimicrobial and antioxidant were screened.

Preliminary Phytochemical Screening

The preliminary phytochemical analysis of the extracts revealed the presence of alkaloids, tannins and phenolic compounds, triterpenes, carbohydrate, steroids, proteins and flavonoids in methanolic extract (Table 1).

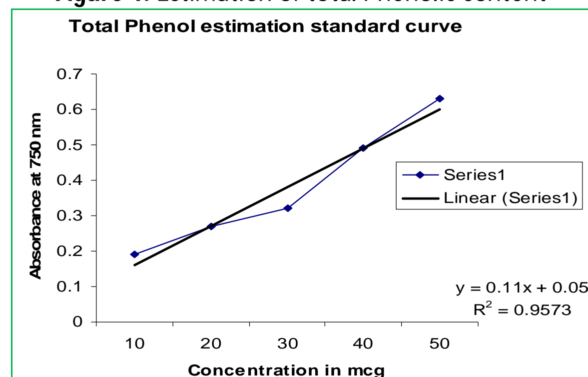
Table 1: Preliminary Phytochemical Analysis

Chemical constituents	Methanolic extract
Alkaloids	+++
Tannins and Phenolic compounds	+++
Saponins	_
Triterpenes	++
Carbohydrates	+
Steroids	++
Gums and mucilages	_
Proteins	+++
Flavonoids	+++

Estimation of phenolic content

It is reported that phenols are responsible for the variation in the antioxidant activity of the plant[25]. They exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals[26]. A total phenolic compound is reported as pyrocatechol equivalents. As the concentration of test compound was increased, the absorbance was increased. It is represented graphically in Figure 1. The total phenolic contents of the test plant were found to be 3.54 ± 0.01 mg / gram of dried extract equivalent to Gallic acid.

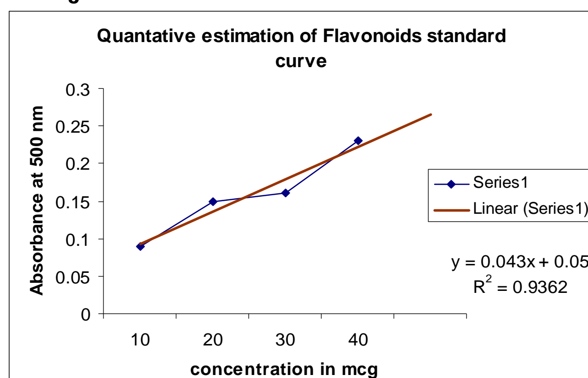
Figure 1: Estimation of total Phenolic content



Estimation of Flavonoids content

The flavonoids content was determined as phloroglycinol equivalent. As the concentration of test compound was increased, the absorbance was increased. It is represented graphically in Figure 2. The Flavonoid content was found to be 3.25 ± 0.10 (MESI) and 4.41 ± 0.02 (MIM) mg / gram of dried extract equivalent to Phloroglycinol.

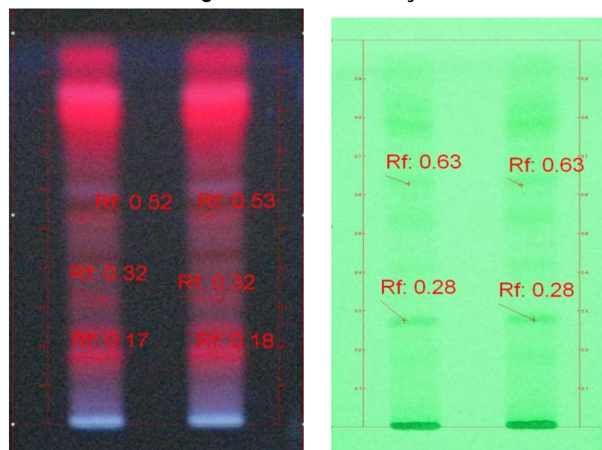
Figure 2: Estimation of total Flavanoid content



HPTLC Analysis

HPTLC profile of methanolic extract of *Samadera indica* is recorded in figure 3.

Figure 3: HPTLC Analysis



In-vitro Antioxidant Activity

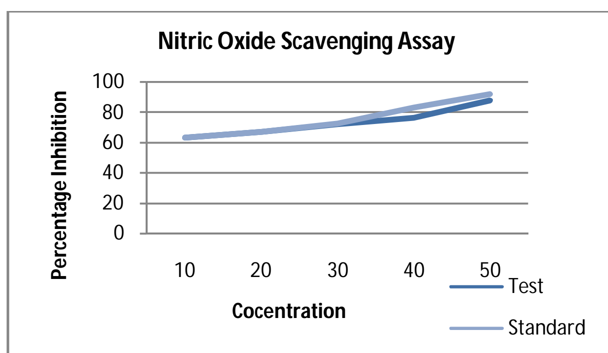
The *in-vitro* antioxidant activity was evaluated by nitric oxide and superoxide scavenging method.

In nitric oxide method, the extract effectively reduced the generation of nitric oxide from sodium nitroprusside. In vitro inhibition of nitric oxide radical is a measure of antioxidant activity of plant drugs. Scavenging of nitric oxide radical is based on the generation of nitric oxide from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent[27]. The methanolic extract decreased the amount of nitrite generated from the decomposition of sodium nitroprusside which may be due to the presence of antioxidant principles in the extract. The percentage scavenging activity increased with increasing concentration of the extract. This represented in table 2 and figure 4.

Table 2: Nitric Oxide Scavenging Assay

Concentration (µg/ml)	Percentage Inhibition (Extract)	Percentage Inhibition (Quercitin)
10	63.28±1.421	63.60±1.325
20	67.27±1.001	67.32±1.478
30	72.06±1.541	72.69±1.245
40	76.56±1.245	83.13±1.548
50	87.84±1.054	92.15±1.245

Figure 4: Nitric Oxide Scavenging Assay

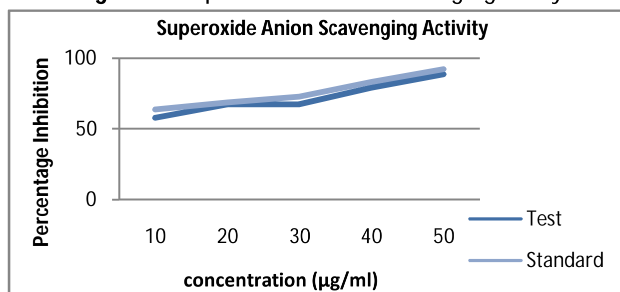


Superoxide radical is known to be a very harmful species to cellular components as a precursor of more reactive oxygen species. The superoxide radical is produced *in vivo* and can result in the formation of H_2O_2 via dismutation reaction. Moreover, the conversion of superoxide and H_2O_2 into more reactive species, e.g., the hydroxyl radical, has been thought to be one of the unfavorable effects caused by superoxide radicals[30]. Figure 5 and table 3, indicates that the percentage scavenging activity increased with increasing concentration.

Table 3: Superoxide anion scavenging activity

Concentration (µg/ml)	Percentage Inhibition (Extract)	Percentage Inhibition (Quercitin)
10	57.76±1.584	63.60±1.325
20	67.32±1.478	68.67±1.145
30	67.27±1.012	72.69±1.245
40	79.04±1.189	83.13±1.548
50	88.28±1.144	92.15±1.245

Figure 5: Superoxide Anion Scavenging Assay

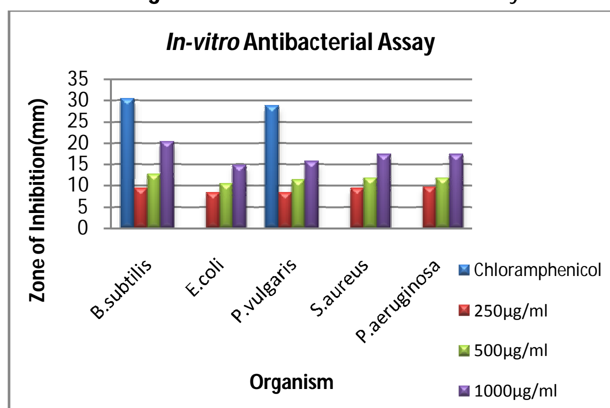


The results of antioxidant activity reveal that the activity is shown due the presence of phenolic and flavonoid content.

In-vitro Antibacterial Studies

Table 4 and figure 6 shows the antibacterial activity of the various concentration of the methanolic extracts (250, 500, 1000 µg/ml) of *Samadera indica* against various strains of bacteria such as *Proteus Vulgaris, Staphylococcus aureus, Bacillus Subtilis, Pseudomonas aerueginosa, Escherichia coli*. The methanolic extracts of *Samadera indica* showed significant activity all the selected species and comparatively more activity against *Bacillus subtilis*. Chloramphenicol (30 µg/ml) standard antibiotic showed a significantly higher ($p<0.05$) activity against *Bacillus Subtilis*, *Proteus Vulgaris*, *Staphylococcus aureus* compared to the different concentration of extract but the standard was found resistant against *Pseudomonas aerueginosa*, *Escherichia coli*.

Figure 6: *In-vitro* Antibacterial Assay



In-vitro Antifungal Studies

The antifungal activity of the various concentrations of methanolic extracts of *Samadera indica* was compared with standard Amphotericin against the various strains of fungi such as *Candida albicians*, *Aspergillus niger* and *Aspergillus fumigates*. The methanolic extract of *Samadera indica* showed activity against *Candida albicians* only, but no clear zone of inhibition was showed against *Aspergillus fumigates and Aspergillus niger*. Hence the extract is resistant to *Aspergillus fumigates and Aspergillus niger*. Table 5 and figure 7 also showed the Amphotericin standard antibiotic showed a significantly higher ($p<0.05$) activity against *Candida albicians* compared to the various concentration of extract. The minimum inhibitory concentration of extract was 250µg/ml.

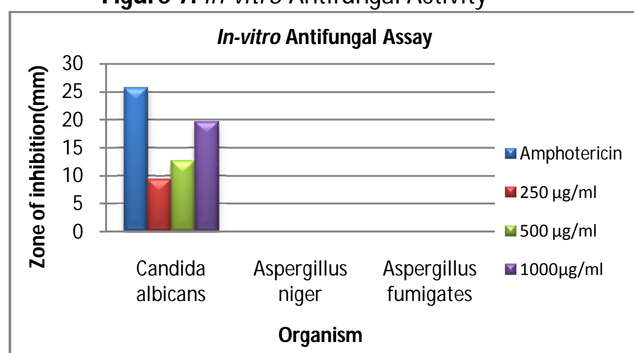
Table 4: *In-vitro* Antibacterial Assay

Organism	Zone of inhibition (mm)			
	Chloramphenicol (30 µg/ml)	Methanolic Extract		
		250 µg/ml	500 µg/ml	1000 µg/ml
B.subtilis	30.33±0.57	9.33±0.58	12.67±0.58	20.33±0.58
E.coli	-	8.33±0.58	10.33±0.58	14.67±0.58
P. Vulgaris	28.66±0.57	8.33±0.58	11.33±0.58	15.67±0.58
S. aureus	28.33±0.577	9.33±0.58	11.67±0.58	17.33±0.58
P. aeruginosa	-	9.67±0.58	11.67±0.58	17.33±0.58

Table 5: *In-vitro* Antifungal Activity

Organism	Zone of inhibition (mm)			
	Standard (Amphotericin)	Methanolic Extract		
		250 µg/ml	500 µg/ml	1000 µg/ml
C.albicans	25.67±0.58	9.33±0.58	12.67±0.58	19.67±0.58
Aspergillus niger	-	-	-	-
Aspergillus fumigates	-	-	-	-

Figure 7: *In-vitro* Antifungal Activity



CONCLUSION

In the present study, methanolic extract of *Samadera indica* showed significant antimicrobial activity. The results from phenolics and flavonoid content revealed that the extract could be a potent natural antioxidant. Hence, we reached the conclusion that the isolates from the extract can be a good antioxidant as well as antimicrobial compound, which can be further used to study the wound healing activity.

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