



In-Vitro Antimicrobial and Antioxidant Screening of Medicinally Used *Sarcococca saligna* Against Human Pathogens

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ABSTRACT

The antibacterial, antifungal and antioxidant study is done for Sarcococca saligna crude extract (Ss. Cr.) and its various fractions. The crude ethanolic extract and various fractions showed good, moderate and significant antibacterial activity against Proteus vulgaris, Escherichia coli, and Staphylococcus aureus respectively. The crude ethanolic extract and rest of the fractions were found inactive against Pseudomonas aeruginosa. Antifungal activity is significant for Ss. Cr. Extract and methanolic extract while poor for Ethyl acetate and petroleum ether fractions. Antioxidant activity was shown by petroleum ether, crude ethanolic extract, ethyl acetate and methanolic fractions. Crude extract and methanolic fractions were found strong DPPH scavenging activity.

Keywords: *S. saligna*, Buxaceae, antibacterial, antioxidant, Antifungal activity.

INTRODUCTION

On the earth, out of 4, 22,127 plant species, about 35,000 to 70,000 species are used as medicinal plants [8]. In the third world countries, 20,000 plants species are believed to be used medicinally [11]. At present, the pharmaceutical sector in India is making use of 280 medicinal plant species, of which 175 are found in the IHR [4]. *S. saligna*, a glabrous shrub with green terete stem belongs to family *Buxaceae*, is a small family of 5 genera and about 100 species, distributed throughout the tropics and temperate regions of India and at altitude of 1,000 to 2,800 meter. The family is represented here by 2 genera and 7 species [5]. In traditional medicine the plants of this family are widely used. Aqueous extract of *S. saligna* is used as antipyretic and calnative [15]. Compounds of this family have shown interesting biological activities such as cholinesterase inhibition as well as antibacterial and antileishmanial activities. 3, 20- diamino-5 α -pregnane or 3/20-amino-5 α -pregnane skeleton is found commonly in the genus *Sarcococca*. *S. ruscifolia* extracts has been reported to have antiulcer, anti-gastritis and antitumor activities [1, 3]. The plant is found to be extensively used in the indigenous system of medicine for the treatment of pain and rheumatic fever, hyperactive states of the gastrointestinal tract, liver diseases, syphilis, infections, fever, pain,

inflammations, as a laxative, blood purifier and for the relief of muscular pain and cardio-suppressant, vasodilator and tracheal relaxant activities [2, 6, 7, 10, 16, 17].

The aim of the present work was to screen the crude ethanolic extract and fractions of *S. saligna*, *in vitro* for antimicrobial and antioxidant activity.

MATERIALS AND METHODS

Collection and identification of plant materials: *S. saligna* (Buxaceae) whole plants were collected from the Mandal, Chamoli Uttarakhand India in October 2012. The plant was identified from Department of Botany, HNB Garhwal University Uttarakhand. A voucher specimen (**Guh s.n.8943**) was deposited in the same section.

Preparation of crude extract : The shade dried whole plant was crushed and boiled in ethanol at 40-50 °C temperature for 16-18 h and then ethanol soluble fraction was filtered off. The filtrate was concentrated under vacuum at low temperature (40°C) with the help of a rotary evaporator (Perfit India). A crude extract (400 g) was obtained from the filtrate.

Fractionation: The crude extract was fractionated with petroleum ether and ethyl acetate by soxhlet apparatus to yield petroleum ether (20g), ethyl acetate (250g), ethyl acetate insoluble (200g) and 30g crude extract was reserved for the pharmacological /biological activities.

Determination of Antibacterial activity

Collection of test organism and preparation of stock culture: Four species of bacteria, one gram-positive (*S. aureus*) and three gram-negative (*E. coli*, *P. aeruginosa* and *P. vulgaris*) were isolated from infected sites of patients attending OPD of V.C.S.G Base Hospital (a tertiary healthcare centre) Srinagar for testing. These were cultured in nutrient broth for 24 h and the fresh inoculums were taken for the test and reconfirmed by gram staining and sub culturing in appropriate selective media.

Preparation of standard culture inoculums of test organism: Three to four isolated colonies were inoculated in 2 mL nutrient broth and incubated till the growth in the broth was equivalent with MacFarland standard (0.5%) as recommended by WHO at which the number of cells was assumed to be 1.5×10^8 cfu mL⁻¹.

Determination of Zone of Inhibition (ZOI): The antibacterial activity was assessed by agar well diffusion method. Muller Hinton agar medium was prepared by using 15g agar dissolved in 1L distilled water. Muller Hinton agar medium was poured into each Petri plate of 20 x 90mm and allowed to cool to 45°C to solidify. The freshly prepared inoculums were swabbed all over the surface of the MHA plate using sterile cotton swab. Wells of 8 mm diameter were made in the agar with a sterile cork borer. 100 µL of the working suspension/solution of different plant extracts were loaded in each well and same volume of extraction solvent for control was filled in the wells with the help of micropipette. Plates were left for some time till the extracts diffused in the medium with the lid closed and incubated at 37°C for 24 h. The tests were performed three times and the zones of inhibition were measured for each extract using a ruler and the results were recorded (Table 1).

Table 1 ZOI (mm) of Ss. Cr. extract and its various fractions tested for antibacterial activity

Micro- organism (0.1ml)	Zone of Inhibition (mm)						
	EASS (10mg/ml)	PESS (10mg/ml)	MASS (10mg/ml)	ECSS (10mg/ml)	Tetracycline (1mg/ml)	Streptomycin (1mg/ml)	Ampicillin (1mg/ml)
<i>P. vulgaris</i> (Pv)	15	—	—	13	—	9.1	20.2
<i>E. coli</i> (Ec)	13	10	25	26	20.3	17.2	10.2
<i>S. aureus</i> (Sa)	20	15	22	36	24.6	10.1	8.1
<i>P. aeruginosa</i> (Pa)	—	—	—	—	—	10	—

Abbreviation: EASS= Ethyl acetate soluble *S. saligna* extract; PESS: Petroleum ether soluble *S. saligna* extract; MASS: Methyl alcohol soluble *S. saligna* extract; ECSS: Ethyl alcohol crude *S. saligna*.

Determination of Antifungal Activity: The minimum inhibitory concentration (MIC), of plant extracts against the toxigenic strain of *A. flavus* (LHPpv1) was determined by poisoned food technique reported [18]. Requisite amount of the extracts were prepared in different solvent system and incorporated to 9.5 mL PDA (potato dextrose Agar) Petri dish to achieve different concentrations at (0.50 to 5.0 $\mu\text{L mL}^{-1}$). Thereafter, 25 μL spore suspension (containing 10^6 spores mL^{-1}) of toxigenic strain of *A. flavus* (LHPpv1) was added to the control as well as to the treated sets. The PDA plate, without extract was treated as control set. The Petri dishes were kept in BOD incubator for 10 days incubation period ($27 \pm 2^\circ\text{C}$). The lowest concentration of extracts that inhibited the complete growth of test mould was taken as MIC.

Determination of Antioxidant Activity: The radical-scavenging capacity of Ss. Cr. extract and its various fractions of *S. Saligna* was determined using the DPPH radical method. A 2 mL aliquot of test solutions was added to 2 mL of $2 \times 10^{-4} \text{ mol L}^{-1}$ ethanolic DPPH solution. The mixture was shaken vigorously and the absorbance was measured at 517 nm immediately. All the tests were performed in triplicate and mean values calculated. The antioxidant activity was expressed according to the ability of an extract to scavenge DPPH free radicals and was determined using the following equation:

$$\% \text{ Inhibition} = [1 - (A_1 - A_2)/A_0] \times 100$$

Where A_0 is the absorbance of negative control (original DPPH sample without sample), A_1 is the absorbance of test sample (DPPH sample in presence of sample) and A_2 is the absorbance of sample without DPPH [19]. The IC_{50} value is the concentration ($\mu\text{g mL}^{-1}$) of extract/standard necessary to reduce the absorbance of DPPH by 50% compared to the negative control. The IC_{50} was determined by interpolation from linear regression analysis of the antioxidant activity (% Inhibition) against sample concentration ($\mu\text{g mL}^{-1}$) and the IC_{50} value decreases as a function of increasing antioxidant activity of samples.

Statistical analysis: Antifungal and Antioxidant Activity experiments were performed in triplicate and data analyzed were mean \pm SE. Data obtained were subjected to one way ANOVA. Means were separated by Tukey's multiple range tests when ANOVA was significant ($p < 0.05$) (SPSS 10.0; 241 Chicago, IL, USA).

RESULTS AND DISCUSSION

Antibacterial activity: The antibacterial activities of Ss. Cr. extract and its various fractions gave different zones of inhibition on the organisms tested (Table 1). The Ss. Cr. extract and ethyl acetate extracts inhibited the growth of three isolates of bacteria. The Ss. Cr. extract and ethyl acetate extract showed more potent against *P. vulgaris*, *E. coli* and *S. aureus*. The methanolic and petroleum ether extract showed potent against *E. coli* and *S. aureus*. All the extracts did not showed any effect on species of *P. aeruginosa*.

The antibacterial activities of various fractions of *S. saligna* compare with different standard shown in (Figure 1).

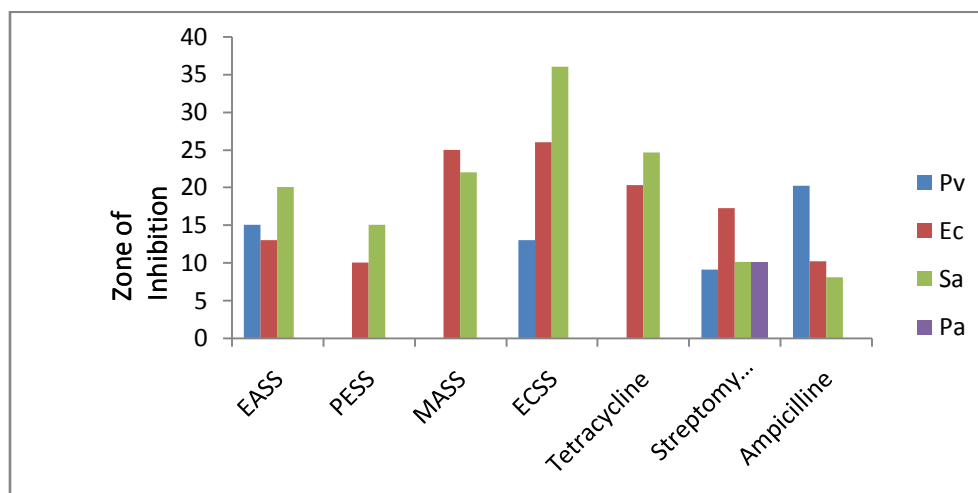


Figure 1. Antibacterial activity of *Ss. Cr.* extract and its various fractions against the test organisms.

Antifungal Activity: During antifungal assay, all the concentrations of *Ss. Cr.* extract and its various fractions were found significantly effective over control after Ten days according to ANOVA and tukey's comparison tests (Table 2). A corresponding decrease in mycelial growth was recorded with increased concentration of *Ss. Cr.* extract and its various fractions. At 1000 ppm, *A. Flavus* LHPpv1 was inhibited 53.971%, 47.367% and 3.270% and 3.012% against MASS, ECSS, EASS and PESS respectively and at different concentration is given in Table 2. Complete inhibition of *A. Flavus* for *Ss. Cr.* extract and its various fractions are given in (Table 3).

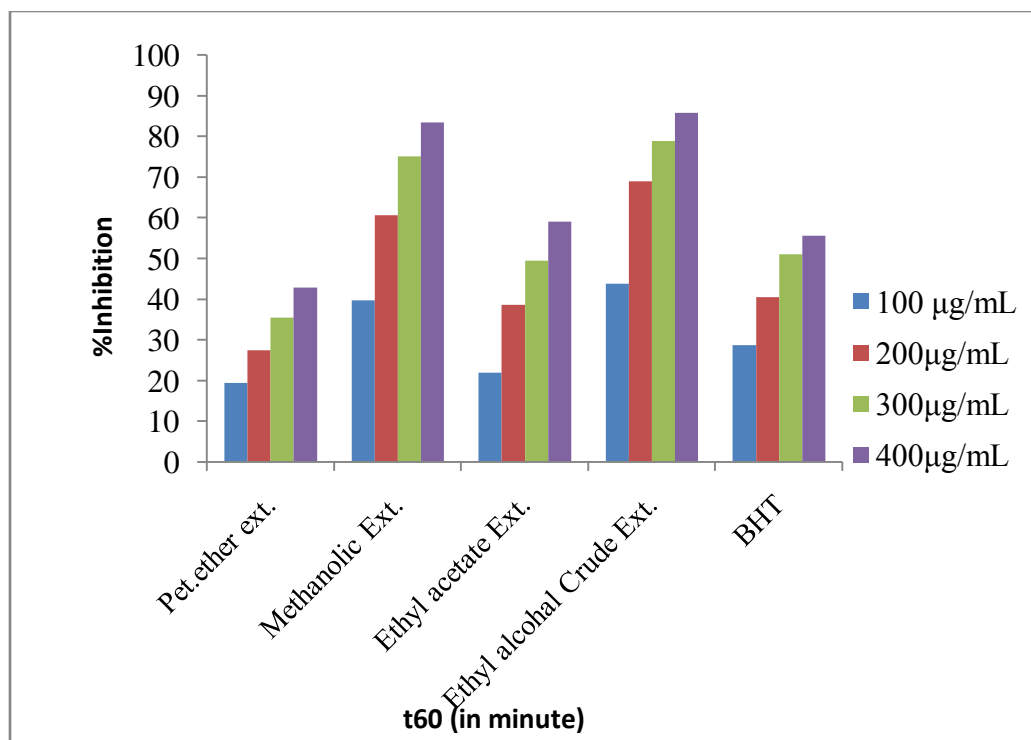
Table 2 . Average diameter of colony of *Aspergillus flavus* LHPpv1 and percentage mycelial inhibition by different plant extracts.

concent ration (ppm)	Diameter (Mean± S.E.) of colony of <i>Aspergillus flavus</i> LHPpv1 (mm)				% Mycelial Inhibition			
	EASS	PESS	MASS	ECSS	EASS	PESS	MASS	ECSS
control	7.033±0.088	6.789±0.077	6.900±0.152	6.967 ± 0.033	-	-	-	-
500	6.900±0.000	6.700±0.056	5.600±0.152	5.967 ± 0.088	1.849	1.746	8.840	4.353
1000	6.800±0.057	6.660±0.051	3.176±0.0233	3.667 ± 0.133	3.271	3.012	3.971	7.367
1500	6.400±0.057	6.200±0.051	0.800±0.000	0.00 ± 0.00	8.961	8.697	8.405	00.00
2000	6.000±0.057	6.200±0.051	0.000±0.000	0.00 ± 0.00	14.651	14.265	00.00	-
2500	5.766±0.033	5.800±0.030	0.000±0.000	0.00 ± 0.00	18.065	17.342	-	-
3000	5.200±0.057	4.9720±0.051	0.000±0.000	0.00 ± 0.00	26.031	25.034	-	-
3500	4.566±0.066	4.254±0.059	0.000±0.000	0.00 ± 0.00	35.049	34.787	-	-
4000	4.166±0.033	3.734±0.010	0.000±0.000	0.00 ± 0.00	40.739	40.012	-	-
4500	3.733±0.033	3.311±0.010	0.000±0.000	0.00 ± 0.00	6.941	6.342	-	-
5000	2.966±0.088	2.000±0.056	0.000±0.000	0.00 ± 0.00	7.809	7.203	-	-

Table 3 MIC values of Ss. Cr. extract and its various fractions

Extracts	MIC (ppm)
Ss. Cr. Extract	1500
MASS	2000
EASS	>5000
PESS	>5000

Antioxidant activity : Our results found that the various plant extracts of *S. saligna* is effective at reducing the stable radical DPPH \square to the yellow-coloured di phenyl picryl hydrazyl indicating that the extract is active in DPPH \square radical scavenging. The Ss. Cr. extract and methanolic fraction had significant scavenging effects with increasing concentrations in the range of 100–400 $\mu\text{g mL}^{-1}$. At a concentration of 400 $\mu\text{g mL}^{-1}$, the scavenging activity of Ss. Cr. extract and methanolic extracts reached 85.88% and 83.52% respectively while at the concentration, that of ethyl acetate and petroleum ether extract was 59.11% and 42.91%. (Figure 2).

**Figure 2.** DPPH scavenging activities of the different solvent extracts of *Sarcococca Saligna*

APPLICATIONS

Antimicrobial medicinally plants are most potential candidate for providing novel drug with new mechanism of action. They kill or inhibit the growth of micro-organism such as bacteria, fungi or protozoan's. The antioxidant supplements or foods rich in antioxidants may be used to help the human body in reducing oxidative damage by free radicals and active oxygen. The role of active oxygen and free radicals in tissue damage in such diseases, are becoming increasingly recognized [20]. Cancer,

emphysema, cirrhosis, arteriosclerosis, and arthritis have all been correlated with oxidative damage. Lipid peroxidation is an important deteriorate reaction in food during storage and processing. It not only causes a loss in food quality but also is believed to be associated with some diseases such as carcinogenesis, mutagenesis, ageing, and arteriosclerosis [21].

CONCLUSIONS

S. saligna was screened for the first time for antioxidant activity. Strong antioxidant properties were confirmed in the Ss. Cr. extract and methanolic extract fractions. We found strong antibacterial activity specifically in the Ss. Cr. extract and ethyl acetate extract of *S. saligna* against the *P. vulgaris*, *E. coli*, *S. aureus* and strong antifungal activity for methanolic, Ss. Cr. extract against *Aspergillus flavus* LHPv1. The present work revealed that the plant could be used for Herbal medicine. In conclusion, *S. saligna* is an important medicinally plant and can be a potential candidate for further bio-assays which would lead to the synthesis of safe herbal drugs of global interests.

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