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Antimicrobial Efficacy of Callus and in Vitro Leaf Extracts of Sapindus Mukorossi Gaertn. Against Pathogenic Microbes

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ABSTRACT

Sapindus mukorossi is well known medicinal and economical plant. Antibacterial and antifungal activities were evaluated from various extracts (ethanolic, methanolic and aqueous) of callus and in vitro leaf against several Gram positive and Gram negative bacteria and clinically isolated fungus. Antimicrobial activities were more in *in vitro* leaf extracts. Callus extract have showed antibacterial activity against *S. aureus and E. coli*. Maximum inhibition zone (8.06±0.17 mm) was observed against *C. tropicalis* from methanolic extracts of *in vitro* leaf. In vitro leaf extracts have shown efficient antifungal activity. Callus and in vitro leaf extract can be used for production of different phytochemicals and plant based antibiotics.

KEYWORDS

Antimicrobial; Medicinal Plant; *Sapindus Mukorossi*; Plant Extract.

INTRODUCTION

Plants are rich source of secondary metabolites and have great therapeutic potential to treat various diseases. A large population of world uses plant based principles for their primary health care [1]. Medicinal plants may be proved as best source of a variety of drugs [2]. About 20% of plants present in the world have been examined for pharmaceuticals and biological tests. The commonly present antibiotics are either derived from natural or synthetic resources [3]. Several researchers have reported the various biological activities such as antimicrobial, anti-inflammatory, anti-HIV, anti-cancer, anti-diabetic etc. from different group of plants[4-9]. Current research shows that researchers and medical professionals are more interested in the use of indigenous drugs for the cure of human health ailments [10]. Due to expensiveness and more side effects of synthetic drugs clinical microbiologists are paying their attention for screening of medicinal plants for phytochemicals and antimicrobial activity as potent therapeutics. Antimicrobial activities of medicinal plants are due to presence of different groups of phytochemicals such as alkaloids, polyphenolic compounds, terpenoids, lignans etc. Human pathogens are developing drug resistance against commonly used antibiotics have compelled researchers for searching new antimicrobial substances from other natural sources including plants [11]. So, there is an essential need to develop a new generation of antibiotics from natural source.

S. mukorossi Gaertn. is a popular medicinal and economical tree and well-known for their therapeutic values. *S. mukorossi* plant is rich in antioxidants and polyphenolic compounds and exhibiting antioxidants properties [12]. Earlier, Aneja et al. [13] have assessed the antibacterial activity of fruit extract against dental caries causing pathogen. Antimicrobial activities of leaf and fruit extracts of this plant were also evaluated on some bacteria and fungus [14,15]. For the validation of antimicrobial potential of this plant this research study was undertaken. This is the first report of antimicrobial activity by using callus and *in vitro* leaf (IVL) extracts.

MATERIAL AND METHODS

Collection of Plant Materials and Preparation of Extracts

Leaves of *S. mukorossi* were collected from the campus of Banaras Hindu University, Varanasi, in the month of April. Callus and IVL were regenerated by following the protocol of Singh et al. [16]. Callus was shade dried at room temperature for 4-5 days and at 40-45°C for 2 h and grinded in mechanical grinder to make coarse powder. Five gram of callus powder was extracted in 150 ml of solvents for 10 h using Soxhlet apparatus. Ethanol, methanol and double distilled water were used as solvents for the extraction. Extracts were then dried at 40°C in rotary evaporator and stored at -20°C for further use. Test samples were prepared in different concentrations for further experiments in their respective extraction solvents. *In vitro* leaf (200 mg) was collected and grinded in mortar and pestle by adding the solvents. Finally, volume of extract was maintained about 20 ml.

Preparation of Samples

Stock samples were prepared in the concentration of 100 mg ml^{-1} in dimethyl sulphoxide (DMSO). About 5 µl extracts was dispensed onto sterile disc for susceptibility test.

Test Microorganism

Total ten microorganisms (Gram positive, Gram negative bacteria and fungus) were subjected for screening of antimicrobial activity. Staphylococcus aureus ATCCC25323, *Enterobacter aerogenes* (Gram positive) *Salmonella* Typhimurium, *Klebsiella pneumoniae*, *Escherichia coli* ATCC35218, *Vibrio cholerae*, *Pseudomonas aeruginosa* ATCC27893 and three fungal strains namely *Candida albicans* ATCC90028, *Candida tropicalis* ATCC750, *Candida parapsilosis* ATCC22019 were used for investigation. Microbial cultures were obtained from Department of Microbiology, Institute of Medical Sciences, BHU, Varanasi, India.

Media

Media was prepared by dissolving Mueller-Hinton agar 38 g l^{-1} and 10 g l^{-1} agar-agar in double distilled water. Saline was prepared by dissolving NaCl (8.5 g l^{-1}) in double distilled water and autoclaved for 15 min at 1.1 Kg/cm^2 and 121 °C. The plating was done by pouring approximate 20 ml of sterile media.

Preparation of Inoculums

Young bacterial and fungal inoculums were prepared by growing cells on MHA (Himedia, Mumbai) for 24 h at 37°C. The turbidity of the bacterial suspension was adjusted to about 0.5 McFarland turbidity standard (~1 x 10^7 CFU/ml).

Antibacterial and Antifungal Sensitivity Test

Antibacterial activity was screened through disc diffusion method [17]. The test cultures were swabbed on solidified media and dried for 5 min. About 5 µl of extract was loaded to each disc. The loaded discs were placed on the surface of the medium. Dimethyl sulphoxide (DMSO) was used as negative control. The plates were incubated at 37°C for 24 h (for bacteria) and at 28°C for 48 h (for fungi). Zones of inhibition (diameter) were recorded in millimeters.

Statistical Analysis

All the above experiments were performed in triplicate and repeated thrice in independent manner. Statistical analysis was done by using SPSS software (version 16, Chikago, USA). Analysed data was represented as mean ± SE.

RESULTS AND DISCUSSION

Both plant extracts of *S. mukorossi* have shown antimicrobial activity. The antibacterial and antifungal activities from callus and IVL extract have been presented in Tables 1 and 2. *S. mukorossi* is a good source of phytochemicals (phenolics, flavonoids, antioxidants, alkaloids, tannins etc.), these classes of phytochemicals played important role in antimicrobial activity and can be used for cure of various ailments [18,19].

Table 1: Antimicrobial activity from in vitro leaf extracts.

Test organisms	Inhibition zone diameter (mm)			
	Ethanolic extract	Methanolic extract	Aqueous extract	Standard drugs (5µl/disc)
Gram positive bacteria				Ampicilin
S. aureus	6.17±0.09	-ve	-ve	21.00 ± 0.28
E. aerogens	-ve	7.03±0.09	-ve	23.33 ± 0.16
Gram negative bacterial				Ciprofloxacin
S. Typhimurium	6.17±0. 09	7.06±0.07	-ve	23.86±0.59
V. cholera	8.00±0.06	7.07±0.06	7.23±0.14	20.26±0.37
E. coli	-ve	7.8±0.11	-ve	24.80±0.35
K. pneumoniae	6.66±0.29	6.53±0.31	6.16±0.08	20.96±0.26
P. aeruginosa	-ve	-ve	-ve	24.43 ±0.47 (Tobramycin)
Fungus				Fluconazole
C. albicans	-ve	-ve	-ve	21.8±0.23
C. tropicalis	7.16±0.12	8.06±0.17	6.56±0.23	18.26±0.81
C. parapsilosis	-ve	-ve	-ve	23.36±0.44

-ve = activity not found

Table 2: Antimicrobial activity from callus extracts.

Test organisms	Inhibition zone diameter (mm)			
	Ethanolic extract	Methanolic extract	Aqueous extract	Standard drugs (5 µl/disc)
Gram positive bacteria				Ampicilin
S. aureus	-ve	6.23±0.14	-ve	21.00 ± 0.28
E. aerogens	-ve	-ve	-ve	23.33 ± 0.16
Gram negative bacterial				Ciprofloxacin
S. Typhimurium	-ve	-ve	-ve	23.86 ± 0.59
V. cholerae	-ve	-ve	-ve	20.26 ± 0.37
E. coli	8.20±0.11	7.3±0.15	6.20±0.11	24.80 ± 0.35
K. pneumoniae	-ve	-ve	-ve	20.96 ± 0.26
P. aeruginosa	-ve	-ve	-ve	24.43 ± 0.47 (Tobramycin)
Fungus				Fluconazole
C. albicans	-ve	-ve	-ve	21.8 ± 0.23
C. tropicalis	-ve	-ve	-ve	18.26 ± 0.81
C. parapsilosis	-ve	-ve	-ve	23.36 ±0.44

-ve = activity not found

Antioxidant activity and reducing power of leaf and fruits extracts were reported in this plant [12]. Both *in vitro* plant material (callus and IVL) extracts showed antibacterial activities. *In vitro* leaf extract has shown more potential towards antimicrobial properties. Callus extract was effective against *S. aureus and E. coli*, only. In callus extract, antifungal activity was absent. *In vitro* leaf extract showed more antibacterial activity against Gram negative bacteria. Ethanolic extract of IVL showed highest inhibition zone (8.00±0.06) against *V. cholerae*. All extracts of IVL showed potent antifungal activity against *C. tropicalis*. Other researchers have also reported the antimicrobial activity from callus extracts [20, 21]. The PE, which formed inhibition zone more than 10 mm in diameter, can be considered active [19]. Fruits and leaf extracts of this plant have shown more potential antibacterial and antifungal activies [14]. Inhibition zone can be enhanced by increasing the concentration of PE. Antimicrobial potential of plant extracts on pathogenic microbes was also observed by other workers [14, 20-22]. Quantity of various phytochemicals or metabolites may be increased by optimizing in vitro plant culture conditions [23].

CONCLUSION

Phytomedicines and its derived products open a new era of traditional medicines around the world. In vitro plant material extracts of *S. mukorossi* showed presence of antimicrobial activity. In some extracts, although the antimicrobial activity was less but it can be increased by increasing the concentration of sample or by purification of extracts. Plant extracts based holistic approach for the cure of various ailments will be new avenue in medical sciences and will provide a cost effective therapy.

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