

Original Research Article

Free radicals scavenging activity and antimicrobial potential of leaf and fruit extracts of *Sapindus mukorossi* Gaertn. against clinical pathogen

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Abstract

Antioxidant activity of fruit and leaf extracts of *Sapindus mukorossi* Gaertn. was evaluated through β -carotene/linoleic acid bleaching assay and hydrogen peroxide scavenging activity. Antimicrobial potential of extracts was also assessed against human disease causing pathogens. In fruit extract, highest antioxidant activity (76.64 ± 1.06) was found in aqueous extract and leaf extract showed maximum antioxidant activity (90.82 ± 1.11) in methanolic extract. In fruit, maximum H₂O₂ scavenging activity was found in methanolic extract and in leaf maximum H₂O₂ scavenging activity (10.53 ± 0.72) in ethanolic extract. Both, fruit and leaf extract have showed significant antimicrobial activity against most of the pathogens. In fruit extract, methanolic and aqueous extract showed potent antimicrobial activity against all tested organisms except *Candida albicans*. All extract of fruit showed maximum inhibition zone (diameter) against *Candida tropicalis*, methanolic extract (12.50 ± 0.29), aqueous extract (11.66 ± 0.33) and ethanolic extract (10.16 ± 0.16). In leaf extract, maximum inhibition zone (9.66 ± 0.52) was formed by ethanolic extract against *Salmonella Typhimurium*. Fruit and leaf extracts have shown more activity against Gram negative bacteria.

Keywords: *Sapindus mukorossi*, medicinal tree, plant extracts, antioxidants, antibacterial, antifungal,

Introduction

Plants are rich source of novel bioactive compounds due to their secondary metabolites and have great therapeutic potential to treat various diseases. World health organization (WHO) reported that about 80 % of world population depends on the plant extract and their active components for their primary health cure, and rely on traditional medicine system [1]. Antioxidants (natural and synthetic origin) have been recommended to use for treatment of various human diseases [2]. Reactive oxygen species (ROS) are known to be responsible for many cell disorders and also cause for many diseases including cardiovascular diseases, atherosclerosis, chronic inflammation etc. [3]. Hydrogen peroxide (H₂O₂), superoxide ion and hydroxide radical (OH⁻) are considered as the most common ROS. The antioxidants are used to prevent ROS concentrations from reaching upto toxic level within a cell that cause damage [4]. Nowadays, uses of synthetic antioxidants are avoided due to their side effects, toxic and carcinogenic effects. Thus, finding of natural antioxidants has increased greatly because of their fewer side effects [5-6]. Oxygen and water is produced rapidly by decomposition of hydrogen peroxide (H₂O₂) and in this reaction, hydroxyl ion may be produced that can initiate lipid peroxidation and also cause DNA damage [7].

Sapindus mukorossi is a very important medicinal tree and distributed from temperate to tropical and sub-tropical regions of Asia. Many pharmacological properties have been reported from

different parts of this plant such as anticancer [8], hepatoprotective [9], antifungal [10], anti-microbial [11] and spermicidal activities [12]. Antibacterial activity have evaluated from the fruit extracts against dental caries causing pathogen [13]. Antimicrobial activity from fruit extract was also assessed against some bacteria and fungus [14]. Different types of flavonoids such as quercetin, apigenin, kaempferol and rutin were reported from leaf extracts of *S. mukorossi* [15]. Antioxidant activity, polyphenolic content and lipid peroxidation activity was evaluated from both leaf and fruit extract [6].

The objective of present work is to evaluate and compare the antioxidant activity through β -carotene/linoleic acid bleaching assay and hydrogen peroxide free radical scavenging assay and antimicrobial activity from different extract (ethanolic, methanolic and aqueous) of both leaf and fruit of *S. mukorossi*. This is first report of antioxidant studies through β -carotene linoleic acid method and hydrogen peroxide scavenging activity and antibacterial activity against clinical isolates from leaf and fruit extracts of this plant.

Material and methods

Material collection and preparation of extracts

Plant specimen was submitted to herbarium of Botanical Survey of India (BSI), Allahabad. Collection number BHU 101 and reference



number BSI/CRC/Tech./2012-13 were given by BSI to the plant specimen.

Leaves of *S. mukorossi* were collected from the campus of Banaras Hindu University, Varanasi, in the month of April and fruits during June. Leaves and fruits were washed under running tap water for removing dust. Leaves and fruit's pericarp were grinded in mechanical grinder to make coarse powder after shade dried for 4-5 days at room temperature and oven dried (40-45 °C) for 2 h. Extraction was done from 25 g leaf powder in 250 ml of solvents and 5 g fruit's pericarp powder in 150 ml solvents using soxhlet apparatus for 10 h. Ethanol, methanol and double distilled water were used as solvents for the extraction. Extracts were then dried at 40-45 °C in rotary evaporator. Extracts were stored at -20°C till use. Test samples were prepared in various concentrations for further experiments in their respective extraction solvents.

Preparation of samples

For antioxidant assay, stocks of samples was prepared by dissolving 50 mg extract in 25 ml of respective solvent, final concentration was 2 µg/µl. From this stock, different volume of samples was taken for various experiments.

For antimicrobial activity stock sample was prepared in concentration of 100 mg/ml in dimethyl sulphoxide (DMSO). About 5 µl extracts was dispensed onto sterile disc for susceptibility test.

Antioxidant activity through β-carotene and linoleic acid assay

For this assay, the method of Miller was followed with some modifications [16]. Stock solution of β-carotene was prepared in concentration of 2 mg/ml of chloroform. One ml β-carotene solution was mixed properly with linoleic acid (20 µl) and 200 µl of Tween 80 in a round bottom flask. The chloroform was completely evaporated upto dryness. In the residue 50 ml double distilled water was added and stirred vigorously to form an emulsion. Emulsion (2400 µl) was mixed with 400 µl or 800 µl of extract separately in test tube and just after mixing absorbance was recorded. Then, the test tubes were incubated for 2 h at 50°C and absorbance was also recorded immediate after incubation. Similarly, same volume of emulsion (2400 µl) was mixed with same volume of DMSO (400 µl or 800 µl) instead of PE, served as control. Absorbance was recorded at 470 nm. Percent inhibition was calculated as:

$$I (\%) = \frac{(\text{Absorbance } \beta - \text{ carotene after 2 h assay})}{(\text{Absorbance of } \beta - \text{ carotene initial})} \times 100$$

Hydrogen peroxide (H₂O₂) scavenging assay

The capacity of plant extracts in scavenging hydrogen peroxide was evaluated by the method of Ruch et al. with some modifications [17].

Phosphate buffer (50 mM, pH 7.4) was used to make the solution of hydrogen peroxide (80 µM). Plant extract in concentration 50 µg/ml (3 ml) were added in hydrogen peroxide solution (0.6 ml, 80 µM). Plant extract was prepared in double distilled water. Reaction mixture was incubated for 50 min and after incubation OD was measured at 230 nm. Phosphate buffer without H₂O₂ was used as blank and hydrogen peroxide solution without extract served as control. Hydrogen peroxide scavenging activity was calculated by following formula:

Hydrogen peroxide scavenging activity (%)

$$= \frac{(A_i - A_t)}{(A_i)} \times 100$$

Where, A_i = absorbance of control, A_t = absorbance of test sample

Test microorganism

Total ten microorganisms (Gram positive, Gram negative bacteria and fungus) were subjected for screening of antimicrobial activity. *Staphylococcus aureus* ATCC 25323, *Enterobacter aerogenes*, *Enterococcus faecalis* (Gram positive) *Salmonella Typhimurium*, *Klebsiella pneumoniae*, *Escherichia coli* ATCC 35218, and three fungal strains namely *Candida albicans* ATCC 90028, *Candida tropicalis* ATCC 750, *Candida parapsilosis* ATCC 22019 were used for investigation. Microbial cultures were obtained from Department of Microbiology, Institute of Medical Sciences, BHU, Varanasi, India. The young bacterial broth cultures were prepared for screening experiments.

Media used

Media was prepared by dissolving Muller Hinton agar 38 g/l and 10 g/l in double distilled water. Saline was prepared by dissolving 8.5 g/l in double distilled water and autoclaved for 15 min at 1.1 kg/cm² and 121 °C. The plating was done by pouring approximate 20 ml of sterile media.

Preparation of inoculums

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 0.5 McFarland turbidity standards (~1 x 10⁷ CFU/ml).

Antibacterial and antifungal sensitivity test

Antibacterial activity was tested using disc diffusion method [18]. The test cultures were swabbed on the top of the 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 for 24 h at 37 °C for bacteria and for 48 h at 28 °C 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. Data was analysed using SPSS software (version 16, Chikago, USA). Analysed data was represented as mean \pm SE.

Results and Discussion

Antioxidant activity though β - carotene/ linoleic assay

This assay is one of the most rapid methods to screen antioxidant activity. In this assay, linoleic acid gets oxidized by reactive oxygen

species generated by oxygenated water. The products formed will initiate the β -carotene oxidation [7]. All extracts showed significant antioxidant potential. All extracts, in 400 μ l volume showed lower activity than 800 μ l volume of extract. Fruit's extract showed less antioxidant potential than leaf extract. In fruit, maximum antioxidant activity (76.64 ± 1.06) was observed in aqueous extract and minimum in ethanolic extract (40.86 ± 0.87) with 800 μ l of extract. In 400 μ l of extract, maximum antioxidant activity was 55.11 ± 0.69 in aqueous extract and minimum (30.64 ± 1.62) in ethanolic extract (Figure. 1). In leaf, highest free radical scavenging activity 90.82 ± 1.11 was observed in methanolic extract and minimum 84.09 ± 0.93 in aqueous extract with 800 μ l extract. In 400 μ l of extract, maximum antioxidant activity (80.07 ± 0.49) was observed and minimum (56.25 ± 1.38) in methanolic extract (Figure. 2). Antioxidant activity through β - carotene/ linoleic assay was also evaluated in several plants by other researchers [19]. Highest antioxidant activity was found in unripe fruit ($90.67 \pm 0.29\%$) followed by young leave, ripe fruit and the seed of *Carica papaya* through β -carotene bleaching assay [20].

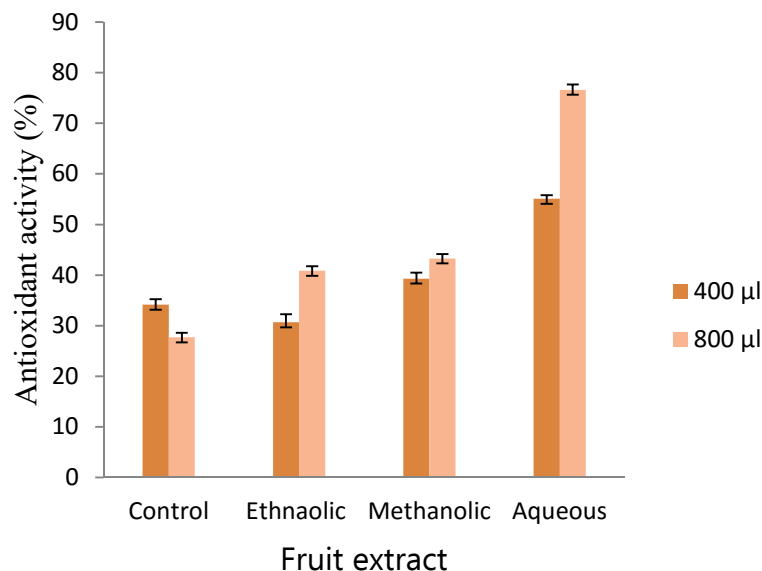


Figure. 1. Antioxidant activity through β -carotene/linoleic acid bleaching assay from fruit extract of *Sapindus mukorossi*

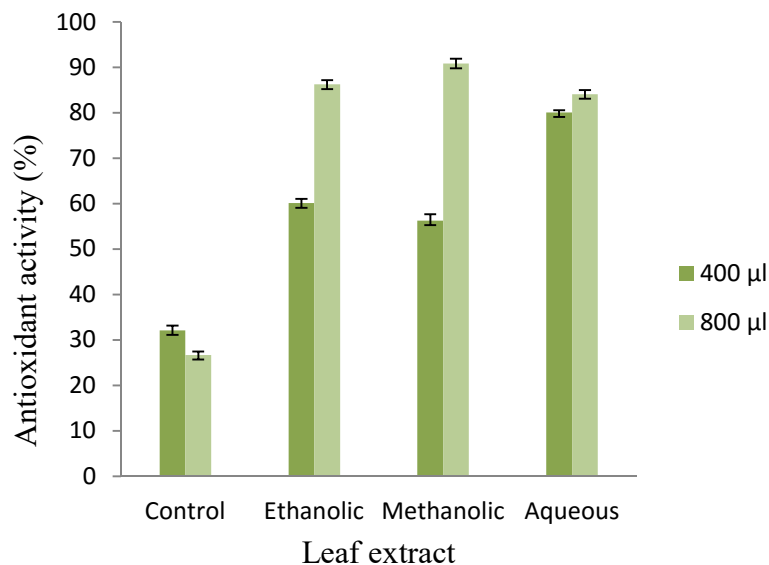


Figure. 2. Antioxidant activity through β -carotene/linoleic acid bleaching assay from leaf extract of *Sapindus mukorossi*

Hydrogen peroxide scavenging activity

Fruit extracts has more efficiency than leaf extracts towards H₂O₂ scavenging activity. In fruit extracts, maximum scavenging activity was found in methanolic extract (23.73 ± 0.53) and minimum in aqueous extract (1.46 ± 0.29). H₂O₂ scavenging activity in ethanolic extract of fruit was 19.94 ± 0.33 (Figure.3). In leaf extract, highest

H₂O₂ scavenging activity was observed in ethanolic extract (10.53 ± 0.72) and lowest in aqueous extract (0.63 ± 0.08). In ethanolic extract H₂O₂ scavenging activity was 1.02 ± 0.26 (Fig.3). High H₂O₂ scavenging activity of fruit extract is may be due to presence of high amount of saponin. Several authors also reported the H₂O₂ scavenging activity from the plant extract [21-23]. H₂O₂ scavenging activity was 30.13 % with 100 µg/ml of ethanolic leaf extract of *Crataegus monogyna* [21].

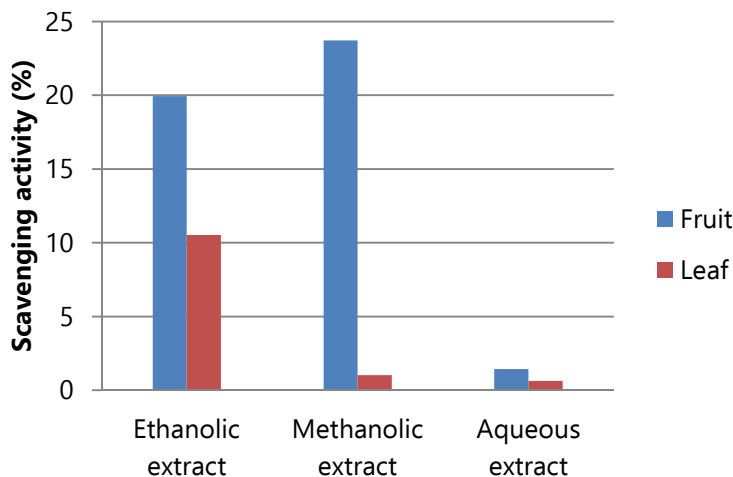


Figure. 3. Hydrogen peroxide scavenging activity from fruit and leaf extract of *Sapindus mukorossi*.

Antimicrobial activity

In vitro antibacterial and antifungal assay results from leaf and fruit extract presented in table 1 and 2. Both, fruit and leaf extract showed potent antimicrobial activity against most of the tested pathogens. But, fruit's extract have more antimicrobial activities than leaf extracts. *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 [24-25]. All extract of leaf showed highest activity against all tested Gram negative bacteria; ethanolic extract was most effective with maximum inhibition zone (9.60 ± 0.52) against *S. Typhimurium* (Table 1). All extracts of leaf have shown antifungal activity against *C. parapsilosis* (Table 1).

Fruit extract was most effective against *Candida* species than different strains of bacteria. Among bacteria, fruit extract showed highest activity against Gram negative bacteria. In Gram negative bacteria, maximum inhibition zone (11.66 ± 0.50) was observed in aqueous extract against *S. Typhimurium* and in Gram positive bacteria maximum inhibition zone (9.00 ± 0.57) was found in *E. aerogens* (Table. 2). The PE, which formed inhibition zone more than 10 mm in diameter, can be considered active [25]. Inhibition zone can be enhanced by increasing the concentration of PE. In antifungal study, PE was highest effective *C. tropicalis* with maximum inhibition zone (12.50 ± 0.29) and moderate effective against *C. parapsilosis* (Table. 2). All the extracts of leaf and fruit were found ineffective against *C. albicans* (Table 1, 2). Ethanolic extract of fruit showed highest activity against Gram positive bacteria, while aqueous extract was highly responsive against Gram negative bacteria. Antimicrobial potential of plant extracts on clinical strain of microbes was also observed by other researchers [26-27].

Table 1. Antimicrobial activity of leaf extract of *Sapindus mukorossi*

Test organisms	Inhibition zone diameter (mm)				Standard drugs (5 μ l/disc)
	Ethanolic extract	Methanolic extract	Aqueous extract	Control	
Gram positive					Ampicilin
<i>S. aureus</i>	8.66 ± 0.33	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	20.83 ± 0.44
<i>E. aerogens</i>	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	23.93 ± 0.58
<i>E. faecalis</i>	8.33 ± 0.46	8.60 ± 0.23	0.00 ± 0.00	0.00 ± 0.00	23.66 ± 0.44
Gram negative					Ciprofloxacin
<i>S. Typhimurium</i>	9.66 ± 0.52	9.23 ± 0.67	8.20 ± 0.75	0.00 ± 0.00	24.26 ± 0.63
<i>E. coli</i>	8.16 ± 0.16	9.33 ± 0.33	8.00 ± 0.28	0.00 ± 0.00	25.0 ± 0.29
<i>K. pneumoniae</i>	8.33 ± 0.33	8.66 ± 0.33	0.00 ± 0.00	0.00 ± 0.00	21.16 ± 0.44
Fungus					Fluconazole
<i>C. albicans</i>	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	22.66 ± 0.88
<i>C. tropicalis</i>	8.83 ± 0.44	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	18.33 ± 0.88
<i>C. parapsilosis</i>	9.33 ± 0.60	8.4 ± 0.46	8.6 ± 0.67	0.00 ± 0.00	22.83 ± 0.16

Table 2. Antimicrobial activity of fruit extract of *Sapindus mukorossi*

Test organisms	Inhibition zone diameter (mm)				Standard drugs (5 μ l/disc)
	Ethanolic extract	Methanolic extract	Aqueous extract	Control	
Gram positive					Ampicilin
<i>S. aureus</i>	0.00 ± 0.00	8.00 ± 0.00	7.66 ± 0.66	0.00 ± 0.00	21.00 ± 0.58
<i>E. aerogens</i>	7.00 ± 0.57	9.00 ± 0.57	8.33 ± 0.88	0.00 ± 0.00	25.00 ± 0.58
<i>E. faecalis</i>	07.50 ± 0.28	8.66 ± 0.33	7.80 ± 0.42	0.00 ± 0.00	23.66 ± 0.67
Gram negative					Ciprofloxacin
<i>S. Typhimurium</i>	09.00 ± 0.57	8.00 ± 0.58	11.66 ± 0.50	0.00 ± 0.00	25.16 ± 0.60
<i>E. coli</i>	7.66 ± 0.00	8.00 ± 0.57	8.00 ± 0.57	0.00 ± 0.00	25.5 ± 0.29
<i>K. pneumoniae</i>	7.5 ± 0.28	7.66 ± 0.66	8.66 ± 0.33	0.00 ± 0.00	22.33 ± 0.33
Fungus					Fluconazole
<i>C. albicans</i>	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	0.00 ± 0.00	22.33 ± 1.20
<i>C. tropicalis</i>	10.16 ± 0.16	12.50 ± 0.29	11.66 ± 0.33	0.00 ± 0.00	18.33 ± 0.88
<i>C. parapsilosis</i>	08.00 ± 0.00	8.60 ± 0.33	10.00 ± 0.57	0.00 ± 0.00	24.16 ± 0.60

Conclusions

S. mukorossi is an important medicinal plant. This plant is rich source of variety of phytochemicals. Antibacterial and antifungal activity of various extracts of fruit and leaf shows that this plant would be a better source for a new plant based antibiotics and may be beneficial for the treatment of various ailments.

Author's contribution

Experiments were performed by RS. Microbial culture was provided by GN. Manuscript was written by RS. Data analysis and editing in manuscript was done by RS and NK. Manuscript was read and approved by all authors.

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