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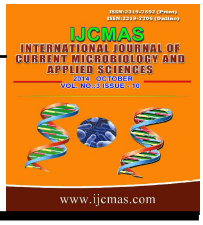

Original Research Article

Phytochemical screening and antimicrobial activity of fruit extract of *Sapindus mukorossi*

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Keywords

Antimicrobial, Antifungal, FTIR, Phytochemical

A B S T R A C T

Sapindus mukorossi is an extremely valuable medicinal plant distributed in tropical and subtropical regions of Asia. Plant extracts appear to be one of the better alternatives as they known to have minimal environmental impact and danger to consume in contrast to synthetic pesticide. The Phytochemical screening of both ethanol and aqueous plant extract revealed the presence of various secondary metabolise such as alkaloids, phytosterols, phenolic compounds, tannins, flavonoids, glycosides and saponins. The FTIR analysis of the ethanol extract of the plant is information about the distribution of functional groups. The antimicrobial activity was done for both ethanol and aqueous extracts. The study revealed that the ethanolic extract of the pericarp of Sapindus mukorossi showed more prominent antibacterial activity against *E.Coli, Staphylococcus aureus* than aqueous extract. The ethanol extract showed antifungal activity against *Aspergillus fumigates* and *Aspergillus Niger* at 100% plant extract.

Introduction

Sapindus mukorossi is a deciduous tree belongs to the family sapindaceae widely grown in upper reaches of Indo-Gangetic plains, Shivaliks and sub-Himalayans tracks at altitudes from 200m to 1500m. Also known as soap-nut tree, it is one of the most important trees of tropical and subtropical regions of Asia. It flowers during summer. The fruit appears in July- August and ripens by November – December. The seeds are 0.8 to 1.3cm in diameter, globosely smooth, black and loosely placed in dry fruit (Chopra and Ghosh, 1946). The fruit is valued for the saponins (10.1%) present in the pericarp constitutes up to 56.5% of the drupe known for inhibiting tumour cell growth (Tanaka *et al.*, 1996). Recently many of the pharmacological actions of this plant have been explored which includes the antimicrobial (Ibrahim *et al.*, 2006) cytoxoic (Sengupta *et al.*, 2004) molluscicidal (Huang *et al.*, 2003) insecticidal (Geyter *et al.*, 2007) and fungicidal (Tanaka *et al.*, 1996). One of the most talked about activities of this plant is the contraceptive of the saponins extracted from the pericarp of the fruit (Garg *et al.*, 1993). Sapindus mukorossi is well known for its folk medicinal values

(Sharma *et al.*, 2011). Pericarps of Sapindus mukorossi have been traditionally used as an expectorant as well as a source of natural surfactant (Kasai *et al.*, 1986). Due to the presence of saponins soap-nut is well known for its detergent and insecticidal properties and is traditionally used for removing lice from the scalp.

The fruits are of considerable importancefor their medicinal value for treating a number of diseases like excessive salivation, pimples, epilepsy, chlorosis, migranes, eczema and psoriasis (Kirtikar *et al.*, 1991). The poedered seeds are employed in the treatment of dental caries, arthritis, common cold, constipation and nausea (Dhar *et al.*, 1989). The seeds of Sapindus mukorossi are used in Ayurvedic medicine to remove tan and freckles from the skin. Its cleanses the skin of oily secretion and is even used as a cleanser for washing hair as it forms a rich, natural lather.

The leaves are used in the baths to relieve joint pain and the roots are used in the treatment of gout and rheumatism. Since ancient times Sapindus mukorossi has been used as a detergent for shawls and silks. The fruit of Sapindus mukorossi was utilised by Indian jewellers for restoring the brightness of tarnished ornaments made of gold, silver and other precious metals (Singh *et al.*, 2010)

The present study was carried out to evaluate the phytochemical screening, FTIR analysis and fruit pericarp of Sapindus mukorossi.

Materials and methods

Chemicals

All the chemicals used for our studies are ARgrade only.

Plant material

The plant samples were procured from local markets and then powdered and stored in air tight bottles.

Preparation of plant material

The powdered material (10g) was successfully extracted with ethanol and water (hot extraction using soxhlet apparatus) and stored in air tight container and kept in refrigerator until use.

Phytochemical screening

Different qualitative chemical tests can be performed for establishing profile of ethanol and aqueous extract for its chemical composition. The following tests were performed on extracts to detect various phytoconstituents present is them (Table 1).

Detection of Alkaloids

Mayer's test

To a few ml of filtrate, a drop or two of Mayer's reagent are added by the side of the test tube. A white or creamy precipitate indicates the test as positive.

Wagner's test

To a few ml of filtrate, few drops of Wagner's reagent are added by the side of the test tube. A reddish brown precipitate confirms the test as positive.

Dragendorff's test

To a few ml of filtrate, 1 or 2 ml of Dragendorff's reagent are added. A prominent yellow precipitate indicates the test as positive.

Detection of carbohydrates

Molish's Test

To 2ml of filtrate, two drops of alcoholic solution of alpha naphthol are added, the mixture is shaken well and 1ml of concentrated sulphuric acid is added slowly along the sides of the tube and allowed to stand. A violet ring indicates the presence of carbohydrates.

Detection of saponins

The extract (50mg) is diluted with distilled water and made up 20ml. the suspension is shaken in a graduated cylinder for 15min. A 2cm layer of foam indicates the presence of saponins.

Detection of Amino acids

Millon's test

To 2 ml of filtrate, few drops of Millon's reagent are added. A white precipitate indicates the presence of proteins.

Biuret test

An aliquot of 2ml filtrate is treated with one drop of 2% copper sulphate solution. To this, 1ml ethanol (95%) is added, followed by excess of potassium hydroxide pellets. Pink colour in the ethanolic layer indicates the presence of proteins.

Ninhydrin test

Two drops of ninhydrin solution (10mg of ninhydrin in 200ml of acetone) are added to 2ml of aqueous filtrate. A characteristic purple colour indicates the presence of amino acids.

Test for sterol

Liebermann – Burchard's test

The extract (50mg) is dissolved in 2ml acetic anhydride. To this, one or two drops of concentrated sulphuric acid are added slowly along the sides of the test tube. An array of colour changes shows the presence of phytosterols.

Detection of phenolic compounds and Tannins

Ferric chloride test

The extract (50mg) is dissolved I 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride Solutions is added. A dark green colour indicates the presence of phenolic compounds.

Lead Acetate test

The extract (50mg) is dissolved in distilled water and to this; 3ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

Alkaline reagent test

An aqueous solution of the extract is treated with 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

Detecting of phlobatanins

The extract (0.5g) was dissolved in distilled water and filtered. The filtrate was boiled with 2% Hydrochloric acid solution. Red precipitate shows the presence of phlobatanins.

Fourier transforms infrared spectrophotometer (FT-IR)

FT-IR is perhaps the most powerful tool for identifying types of chemical bonds

(functional groups). The wavelength of light absorbed is characteristic of the chemical bond as can be seen in this annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. Dried powder of ethanolic extract of each plant materials was considered for instrumental analysis. For the FT-IRstudy dried power of ethanolic extract 10mg of each plant material was encapsulated in 100mg of KBR pellet, in order to prepare translucent sample discs. The powdered sample each plant specimens, were treated for FTIR spectroscopy (Shimadzu, IR Affinity I, Japan). Scan range: from 400 to 4000 cm^{-1} with a resolution of 4 Cm^{-1}.

Antimicrobial activity of various extract

The antimicrobial activity of all the extracts were carried out by disc diffusion method using following organism

Microorganisms used

Bacterial pathogens :
(i) E.coli
(ii) Staphylococcus aureus
Fungal pathogens:
(i) Aspergillus niger
(ii) Aspergillus fumigate

A suspected antimicrobial compound or treatment is present within a reservoir created on an inoculated plate of agar medium; Following diffusion of the compound(s) through the agar, "zone of inhibition" forms where concentration of the diffused molecules are sufficient to inhibit microbial growth. Diffusion of antimicrobial compounds from a reservoir over time produces an outward gradient of decreasing concentration of the compound. Where concentration of the compound is sufficient to inhibit the growth of the microbes, the growth is blocked, resulting in the observed zone, which extends outwards from the reservoir (with a corresponding decrease in concentration) to the distance from the reservoir at which the concentration required for inhibition exists.

Antimicrobial test

This method is suitable for organism that grows rapidly over night at 35ºC -37ºC. The antibiotic (specific concentration) impregnated disc absorbs moisture from the agar and antibiotic diffuses into the agar medium. The rate of extraction of the antibiotic from the disc is greater than the rate of diffusion. As the distance from the disc sees there is a logarithmic reduction in the antibiotic concentration. Zone of inhibition of microbial growth around each disc is measured and the susceptibility measured.

Media for test organism were prepared by 33.6g of muller hinton agar was added to 90ml of sterile distilled H_2O and autoclaved at 121ºC for 15'at 15 lbs. 1.0g of dextrose was added to 10ml of sterile dis. H_2O and steam sterilized for 15' after cooling both the content was mixed and poured into sterile petri plates approximately 4mm and allowed to set at ambient temperature and used.

Assay of antibacterial activity

Antibacterial activity of plant extracts were carried out against bacterial pathogens, such as E.coli and staphylococcus aureus using agar well diffusion method (Baurer *et al.,* 1996). Initially, the stock cultures of bacteria were revived by inoculating in broth media and growth at 37ºCfor 18hrs. The Nutrient agar plates were prepared and wells were made in the plate (Mustika *et al.,* 1984). Each plate was inoculated with 18h old

cultures (100 µl, 10^{-4}cfu) and spread evenly on the plate. After 20 min, the wells were filled with ethanol plant extracts (25%, 50%, 75%, 100%), and aqueous plant extract (25%, 50%, 75%, 100%). The control wells with ethanol and water prepared. All the plates were incubates at 37ºC for 24 h and the diameter of inhibition zone in mm were noted .A a mixture of plant extract (75%) and lime juice (20%) in the proportion (50%,70%,90%,100%) was also filled in another two plates spread with bacteria *(staphylococcus aureus and E.coli)*

Assay of antifungal activity

Antifungal activity of plant extract was carried out against fungal pathogens such as *Aspergillus niger and Aspergillus fumigate* using agar well diffusion method. The stock cultures of fungal pathogens were revived by inoculating in broth media and growth 27ºC for 48 hrs. The potato Dextrose agar plates of the above media were prepared and wells are made in the plate. Each plate was inoculated with 18 h old cultures (100µl 10^{-4} FCU and spread evenly on the plate. After 20 min, the wells were filled with ethanol plant extracts (25%,50%,75%,100%) and Aqueous plant extract (25%,50%,75%,100%). The control plates with water and was also prepared. All the plates were incubated at 27ºC for 48 hours and the diameter of inhibition zone in mm were noted.

Results and Discussion

Phytochemical screening

The phytochemical screening for ethanol plant extract of the above plant parts showed presence of alkaloids, phytosterols, phenolic compounds, tannins, flavonoids, and saponins. Among the two different extracts, ethanol extract show the presence of maximum number of compound.

Table.1 Qualitative phytochemical analysis of plant extracts (Fruit pericarp)

Phytochemical test	Ethanol extract	Aqueous extract
Alkaloid test		
Mayer's test	+	-
Wagner's test	+	-
Dragendorff's	+	-
Carbohydrate		
Molisch's test	-	-
Saponin test	+	-
Amino acids		
Millon's test	+	-
Phytosterols	+	-
Test for Sterols		
Libermann and Buchards test	+	+
Phenolic compounds& Tannins		
Ferric chloride	+	+
Lead acetate	+	+
Alkaline reagent test	+	+
Flavonoids	+	+
Tannis	+	+

Table.2 Antimicrobial activity

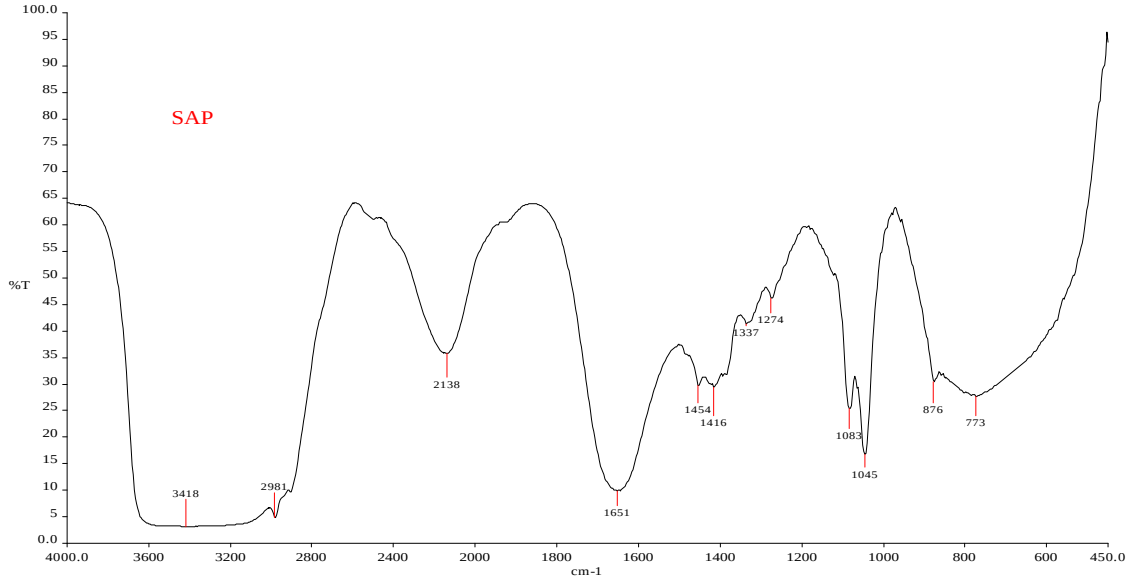
Micro organisms	Zone of Inhibition (*mm*)							
	Aqueous extract				Ethanol extract			
	25%	50%	75%	100%	25%	50%	75%	100%
S.Aureus	NA	NA	NA	6	NA	6	7	9
E.Coli	NA	NA	6	6	NA	7	9	11
A. Fumigate	NA	5	6	8	NA	7	8	10
A. Niger	NA	NA	5	5	NA	NA	7	10

NA= No Activity

Table.3 FTIR for *S.Mukorossi* Fruit

Frequency(cm^{-1})	Functional groups corresponding to the peaks
3418	Alcohols
2981	Alkanes
2138	Carboxylic acids
1651	C=o
1454	Aromatics
1461	Alkanes
1337	Esters
1274	Ethers
1084	Alkylhalide
1045	Alkyl halide
876	Alkyne groups
773	Alkyne groups

Fig.1 FTIR for *Sapindus mukoross* fruit



Functional groups identification

The FTIR spectrum was used to identify the functional groups of the active components present in extract based on the peak values in the region of IR radiation (Fig. 1).

In sapindus pericarp, IR –spectrum shows strong absorption peaks at 3418, 2981, 2138, 1651,1451, 1416,1337,1274, 1083, 1045, 876 and 773which corresponds to alcohols, alkanes, carboxylic acids, C=O, aromatics, alkanes, esters, ethers, alkylhalide and alkyne groups.

Antibacterial activity

The antibacterial assay shows that ethanol extracts of S.Mukorossi pericarp shows maximum zone of inhibition without dilution.The ethanol plant extract shows prominent inhibition against *E.coli* than aqueous extract (Table 2).

Antifungal activity

The ethanol plant extract shows significant zone of inhibition against the fungi without dilution. The fungi is less sensitive to aqueous extract than ethanol extract.

The present investigation indicates that the sapindus mukorossi pericarp has contain more phytochemical constituent. The alcoholic extract is very active against bacteria and fungi when compared to the aqueous extract. FTIR studies has confirmed the presence of various functional groups present in the sapindus mukorossi, Further research has to be done to characterize active metabolite present in the sapindus (Table 3).

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