

## Study on Phytoconstituents and Antimicrobial Potential of *Sapindus mukorossi* Fruit Extract

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DOI 10.51129/ujpah-2020-28-1(2)

**Abstract**-*Sapindus mukorossi* is well known for its folk medicinal values. The fruit is valued for the saponins (10.1%) present in the pericarp which constitutes up to 56.5% of the drupe known for inhibiting tumour cell growth. Recently many of the pharmacological actions of this plant have been explored which include the antimicrobial molluscicidal and insecticidal actions. The fruits are of considerable importance for their medicinal value for treating several diseases like excessive salivation, pimples, epilepsy, chlorosis, migranes, eczema and psoriasis etc. The powdered seeds are employed in the treatment of dental caries, arthritis, common cold, constipation and nausea. Keeping in view the medicinal importance of the fruit, it was thought worthwhile to carry out systematic study on phytochemical investigation and antimicrobial potential of fruit extract of *Sapindus mukorossi*. Fruit were dried, powdered and extracted with different solvent systems with increasing polarity. The extracts were screened for the antibacterial and antifungal activity. Predominance of antibacterial activity was observed at dose level of 50µg/ml of petroleum ether extract. Maximum zone of inhibition (19mm) &(18mm) was observed against *Salmonella typhii* and *Shigella dysenteria* respectively as compared to standard drug Ampicillin 20µg/ml. Since antibacterial activity was observed in 50µg/ml concentration of petroleum ether extract, it was chosen for

exploring its antifungal potential also. Maximum zone of inhibition was observed against *Aspergillus sulfurous* as compared to standard drug ketoconazole 20µg/ml. From the above study, it could be concluded that *Sapindus mukorossi* petroleum ether fruit extracts(50µg/ml) have good antimicrobial potential and can be explored further to isolate active principles from the same.

**Key words:** Epilepsy, Phytochemical Screening, Zone of Inhibition, Antimicrobial potential

### Introduction

The traditional knowledge and use of medicinal plants for curing of disease has been widely established across the globe. 80% of the world's population in developing countries uses traditional medicine as per WHO guidelines. Furthermore, the traditional knowledge with its holistic and systematic approach supported by experimental base can serve as an innovative and powerful discovery engine for newer, safer and affordable medicines. *Sapindus mukorossi* also known as soap-nut 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. <sup>1</sup>The fruit is valued for the saponins (10.1%) present in the pericarp which constitutes up to 56.5% of the drupe known for inhibiting tumor cell growth.

The fruits are of considerable importance for their medicinal value for treating several diseases like excessive salivation, pimples, epilepsy, chlorosis, migranes, eczema and psoriasis. The powdered seeds are employed in the treatment of dental caries, arthritis, common cold, constipation and nausea.<sup>1</sup> The fruit 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 a detergent for shawls and silks. Recently many of the pharmacological actions of this plant have been explored which includes the antimicrobial molluscicidal and insecticidal actions. The fruit of *Sapindus mukorossi* was utilized by Indian Jewellers for restoring the brightness of tarnished ornaments made of gold, silver and other precious metals<sup>2-4</sup>. Various types of triterpene, saponins of oleanane, dammarane and tirucullane type were isolated from the galls, fruits<sup>5-9</sup> and roots of *Sapindus mukorossi*. Oleanane type triterpenoid saponins named Sapindoside A and B were reported from the fruits of<sup>9-11</sup> *Sapindus mukorossi*. Sapindoside C-11, Sapindoside D-12, which is a hexaoside of hederagenin, and Sapindoside, a nonaoside of Hederagenin was isolated and identified by Chirva et, al from the methanolic extract of the fruits of *Sapindus mukorossi*<sup>12</sup>. Keeping in view medicinal importance of the fruit, it was thought worthwhile to carry out phytochemical investigation and antimicrobial potential of fruit extract of *Sapindus mukorossi*.

### **Material and Methods**

All the solvents and reagents used in this study were of analytical grade and were always freshly prepared before use (Merck India Ltd. & Ranbaxy Fine Chemicals Ltd.). Fehling reagent, Mayer's reagent, Dragendroff reagent, Hager reagent, Wagner reagent, Molish reagent, Millon reagent, Ninhydrin reagent, NaOH pellets, FeCl<sub>3</sub> were purchased from Ranbaxy

Fine Chemicals Ltd, New Delhi. Picric acid and Hydrochloric acid was purchased from S.D. Fine Chemicals Ltd. Mumbai.  $\alpha$ -naphthol, H<sub>2</sub>SO<sub>4</sub> was purchased from Merck India Ltd. Mumbai. Mueller Hinton Hi Veg Agar, Peptone, Beep extract and DPPH were obtained from Himedia.

### **Plant Material**

The fruits were procured from garden of SBS University and authenticated by Deptt. of Pharmacognosy, SBS University and kept as voucher specimen in herbarium of the department. Fruit was dried, powdered and stored in airtight bottles.

### **Extraction and Fractionation**

The powdered material (50g) was successfully extracted with solvents of increasing polarity like petroleum ether, chloroform and ethanol using soxhlet apparatus. The extracts so obtained were concentrated and dried concentrates were stored in airtight container and kept in refrigerator until use.

### **Qualitative phytochemical analysis**

The various solvent extracts of *Sapindus mukorossi* fruit were subjected to qualitative chemical tests for the identification of various phytoconstituents viz, alkaloids, carbohydrates, glycosides, proteins, tannins, sterols, saponins, amino acids etc. These phytoconstituents are solely responsible for biological activity of these plants.

### **Antibacterial Activity**

Antibacterial bioassay<sup>13</sup> were evaluated against gram positive bacterial strains, *Staphylococcus aureus* (ATCC 11633S. and gram negative bacterial strains, *Salmonella typhi* (MTCC 733) & *Shigella dysenteriae* (ATCC3313) by disc diffusion method. Standard inoculums (1ml/100 ml of medium) with suspension (10<sup>5</sup>cfu/ml) were introduced on to the surface of sterile agar plates, and a sterile bent glass spreader was used for even

distribution of the inoculum. The discs measuring 6 mm in diameter and 2 mm thickness were prepared from Whatman (grade no. 1) filter paper and sterilized by dry heat for 1 hr. Three discs of test samples were placed on three portion together with one disc with reference drug, Ampicillin and disc impregnated with solvent (DMF) as negative control. The sterile discs previously soaked in a known concentration of the *Sapindus mukorossi* fruit extracts samples (petroleum ether, chloroform and ethanol extracts in 25µg/ml, 50µg/ml, 75µg/ml in dimethyl formamide were placed in nutrient agar medium. Ampicillin (20µg/disc) was used as positive control for bacteria. Plates were inverted and incubated for 24 h at 37±20°C. Diameters of zone of inhibition (mm) were determined and average diameter of test samples were calculated in triplicate sets. Zone of inhibition of test samples were compared with that produced by standard.

#### Antifungal Activity

Fungal infections are most common among the human population and several therapeutic agents are also available in the market but most of them are effective as topical applications. Rare drugs are available for deep mucosal infections. We chose petroleum ether fruit extract of *Sapindus mukorossi* to inspect the antifungal activity, as it has shown good antibacterial effect. Strains chosen were *Aspergillus tarrius*, *Aspergillus sullitrius*, *Aspergillus niger*, *Aspergillus fumigatus*, *Trichophytom* and *Aspergillus flavus*. Medium employed for antifungal activity was potato dextrose agar (PDA) for isolation of fungal culture and sabraud's agar medium for antifungal assay by plate diffusion method<sup>14</sup>. Readily available PDA powder was used having the following composition and pH: Dextrose 2%, Potato peel off-200gm/lit., water, Agar-1.5%, pH-5.

## Results and Discussion

### Qualitative chemical examination of extracts of the fruit of *Sapindus mukorossi*

The different extracts of fruit of *Sapindus mukorossi* were chemically analyzed for the presence of various chemical constituents. Petroleum ether extract shows presence of steroids. Chloroform shows presence of carbohydrates, phenols and saponins while ethanolic was found to be rich in alkaloids saponins, phenols, amino acids and carbohydrates summarized in Table-I. Antibacterial bioassay of three extracts namely petroleum ether, chloroform and ethanol (25,50 75 µg/ml) each were evaluated against gram positive bacterial strains, *Staphylococcus aureus* (ATCC 11633) and gram-negative bacterial strains, *Salmonella typhi* (MTCC 733) & *Shigella dysenteriae* (ATCC3313) by disc diffusion method. From antimicrobial activity (Table- II, fig. I) It was observed that increase in concentration did not have much impact on zone of inhibition. Good and moderate effect was exhibited by petroleum ether and ethanolic extract of the fruit at dose level of 50µg/ml respectively while chloroform extract did not show any prominent results. Maximum zone of inhibition (19mm & 18mm) was observed against *Salmonella typhi* and *Shigella dysenteria* respectively in 50µg/ml concentration of petroleum extract as compared to standard drug Ampicillin 20µg/ml. Since antibacterial activity was observed in 50µg/ml concentration of petroleum ether extract, it was chosen for exploring its antifungal potential also against *Aspergillus tarrius*, *Aspergillus sullitrius*, *Aspergillus niger*, *Aspergillus fumigatus*, *Trichophytom* and *Aspergillus flavus*. Maximum zone of inhibition (24.5 mm) was observed against *Aspergillus sulfurous* as compared to standard drug ketoconazole 20µg/ml (Table-III, fig. II).

**Table-I Results of Phytoconstituents present in various of *Sapindus mukorossi* fruit extracts**

S. No	Plant constituent/Test/ reagent used	Petroleum ether Extract	Chloroform Extract	Ethanollic Extract
1	Alkaloids			
	Mayer's test	+	+	+
	Wagner's test	+	-	+
	Dragendroff's test	+	-	+
2	Carbohydrates			
	Molish' test	+	-	+
3	Saponins			
	Foam test	+	+	+
4	Amino acids			
	Million's test	+	+	+
	Biuret test	+	+	+
	Ninhydrin test	-	-	+
5	Sterol			
	Libermann- burchard test	+	+	+
6	Phenolic compounds/ tannins			
	Ferric chloride test	+	+	+
	Lead acetate test	+	+	+
	Alkaline test	+	+	+

**Table-II Antibacterial effect of *Sapindus mukorossi* fruit extracts**

	Zone of inhibition			Zone of inhibition			Zone of inhibition			Zone of inhibition
	Petroleum ether			Chloroform			Ethanol extract			AMP(20µg/ml)
	Extract (con in %)			extract (con in %)			(con in %)			
	25	50	75	25	50	75	25	50	75	
<i>Salmonella typhi</i>	1	19	15	2	5	5	1	14	14	27
<i>Staphylococcus aureus</i>	0	9	15	3	5	6	3	16	16	25
<i>Shigella dysenteria</i>	2	18	17	2	5	7	0	10	12	26
DMF	-	-	-	-	-	-	-	-	-	

- AMP = Ampicillin
- DMF = Dimethyl formamide
- SA = *Staphylococcus aureus* (ATCC 11633)
- ST = *Salmonella typhi* (MTCC 733)
- SD = *Shigella dysenteriae* (ATCC3313)

*Salmonella typhi**Shigella dysenteria*

**Fig. I** Plates showing Antibacterial effect of *Sapindus mukorossi* petroleum ether fruit extracts(50µg/ml)

**Table-III** Antifungal activity of *Sapindus mukorossi* petroleum ether fruit extracts (50µg/ml)

Sr.NO.	TEST ORGANISM	ZONE OF INHIBITION
1	<i>Aspergillus tarrus</i>	14mm
2	<i>Aspergillus sulfurius</i>	24.5mm
3	<i>Aspergillus niger</i>	21mm
4	<i>Aspergillus flavus</i>	14.5mm
5	<i>Aspergillus fumigatus</i>	20mm
6	<i>Trichophyton</i>	21mm
7	ketoconazole20µg/m	12mm



**Fig.II Plates showing Antifungal effect of *Sapindus mukorossi* petroleum ether fruit extracts(50 $\mu$ g/ml)**

## Conclusion

From the present study, it was observed that petroleum ether fruit extract of *Sapindus mukorossi* rich in steroids showed maximum zone of inhibition against *Salmonella typhi* and *Shigella dysenteria* respectively at dose, 50µg/ml as compared to standard drug, *Ampiciline* 20µg/ml. Since antibacterial activity was observed in 50µg/ml concentration of petroleum ether extract, it was chosen for exploring its antifungal potential also. Maximum zone of inhibition was observed against *Aspergillus sulfurous* as compared to standard drug ketoconazole, 20µg/ml. Thus, from the above study, it could be concluded that *Sapindus mukorossi* petroleum ether fruit extracts(50µg/ml) has good antimicrobial potential and can be explored further to isolate active principle from the same.

## Conflict of Interests

The authors declare the absence of conflict of interests.

## Acknowledgement

The authors gratefully acknowledge Management of Dolphin (PG) Institute of Biomedical & Natural Sciences, Dehradun, India for providing the necessary facilities during this experimental study.

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