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Physicochemical, Phytochemical Studies and Haemolytic Activity of Different Extracts of *Sapindus mukorossi*

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ABSTRACT

Herbal medicine is an oldest form of medical treatment that is using to treat specific conditions and many diseases. *Sapindus mukorossi* Gaertn. (Sapindaceae) known as "Reetha" has the reputation of being used as a medicinal herb for curing various ailments. In present study various physicochemical parameters and Haemolytic activity of fruits of *Sapindus mukorossi* were carried out.

INTRODUCTION

The genus Sapindus consists of different deciduous and evergreen species of shrubs and small trees in the family Sapindaceae. These are native of temperate to tropical regions of world. Members of the genus are commonly known as soapberries or soapnuts as the pericarp of fruits is used to make soap.

Figure 1: Fruits of *Sapindus mukorossi*

Sapindus mukorossi is commonly known as reetha in Nepal[2, 3]. It is large, deciduous tree, usually up to 12 m and sometimes of 20 m height. Fruit is a fleshy drupe having globose shape, and is single-seeded. The fruit is commonly known as a washnut[4], have the highest saponin content. Soapnuts have been used medically as an expectorant, emetic, contraceptive, and for treatment of excessive salivation, epilepsy, psoriasis, anti-tumour and migraines. Reetha has hold deep roots in Ayurveda and used as main ingredient in Ayurvedic medicines and cosmetics as cleansing lotion, protein shampoo, and conditioner. The ayurvedic preparations are used for eczema and psoriasis and are traditionally used for removing lice from the scalp due to its insecticidal properties. Moreover *Sapindus mukorossi* is useful for treating a number of other diseases like common cold, pimples, constipation, nausea, contraceptive, and chlorosis[5, 6, 7].

Moreover the saponin from the soapnuts is used as an auxiliary and in preparation of toothpaste. It also has antiviral, reducing blood pressure functions[8,9,10,11,12,13]. So it is widely used in chemical industries, food and textile industries.

The major constituents of *Sapindus mukorossi* fruit are sugars, mucilage, [14]saponins, mainly triterpenoids[15,16,17], fatty acids[18] and flavonoids[19]. Different types of triterpene, saponins of oleanane (Sapindoside A & B)[20], dammarane and tirucullane type were isolated from the galls, fruits and roots of *Sapindus mukorossi*.

MATERIALS AND METHODS

The fruits of *Sapindus mukorossi* Gaertn were procured from Dhari Shah Pharmacy, Ambala Cantt and were authenticated.

Requirements

Silica crucible, Ashless filter paper (Whatman no. 44), Petri dish, stoppered conical flask, Magnetic stirrer, Alcohol (95%), chloroform, ethyl acetate, distilled water, reagents, ELISA plate, Micropipettes, Pipettes, Volumetric flasks, RBC, Phosphate buffer pH 7.4.

Physicochemical parameters

The various physicochemical parameters like Extractive values, Ash value, Loss on drying and preliminary phytochemical screening were performed as per IP, 1996.

Preparation of extracts

The powdered fruits of *Sapindus mukorossi* were extracted four times with methanol, ethyl acetate, chloroform and water separately for one hour each time at 70°C. All the extracts were filtered through the muslin cloth, pooled and dried under vacuum in a distillation assembly. Foam was produced during water extraction so 2-3 ml Liquid paraffin was used as antifoaming agent. It was filtered through the muslin cloth. The aqueous extract was pooled and was shaken with Hexane and allowed to stand for some time for the separation of liquid paraffin. Afterwards it is dried under vacuum. The waxy material was obtained.

Haemolytic activity

Preparation of blood reagent: 10 ml of 3.6% sodium citrate solution was added to 90 ml fresh bovine blood. 2 ml blood was mixed with 30 ml phosphate buffer pH 7.4.

Preparation of phosphate buffer: 6.8 g of potassium dihydrogen phosphate was dissolved in 250 ml distilled water and added 393.4 ml of sodium hydroxide solution in it (0.1 mol/l prepared by dissolving 4.0 g of sodium hydroxide in 1000 ml distilled water).

Determination of Total Saponins by gravimetric method

Method 1

5 gm of the extract was accurately weighed and dissolved it into 100 ml of water. The solution was partitioned 3 times with 50 ml benzene each time. The benzene fractions were discarded. The aqueous layer was further partitioned 3 times with 50 ml n-butanol each time. The butanol fractions were collected and it was shaken with 50 ml of water. The butanol layers were collected and filtered to remove the traces of water. The butanol fractions were dried under vaccum and kept it in desiccator and weighed.

% of total saponins = weight of residue / weight of extract taken X 100

Method 2

5 gm of the extract was accurately weighed and dissolved in 100 ml of water. The solution was partitioned 3 times with 50 ml Diethyl ether each time. The ether layers were discarded. The aqueous layer was further partitioned 3 times with 50 ml n-butanol each time. The butanol fractions were collected and shaken with 50 ml of water. The butanol layers were collected and filtered it to remove the traces of water. The butanol fractions were pooled, dried under vacuum and kept in desiccator and weighed.

% of total saponins = weight of residue / weight of extract taken X 100

Method 3

2 gm of the powdered material was weighed in a beaker and 50 ml of petroleum ether was added and gently heated to 45°C on a water bath for 5 min and shaken at regular intervals. It was filtered and the operation repeated further with 2 X 50 ml of petroleum ether. The petroleum ether layer was discarded and the marc was preserved. The marc was extracted at 40°C four times with 60 ml methanol each time. The methanol layers were pooled, filtered and concentrated to 25 ml. The concentrate was slowly added to 150 ml acetone to precipitate the saponins. The saponins were filtered and dried at 100°C to a constant weight.

% of total saponins = Weight of residue / weight of sample taken X 100

RESULTS AND DISCUSSION

Table 1: Physicochemical Constants of the fruit of *Sapindus mukorossi*

Physicochemical parameters	Value determined (%w/w)	Limits (Anonymous, 2005)
Total ash	1.69	≥8.4
Acid-insoluble ash	0.002	≥0.73
Loss on drying	6.8	≥10.0
Water-soluble extractive value		
By cold maceration	67.04	≤36.0
By hot percolation	75.2	≤36.0
Ethanol-soluble extractive value		
By cold maceration	49.6	≤33.0
By hot percolation	62.24	≤33.0

Table 2: Extractive value of fruit of *Sapindus mukorossi* by successive solvent extraction

S. No.	Solvent	Color of extract	Extractive value (%w/w)
1	Alcohol	Light brown	46.4
2	Ethyl acetate	Pale yellow	12.77
3	Chloroform	Greenish yellow	22.43
4	Water	Dark brown	70.11

Table 3: Qualitative chemical examination of extracts of the fruit of *Sapindus mukorossi*

S. No.	Plant constituent/Test/ reagent used	Alcohol extract	Ethyl acetate extract	Chloroform extract	Water extract
1.	Carbohydrates				
	Molish's reagent	+	+	+	+
	Fehling solution	+	+	+	+
2.	Proteins				
	Biuret test	-	-	-	-
	Millons test	-	-	-	-
3.	Phenolic compounds/ Tannins				
	Ferric chloride solution	-	-	-	-
	Lead acetate	-	-	-	-
4.	Steroids and triterpenoids				
	Salkowski reaction	-	-	-	-
	Liebermann – Burchard reaction	+	+	+	+
	Liebermann reaction	-	-	-	-
5.	Glycosides				
	Borntrager's test	-	-	-	-
	Modified Borntrager's test	-	-	-	-
6.	Flavonoids				
	Shinoda test	+	+	-	-
	Lead acetate solution	+	+	-	-
7.	Alkaloids				
	Mayer's reagent	-	-	-	-
	Dragendorff's reagent	-	-	-	-
	Hager's reagent	-	-	-	-
	Wagner's reagent	-	-	-	-
8.	Saponins				
	Foam test	+	+	+	+

Table 4: Haemolytic Test of Alcoholic Extract of Reetha Powder

Blood suspension(µl)	Phosphate buffer (µl)	Sample solution (µl)	Concentration(µl/µg)	Results
100	90	10	100	-
100	85	15	150	+
100	80	20	200	+
100	50	50	500	+
100	-	100	1000	+

Table 5: Preliminary Test, Haemolytic Test of Ethyl acetate Extract of Reetha Powder

Blood suspension(µl)	Phosphate buffer (µl)	Sample solution (µl)	Concentration(µl/µg)	Results
100	90	10	10	-
100	80	20	20	+
100	50	50	50	+
100	-	100	100	+

Table 6: Haemolytic Test of Aqueous Extract of Reetha Powder

Blood suspension(µl)	Phosphate buffer (µl)	Sample solution (µl)	Concentration(µl/µg)	Results
100	90	10	10	-
100	80	20	20	-
100	70	30	30	+
100	50	50	50	+
100	-	100	100	+

Table 7: Haemolytic Test of Chloroform Extract of Reetha Powder

Blood suspension(µl)	Phosphate buffer (µl)	Sample solution (µl)	Concentration(µl/µg)	Results
100	90	10	10	-
100	80	20	20	+
100	50	50	50	+
100	-	100	100	+

Table 8: Determination of Total Saponins by Hemolytic Values

Sample taken	Lowest Concentration (µg/µl) showing haemolysis
Alcoholic Extract	150
Ethyl Acetate Extract	20
Aqueous extract	30
Chloroform Extract	20

Table 9: Determination of Total Saponins:

Sample taken	% Total saponins		
	Method 1	Method 2	Method 3
Aqueous extract	62	-	58
Alcoholic extract	33.52	14.05	31.26
Ethyl acetate extract	64.56	0	62.21
Chloroform extract	76.0	0	73.29

Table 10: Comparative Table

Sample taken	Lowest Concentration(µg/µl) showing Haemolysis	% Total saponins (Mean of method 1 and 2)
Alcoholic extract	150	32.39
Ethyl acetate extract	20	63.38
Aqueous extract	30	60.00
Chloroform extract	20	74.645

CONCLUSION

The different extracts of fruit of *Sapindus mukorossi* were chemically analyzed for the presence of various chemical constituents. Gravimetric analysis of the extracts was done which included the estimation of total saponins. Saponins being the main constituent of reetha are present in every extract, so Haemolytic activity of the extracts was checked at different concentrations. It was found that the extract having high saponin content showed haemolysis at lowest concentration. This meets the fact that saponins possess haemolytic activity.

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