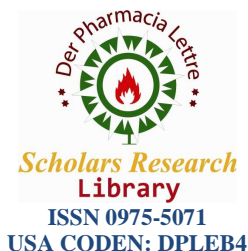




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Botanical and phytochemical evaluation of fruits of *spondias pinnata*

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ABSTRACT

Botanical and phytochemical evaluation of fruits of spondias pinnata. Various parameters like physicochemical constants, fluorescence analysis and phytochemical profile including TLC fingerprint. These studies will provide referential information for correct identification and help in checking adulteration in market samples used in the preparation of various herbal formulations. The salient qualitative and quantitative parameters are reported. The plant is rich in flavonoids and phenolics.

Keywords: *Spondias pinnata*, Anacardiaceae, physicochemical, phytochemical screening, triterpenoids.

INTRODUCTION

Spondias pinnata belonging to the Anacardiaceae family has been previously investigated for the biological activities of the crude extract with special emphasis to the antimicrobial activity, antioxidant activity and anti-inflammatory activity. In this study, the powdered fruit of *Spondias pinnata* was extracted with ethanol and subsequent preliminary phytochemical screening was performed which revealed the presence of alkaloids, carbohydrates, flavonoids, triterpenoids, steroids, tannins, resins and saponins from the same extract. This study includes pharmacognostic evaluation, determination of physicochemical parameters of the extract using TLC fingerprinting. The study or use of medicinal herbs to prevent and treat diseases and ailments or to promote health and healing. Phytochemical studies have yielded flavonoids, tannins, saponins and terpenoids. Essential oil from the pulp yielded carboxylic acids and esters, alcohols, aromatic hydrocarbons. Fruits yield β -amyryn, oeanolic acid, glycine, cystine, serine, alanine, and leucine. Aerial parts yield lignoceric acid, β -sitosterol and its glucoside [5]. The fruits are eaten as a vegetable when green and as a fruit when ripe. They are used for flavoring. The flowers are sour and used in curry as a flavoring and also eaten raw as well as the local people make chutney, jam and pickle. Fruits are very nutritious and rich in vitamin A, minerals and iron content. It is astringent, sour, thermogenic, appetizer and aphrodisiac and is good for rheumatism and sore throat [7]. The bark is useful in dysentery and diarrhea and is also prevent vomiting. The root is considered useful in regulating menstruation. The plant is reported to have anti-tubercular properties. The leaves are aromatic, acidic and astringent [5].

Hence, *Spondias pinnata* is used in both folk and traditional system of medicine. The present investigation is performed to standardize the plant through pharmacognostical and phytochemical analysis.



Fig.1 *Spondias pinnata*, Anacardiaceae

MATERIALS AND METHODS

Collection and authentication

The fruits of *Spondias pinnata* (Anacardiaceae) were collected from local market of Lucknow, in the month of January 2014. The plant material was authenticated by Mr. Muhammad Arif (Assistant Professor) and Dr. Arshad Hussain (Associate Professor) Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Integral University, Lucknow- 226 022, A voucher specimen of *Spondias pinnata* (Anacardiaceae) (IU/PHAR/HRB/14/12) was deposited in the institute for future reference.

Preparation of plant extract

The freshly collected fruits of *Spondias pinnata* (Anacardiaceae) were washed with distilled water to remove dirt and soil and shade dried. Dried plant material was reduced to coarse powder by mechanical grinder and further extraction was carried out with ethanol by Soxhlet extraction method to avoid damage due to heat. The extract was filtered and concentrated under reduced pressure, below $40 \pm 1^\circ\text{C}$ using roteva vaccum rotary evaporator (Model no- UDOIAB-2391 Medica instrument), to dryness to get a constant weight.

Physico-chemical and fluorescence analyses

Analysis of the physicochemical parameters of the fruit powder was done to evaluate the quality and purity of the drug. Various physicochemical parameters like moisture content, extractive values and ash values were calculated as per WHO guidelines.

Fluorescence analysis of the powder sample was carried out by treating with different chemical reagents to observe various colour instances. The extract of the powdered plant was prepared with different polar and non-polar solvents for the study of successive extractive values. Fluorescence analysis of the powder sample was carried out by treating with different chemical reagents in day light and UV light (254 nm and 365 nm). The dry powder was studied on glass slide whereas the different extracts were studied by adsorbing the extracts on Whatmann filter paper [14].

Preliminary phytochemical analysis

Preliminary phytochemical screening for the detection of various chemical constituents was carried out by using standard procedures described by Harborne [14, 15, 16].

TLC identity test

Thin layer chromatography of the petroleum ether, chloroform and methanol was carried out in various solvent system at 30°C using silica gel G as adsorbent and the R_f values were determined [17, 18].

RESULTS AND DISCUSSION**Macroscopic characters**

The fruit is simple, succulent, and fibrous and drupe type of fruit. The Epicarp is thin, greenish yellow when ripe. Mesocarp is soft acidic, juicy when ripe, aromatic, 6-8 celled and Endocarp is tough, fibrous and woody

Shape and size : ovoid or oblong up-to 4-5 cm. in diameter.
 Colour : Fresh fruit is yellowish green and dried fruit is externally dark brown, internally yellowish brown.
 Taste : Astringent
 Odor : Aromatic pleasant
 Texture : Hard, stone semi woody, fibrous with many cavities outside.
 Epicarp and mesocarp is very brittle.

Physico-chemical and fluorescence analyses

Quantitative standards of fruit like moisture content of fresh sample of fruits were determined as usual method. Ash values and Loss on drying of dried sample were determined as per Indian Pharmacopoeia and results are shown in Table 1b. Percentage of moisture content of fresh fruit was determined as follows. Total ash, acid insoluble ash, water soluble ash values of fruit powder were done as per Indian Pharmacopoeia. The results are shown in Table 1b. The fluorescence analysis of powdered drug in day light, short UV and long UV were examined by reported methods. The observations are given in Table 2.

Physico-chemical data like loss on drying, ash values, foreign matter and successive extractive values with different solvents of powdered plant were determined. The percentage of all values in triplicate and their mean values \pm SEM were calculated with reference to the air dried drug (Table 1a).

Table 1. Quantitative standards of powdered fruit parts of *Spondias pinnata*
1(a) Extractive values of different extracts of *Spondias pinnata*

Solvent used	Cold extractive values (g)	Hot extractive values Individual(g)	Successive extractive values (g)
1. <i>Petroleum ether</i>	0.26 \pm 0.56	0.34 \pm 0.115	0.48 \pm 0.163
2. <i>Chloroform</i>	0.42 \pm 0.321	0.74 \pm 0.040	0.52 \pm .062
3. <i>Methanol</i>	1.55 \pm 0.45	2.54 \pm 0.052	2.07 \pm 0.075
4. <i>Water</i>	2.96 \pm 0.08	3.79 \pm 0.11	2.76 \pm 0.413

1(b) Ash values of the powdered fruit parts of *Spondias pinnata*

1.	Ash value	
	Total ash value	5.49 \pm 0.144
	Acid insoluble ash	0.81 \pm 0.020
	Water soluble ash	4.62 \pm 0.053
2.	<i>Loss on drying</i>	13.77 \pm 0.228

1(c) Powder characteristics of the fruit parts of *Spondias pinnata*

S. No.	Reagents+ powdered fruit	Constituents	Colour	Degree of intensity
1.	Phloroglucinol + Conc. HCl	Lignin	Pink	+++
	Aniline hydrochloride	Lignin	Bright yellow	++
	Chlor-zinc-Iodine Solution	Suberin	Yellowish brown	--
	Ruthenium red solution	Mucilage	--	--
	Iodine solution	Starch	Blue	+
2.	Millon's reagent	Protein	Brick red	++
	Dragendorff's reagent	Alkaloids	--	--
	Conce. NaOH (Aq)	Flavonoids	Golden yellow	+++
	Frothing test	Saponins	--	--
	Aqs Ferric Chloride	Phenolics / Tannins	Smoke colour	++
3.	Keddy reagent	Sterol glycoside	Pink colour	++
4.	Salvoski's test	Steroids	Orange color at the junction of two layer	++
5.	5% KOH (Aq)	Anthraquinone glycoside	--	--
	Spot test	Fixed oil	--	--

The fluorescence analysis of the powdered drug in various solvents and chemical reagents was performed under normal and ultraviolet light (Table-2).

Table 2. Fluorescence analyses of the powdered fruit parts of *Spondias pinnata*

S. No.	Treatment	Day light	UV (254nm)	UV (365nm)
1.	Dry powder	Brown yellow	Yellow	Fine particles gives orange colour
2.	Powder + 1M-NaOH alcoholic	Light brown	Flesh	Italic
3.	Powder + 1M-NaOH aqueous	Light pink	Light yellow	Coffee brown
4.	Powder + 1 M-HCl aqueous	Straw colour	Coffee brown	Dark brown
5.	Powder + 50% H ₂ SO ₄	Light yellow	Yellow	Chocolate brown

Preliminary phytochemical analysis

Preliminary phytochemical screening for the detection of various chemical constituents was carried out by using standard procedures described by Harborne. Table 3 [5,14, 15].

TLC fingerprint

For the identification by TLC fingerprint the ethanolic extract was analysed. The solvent system used was Methanol: Ethyl acetate: Acetic acid: in the ratio of (5:4:1) and shows three spots at R_f value 0.32, 0.41 and 0.85 Anisaldehyde sulphuric acid reagent was used as detecting agent. The R_f values were compared with standard drug gallic acid and colours were recorded.

Solvent system used was Chloroform: Ethyl acetate: Formic acid in the ratio of (5:4:1) and R_f value 0.07, 0.65,0.82 and n-butanol: Acetic acid: Water (4:1:5) and R_f value 0.13,0.63,0.78. [11].

Table 3. Qualitative phytochemical analysis of various extractives of fruit parts of *Spondias pinnata*

S.NO	EXTRACT	METHANOL	CHLOROFORM	PETROLEUM ETHER	ETHYL ACETATE
1.	Alkaloid	+ve	-ve	-ve	+ve
2.	Amino acid	-ve	-ve	-ve	-ve
3.	Steroid/Tri-terpenoids	-ve	-ve	-ve	-ve
4.	Tannins	+ve	-ve	-ve	+ve
5.	Flavanoid	+ve	-ve	-ve	+ve
6.	Glycosides	+ve	-ve	-ve	+ve
7.	Reducing sugar	+ve	-ve	-ve	-ve
8.	Saponin	+ve	-ve	-ve	+ve

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