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Research Article

PHYTOCONSTITUENTS AND HPTLC ANALYSIS IN *SARACA ASOCA* (ROXB.)WILDE

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ABSTRACT

Saraca asoca (Roxb.)Wilde, (Ashok) belonging to the family Caesalpiniaceae is a universal panacea in the ayurvedic medicine. This versatile plant has anti-cancer, anti-menorrhagic, anti-oxytocic, anti–microbial activity and has extended uses in ayurveda, unani and homeopathy. The present study attempts to investigate the qualitative phytochemical constituents from extracts of the flower of *Saraca asoca* (Roxb.)Wilde. Tannin, flavonoids, carbohydrates, proteins, steroids have been found in different extracts of the flowers. Phytochemical as well as physicochemical analysis is useful to establish the authenticity of the crude drug form. A sensitive and reliable high performance thin layer chromatographic (HPTLC) method has been developed for qualitative determination of a pharmacologically important active constituent- gallic acid in the dried flowers and leaves of *Saraca asoca* (Roxb.)Wilde. The assay combines the separation of analytes on precoated silica gel 60 F_{254} HPTLC plates as stationary phase and Toluene: Ethyl Acetate: Formic Acid: Methyl Alcohol (6:6:1.6:0.4 v/v/v/v) as mobile phase. Detection has been carried out densitometrically at λ =280 nm for gallic acid. This is the first report of occurrence of gallic acid in leaves of *S. asoca*. Further investigation will be carried out for the quantification of the test compound, gallic acid in *Saraca* leaf.

Keywords: *Saraca asoca,* Gallic acid, Flower, Leaf, HPTLC

INTRODUCTION

Medicinal herbs are moving from fringe to mainstream uses with a great number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. Ashoka is one of the most ancient trees of India commonly known as an "ashok briksh". *Saraca asoca* (Roxb.)Wilde or *Saraca indica* belongs to the family Caesalpiniaceae. All the plant parts are considered to contain medicinal properties. Leaves of *Saraca asoca* are known to contain carbohydrates, proteins, tannins and saponins[1] and shows antibacterial activity[1]. Barks contain glycosides, steroids, saponins, carbohydrates and tannins[2]. The flowers (Figure1) are also regarded as medicinally important plant part and used as therapeutic agent in treatment of diabetes, cancer and hemorrhagic dysentery, uterine infections as menorrhagia and other types of uterine disorders. It is also used in bleeding piles, bacillary dysentery.

Fig. 1: Flowers of *Saraca asoca* (Roxb.)Wilde.

Dried flower buds are reported to have antibacterial activity[3]. Aqueous suspension of *Saraca indica* flower has antiulcer activity in albino rats[4]. *Saraca asoca* bark and flowers exhibit antitumour activity against DLA, S-180 and Ehrlich ascites carcinoma tumour cell lines[5]. Larvicidal activity has also been recorded[6]. Chemo preventive activity of flavonoid fraction of *S. asoca* is reported in skin carcinogenesis[7]. During '*ashoka-sasthi*' the flower buds are taken orally by women. Though phytoconstituents have been reported earlier in case of leaves and bark of the plant[1,2], no detail qualitative phytochemical analysis are found for flowers. The present paper attempts to cover this lacuna by evaluating the phytoconstituent standardization parameters in flowers that can possibly be useful for identification of the drug in the dry form. However, the presence of gallic acid, an antioxidant molecule, has already been reported in *Saraca asoca* flower[2], but there is no reference about its presence in *Saraca* leaf. The detection and qualitative assay has been developed by HPTLC method for the standard gallic acid[8]. Simple HPTLC method is used to confirm the presence of gallic acid in *Saraca asoca* flowers as well as leaves.

MATERIALS AND METHODS

Collection and authentication of plant material

The flowers and leaves of *Saraca asoca* were collected in March 2011 from the campus of Bethune College, Bidhan Sarani, Kolkata, and West Bengal, India. The species was authenticated by Professor Gour Gopal Maity of University of Kalyani , West Bengal.

Preparation of extracts

Plant samples were washed with water and air-dried at room temperature for 7 days, then oven-dried at 40ºC to remove the residual moisture. The dried flowers and leaves were pulverized and stored in air-tight container for future use. Four different solvents were used for preliminary phytochemical screening - chloroform, ethanol, methanol and distilled water. Equivalent amount of powdered samples of flower were extracted with chloroform, ethanol and methanol successively at room temperature for 3 days. Water extraction was done in water bath at 60ºC. The four filtrates were separately concentrated in water bath at 45ºC and evaporated under reduced pressure and then the percent extract yield (%) was calculated.

Phytochemical analysis

The four extracts obtained from the powdered flowers of *Saraca asoca* were subjected to phytochemical tests to determine the presence of active secondary metabolites using standard procedures [9].

Physico-chemical parameter[1]

Loss on Drying: 5 gm of the drug was weighed in an evaporating dish. It was dried at 105°C for 3 hours and weighed again. The drying and weighing was continued at intervals of one hour until difference between two successive weights was not more than 0.25%.

Ash Values: Ash values are useful in determination of crude drug in the powder. The ash values represent the inorganic salt present in the drug. The different ash values such as total ash, acid insoluble ash and water soluble ash were determined from shade dried flower powder.

HPTLC analysis

Chemicals and standard gallic acid

Gallic acid was obtained from Titan Biotech Ltd. and Methanol, Toluene, Ethyl Acetate, Formic Acid were used of analytical grade E-Merck. Silica gel 60 F_{254} precoated Thin Layer Chromatography (TLC) aluminium plate was used of E-Merck.

Preparation of standard and sample solution

5 mg Gallic acid was dissolved in 3 ml of methanol. It was then sonicated for 5 min and the final volume was made upto 5 ml with the same solvent to obtain stock solution containing 1 mg/ml. Air dried samples (0.5g) was extracted with 10 ml of methanol. Extracts were concentrated, filtered and the final volume made upto 10 ml with methanol prior to HPTLC analysis to get stock solution containing 50 mg/ml.

Chromatographic conditions

Chromatography was performed on precoated silica gel 60 F_{254} HPTLC plates (10.0 x 10.0 cm). Methanolic solutions of standard compound (gallic acid) and samples of known concentrations were applied to the plate positioned at 10 mm from the bottom and 19 mm from the side of the plate having 8 mm bandwidth using a CAMAG Linomat 5 automated TLC applicator with the nitrogen flow providing a delivery speed of 150 nl/s from the syringe.

Detection of Gallic acid

Plate was eluted in pre-saturated CAMAG twin trough glass tank with the mobile phase Toluene: Ethyl Acetate: Formic Acid: Methyl alcohol (6:6:1.6:0.4 v/v/v/v) to a distance of 86.2 mm at room temperature. After drying, the spots were visualized under CAMAG UV cabinet (254 and 280 nm). Then the plate was scanned using CAMAG TLC scanner 3 equipped with WINCATS software (CAMAG). The identification of gallic acid in methanolic solution of flower and leaf of *Saraca asoca* was confirmed by superimposing the UV spectra of samples and standards within the same retention factor (R_f) value.

RESULTS

Phytochemical and physicochemical analysis

The experimental yield of chloroform, ethanol, methanol and water extracts of *Saraca* flower were found to be 1.80% 11.90%, 15.10% and 22.00% respectively (Table 2). Water soluble extractive value showed the presence of sugar, acids and inorganic compounds and alcohol soluble extractive values determined the presence of polar constituents. The physicochemical parameters total ash, acid insoluble ash and water soluble ash value were found to be 3.00%, 2.02% and 1.12% respectively (Table 3). Total ash value percentage showed the amount of mineral and earthy material present in the plant sample. The amount of acid insoluble siliceous matter present in the plant sample was 2.02% (Table 3).

Preliminary phytochemical results indicate the presence or absence of phytochemical-constituents in different extracts of the *Saraca* flower (Table 1). Alkaloids were found to be absent in all the four extracts. Carbohydrates, tannin, flavonoid, saponin, glycosides, proteins and steroids were found to be present in methanol and ethanol extracts. The chloroform extract contained only carbohydrates whereas the water in addition to carbohydrates, contain tannin, flavonoid, saponins, and steroids.

Table 1: Table shows preliminary phytochemical screening of *Saraca asoca* flower extracts

Phytoconstituents	Test/ Reagent	Chloroform extract	Ethanol extract	Methanol extract	Aqueous extract
Alkaloids	Dragendorff's test	-	-	-	-
	Hager's test	-	-	-	-
	Wagner's test	-	-	-	-
Proteins	Biuret test	-	+	+	-
	Ninhydrin's test	-	+	+	-
	Millon's test	-	+	+	-
Tannins	Lead Acetate	-	+	+	+
	Potassium dichromate	-	+	+	+
	Ferric Chloride	-	+	+	+
Steroids	Salkowski test	-	+	+	+
Carbohydrates	Fehing's test	+	+	+	+
	Benedict's test	+	+	+	+
	Barfoed's test	+	+	+	+
Flavonoids	Sodium hydroxide test			+	+
	Conc. H_2SO_4 test	-	+	+	+
Glycosides	Borntrager's test	-	+	+	-
	Keller-Killani test	-	+	+	-
Saponins	Foam test	-	+	+	+

(+)Present (-)Absent

Table 2: Extractive values of *Saraca asoca* flowers

Types of solvents	% w/w
Chloroform	1.80
Ethanol	11.90
Methanol	15.10
Water	22.00

Table 3: Ash values and loss on drying of *Saraca asoca* flowers

Total ash	3.00
Acid insoluble ash	2.02
Water soluble ash	1.12
Loss on Drying	12.90

HPTLC analysis

Photograph of chromatograms of the standard Gallic acid at 280 nm obtained in the methanolic extract of dried flower extract and leaf extract of *Saraca asoca* are given in Figure 2. The Gallic acid bands in sample chromatogram of *Saraca* sp. are identified and confirmed by comparing the chromatogram obtained from the reference standard solution (Figures 3- 5) and by comparing retention factor(R_f) of Gallic acid from sample and standard solution. The R_f value of standard gallic acid is 0.44, whereas the R_f value of methanolic extract of the flower and leaf of *Saraca asoca* is 0.43 which almost coincides with standard R_f value of gallic acid (Figure 2).

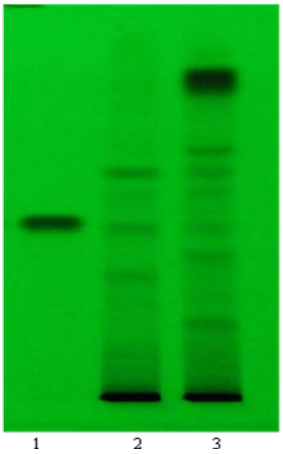

Fig. 2: Photograph of chromatograms obtained at 280nm from standard Gallic acid (1)Flower extract(2) and Leaf extract (3) of *Saraca asoca*

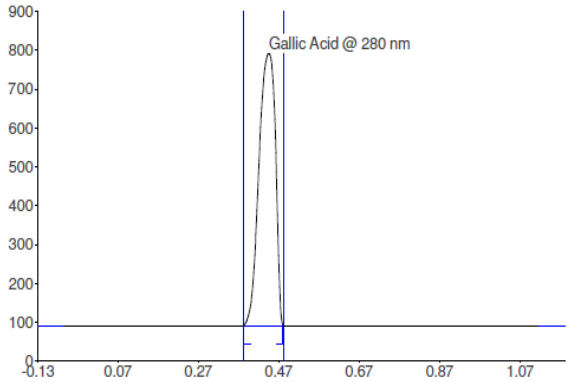

Fig. 3: Chromatogram of standard Gallic acid

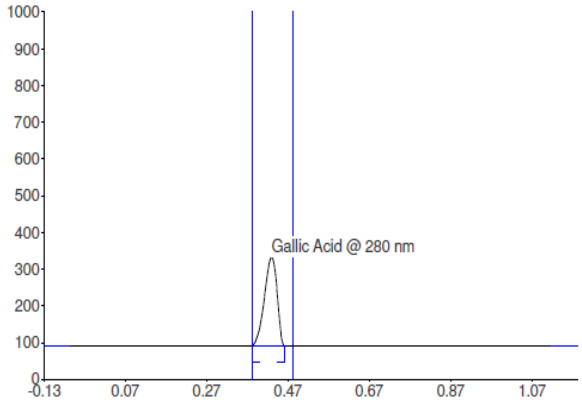

Fig. 4: Chromatogram of Gallic Acid in the methanolic extract of flower of *Saraca asoca*

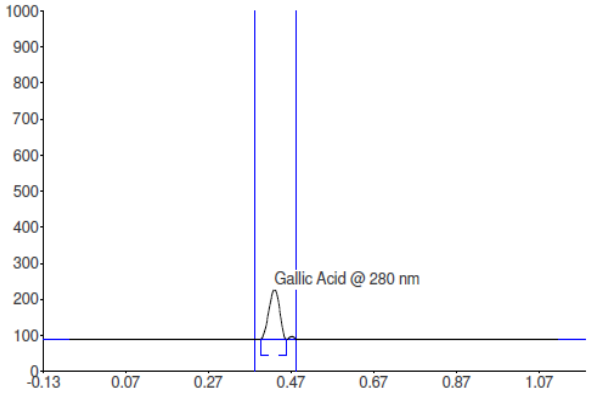

Fig. 5: Chromatogram of Gallic Acid in the methanolic extract of leaf of *Saraca asoca*

DISCUSSION

Preliminary phytochemical screening revealed the presence of tannins, proteins, steroids, glycosides, carbohydrates, saponins, flavonoids in different extracts of the flower of *Saraca asoca.* These results show that flowers of *Saraca asoca* contain a number of chemical ingredients, which may be responsible for the various pharmacological actions although their specific roles remain to be investigated. It has been observed that most active principles present in the flowers are flavonoid, steroids, tannins and glycosides. These phytoconstituents may be responsible for various pharmacological actions of this plant part, like antibacterial, antiulcer, anticancer, larvicidal and chemo protective activities [3,4,5,6,7]. Methanolic extracts of flower and leaves confirmed the presence of gallic acid using HPTLC assay. This is the first report of the presence of gallic acid in *Saraca asoca* leaf. The presence of gallic acid in leaf is very important since flower is only seasonal, while leaf is available all year round. Hence, the amount of gallic acid in *Saraca asoca* leaf can be quantified further for proper utilization of this age old plant. The physicochemical evaluation of this plant is an essential parameter for the detection of adulterant and improper handling of drugs. The present work can serve as a valuable source of information and provide appropriate standards to establish the quality of this plant material in future study or application.

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