

Available online on 15.12.2022 at <http://ajprd.com>

Asian Journal of Pharmaceutical Research and Development

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Research Article

Qualitative and Quantitative Screening of Phytochemicals in Polar and Non Polar Solvent Extracts of Stem Bark and Leaves of *Saraca Indica*

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ABSTRACT

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 prospective study. From the results it is evident that the bark and leaves of *Saraca indica* contain a significant amount of phytochemicals viz. alkaloid, flavonoids, phenolic, saponins and tannin. The bark of *Saraca indica* contained relatively higher amounts of phytochemicals than leaves. The comparative analysis of phytochemicals viz. total alkaloids, flavonoids, phenols, saponins and tannins in different solvent extracts from bark and leaves of *Saraca indica*

Keywords: *Saraca Indica*, Qualitative, Quantitative, Screening, Phytochemicals, Stem Bark

ARTICLE INFO: Received 15 Sept. 2022; Review Complete 28 Oct. 2022; Accepted 26 Nov. 2022; Available online 15 Dec. 2022

Cite this article as:



Gogate S, Mathur R, Raghuvanshi A, Yadav AK, Dhurve D, Sen DK, Raj D, Rathi J, Qualitative and Quantitative Screening of Phytochemicals in Polar and Non Polar Solvent Extracts of Stem Bark and Leaves of *Saraca Indica*, Asian Journal of Pharmaceutical Research and Development. 2022; 10(6):23-26.

DOI: <http://dx.doi.org/10.22270/ajprd.v10i6.1185>

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INTRODUCTION

Ashok is a small evergreen tree of sub family *Caesalpinoideae* of family *Leguminosae*¹. The leaves are paripinnate, oblong and rigidly sub-coriaceous with 6- 7 leaflets². This tree has orange coloured flowers with a beautiful aroma, 7- 8 stamens are found in flower and fruits are smooth, leathery and flat pods including 6- 8 seeds inside³. Bark of this tree is rich in tannins, flavonoids, steroids, volatile oil, glycosides, and various steroidal glycosides⁴. Leaves contain various carbohydrates, tannins, gallic acid⁵. Flowers are rich in sarcasin, sarcadin, waxy substances, proteins, carbohydrates and steroids^{6,7}. Seeds of this plant contain various fatty acids like oleic, linoleic, palmitic and stearic acid⁸. Ashok is also a cardiac tonic that can act as a supportive therapy for people suffering from hypertension, circulatory problems, edema, congestive heart failure etc⁹. Its bark has natural detoxification properties which make it very useful to improve skin complexion and keep the body

free from toxins inside out. Its natural cleansing properties can help the body stay toxin free¹⁰.

MATERIALS AND METHODS

Collection and Drying: Fresh bark and leaves of *Saraca indica* were collected in the month of August from college campus. The collected plant materials were brought to the laboratory on the same day. Plant samples were washed with water and air-dried at room temperature for 7 days, oven – dried at 40 °C to remove the residual moisture. The dried leaves and bark were powdered using a mixer grinder and stored in airtight container for future use.

Extraction: Three different solvents such as Acetone, Ethanol and Distilled water were used for extraction. About 1 gm of the plant samples were added respectively into the test tubes containing 5 ml solvents, and were extracted at room temperature. The extracts in all the three solvents of bark and leaves were tested for the presence of biological compounds following standard methods.

Qualitative estimation of phytochemicals: Qualitative analysis of phytochemicals was done for carbohydrates, phenols, tannins, flavonoids, saponins, glycosides, cardiac glycosides and alkaloids

Test for Carbohydrates:

Fehling's test: Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

Benedict's test: Crude extract when mixed with 2ml of Benedict's reagent and boiled, a reddish brown precipitate formed which indicated the presence of the carbohydrates. A suspension was prepared by dispersing 5 gm of the dried leaves in 10% acetic acid solution in ethanol and kept at 28°C for 4hrs which was further filtered through Whatman No. 42. Thereafter alkaloid was precipitated by concentrating the filtrate to one-quarter of its original volume and drops of conc. aqueous NH₄OH were added. Finally, the precipitate was washed with 1% ammonia solution and dried at 80°C in the oven. The content of alkaloid was calculated and expressed as mg/g of sample.

Determination of Tannins:

The finely powdered leaves and barks of *Saraca indica* were kept separately in a beaker containing 20 ml of 50% methanol covered with parafilm and then heated at 80°C in a water bath for 1 hr with continuous stirring. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper and rinsed with 50% methanol. 1 ml of sample extract was treated with 20 ml distilled water, 2.5 ml Folin-Denis reagent and 10 ml of 17% Na₂CO₃ for the development of a bluish-green colour and was allowed to stand for 20 mins. The absorbance was measured at 760 nm and the amount of tannin was calculated by comparing it with a standard curve prepared in the range of 0-10 ppm.

For measuring tannin the finely powdered bark and leaves of *Saraca indica* were kept separately in a beaker containing 20 ml of 50% methanol covered with parafilm

and then heated at 80°C in a water bath for 1 hr with continuous stirring. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper and rinsed with 50% methanol. 1 ml of sample extract was treated with 20 ml distilled water, 2.5 ml Folin-Denis reagent and 10 ml of 17% Na₂CO₃ for the development of a bluish-green colour and was allowed to stand for 20 mins. The absorbance was measured at 760 nm and the amount of tannin was calculated by comparing it with a standard curve prepared in the range of 0-10 ppm.

Determination of Saponins:

100 ml Isobutyl alcohol was added to 1 gm of the finely powdered sample and stirred for 5 hrs. 20 ml of 40% saturated solution of Magnesium carbonate was added to the mixture and filtered. 2 ml of 5% FeCl₃ solution and 50ml volume of distilled water was added to 1ml of colourless solution and kept for 30 mins for colour (blood red) development. The absorbance of the samples as along with the standard were read at 380 nm and calculated in

mg/g. Standard saponin solution was prepared in the reference range of 0-10 ppm.

Determination of total phenols:

Five gms of the powdered leaves were boiled with 50 ml of ether for 15 mins and distributed in the ratio 1:2 (extract: distilled water). 2ml of ammonium hydroxide followed with 5ml of pentanol was added to it and incubated at the room temperature for 30mins. The absorbance was read at 505 nm wavelength. For measuring alkaloids a suspension was prepared by dispersing 5 gm of the dried bark and leaves separately in 10% acetic acid solution in ethanol and kept at 28°C for 4hrs which was further filtered through Whatman No. 42. Thereafter alkaloid was precipitated by concentrating the filtrate to one-quarter of its original volume and drops of conc. aqueous NH₄OH were added. Finally, the precipitate was washed with 1% ammonia solution and dried at 80°C in the oven. The content of alkaloid was calculated and expressed as mg/g of sample. For determining flavonoids 5 gm of bark and leaves were boiled separately in 2M HCl for 30 min under reflux and filtered after cooling. An equal volume of ethyl acetate was then added drop wise to the filtrate.

Determination of Flavonoids:

The flavonoids content was also determined by Harborne²⁷ (Harborne, 1973) method. 5 gm of leaves were boiled in 2M HCl for 30 min under reflux condition and filtered after cooling. An equal volume of ethyl acetate was then added drop wise to the filtrate. The weight of precipitated flavonoid was determined and recorded as mg/g. The weight of precipitated flavonoid was determined and recorded as mg/g.

For determining saponin content 100 ml Isobutyl alcohol was added to 1 gm of the finely powdered bark and leaf samples separately and stirred for 5 hrs. 20 ml of 40% saturated solution of Magnesium carbonate was added to the mixture and filtered. 2 ml of 5% FeCl₃ solution and 50ml volume of distilled water was added to 1ml of

colourless solution and kept for 30 mins for colour (blood red) development. The absorbance of the samples as along with the standard were read at 380 nm and calculated in mg/g. Standard saponin solution was prepared in the reference range of 0-10 ppm.

Determining total phenolic content:

Five gms of the powdered bark and leaves of *Saraca indica* were boiled with 50 ml of ether for 15 mins and distributed in the ratio 1:2 (extract: distilled water). 2ml of ammonium hydroxide followed with 5ml of pentanol was added to it and incubated at the room temperature for 30mins. The absorbance was read at 505 nm wavelength.

The amount of total phenolics in extracts was determined by the Folin-Ciocalteu method. Gallic acid was used as a standard by using different concentrations of (20-200µg) from which the total phenol content in the extract was expressed in terms of gallic acid equivalent (mg GAE /gm) extract. Different aliquots of 0.1 to 1.0 ml of plant extract were also prepared in methanol and 0.5 ml of each

sample were introduced into test tubes and mixed with 2.5 ml of a 10-fold dilute Folin- Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The mixture was allowed to stand for 30 mins at room temperature. Phenols react with the phosphomolybdic acid in Folin- Ciocalteu reagent in alkaline medium and produce blue coloured complex (Molybdenum blue). The absorbance of the resulting solutions was measured at 760 nm against reagent blank. A standard calibration curve was prepared by plotting absorbance against concentration and it was found to be linear over this concentration range. The concentration of total phenol in the test sample was determined from the calibration graph. The assay was carried out in triplicate

and the mean values with \pm SD are presented. The aluminium chloride colorimetric method was used for flavonoids determination. Each solvent extract (0.5 ml of 1:10 gm ml⁻¹) was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It was kept at room temperature for 30min; the absorbance of the reaction mixture was measured at 418 nm. The percentage of total flavonoids were calculated from the calibration curve of Quercetine equivalent (QE) (200-1000 μ g) plotted by using the same procedure and total flavonoids was expressed as Quercetin Equivalent (QE) equivalents in mg per gm sample.

RESULTS AND DISCUSSION

Qualitative Phytochemical Analysis:

Table 1: Qualitative phytochemical analysis of bark of *Saraca indica*

S. No.	Name of The Test	Acetone	Ethanol	Water
1	Carbohydrate			
	Fehling ,Test	-	-	+
	Benedicts Test	+	+	+
	Iodine Test	+	-	-
2	Phenols &TanninS Test Ferric Chloride Test	+	+	-
3	Flavanoids Test Alkaline Reagent Test	-	-	-
4	Sponin Test Froth Foam Test	-	+	+
5	Glycosides			
	Lieberman 's Test	-	-	-
	Salkowski Test	+	+	+
	Keller Kilani Test	+	+	+
6	Compounds Test Phenolic	+	+	+
7	Steroid	+	+	+

Table 2: Qualitative phytochemical analysis of leaves of *Saraca indica*

S. No.	Name Of The Test	Acetone	Ethanol	Water
1	Carbohydrate			
	Fehling ,Test	-	+	-
	Benedicts Test	+	+	-
	Iodine Test	-	+	-
2	Phenols &TanninS Test Ferric Chloride Test	+	-	+
3	Flavanoids Test Alkaline Reagent Test	-	-	-
4	Sponin Test Froth Foam Test	-	+	+
5	Glycosides			
	Lieberman 's Test	+	-	+
	Salkowski Test	-	+	+
	Keller Kilani Test	+	-	+
6	Compounds Test Phenolic	+	-	-
7	Steroid	+	-	-

DISCUSSION:

Phytochemical analysis conducted on the *Saraca indica* leaves and bark extracts revealed the presence of

constituents which are known to exhibit medicinal as well as physiological activities. The Phytochemical screening of the bark and leaves of *Saraca indica* was done with acetone, ethanol, and distilled water. Among the three extracts show presence of carbohydrate in bark as evidenced by positive Fehling's test. All the three extracts of bark showed positive Benedict's test for carbohydrate. Only the acetone extracts of bark showed positive Iodine test. The phenols and tannins were detected only in acetone and ethanol extracts but not in aqueous extracts. All the three solvent extracts of bark showed negative alkaline reagent test which indicated the absence of flavonoids. Saponin was detected in ethanol and aqueous extracts of bark as evidenced by positive froth foam test. Glycosides were not detected in any of the three solvent extracts of bark in Libermann's test, but in Salkowski and Keller- Kilani tests all the three solvent extracts showed the presence of glycosides. Phenolic compounds were detected in all extracts. The steroids were recorded in all the three solvent extracts of bark.

In the solvent extracts of leaves of *Saraca indica* carbohydrates were detected in all the three solvent extracts. Fehling's test was positive in only ethanol. Benedict's test was positive in acetone and ethanol extract. Similarly, Iodine test for carbohydrates was positive in only ethanol extract. Phenols and tannins were detected in all except ethanolic extracts. Flavonoid was not detected in all and saponins in all except acetone extracts. Ethanolic extract showed negative results for the presence of glycosides in Libermann's, and Keller- Kilani. Phenolic compounds and steroids were detected in acetone and ethanolic extracts of leaves.

CONCLUSION

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 prospective study. From the results it is evident that the bark and leaves of *Saraca indica* contain a significant amount of phytochemicals viz. alkaloid, flavonoids, phenolic, saponins

and tannin. The bark of *Saraca indica* contained relatively higher amounts of phytochemicals than leaves. The comparative analysis of phytochemicals viz. total alkaloids, flavonoids, phenols, saponins and tannins in different solvent extracts from bark and leaves of *Saraca indica* has been presented in Table. In all the three solvent extracts it was found that the bark of *Saraca indica* contained higher amount of phytochemicals in comparison to leaves extract. The extraction of various phytochemicals was seen to be more effectively done when acetone is used as solvents.

CONFLICTS OF INTERESTS

There are no conflicts of Interests

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