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Phytochemical screening of *Saraca asoca* and antimicrobial activity against bacterial species

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ABSTRACT

Saraca asoca is an indigenous plant with lots of traditional importance belonging to the family Caesalpinaceae. These are the wonderful herb that claims to cure several diseases according to ayurvedic medicine. In the present study, phytochemical analysis and antimicrobial activity of dried leaves and flowers of Saraca asoca were done with the samples extracted with Acetone, Diethyl ether, Distilled water, Methanol and Petroleum ether. From the results of the study it may be concluded that some of the extracts showed the presence of alkaloids, flavonoids, tannins, saponins, glycosides and phenolic compounds. TLC profiles of S. asoca flowers and leaves extract gives an idea about the presence of various phytochemicals. The antimicrobial activity of each extract was evaluated by disc diffusion method using some bacterial species such as Klebsiella sp, Pseudomonas aeruginosa, Micrococcus sp , and Bacillus sp. The extracts of leaves and flowers in acetone, distilled water and methanol extracts showed the antibacterial activity against all bacterial species.

Keywords: *Saraca asoca*, TLC, Phytochemicals, Antimicrobial activity, Plant extracts.

INTRODUCTION

Asoka is one of the most legendary and sacred trees of India. Asoka tree, universally known by its binomial Latin name *Saraca asoca* (Roxb.), De. wild or *Saraca indica* belonging family *Caesalpinaceae* .It is a ever green tree called in English Asok tree. It is also known as Kankeli (Sanskrit), Ashoka (Assamese), Ashoka (Bengali), Ashoka (Gujarati), Ashoka (Hindi), Ashokadamara (Kannada)Ashok (Kashmiri), Asokam (Malayalam), Ashok (Marathi), Ashoka (Oriya), Ashok (Punjabi), Asogam (Tamil), Ashokapatta (Telugu). Ashoka is one of the sacred plants of Hindus, and is especially sacred to the Hindu God of Love, Kamadeva, for whom it is worshipped every year on December 27; it is mentioned in Hindu mythology as the Ashoka tree, beneath which the Indian philosopher and founder of buddhism , Gauthama Siddhartha (c.563 - 483 B.C) was said to have been born under this tree. The aim of the present study is to provide complete information about the medicinal & pharmacological importance of the *Saraca asoca*.

Classification [1]

Kingdom	: Plantae
Division	: Magnoliophyta
Class	: Magnoliopsida
Order	: Fabales
Family	: Caesalpinaceae
Genus	: Saraca
Species	: asoca

It is distributed in evergreen forests of India up to an elevation of about 750 meters. It is found throughout India. Specially in Himalaya, Kerala, Bengal and whole south region. In Himalaya it is found at Khasi, Garo and Lussi hills and in Kerala region it is found in Patagiri, Kaikatty & Pothundi of Palakkad district, Thrisur, Kollam and Kannur districts [2].

Saraca asoca has many uses mainly in the medicine to treat the women gynecological disorders , in all types of abnormal discharges per vagina , in uterine inertia, uterine pain, urinary calculus, dysurea, etc. *Saraca asoca* (ashoka) plant contains the presence of glycoside, flavonoids, tannins and saponins [3] .It is used as spasmogenic, oxytocic, uterotonic, antibacterial, anti implantation, anti tumour, anti progestational, anti estrogenic activity against menorrhagia and anti cancer agent. The plant is useful in dyspepsia, fever, burning sensation, colic, ulcer, menorrhagia, leucorrhoea, pimples, etc [4]. *Saraca asoca* dried bark has been used for menorrhagia in India [5,6]. In India *Saraca asoca* dried bark as well as flower is given as a tonic to ladies to treat Uterine disorders. *Saraca asoca* stem bark also used in case of all disorder associated with the menstrual cycle [7,8]. Ashoka is blood purifier & used in all skin diseases, ammenorhea, dysmenorrhea menopause, menorrhagia, painful menstruation blood circulation and purification, cancer, diarrhea, dysentery, edema, heart disease, hepatitis, herpes, jaundice, joint pain, kidney and gall stones, paralysis, skin problems, rheumatoid arthritis, obstructions in urinary passages [9].

MATERIALS AND METHODS

Collection of The Plant Material

The fresh flowers and leaves of *Saraca asoca* were collected in March 2015 from the nearby garden, Valayanchirangara, Perumbavoor. The collected plant materials were brought to the laboratory on the same day.

Extraction of Plant Material

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 flowers and leaves were powdered using a mixer grinder and stored in air-tight container for future use. Five different solvents such as Acetone, diethyl ether, Distilled water , Methanol and Petroleum ether were used for extraction . About 1 gm of the plant samples were added respectively into the test tubes containing with 5 ml solvents, and were extracted at room temperature.

Qualitative Phytochemical Analysis

The extracts in all the 5 solvents of leaves and flower were tested for the presence of biological compounds by using following standard methods [10,11,12].

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.

- **Iodine test**

Crude extract was mixed with 2ml of iodine solution. A dark blue or purple coloration indicated the presence of the carbohydrate.

Test for Phenols and Tannins

Crude extracts were mixed with 2ml of 2% solution of $FeCl_3$. A blue–green or black coloration indicated the presence of phenols and tannins.

Test for Flavonoid

- **Alkaline reagent test**

Crude extracts were mixed with 2ml of 2% solution of NaOH. An intense yellow color was formed which turned colorless on addition of few drops of diluted acid which indicated the presence of flavonoids.

Test for Saponins

Crude extracts were mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponin.

Test for Glycosides

- **Liebermann's test**

Crude extracts were mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H_2SO_4 was added. A colour change from violet to blue to green indicated the presence of steroidal nucleus, i.e., glycone portion of glycoside.

- **Salkowski's test**

Crude extracts were mixed with 2ml of chloroform. Then 2ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown color indicated the presence of steroidal ring, i.e., glycone portion of the glycoside.

- **Keller-kilani test**

Crude extracts were mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2ml of concentrated H_2SO_4. A brown ring at the inter phase indicated the presence of cardiac glycoside.

Test for Steroid

Crude extracts were mixed with 2ml of chloroform and concentrated H_2SO_4 was added sidewise. A red color produced in the lower chloroform layer indicated the presence of steroids.

Test for Phenolic compounds

The extracts were dissolved in 5ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green color indicated the presence of phenolic compounds.

Quantitative Determination of Phytochemicals

Alkaloid determination using Harborne method

2.5g of the sample was added with 100 ml of 5% acetic acid in ethanol and allowed to stand for 4 hour. The filtered extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added until the precipitation was complete and allowed to settle .The precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Flavonoid determination by the method of Bohm and Kocipai- Abyazan

2.5 g of each plant sample was weighed and 50 ml of the 40% aqueous methanol was added at room temperature and shaken for 4 hour . The entire solution was filtered through Whatsman filter paper no.42 and repeat the process . The filtrate as a whole was transferred into a crucible and evaporated to dryness over a water bath and weighed.

Saponin determination

For the determination of saponins, 5g of each plant sample was weighed, and was dispersed in 100 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 hour with continuous stirring at about 55°C. The filtrate and residue were re-extracted with 100 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered and the purification process was repeated , about 30 ml on n - butanol was added. The combined n - butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight. The saponin content was calculated.

Thin Layer Chromatography

Thin layer chromatogram of the extracts were done in TLC plates . 2-5 µg of 1% solution of sample spotted using micro pipette. Various solvents like acetone, butanol and acetone-butanol (1:1). Plate is placed under UV light, dark spots are observed.

The Rf value of sample was calculated by the Formula ,

$$Rf = \frac{\text{Distance moved by solute from the origin}}{\text{Distance moved by solvent from the origin}}$$

The five sequential extracts are used for TLC profiling. Before spotting the extracts are filtered and concentrated, in order to remove the solvents.

Determination of Antibacterial Activity

Collection of Test Organisms

The test organisms were collected from nearby diagnostic laboratories including *Bacillus* sp (AL1and AL2), *Klebsiella* sp, *Staphylococcus* sp, *Pseudomonas aeroginosa*.

Antibacterial Activity Test

Test organisms were uniformly inoculated into Muller Hinton infusion agar plates Filter paper discs were soaked in crude extracts of leaves and flower , placed on MHA plates and zone of inhibition was measured in millimeter after 24 hr incubation at 37˚C.

RESULTS AND DISCUSSION

Phytochemical analysis conducted on the *Saraca asoca* leaves and flower extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. Analysis of the leaf extracts exposed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, phenolic compound and alkaloids. The presence of carbohydrate was found in all extracts of *Saraca asoca* flower and was absent only in acetone for the leaves extracts. Phenolic compounds present in acetone, distilled water and methanol solvents of both flower and leaves extracts. In the case of leaves and flower extracts, flavonoids were present in three solvents such as acetone, distilled water and methanol. Phenols and tannins & steroids are present in distilled water and methanol of leaves extracts. The extracts of flower in acetone, distilled water and methanol were showed the presence of phenol, tannins and steroid. Saponins were present in methanol, distilled water and petroleum ether extracts and terpenoids absent in all solvent extracts.

TABLE. 1. Qualitative phytochemical analysis of *Saraca asoca* leaf sample

Test	Acetone	Di ethyl ether	Distilled water	Methanol	Petroleum ether
Test for Carbohydrates					
Benedict's test	-	-	+	+	-
Fehling's test	-	+	+	+	-
Iodine test	-	-	+	-	+
Test for Flavonoids					
Alkaline reagent test	+	-	+	+	-
Test for Saponins					
Froth foam test	-	-	+	+	+
Test for Phenol & Tannin					
Ferric chloride test	-	-	+	+	-
Test for Glycosides					
Liebermann's test	-	-	+	+	-
Salkowski's test	-	-	+	+	-
Keller-kilani test	-	-	+	+	-
Test for Steroids					
Salkowski's test	-	-	+	+	-
Test for Phenolic Compounds					
Ferric chloride test	+	-	+	+	-

(+ indicates presence) (- indicates absence)

TABLE. 2. Qualitative phytochemical analysis of *Saraca asoca* flower sample

Name of the test	Acetone	Di ethyl ether	Distilled water	Methanol	Petroleum ether
Test For Carbohydrates					
Benedict's test	+	-	+	+	-
Fehling's test	+	+	+	+	+
Iodine test	-	-	+	-	+
Test For Flavonoids					
Alkaline reagent test	+	-	+	+	-
Test for Saponins					
Froth foam test	-	-	+	+	+
Test for Phenol & Tannin					
Ferric chloride test	+	-	+	+	-
Test for Glycosides					
Liebermann's test	+	-	+	+	-
Salkowski's test	+	-	+	+	-
Keller-kilani test	+	-	+	+	-
Test for Steroids					
Salkowski's test	+	-	+	+	-
Test for Phenolic Compounds					
Ferric chloride test	+	-	+	+	-

(+ indicates presence) (- indicates absence)

TABLE. 3. Quantitative phytochemical analysis of *Saraca asoca* leaf

Analysis	Leaf sample (gm)
Alkaloid	0.064
Flavonoid	0.005
Saponin	0.597

TABLE. 4. Quantitative phytochemical analysis of *Saraca asoca* flower

Analysis	Flower sample (gm)
Alkaloid	0.032
Flavonoid	0.004
Saponin	0.115

TABLE. 5. Thin layer chromatography of leaf extract- Rf value

Solvents	Leaf extracts in solvents				
	Acetone	Di ethyl ether	Distilled water	Methanol	Petroleum ether
Acetone	0.6107	0.6964	0.4688	0.5686	0.5789
Butanol	0.9520	0.9272	0.9607	0.9272	0.9615
Acetone: butanol (1:1)	0.8084	0.8609	0.625	0.8776	0.8806

TABLE. 6. Thin layer chromatography of flower extract- Rf value

Solvents	Flower extracts in solvents				
	Acetone	Di ethyl ether	Distilled water	Methanol	Petroleum ether
Acetone	0.05065	0.06738	0.04210	0.04449	0.06862
Butanol	0.08811	0.09038	0.09523	0.09053	0.08771
Acetone: butanol(1:1)	0.086	0.09791	0.06296	0.07602	0.08666

TABLE. 7. Antibacterial activity of *Saraca asoca* (leaf)

Organism	Leaf Extracts in Solvents				
	Acetone	Di ethyl ether	Distilled water	Methanol	Petroleum ether
	Zone of inhibition (mm)				
Klebsiella sp.	16	0	0	16	10
Psudomonas aeroginosa	0	7	0	8	0
Staphylococcus sp.	0	0	0	7	0
Bacillus sp. AL1	17	0	0	9	10
Bacillus sp.AL2	0	0	0	12	0

The glycosides present in leaves extracts in distilled water and methanol and the flower extracts in acetone was additional to leaves extracts (Table1 & Table 2). Based upon the quantitative determination of phytochemical constituents were carried out for the powdered plant material by various standard methods and found that alkaloid 0.064 gm, flavonoid 0.005 gm and saponins 0.597 gm were present in the leaves of *Saraca asoca.* The flowers of *Saraca asoca* contains alkaloid 0.032 gm, flavonoid 0.004 gm and saponin 0.115 gm (Table 3 & Table 4).

TLC profiling of the *Saraca asoca* leaves and flower were carried out using extracts of acetone, diethyl ether, distilled water, methanol & petroleum ether respectively. The different Rf values were observed in each solvents in various extracts of leaf (Table 5) and flower (Table 6). Phytochemical analysis of bark skin of *Saraca asoca* and *Shorea robusta* and were concluded that, the petroleum ether extract of Ashoka exhibited several brownish spots on chromatoplate indicating the presence of different fatty acids in the extract at Rf - 0.25, 0.31, 0.40, 0.50 & 0.64. The methanol extract of Ashoka exhibited several spots on chromatogram at Rf -0.14, 0.20, 0.24, 0.28, 0.37, 0.52 and 0.59 [13].

The antimicrobial activity of acetone extract of leaves of was highest on *Bacillus* sp.AL1 with zone of inhibition of 17 mm and *Klebsiella* sp. with zone of inhibition of 16 mm, while the lowest activity was noticed with acetone extract against *Pseudomonas aeroginosa*, *Staphylococcus* sp., and *Bacillus* sp. AL2. The methanol extract of leaf was highest activity against all microorganisms. the highest on *Klebsiella* sp. with zone of inhibition of 16 mm. The diethyl ether extract was least activity against all bacterial species .Petroleum ether extract showed activity only against *Klebsiella* sp. and *Bacillus* sp.AL1 and distilled water extracts has no antibacterial activity (Table 7). Antimicrobial activity of flower extract, in acetone extract was highest on *Bacillus* sp. AL1 with zone of inhibition is 17 mm and no antimicrobial activity against other microorganisms . Diethyl ether extract showed highest activity against *Pseudomonas aeroginosa* , the zone of inhibition was 7 mm and o activity showed against other microorganisms and distilled water extract has no activity of selected microorganisms. The methanol extract has highest activity against all microorganisms, highest zone was showed against *Klebsiella* sp. with the zone of inhibition of 16 mm and lowest activity showed *Staphylococcus* sp. with the zone of inhibition is 7 mm. Petroleum ether extract exhibited zone only against *Klebsiella* sp. and *Bacillus* sp. AL1 with the zone of inhibition of 10 mm (Table 8).

TABLE. 8. Antibacterial activity of *Saraca asoca* (flower)

Organism	Flower Extracts in Solvents				
	Acetone	Di ethyl ether	Distilled water	Methanol	Petroleum ether
	Zone of Inhibition (mm)				
Klebsiella sp.	19	6	0	10	8
Psudomonas aeroginosa	14	0	0	11	9
Staphylococcus sp.	7	0	19	0	0
Bacillus sp. AL1	0	0	20	6	0
Bacillus sp. AL2	7	0	19	8	0

The bioactive screening and antimicrobial activity of flowers from the selected three medicinal plants on chosen microbes, and concluded that, the flower extracts revealed the presence of carbohydrates, alkaloids, tannins, saponins, flavonoids, anthraquinone, glycosides, steroids, terpenoids and sterols [14]. *Saraca asoca* has an antibacterial activity againt plant pathogens such as *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhosa*, *Staphylococcus aureus* and no activity against *Agrobacterium tumefaciens* [15]. *Saraca indica* dried flower buds tested against *Salmonella viballerup, Shigella boydii, Escherichia coli, Vibro cholera, Shigella flexneri* and *Shigella dyserteriae* results showed active [16]. The antibacterial activity of *Saraca indica* leaves with *Escherichia coli, Staphylococcus aureus* and the results showed, positive for *Escherichia coli* and negative for *Staphylococcus aureus* [17] .

CONCLUSION

Saraca asoca is highly regarded as an universal panacea in the ayurvedic medicine. It is one of the universal plant having medicinal activities and is the source of various types of compounds. The present study revealed that ,the various phytochemical components such as carbohydrates, flavonoids, saponins, phenols, tannins, glycosides and steroids, are present in the leaves and flower of *Saraca asoca*, and has the antibacterial activity against various

microorganisms. Ashoka have many medicinal uses and also a nontoxic traditional medicinal plant. The use of phyto compounds of Asoka against diseases is a challenge in the development of modern drug discovery.

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