

## Phytochemicals and their Medicinal Values of *Saraca asoca* A Research Review

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### ABSTRACT

Botanical drugs or Natural drugs are medicines which are composed of natural substances, having constituents of both medicinal activities and health-enhancing properties. Medicinal potential in these plants remain un-accessed due to a plethora of reasons. With sufficient investment, the entire infrastructure of global botanical drugs can be upgraded. The plant *Saraca asoca* (Roxb.), Wild belongs to Caesalpiniaceae family. The data in this article can be useful in understanding the various phytochemicals present in the bark, stem, flower and seeds. The tree's extracts are numerous and can be extracted by Gas Chromatography - Mass Chromatography (GC-MC), High Performance thin layer chromatography (HPTLC) and Soxhlet. The study shows us these extracts have many medicinal properties and abilities that can be used to treat different diseases in all the functioning systems of the body. The future study of herbal plants is crucial to improve medicinal care so that botanical plants can be used extensively in ayurvedic, homeopathy and natural-based medicine.

### Keywords

*Saraca asoca*

Epicatchin

Procyanidin

Phytochemicals

HPTLC

GC-MS

### INTRODUCTION

Herbal medicine has been used extensively throughout the world. Indigenous drugs extracted from plant origin forms a major part of complementary and alternative medicine/traditional medicine (TM/CAM). India is referred to as the medicinal garden of the world as approximately 25% of the prescription drugs are derived from trees, herbs and shrubs. Nature has bestowed our country with enormous wealth of medicinal plants which are having various uses in improving human health. Genus *Saraca* comprises 20 different species out of which majorly four are endowed in India. They are *Saraca asoca* (Roxb.) Willd., *Saraca indica* L., *Saraca declinata*, *Saraca thaipingensis* [1]. Out of the four, *Saraca asoca* is the only one that is a wild type and grown in a botanical garden. This species has a domestic demand for more than 5000 metric tons during 1999-2000 and 1500 metric tons during 2007-2011. However wild population of the plant is scattered in small patches and red listed making it highly valued and endangered medicinal plant, which has been classified as "vulnerable"

under IUCN list due to destructive harvesting from its natural habitats [2]. *Saraca asoca* (Roxb.) Wilde is an important plant in the medicinal world and Indian culture. It belongs to the order (Fabaceae) commonly known as 'Ashoka'. The plant is mainly used in Herbal and Ayurvedic medicine. It is an evergreen, perennial and rainforest tree which is widely found in the middle section of Western Ghats, central area of Deccan plateau and coastal zone of Indian subcontinent. *Saraca asoca* is the most legendary and sacred tree with ayurvedic qualities. The earliest chronicled mention of this tree is in the ayurvedic treatise and later in Charaka Samhita (100 A.D). Ashokarista is a famous formulation from the bark of the plant which is used in ayurvedic medicinal preparation and commercially by many reputed companies. *S. asoca* has been recommended in formulations for the management of gynecological disorders and anodynes. The studies have revealed that alcoholic and aqueous extracts of the stem bark of *Saraca asoca* have reported phenolic glycoside (P2) from phenolic glycoside fraction and also non-phenolic glycosides which has stimulant action on isolated human uterus, the extracts have further more

## Research Article

stimulated effects on the ovarian and endometrial tissue [3]. It has diverse pharmacological activities like menorrhagia during uterine fibroids [4] anti-estrogenic, anti-cancerous [5] antibacterial, antimutagenic, antimicrobial, genoprotective, antihyperglycemic, CNS depressant, antipyretic, analgesic, larvicidal, antidiabetic, antioxidant, oxytocic and anti-inflammatory properties have been reported from various parts of the plant [6].

### Habitat

The tree is found in India up to an altitude of 750 meters, occurrence is throughout the country in eastern and central Himalayas, Khasi, Garo and Lushai hills and in Kerala region it is found in Kotagiri, Kaikatty and Pothundi of Palakkad district, Thrissur, Kollam and Kannur districts. Figure 1, 2 and 3 showing Habitat, Leaves and Bark of *Saraca asoca*.

### Morphology

*Saraca asoca* can grow up to a height of 6-9 meters, branches are glabrous, stems are ascending or erect more than 2 meters tall, solid, sparingly glabrate or glabrous. The leaves are 16-25 cm long, drooping, paripinnate, corky at base, petioles very short, leaflets 4-6 pairs, oblong-lanceolate, opposite, acute, base rounded, stipules interpetiolar, young leaves are thin, flaccid, hairy, vertically down and dark green in color. Stem bark is uneven and rough due to the presence of rounded or projecting lenticels [7]. Bark is smooth,

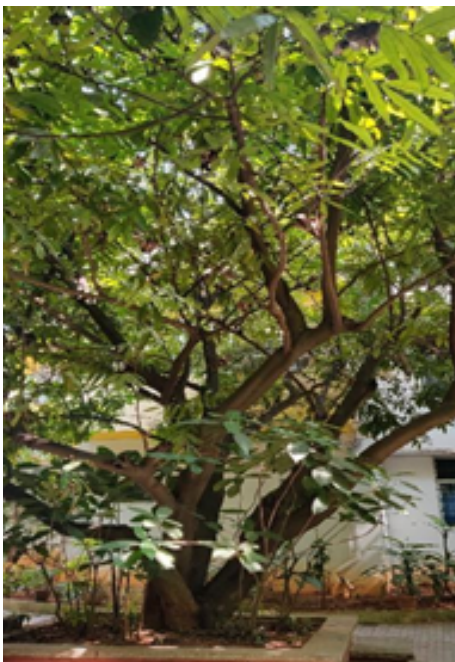


Figure 1: Habit of *Saraca asoca*.



Figure 2: Leaves of *Saraca asoca*.



Figure 3: Bark of *Saraca asoca*.

channeled with circular lenticels and transversely ridged, sometimes cracked. Fracture splitting exposing a striated surface, a thin whitish and continuous layer is seen beneath the cork layer. Inflorescence is axillary corymbs, the flowers are actinomorphic, somewhat irregular fragrant, numerous, dense in axillary corymbs, peduncles stout, pedicels 8-13 mm long, pedicellate, pedicels is red, bracteates, bracts is red, ovate, and two bracteoles which are also red in color. Calyx is petaloid, tubular, changing from yellow to orange to red, segments of 4, imbricate. It has no petals, stamens 7-8 which are much exerted, filaments filiform, thrice as long as calyx segments, anthers are purple, versatile. Ovary is pubescent, style curved, long and stigma capitate. Fruit is pod-like liner-oblong, compressed and 4-8 seeds are found which are ellipsoid-oblong, long and slightly compressed. Usually the flowering and fruiting time is January-May [8].

### MEDICINAL VALUES OF SARACA ASOCA

Since ages medicinal plants have played a very crucial role in the ancient civilization and it's been carried on since. These medicinal plants are much more mingled with the rural culture rather than urban, due to the availability of varieties of plants which grow in rural areas and in a way they are forced to adapt to these plant based medicine because they lack the facility of English medicine. According to the World Health Organization (WHO) more or less 80% of the globe population use medicinal plants to treat various diseases [9110].

One of the prominent plants that plays a huge part in the herbal medicinal field is *Saracaasoca*. In recent years many research studies have been carried out on this plant that showed a promising result of its medicinal uses. These plants are mainly used to treat gynecological disorders like uterine pain, and uterine discharge, dysuria, urinary calculus etc. Other than this it is also used to treat cancer, Arthritis and it acts as antimicrobial [11,12].

The parts of the tree used are bark, flower and seeds. The extract can be used by the patient both externally and internally. The effect of the medicinal property can play a crucial role in various systems of the body like, in Cardiovascular system the flower extract affects edema and hemorrhages, In Gastro-intestinal tract it cures diarrhea, dysentery, worm infestation and thirst, In Reproductive system it cures menorrhagia, dysmenorrhoea, leucorrhoea and other uterine disorders, in Urinary system powdered seed is used in dysuria and stones in the urinary tract<sup>13</sup>. Its bark acts as haemostatic refrigerant, alexiteric, anthelmintic, antibacterial, diuretic [14,15,16].

A number of polyphenolic compounds, including (+)-catechin (CA), (+)-epicatechin (EC), (-)-epigallocatechin (EGC),

leucocyanidin, procyanidin B-2, 11'-deoxy procyanidin B, leucopelargonidin,  $\beta$ -sitosterol, gallic acid, quercetin apigenin-7-O- $\beta$ -D-glucoside, cyanidin-3, 5-diglucoside, kaempferol 3-O- $\beta$ -D-glucoside, pelargonidin-3,5-diglucoside, quercetin and its 3-O- $\beta$ -D-glucoside, *n*-octacosanol, (-)-procyanidin derivatives, methyl- and ethyl cholesterol derivatives have been reported from the plant. These compounds are widely distributed in plant derived foods and herbal medicines. Out of the identified compounds gallic and epicatechin are major polyphenolic bioactive molecules. Gallic acid shows evidence of suppression of a high-fat diet-induced dyslipidemia, hepatosteatosis and (+)-Catechins are well-known flavonoids known for antioxidant activity and also used for the symptomatic treatment of several gastrointestinal, respiratory and vascular diseases [17].

### DISEASES CURED BY S. ASOCA

#### Anticancer

*Saracaasoca*'s ethnobotanical studies revealed its flavonoid fraction from plants to prevent two-stage skin carcinogenesis act against Sarcoma-180 tumor cells and Dalton's lymphoma ascites while being nontoxic to healthy lymphocytes [18]. The ethanolic extract inhibits breast cancer and lectin 'saracin' isolated from seed integument induce apoptosis in humans [19]. Antioxidants can reduce the risk of mutagenesis in Salmonella strains. Lignin glycoside 'saracoside' acts as a protein inhibitor of DNA topoisomerase IB [20].

#### Anti-menorrhagic, uterine tonic and anti-oxytocic

By the usage of dried roots, barks and flowers many uterine abnormalities, amenorrhea, menorrhagia, painful periods, endometriosis and menstrual cycle disorders can be treated [21,22]. The bark extract is used as uterine contraction prolonger, uterine sedative, estrogenic stimulant on ovaries and endometrium [23]. Ethanolic extract has an effect on estrogen primed and gravid uteri. Phenolic glycoside P2 isolated produces both oxytocic activity in vivo and in vitro respectively in animals and humans [24].

#### Antibacterial and Antifungal

Ethanolic, methanolic, acetone and aqueous extracts have been tested against many pathogenic bacteria like *Escherichia coli*, *S. typhimurium*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *P. vulgaris*, *Proteus vulgaris*, etc. The antifungal activity of hot aqueous and methanolic is used against *Helminthophobiasp*, *Bipolaris sp*, *lunata Aspergillus flavus*, *Colletotrichum gloeosporioides* in future this leads to detailed investigation for uncovering bio-active principles [25].



## Research Article

### Anti-inflammatory, antiarthritic and cardioprotective effect

Extracts exhibit anti-inflammatory potential by inhibiting various transcription factors like NF- $\kappa$ B, AP-1, GATA-1 and also reduces pro-inflammatory cytokines of IL-1 and TNF- $\alpha$  [26]. Constituents from the plant decrease the level of liver and serum collagen, lysosomal enzymes and restoring of normal histological architecture of joints, thereby reducing rheumatoid arthritis [27] and also show hemopurificatory effects.

### Antioxidant, hypolipidemic and antidiabetic

Various extracts like catechin, lignin glycosides, ascorbic acid, and polyphenolics like gallic acid acts as antioxidants. It inhibits  $\alpha$ -glucosidase and  $\alpha$ -amylase enzymes linked to type-2 diabetes and prevents LDL oxidation [28].

### Other uses of *Saraca asoca*

The extract from the bark of the tree *Saraca asoca* has many important effects on various parts of the body. In the central nervous system, it helps to overcome the patient's depression by producing huge amounts of CNS depressant which in turn controls mental health, prolongation of sleep, muscle relaxation and sedation [29]. It also helps in dissolution of kidney stones/ oxalic acid stones in urinary passage and Acts as derma protective in treatments like acne, dermatitis, eczema, psoriasis, scabies. *Tinea pedis* are shown to reduce skin tumors induced by 7,12-dimethylbenzanthracene [30]. *Saraca asoca* extract has other qualities like analgesic, larvicidal, anti-helminthic, and genoprotective effects. Useful in treating paralysis, hemiplegia and visceral numbness showing its effect through the parasympathetic and autonomic nervous systems delays bone consolidation and calcium deficiency. Various extracts have been reported to act as an antidote in snake bite, an anti-leucorrhea agent, a contraceptive, and in the treatment of dysentery, worm infestations and stomach pain [31].

## MATERIALS AND METHOD

Plant sample extracted from bark was washed with water and air-dried at temperature of 20°C-21°C for 7 days, later Oven dried at 40°C to remove the residual moisture. The pieces of bark were kept away from sunlight to avoid destruction of active compounds. The dried bark and flowers were powdered using a mixer grinder and stored in an air-tight container for further use. Many different types of solvents such as diethyl ether, acetone, ethanol, distilled water and petroleum benzene were used for extraction. Small quantities about 1 gm of plant sample were added respectively into different test tubes containing 5 ml solvents and extracted at room temperature.

### Gas Chromatography - Mass spectrometry (GC-MS)

GC-MS is an analytical method used to identify different substances with various test samples [32]. GC-MS applications include fire investigation, drug testing, environmental analysis and explosives investigation. It's been regarded as the "golden standard" because of 100% accurate tests.

Analysis of the chemical composition of the oil was done by Gas chromatography and Mass spectrometry. It can be done with the help of GC-MS (QP 2010 series, Shimadzu, Tokyo, Japan) equipped with a ZV 5 silica capillary column of 30 m length, 0.25 mm diameter and 0.25  $\mu$ m film thickness. The analysis was done using an ion trap technique operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas. An injection volume of 1  $\mu$ l was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature 220°C. The oven temperature was programmed from 60°C (isothermal for 2 min), with an increase of 10°C/min, to 110°C, then 5°C/min to 260°C, ending with a 10 min isothermal at 260°C. Mass spectra were taken at 70 eV; a scan interval of 0.5seconds and fragments from 40 to 450 Da. Total GC running time is 49 mins. The plant extract was dissolved in methanol [1:10] and filtered with the HPLC method and analyzed in GC-MS for different components [33]. The extract of *Saraca asoca* revealed existence of many compounds like 1,3-Benzenediol, 4-propyl- (30.03%), 2-Hydroxy-5-methyl isophthalaldehyde (22.06%), Homovanillic acid (8.60%) etc.

### High-performance thin-layer chromatography (HPTLC)

In the process of HPTLC fingerprinting methanolic extract was prepared by extracting 2 g of the powdered flowers of *Saraca asoca* with 10 ml methanol by maceration at room temperature. The procedure was repeated thrice with methanol (10 ml) at room temperature. The extracts were combined together, filtered through Whatman No.1 filter paper. The combined filtrate was concentrated under vacuum in a rotary evaporator at 40°C. 10 mg of this dried extract was redissolved in 1 ml of methanol. Stock solution of standard marker  $\beta$ -sitosterol was prepared by dissolving 1 mg  $\beta$ -sitosterol in 10 ml methanol to get 0.1 mg.ml<sup>-1</sup> solution of standard marker. Chromatography was performed on Merck HPTLC precoated Silica gel G60 F254 (10x10) TLC plate of uniform thickness of 0.2 mm. 15 $\mu$ l of sample and 10  $\mu$ l of standard were implicated with CamagLinomat V sample applicator on Plate. The plate was developed to a distance of 8 cm from the lower edge of the plate in solvent system Toluene: Ethyl acetate: Formic acid (7:3:1). The plate was visualized under UV 254 nm, UV 366 nm and under visible light after dipping

in anisaldehyde-sulphuric acid reagent followed by drying and heating at 110°C for 5 minutes in order to develop the chromatogram. The plate was documented in visible light after derivatization [34]. Quantitative High-performance thin-layer chromatography analysis of  $\beta$ -sitosterol in ethanolic extract of *S. asoca* shows a densitogram and banding pattern obtained from extract shows  $\beta$ -sitosterol 0.06 %. Rf value of  $\beta$ -sitosterol was recorded 0.80 [35].

### Soxhlet Extraction

Soxhlet extraction is used when the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. It allows for unmonitored and unmanaged operation while efficiently recycling a small amount of solvent to dissolve a larger amount of material. For the extraction the bark was desiccated at room temperature for 1 week until completely dry and ground in an electric grinder. The powder obtained was macerated in 100% methanol for 24 h followed by extraction at 50°C–55°C using hot percolation method by Soxhlet extraction. After 2 to 3 cycles, the methanolic extract obtained was evaporated to dryness under reduced pressure using rotavapor at 50°C–55°C. This process was repeated 4 to 5 times until all the components were extracted. The obtained extract is concentrated and dried at 37°C. Traces of methanol were evaporated by lyophilization at –48°C. The extract was stored in the desiccator until use [36,37]. The advantages of this extraction is that it can be repeated multiple times until high efficiency, small solvent dosage and complete extraction is obtained.

## PHYTOCHEMICALS

The phytochemical screening of *Saracaasoca* showed the presence of Saponins, glycosides, tannins, steroids, terpenoids, reducing sugar, flavonoids, alkaloids, resins etc. [38]. The experimental yield of HPTLC and phytochemical analysis of *Saracaasoca* was found to be, Chloroform-1.80%,

Ethanol-11.90%, Methanol-15.10% and water-22.00% [39]. Barks and flower of *Saracaasoca* is considered to be one of the most valuable ingredients to ayurvedic medicines because of the presence of phytochemicals like carbohydrates, phenols, tannins, saponins, glycosides [40]. Qualitative analysis for the phytochemicals are conducted by the following standard procedures [41]. Mentioned in table 1.

### Test for Carbohydrates

**Fehling's test:** Fehling reagent A and Fehling B were mixed in equal volume together and 2ml of it was added to crude extract and boiled gently. The appearance of brick red precipitate at the bottom of the test tube indicated the presence of reducing sugars.

**Benedict's test:** To 2ml of Benedict's reagent crude extract was mixed and boiled, the appearance of reddish-brown precipitate indicated the presence of the carbohydrates.

**Iodine test:** To 2ml of iodine solution Crude extract was mixed. The presence of the carbohydrate is indicated by the formation of purple or dark blue color

### Test for Phenols and Tannins

To 2ml of 2% solution of  $\text{FeCl}_3$  crude extract was mixed. A blue–green or black coloration indicated the presence of phenols and tannins.

### Test for Flavonoid

**Alkaline reagent test:** To 2ml of 2% solution of NaOH crude extract was mixed. An intense yellow color was formed which turned colorless in addition to a 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.

**Table 1:** Uses of Phytochemicals.

Sl. No	Phytochemicals	Uses	Reference
1	Carbohydrates	Nutrition and Energy producer	[43]
2	Tannins	Ulcer, dysentery, asthma, bad throat, diarrhea, urinary disease, viral infection.	[44][45]
3	Flavonoids	Anti-oxidative, anti-inflammatory, anti-mutagenic, anti-carcinogenic and activate cellular enzyme function.	[46][47]
4	Glycosides	Anti-cancer activity	[48]
5	Steroids	Anti-inflammatory agent	[49]
6	Phenolics	Defense against UV radiations, pathogens and parasites.	[50]

## Research Article

### Test for Glycosides

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

**Salkowski's test:** To 2ml of chloroform crude extract was mixed. Then 2ml of concentrated  $H_2SO_4$  was added carefully and shaken gently. A reddish-brown color indicated the presence of a steroidal ring, i.e., the glycone portion of the glycoside.

**Keller-kiliani 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  $H_2SO_4$ .

### 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 [42].

## RESULT AND DISCUSSION

As discussed in the above article it is evidently proved that the medical importance of the tree *Saracaasoca* is most prominent in the field of ayurvedic, therapeutic and botanical sectors as it is used as anticancer, antihemorrhagic, uterine tonic and oxytocic, antibacterial, antifungal, anti-inflammatory, antiarthritic, cardioprotective effect, antioxidant, hypolipidemic, antidiabetic, dysentery, worm infestations, stomach pain, CNS depressant, derma protective, analgesic, larvicidal, anti-helminthic, genoprotective effects and it is also used to cure gastro-intestinal, uterine diseases. It is necessary that in the future the studies on stabilization and standardization on *Saracaasoca* should be implemented to bring out the most affordable medicine for all the people in the country as the cost of ayurvedic medicine is comparatively less than English medicine and as to escape from the side effects of allopathic medicine.

Phytochemical analysis conducted on *Saraca asoca* was found to contain constituents which exhibit physiological and medicinal values. Phytochemical analysis of leaf extract was found to contain flavonoids, phenols, glycosides,

steroids, saponins, tannins and alkaloids [40]. Carbohydrates was found in all the extracts of *Saraca asoca* flower and it is absent only in acetone for *Saraca asoca* leaves extract. These phytochemicals which are present in this plant which is essential for its own growth and also gives its medicinal values.

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## CONFLICT OF INTEREST

No conflict of interest.

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