

## REVIEW ARTICLE

# Plant profile, phytochemistry, pharmacology and genetic diversity of *Saraca asoca*: a vulnerable medicinal tree of India

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## Abstract

*Saraca asoca*, commonly known as 'Ashoka', is an important ancient and indigenous medicinal plant, found throughout India. The word 'Ashoka' means 'remover of sorrow' in Sanskrit, with reference to its bark keeping women healthy and youthful. It is also an important sacred and religious tree. Although the bark is most useful, every part of the plant including flower, leaves, root and seeds has medicinal properties and has been used in Ayurvedic medicinal preparations for centuries. The bark contains glycoside, tannins, flavanoids, saponins, esters and primary alcohols which contribute to its medicinal properties. Overexploitation, unethical harvesting and deforestation of natural habitats have reduced its population in the wild, and consequently the species is being listed in the 'globally vulnerable' category by the IUCN. Increased market demand has resulted in adulteration with substitutes. This review is an attempt to consolidate the current knowledge of scientific information on the therapeutic properties, phytochemistry, pharmacology, relationship among related taxa and genetic diversity of the species.

**Keywords:** Alternative medicine, Ashoka, genetic variation, medicinal plant, pharmacology, phytoconstituents.

## Introduction

Medicinal plants have been a part of our lives and utilized in traditional medicines from time immemorial. A large number of herbal medicines and plant-based drug formulations of immense therapeutic value are described in Indian folk medicines. Even today the plant-based drugs are popular over modern synthetic drugs mainly due to their negligible side-effects, low-cost and easy availability. *Ayurveda*, a traditional medicinal practice based on human physiology, employs a large number of medicinal plants and herbs, either exclusively or in combination for overall health. According to WHO (2005), in developing countries like India, almost 70% of the population is dependent on traditional herbal medicines for their primary health care needs.

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*Saraca asoca* (Roxb.) W. J. deWilde belonging to family Fabaceae, is an indigenous, ancient, venerated and an important medicinal tree of India. It is very popular and has been used in many traditional medical therapies for its extra-ordinary properties, with many health benefits and cures especially for women related disorders (Sharma *et al.*, 2000). The Ashoka tree finds mention in the *Charka Samhita* as anodynes in gynecological disorders (Mohan *et al.*, 2016). The tree is also known as 'Sita Ashoka', which literally means 'without sorrow' in Sanskrit language owing to its manifold health benefits especially on keeping women healthy and youthful. It is an important sacred tree of Hindus and Buddhists. The Ashoka tree is referred in the legendary Ramayana in connection with the 'Ashoka Vatika', where Hanuman first met Sita, and Lord Buddha was said to be born under this tree species (Pradhan *et al.*, 2009). It is a popular tree with many vernacular names - Ashoka, Sita-ashoka, Aganpriya (Hindi), Kankeli, Gandhpushp (Sanskrit), Oshok (Bengali), Jashundi (Marathi), Asupala, Ashopalav (Gujarati), Asokada, Kankelimara (Kannada) and Asokam (Tamil and Malayalam) (Yadav *et al.*, 2019).

The genus *Saraca* comprises about 20 species (Santapau & Henry 1998). Of these, *S. asoca* (Roxb.) W. J. deWilde, *S. declinata* Miq. (Red Saraca), *S. indica* L. and *S. thaipingensis* Prain (Yellow Saraca) have been reported from India (Begum *et al.*, 2014). Whereas *S. asoca* is found in the wild besides

in gardens and avenues, the other three species are found only in botanical gardens (Begum *et al.*, 2014). Though they have immense medicinal value, these are also grown for ornamental purpose (Sil *et al.*, 2018).

The most popularly used part of the *Saraca asoca* is the bark, even though all the other parts such as leaves, roots, flowers, and seeds exhibit medicinal properties with extensive use in anti-microbial, anti-cancer, anti-inflammatory, anti-arthritic and many more medications (Yadav *et al.*, 2019). The bark is useful in treating excessive menstrual bleeding, irregular mensuration, pain, dysmenorrhea, congestion, uterine spasms, and leucorrhoea and as a uterine tonic with a stimulatory effect on the ovarian tissue endometrium (Baranwal 2014). *Ashokarishta*, *Ashokaghritha*, *Ashoka kwath*, *Asoka arishtam* and *Madhukadya* are some of the well-known indigenous preparations commonly available, which uses *S. asoca* as the primary raw constituent (Smitha & Thondaiman 2016). The plant parts contain many secondary metabolites including tannins, flavonoids, alkaloids, steroids, saponins, glucosides, terpenoids, triterpenoids, phenolics, catechol and other phytochemicals which contribute to its medicinal properties (Ahmed *et al.*, 2016).

Ashoka flowers are used to decorate Buddhist monasteries and Hindu temples (Boroker & Pansare 2017). The two important rituals associated with the tree are 'Ashoka tri-ratri' and 'Asokasathi', which represents its cultural significance (Pradhan *et al.*, 2009). As a ritual in traditional culture, the flower buds are eaten by married Hindu women on 'Ashok Shashti day' to seek protection for children against negativity, grief and sorrow and to invoke the deities (Pradhan *et al.*, 2009). Many tribal womenfolk from Chhattisgarh drink a decoction of the bark in cow's milk and sugar for three days after every three months to prevent gynecological problems and diabetes (Boroker & Pansare 2017).

Unethical harvesting, indiscriminate use of the plant parts, increasing demand and unscientific management practice have resulted in severe shortage of the genuine plant product which in turn has resulted in an increased cost and rampant substitution and adulteration (Smitha & Thondaiman 2016). Over-exploitation due to its high medicinal value, increasing market demand, habitat loss due to deforestation, habitat fragmentation, narrow ecological niche, illegal encroachments of its natural habitats and poor seed viability has reduced its natural population in the wild (Devan & Warriar 2021). An assessment based on category B i.e., based on extent of occupancy and area of occupancy, no sub populations have been reported in Sri Lanka in the forest surveys conducted during 1991 and 1996 for National Conservation Reviews indicating *Saraca* to be extremely rare or possibly extinct ([www.iucnredlist.org/species/34623/9879360#population](https://www.iucnredlist.org/species/34623/9879360#population)). The species is presently listed as 'a globally vulnerable species' in the

threatened species list of IUCN (<https://www.iucnredlist.org/species/34623/9879360>). This review is an attempt to compile different research works on the phytochemical constituents and pharmacological activities of the various plant parts, relationship among related taxa and molecular marker studies for its molecular characterization which may help in understanding the genetic diversity within the population that may be beneficial for future conservation and improvement strategies of the tree. A brief account of distribution and botanical description /morphology is also included. The study may provide a comprehensive view for the management of the species.

### Morphology

*Saraca asoca* is a small to medium-size evergreen tree with a dense and spreading crown (Figure 1). The tree can grow up to 7 - 10 m in height. The bark is grey or dark brown covered with a warty surface. It is uneven and rough to touch, due to the presence of projecting lenticels and circular lenticels and is channelled or sometimes cracked (Boroker & Pansare 2017). The leaves are alternate, paripinnate, 15-20 cm long, copper red when young and green on maturity. The leaves have 4 - 6 pair of leaflets which are oblong-lanceolate, obtuse or acute, glabrous with rounded base; petioles are short (Boroker & Pansare 2017). Flowers are fragrant, and borne in dense axillary corymbs. They are bisexual, aromatic, staminate and astringent in taste. Young flowers are yellow which gradually turn to orange and scarlet red on maturity (Mishra & Vijaykumar 2015). Pods are linear, oblong, compressed, dehiscent, tapering to both ends and are green and leathery which turns to brownish purple or black on maturity. Each pod contains 4 - 8 seeds which are ellipsoid, oblong, compacted and covered with a seed-coat. The seeds turn black on maturity and are recalcitrant type (Chauhan 2019).



**Figure 1:** *Saraca asoca*. A. Tree in flowering stage. B. A close view of the twig. C. An enlarge view of inflorescence. D. Pods. E. Pod with seeds. (Photo credit: Prof. S. R. Yadav).

### Distribution

*Saraca asoca* is native to the Indian sub-continent, though also found in Indo-Malaysian region and Sri Lanka. In India, it is mainly found in peninsular region extending into the Western Ghats, Eastern Ghats and the sub-Himalayan tracks, growing at an elevation of approximately 750 m (Patwardhan *et al.*, 2014). Its original distribution was in the Deccan Plateau and distributed in Central and Eastern Himalaya, Burma, East Bengal, Western Peninsula, and Sri Lanka (Preeti *et al.*, 2012). It is also found in semi-evergreen and in moist deciduous forests along the river streams (Patwardhan *et al.*, 2016). The most suitable growth condition is red laterite alluvial soil with an annual rainfall of 2000 - 4000 mm (Yadav *et al.*, 2019).

### Phylogeny

Phenetic study based on morphological characters and ternary plot analysis has shown *S. declinata* to be closer to *S. thaipingensis* compared to *S. indica* (Sil *et al.*, 2018). Traits such as ovary texture, the pattern of distribution of trichomes on anther and hooded anther, exine surface, bract, stigma morphology and shape and size of the seed have been suggested to be crucial morphological markers for delimiting the three species of *Saraca*. A molecular analysis of the intra and inter specific diversity of six species of *Saraca* using *matK* sequences showed *Saraca* as a diverse genus with different species. The phylogenetic interrelationships have indicated *S. declinata* to be the most evolved and *S. dives* with the foremost evolution. *Saraca indica* and *S. asoca* showed firm molecular resemblance yet their molecular diversity has placed them as separate species while *S. declinata* and its synonym *S. palembanica* exhibit distinct molecular identities (Sil *et al.*, 2021).

### Phytochemistry

The biological activities of the medicinal plants are generally due to the presence of secondary metabolites. Ashoka bark, leaves and flowers which show therapeutic importance have a variety of phytochemical constituents including flavonoids, alkaloids, triterpenoids, phenolics, tannins, steroids, glycosides, quinones and volatile organic compounds (Singh *et al.*, 2015, Ahmad *et al.*, 2016) (Table 1). Minerals such as sodium, potassium, calcium, aluminum, iron, magnesium, strontium and phosphate are present in the bark (Saha *et al.*, 2012). The wax from the bark also contains n-alkanes, primary alcohol, esters and n-octacasanol (Boroker & Pansare 2017). The plant seeds and fruits contain a lectin named Saracin (Ghosh *et al.*, 1999). The chemical structures of some of its phytochemicals drawn on software ChemSketch 2015 are shown in Figure 2.

### Adulterants

Overexploitation, destructive extraction and non-availability of Ashoka trees due to increased market demand have caused increased adulteration or substitution of the drug.

The commonly used adulterants are the barks of *Polyalthia longifolia*, *Shorea robusta*, *Trema orientalis*, *Bauhinia variegata*, *Afananexis polystakis* and *Caesalpinia pulcherrima* (Khatoon *et al.*, 2009, Urumarudappa *et al.*, 2016). Adulteration and/or substitution with *Polyalthia longifolia* is common as both have the same vernacular name 'Ashoka' resulting in misidentification and confusion (Khatoon *et al.*, 2009). A comparison of transverse and longitudinal sections of stem bark of *Saraca asoca* and *Polyalthia longifolia* which has smaller epidermal cells along with specialized cells in the cortical region can help in identification of the two barks by microscopy (Khatoon *et al.*, 2009). Some of the identifying features of *S. asoca*, *S. declinata*, and *Polyalthia longifolia* are listed in Table 2 (Devan & Warriar 2021) (Table 2). Adulteration or substitution can adversely affect the health and safety of the consumer and reduce the therapeutic efficacy of the traditional medicine. The NMR spectroscopic analysis to validate market samples with *S. asoca*, has shown high level of adulteration in the market samples (Urumarudappa *et al.*, 2016). Kumar (2016) used RAPD derived SCAR markers to identify and differentiate *S. asoca* from *P. longifolia*. Hedge *et al.*, (2017) isolated and used Start Codon Targeted (SCoT) and Inter Simple Sequence Repeat (ISSR) markers and phytochemical profiling by RP-HPLC analysis to identify bark samples for validation.

### Biological and pharmacology activities of *Saraca asoca*

*Saraca asoca* has multiple pharmacological activities attributed to its rich phytochemicals (Table 3). Some of the activities are discussed below.

#### Anti-menorrhagic and uterine tonic

The dried bark, root and flowers are especially useful for the treatment of gynecological problems, mainly associated with absence of periods (amenorrhea), irregular periods, painful periods (dysmenorrhea), excessive menstrual bleeding (menorrhagia), premenstrual syndrome, leucorrhoea and endometriosis. It has an estrogenic stimulatory effect to the endometrium and ovarian tissues acting as a uterine tonic (Baranwal 2014). Swar *et al.*, 2017 treated ovariectomized female albino Wistar rats with ethanolic flower extract and observed that the flowers has estrogenic potency which may be due to phytoestrogens such as  $\beta$ -sitosterol, kaempferol, luteolin and quercetin present in the flower.

#### Effect on menstrual cycle

An investigation to study the restoring capacity of the bark extract on reproductive cycle, Progesterone (P4), Estrogen (E2) levels over disruptions caused due to the synthetic contraceptive Levonorgestrel (LNG) in female albino rats, showed that the methanol bark extract significantly reduced the hormone levels to normal (Hazarika *et al.*, 2021).

**Table 1:** Various phytoconstituents from different plant organs.

Class	Compound	Plant organ	Reference
Flavonol	Catechin	Bark, seed, pod	Mukhopadhyay <i>et. al.</i> , 2017
	Epicatechin		Pradhan <i>et. al.</i> , 2009
	Leucocyanidin		Ahmad <i>et. al.</i> , 2016
	Leucopelargonidin	Bark, leaves	Ahmad <i>et. al.</i> , 2016,
	Epigallocatechin	Bark	Ahmad <i>et. al.</i> , 2016
	Gallocatechin		Ahmad <i>et. al.</i> , 2016
	epiafzelechin-(4 $\beta$ →8)- epicatechin		Pradhan <i>et. al.</i> , 2009
	procyanidin B2		Pradhan <i>et. al.</i> , 2009
	3',5'-dimethoxy epicatechin		Ahmad <i>et. al.</i> , 2016
	Deoxyprocyanidin B		Singh <i>et. al.</i> , 2015
	Haematoxylin		Singh <i>et. al.</i> , 2015
Flavonol glycosides	Quercetin-3-O-P-D-glucoside	Flowers	Pradhan <i>et. al.</i> , 2009
	Cyanidine-3,5-diglucoside		Pradhan <i>et. al.</i> , 2009
	Pelargonidin-3,5-diglucoside		Pradhan <i>et. al.</i> , 2009
	3'-deoxycatechin-3-O- $\alpha$ -L-rhamnopyranoside	Bark	Ahmad <i>et. al.</i> , 2016
	3'-deoxyepicatechin-3-O- $\beta$ -D-glucopyranoside		Ahmad <i>et. al.</i> , 2016
Lignin glycosides	Lyonside	Bark, leaves, flowers	Ahmad <i>et. al.</i> , 2016
	5-methoxy-9- $\beta$ -xylopyranosyl isolariciresinol	Bark	Singh <i>et. al.</i> , 2015
	Icariside E		Sadhu <i>et. al.</i> , 2007
	Nudiposide		Sadhu <i>et. al.</i> , 2007
	Schizandriside		Sadhu <i>et. al.</i> , 2007
Phenolic acid	Gallic acid	Bark, leaves, flowers	Saha <i>et. al.</i> , 2013
	Quercetin	Bark, flowers	Saha <i>et. al.</i> , 2013, Swar <i>et. al.</i> , 2017
	Ellagic acid	Bark	Saha <i>et. al.</i> , 2013
	Acetyl salicylic acid	Seed	Gupta <i>et. al.</i> , 2014
Sterol	$\beta$ -sitosterol	Bark, leaves, flower	Swar <i>et. al.</i> , 2017
Steroidal glycosides	$\beta$ -sitosterol glucoside	Bark, leaves	Sadhu <i>et. al.</i> , 2007
Fatty acid	Oleic acid	Pod, seed	Singh <i>et.al.</i> ,2015, Yadav <i>et.al.</i> , 2019
	Stearic acid		Singh <i>et.al.</i> ,2015, Yadav <i>et.al.</i> , 2019
	Linoleic acid		Singh <i>et.al.</i> ,2015, Yadav <i>et.al.</i> , 2019

**Anti-keratinizing**

Shahid *et al.*, (2015) investigated the effect of methanolic barkextract in artificially estrogen induced proliferation of endometrial thickening and keratinized metaplasia in rat uterus. The methanol extract of bark reduced the endometrial thickening to 2 -4 layers of stratified epithelium and significantly lowered serum estrogen levels. It also reduced the formalin-and carrageenan-induced paw edema, lipopolysaccharides induced COX-2 enzyme in rat uterus, thus indicating it's anti-proliferative and anti-keratinizing

potential manifested through an anti-estrogenic and anti-inflammatory activity.

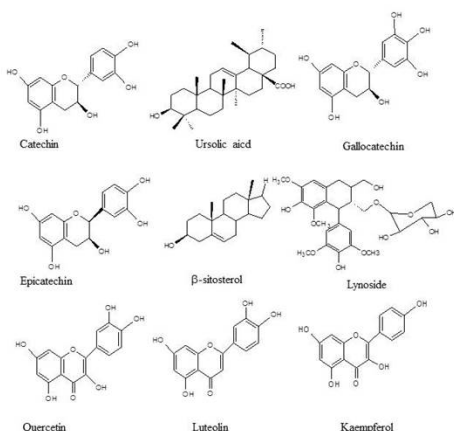
**Anti-oxidative and Anti-cancer**

Several reports have elaborated the presence of different anti-oxidant compounds in thebark, leaves, and flowers of *S. asoca* which are effective against a number of disorders including cancer (Ahmad & Ghosh 2022). Methanol bark extract of the trees has been shown to reduce growth in HeLa cells (human cervical cancer cell lines) showing important



**Table 2:** Contradistinctive traits for identification between two species of *Saraca* and *Polyalthia longifolia* (Adapted from Devan & Warrier 2021)

Trait	<i>S. asoca</i>	<i>S. declinata</i>	<i>Polyalthia longifolia</i>
Stem bark	channeled	curved	curved
Distribution of fibers in phloem region	group of 3-24	solitary or in groups of 3-6	present as broad concentric band which alternates with parenchymatous bands
Phelloderm and stone cells	phelloderm of 2-3 continuous bands of stone cells	phelloderm as outer and inner regions with groups of 6-7 stone cells	stone cells present throughout phelloderm
Medullary rays	uni-biseriate	uniseriate - triseriate	multi-seriate
Mucilage canals and oil cells	absent	absent	present

**Figure 2:** Chemical structures of some of the phytoconstituents of *Saraca asoca* (Adapted from Ahmad *et al.* 2016, Swar *et al.* 2017).

cytotoxic and anti-tumor activity (Asokan & Thangavel 2014). The MTT and SRB assays showed significant inhibition of HT-29 colon cancer lines showing *in vitro* cytotoxicity of the bark extract (Jeenathunisa & Rajan 2021). The presence of natural flavonoids (catechin) in methanolic bark extract has shown promising results against acute myeloid leukemia (Mukhopadhyay *et al.*, 2017) in benzene induced swiss albino mice models. The mice treated with catechin showed reduced DNA fragmentation, up-regulation of p53, p21 and bc12, CYP2E1, caspase 11 and myeloperoxidase. Catechin showed modulatory influence on cell cycle deregulation and hematological abnormalities which are associated with benzene induced leukemia. Thus, indicating that catechin is similar to doxorubicin (chemotherapeutic drug) to give stability and protection towards cancer (Mukhopadhyay *et al.*, 2017). Banerjee *et al.*, (2022) synthesized phyto-fabricated silver nanoparticles from an aqueous bark extract of *S. asoca*. They checked the effectiveness of the nanoparticles on acute myeloid leukemia (AML) and compared to doxorubicin, a chemotherapeutic drug involving *in vivo* studies on AML mice along with *in vitro* studies on lymphocytes and leukemia cells to check the cytotoxic and genotoxic effects. The nanoparticles were selectively cytotoxic towards the leukemia lymphocytes cells through oxidative damage, suggesting potential avenues of phyto-fabricated nanoparticles.

### Anti-microbial (anti-bacterial and anti-fungal)

Numerous investigations have demonstrated the anti-bacterial activity in aqueous, alcoholic (methanol and ethanol) and acetone extracts of the bark, leaves and dried flower buds against a number of bacteria such as *Escherichia coli*, *Klebsiella pnemone*, *Klebsiella aerogenes*, *Salmonella typhosa*, *Salmonella typhimurium*, *Salmonella typhii*, *Salmonella enteritis*, *Bacillus subtilis*, *B. cereus*, *Pseudomonas vulgaris*, *Pseudomonas aeruginosa*, *Vibrio chlorae*, *Proteus vulgaris*, *Staphylococcus aureus* (Shirolkar *et al.*, 2013, Mohan *et al.*, 2016). The anti-fungal activity of aqueous and methanol extracts of various parts of the plant have been reported against a number of fungi like *Alternaria alternate*, *Alternaria cajani*, *Colletotrichum gloeosporioides*, *Helminthosporium sp.*, *Curvularia lunata*, *Bipolaris sp.*, *Fusarium sp.*, *Candida albicans*, *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus fumigatus* (Shirolkar *et al.*, 2013, Chakraborty *et al.*, 2014). Due to its antibacterial efficacy this tree may be a beneficial resource in the future for a single molecular antibiotic against increasing antibiotic resistance of a number of human pathogenic bacteria.

### Anti-depressant

Methanolic bark extract was used to evaluate the anti-depressant activity in rats by inducing Chronic Unpredictable Mild Stress (CUMS) and estimating the associated antioxidant enzymes such as catalase, superoxide dismutase activities, glutathione malondialdehyde levels in the brain. The extract showed decrease in the stress parameters along with an increase in the associated antioxidant enzyme levels. This improved the behavioral and neurochemical response, modulating neurotransmitter activity associated with depression, thus showed potential anti-depressant characteristics (Gill *et al.*, 2018).

### Anti-epileptic

Ethanol extracts of *S. asoca* flower were used to investigate anti-epileptic activity in swiss albino mice by using Maximum electroshock (MES) and pentylenetetrazole (PTZ) induced seizure models. Decrease in tonic hind limb extension (THLE) phase and increase in the latency period of seizures were measured against the induced convulsions. The ethanol

**Table 3:** Biological activities in different plant organs.

<i>Plant parts</i>	<i>Chemical class</i>	<i>Biological activity</i>	<i>Reference</i>
Bark	Flavonoids (catechin)	anti-cancer	Mukhopadhyay <i>et al.</i> , 2017
		cardioprotective	Swami <i>et al.</i> , 2013
	polyphenols	antioxidant	Singh <i>et al.</i> , 2015
		anti-depressant	Gill <i>et al.</i> , 2018
		regulation of menstrual cycle	Hazarika <i>et al.</i> , 2021
Leaf	tannins, triterpenoids, saponins, flavonoids and glycosides	anti-keratinizing	Shahid <i>et al.</i> , 2015
		analgesic activity	Verma <i>et al.</i> , 2010
	alkaloids, tannins, flavonoids and saponins	anti-helminthic	Sharma <i>et al.</i> , 2011
		anti-ulcer	Verma <i>et al.</i> , 2020
Flower	phytoestersols	estrogenic stimulatory	Swar <i>et al.</i> , 2017
	polyphenols, phytoestersols, flavonoids, and saponins	anti-epileptic	Srinivasa 2018
		anti-hyperglycemic and anti-hyperlipidemic	Mishra and Vijaykumar 2014
	flavonoids	anti-diabetic	Thilagam <i>et al.</i> , 2021
Seed	phenolic compounds, flavonoids and terpenoids	anti-arthritic and anti-inflammatory	Gupta <i>et al.</i> , 2014
	flavonoids, tannins and saponins.	anti-pyretic	Sasmal <i>et al.</i> , 2012

extract of the flowers significantly decreased the THLE and seizure latency in MES and PTZ models, thereby exhibiting the activity (Srinivasa *et al.*, 2018).

#### **Anti-hyperglycemic and hypolipidemic**

The methanol, petroleum ether and chloroform extract of *S. asoca* leaves have exhibited a significant potential for anti-hyperglycemic and antioxidant ability in Streptozotocin induced diabetic rat model, as the oral administration of the extracts significantly reduced the blood glucose levels; the methanol extract was most potential for its anti-hyperglycemic activity (Kumar *et al.*, 2012). In another study, the ethanolic extract of Ashoka flowers showed remarkable capability for its anti-hyperglycemic and antioxidant activities in Streptozotocin-nicotinamide induced diabetic rats (Mishra & Vijaykumar 2014). The flower extract is rich in phenolic compounds phytoesterol, flavonoids and saponins which lowers the blood glucose levels and those of the antioxidant enzymes including catalyst glutathione peroxidase, superoxide dismutase, glutathione reductase to near normal. The flavonoids in the flower extract, known for their antioxidant properties scavenged the free radicals produced during diabetes (Mishra & Vijaykumar 2014). Thilagam *et al.*, (2021) reported ethanolic extracts of *S. asoca* flowers containing polyphenols, flavonoids phytoestersols and saponins significantly increased serum insulin levels along with a decrease in blood glucose levels, alkaline phosphatase, HbA1C, serum triglyceride and total cholesterol levels in alloxan-induced diabetic rats suggesting anti-diabetic along with anti-hyperlipidemic activity.

#### **Anti-inflammatory and anti-arthritic**

Treatment with *S. asoca* methanol extract to adjuvant-induced arthritis in female wistar rats decreased the paw thickness and raised the body weight, along with significant lowering of the plasma and liver lysosomal enzyme levels, urinary collagen, pro-inflammatory cytokines levels, protein bound carbohydrates, and restored the histological architecture of the joints, thus decreasing the rheumatoid arthritis in rats (Saravanan *et al.*, 2011).

In another study with Freund's adjuvant-induced arthritis in wistar albino rat model, the seed acetone extract with high concentration of phenolic compounds, flavonoids and terpenoids were shown to exhibit anti-arthritic and anti-inflammatory potential by significantly reducing glucosamine and hydroxy proline concentration in the urine, ankle joint inflammation and paw edema along with normal radiological images of the joint (Gupta *et al.*, 2014).

#### **Cardioprotective**

Cardioprotective activity was demonstrated by Swami *et al.*, (2013) using cyclophosphamide induced cardiotoxicity in rats. Cyclophosphamide caused changes in ECG, increased biomarker enzymes such as creatine kinase, lactate dehydrogenase, alkaline phosphatase, and increased cholesterol and triglyceride levels. Treatment with ethanol bark extract revoked the changes caused by cyclophosphamide by restoring ECG, cardiac biomarkers, oxidative enzymes, lipid profile and lowered the severity of myocardium cellular damage.

### Analgesic

*Saraca asoca* leaf extracts in water, methanol, chloroform and petroleum ether were examined for phytoconstituents and analgesic activity. The analgesic activity was carried out in albino rats employing tail immersion method and formalin induced methods. Formalin test is an effective analytic method to correlate with clinical pain. The methanol leaf extracts showed highest analgesic activity, relieved pain in the animals injected with formalin in a dose dependent manner by acting on both peripheral and central nervous system. The phytoconstituents in the leaf extract such as tannins, triterpenoids, saponins, flavonoids and glycosides may be involved in the analgesic activity (Verma *et al.*, 2010).

### Anti-ulcer

The ethanol extracts of *S. asoca* leaves produced remarkable protection against ulcers which were induced by ethanol, pylorus ligation and indomethacin in albino rats, by reducing ulcer index, increasing gastric pH and reducing total acidity gastric volume showing anti-ulcer activity (Verma *et al.*, 2020).

### Anti-pyretic

Anti-pyretic action of acetone seed extract of *S. asoca* was demonstrated in the rat models with artificially induced pyrexia using Brewer's yeast (Sasmal *et al.*, 2012). The anti-pyretic activity could be attributed to the phyto-constituents such as flavonoids, tannins and saponins.

### Anti-helmintic

Parasitic worms are common infections in man leading to anaemia, malnutrition, eosinophilia and pneumonia (Sharma *et al.*, 2011). The *S. asoca* leaf extracts in aqueous, methanol and chloroform are reported to show anti-helmintic activity due to paralysis and death of adult earthworm, *Pheretima posthuman*, which resembles the human round worm parasite. The methanol extract was most effective as compared to standard drug albendazole. The phytoconstituents of the methanol extract such as alkaloids, tannins, flavonoids and saponins may be responsible for the anti-helmintic activity (Sharma *et al.*, 2011).

### Genetic Studies

Karyomorphological studies using root tip have showed somatic cells with 17 sets of homologous pair of chromosomes (Deepa *et al.*, 2013).

The complete chloroplast (cp) genome/plastome was isolated and sequenced, and showed a total of 5,206,216,851 paired-end filtered reads with 151 base pairs. The total plastome length was 137,743 base pairs with GC content of 35.26% and 126 coding genes. This molecular signature can be employed to distinguish the genuine *Saraca asoca* samples from its adulterants (Ali *et al.*, 2020). The chloroplast (cp) genome/ plastome contains helpful genetic information due to its conservative rate of evolution, as it has uniparental

inheritance. The *rbcl* and *matK* genes were established for molecular typing of *S. asoca* in molecular phylogenetic analysis (Ali *et al.*, 2021).

The phylogenetic analysis and evolutionary inter-relationships of *S. asoca* was investigated with other members of the Detarieae tribe belonging to the sub family Caesalpinioideae, using the *matK* chloroplast gene. The analysis implied that *S. asoca* may have undergone synonymous substitutions during evolution and it showed close relationship with *Saraca palembanica*, *S. declinata*, *Enderbia spectabilis* and *Lysidice rhodostegia* (Saha *et al.*, 2013).

### Genetic Diversity

Molecular marker-based methods can be employed for characterization of the DNA and identification of genetic diversity analysis.

Inter-simple Sequence Repeat (ISSR) markers have been employed to evaluate the genetic variation between 11 populations of the tree species with 106 accessions gathered from Western Ghats (Hedge *et al.*, 2018). Using UPGMA and Bayesian model, 2 clusters of comparable grouping styles were constructed. UPGMA dissimilarity analysis revealed close association of genotypes based on the geographical locations (Hedge *et al.*, 2018). These genetic diversity and genetic relationships were further supported using ISSR and found genotype collected from different geographical locations showed high genetic variability among them (Hedge *et al.*, 2019). In another study involving ISSR, 15 genotypes were collected from different geographical locations, from a total of 40 ISSR markers analyzed 28 markers showed polymorphic bands. A total of 149 bands were amplified, of which 133 were seen to be polymorphic. This showed higher genetic variability in the genotypes which were collected from different geographical locations (Yadav *et al.*, 2020).

Random Amplified Polymorphic DNA (RAPD) profiling was conducted in a total of 165 individuals from 5 natural populations in Odisha, which showed a comparatively high genetic diversity and polymorphism among the population, hence recommending *in situ* conservation (Senapati *et al.*, 2012). Florescent labeled RAPD primer analysis based on 6 accessions from Hyderabad exhibited high polymorphism, indicating even a small population size can show high genetic diversity within the species (Mohan *et al.*, 2017). RAPD fingerprinting analysis on 7 wild and 3 cultivated populations from Western Ghats showed substantial genetic diversity amongst the natural populations (Saini *et al.*, 2018).

DNA fingerprinting and genetic profiling by Amplified Fragment Length Polymorphism (AFLP) technique was employed to compare and differentiate *Saraca asoca* from adulterants like *Polyalthia longifolia* bark samples. *Polyalthia longifolia* showed high level of genetic diversity as compared to *Saraca asoca* (Sharma *et al.*, 2018). Sumangala *et al.*, (2013) identified 10 novel microsatellite markers which could be used for studying population genetics of the species.

### DNA Barcoding

DNA barcoding technique has been used to differentiate adulterants in 25 samples available in the market. The market and *S. asoca* specimens were barcoded employing 2 chloroplastic regions *rbcl* and *psbA-trnH* to develop Biological Reference Material (BRM) i.e. sample with the unique identification number. From the amplified DNA barcoded region of BRM sequences only 3 matched with the *rbcl* region and only one matched with the *psbA-trnH* region, thereby indicating prevalence of widespread adulteration (Urumarudappa *et al.*, 2016).

### Conclusions

*Saraca asoca* offers many promising prospects in traditional herbal therapy and modern medicine. The tree is apparently a universal panacea for its usefulness in many different ailments. The versatile plant has a variety of phytochemical constituents which are responsible for its many fold activities. However, further studies and endeavors are required, especially to evaluate the *in vivo* biological activities for better validation of the efficacy of its different constituents. Further, detailed phytochemical studies on leaves and flowers could help to identify them as prospective substitutes for the bark and also help to develop a probable phytomarker which may lead to the identification of genuine *Saraca asoca* and prevent adulteration. Owing to its varied uses, the demand for genuine bark is constantly on the rise. Overexploitation, indiscriminate use along with habitat destruction has led to sharp reduction of its population. Molecular characterization using genetic markers may help in identifying different genotype pools which may be useful for future conservation, improvement and management of the species. Transcriptome analysis may help to identify genes involved in seed viability and could also help in improving and increasing its shelf life. Thus, the conservation practice will require innovative biotechnology tools. Lastly government initiatives, experts support and creating awareness among the public may be beneficial to the already dwindling population. In summary, *S. asoca* has been extensively studied with reference to its profile, phytochemistry and various biological activities. However, newer studies and findings would enhance the existing therapeutic significance of *S. asoca* and encourage its future use in modern medicine, whereas novel biotechnological and genetic approaches may help in its conservation.

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### Declarations

Authors declare no conflict of interest

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