

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2016.503.036>

Biological Activities of Different Parts of *Saraca asoca* an Endangered Valuable Medicinal Plant

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A B S T R A C T

Keywords

Saraca asoca,
Phytochemical screening,
Antibacterial activity,
Antioxidant activity, DPPH.

Article Info

Accepted:
15 February 2016
Available Online:
10, March 2016

This report describes the phytochemical relation of the antibacterial, antioxidant activity of different explants extract of *Saraca asoca* (Roxb.) De Wilde. In the present study preliminary phytochemical analysis of different extracts (bark, flower and leaves) was conducted, which revealed the presence of Alkaloids, Flavonoids, Glycosides, Saponins, Phenols, Steroids, Tannins and Triterpenoids. Quantitative estimation of Flavanoids and Phenols was conducted using methanolic, ethanolic and aqueous extracts. Screening for antibacterial activity of extracts of different *S. asoca* explants were performed against gram positive and gram negative bacteria by the disk diffusion method. The activities of the compounds were compared with standard strain for antibacterial properties of the imine base and its solvent extract evaluated and presenting in indicate that the compounds are active in exhibiting antibacterial role. Methanol bark extract showed maximum DPPH, radical scavenging activity which is the measure of antioxidant property at the concentration of above compounds at 200 µg/µL⁻¹ follows the order Ascorbic acid > Bark extract of *S. asoca* while at higher concentration the same order is followed by bark extraction exchanged their position. There present study confirms of photochemical, antibacterial and antioxidant activity of all plant extract of *S. asoca*.

Introduction

Undoubtedly plants are a good source of biologically active natural products. While investigating the bioactive natural compounds, it is essential to have access to simple biological tests in order to locate required activities (Sener *et al.*, 1994). This species is currently listed as a 'globally vulnerable' species by the IUCN 2013. (<http://www.iucnredlist.org/apps/redlist/details/34623/0>).

Saraca asoca is a medicinally important and globally vulnerable plant species found in the evergreen forests of India (Thakur *et al.*, 1989). India has often been referred to as the medicinal garden of the world and the medicinal plant *Saraca asoca* has been regarded as one of the foremost plants utilized from antiquity till date. *Saraca asoca* (Roxb.) De Wilde, is a small evergreen tree, belongs to the family

Caesalpiniaceae is commonly known as Asoka, Sita Asoka and Haempushpam. It is an evergreen tree which is 9 m in height. The flowers are orange yellow in colour and arranged in dense corymbs. It occurs throughout India up to an altitude of 750m in central and eastern Himalayas. Leaves are parpinnate 15-20 cm long and the leaflets 6-12, oblong and rigidly sub-coriaceous. Leaves are narrowly lanceolate, cork like at the base and with a shot pestistipules are intra-petiolar and completely united. The bark is dark brown or grey or almost black with warty surface. Stem bark are rough and uneven due to the presence of rounded or projecting lenticles. Bark channeled, smooth with circular lenticles and traversely ridged, sometimes cracked. Fracture splinting exposing striated surface, a thin whitish and continuous layer is seen beneath the cork leaver. Flowers are fragrant. Flowers are Polygamous apetalous, yellowish orange turning to scarlet, in short laterally placed corymbose, auxillary panicles, bract small, deciduous and calyx petaloid. Seeds are 4-8, ellipsoid-oblong and compressed (Ali *et al.*, 2008 and Jain *et al.*, 1968).

Useful parts of the plant are barks, leaves, flowers and seed. The earliest chronicled mention of this tree is in the ayurvedic treatise and later in Charka Samhita (100 A.D.) in which Ashoka has been recommended in formulations for the management of gynecological disorders as anodynes. Ashokarista is a very famous formulation from the bark of this plant which is available commercially from various reputed companies and used to treat menstrual disorders. Different parts of *Saraca asoca* plant have been attributed with high medicinal value. *Saraca asoca* bark extracts are often used in Leucorrhea (Shukla *et al.*, 2008). Flowers have shown encouraging anti-ulcer activity in albino rats (Maruthappan *et al.*, 2010). Saracin, a lectin

purified from *Saraca indica* seed integument, has been found to agglutinate human lymphocytes and erythrocytes irrespective of the blood group; it causes agglutination of Ehrlich ascites carcinoma (EAC)3 cells as well as animal erythrocytes (Cibin *et al.*, 2010). Moreover, chemopreventive activity of flavonoid fraction of *Saraca asoca* flower was reported in skin carcinogenesis (Ghosh *et al.*, 1999). Larvicidal activity has also been recorded by using *Saraca* bark and leaves (Methew *et al.*, 2008). Biochemical analyses have shown that leaves of *S. asoca* contain carbohydrates, proteins, glycosides, flavonoids, tannins and saponins (Pradhan *et al.*, 2010). Different plant parts of *S. asoca* provide antibacterial (Nayak *et al.*, 2011) CNS depressant, anti-pyretic, anthelmintic and analgesic activities (Nayak *et al.*, 2010 and Varma *et al.*, 2010).

All the plant parts are considered to contain medicinal properties. Leaves of *Saraca asoca* are known to contain carbohydrates, proteins, tannins and saponins and shows antibacterial activity (Pradhan *et al.*, 2010). Barks and Flowers contain glycosides, steroids, saponins, carbohydrates and tannins (Pal *et al.*, 1985). The flowers are also regarded as medicinally important plant part and used as therapeutic agent in treatment of diabetes, cancer and hemorrhagic dysentery, uterine infections as menorrhagia and other types of uterine disorders. It is also used in bleeding piles, bacillary dysentery.

Dried flower buds are reported to have antibacterial activity. Aqueous suspension of *Saraca indica* flower has antiulcer activity in albino rats (Maruthappan *et al.*, 2010). *Saraca asoca* bark and flowers exhibit antitumour activity against DLA, S-180 and Ehrlich ascites carcinoma tumour cell lines, Larvicidal activity has also been recorded

(Methew *et al.*, 2008). Chemo preventive activity of flavonoid fraction of *S. asoca* is reported in skin carcinogenesis. During ‘ashoka-sasthi’ the flower buds are taken orally by women. Though phytoconstituents have been reported earlier in case of leaves and bark of the plant (Pradhan *et al.*, 2010), no detail qualitative phytochemical analysis are found for flowers.

The antimicrobial activity of the stem and bark of *Saraca indica* have been evaluated against standard strain of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium* (Sainath *et al.*, 2009). The leaves of *Saraca indica* also evaluated for anthelmintic activity (Nayak *et al.*, 2011), analgesic and antipyretic activities (Pradhan *et al.*, 2010), CNS depressant activity (Yadav *et al.*, 2008). The reports of quantitative estimation of different antioxidants of the bark of *Saraca asoca* are hardly available. The antioxidant property is also related to the condition of soil and environment where the plant is grown. So in this investigation we are quantitatively estimate different phytochemicals such as total polyphenols, flavonoids, ascorbic acid and tannins and free radical scavenging activities of DPPH to evaluate antioxidants properties of the bark of *Saraca asoca*. In the Present study was phytochemical analysis, antibacterial activity and antioxidant study of the different parts of methanol extracts of *Saraca asoca* bark, flower and leaves was carried out.

Materials and Methods

Plant material used various plant parts (bark, flowers and leaves) of *S. asoca* were collected from the Botanical garden, Osmania University, Hyderabad. The collected plant materials were identified and voucher specimens were kept at the medicinal plant museum (Herbarium) of the Department of Botany.

Preparation of Extracts

Plant samples (bark, flowers and leaves) were washed with distilled water and air-dried at room temperature for 7-10 days, then oven-dried at 40 °C to remove the residual moisture. The dried plant parts were pulverized and stored in air-tight containers at 4 °C for future use. 50 g of powdered samples of bark, flowers and leaves were extracted with methanol by soxhlation method at 60 to 80 °C. The three filtrates were separately concentrated in water bath at 40 °C and evaporated under reduced pressure.

Phytochemical Analysis

The four extracts obtained from the powdered flowers of *Saraca asoca* were subjected to phytochemical tests to determine the presence of active secondary metabolites using standard procedures (Mohan *et al.*, 2014).

Antibacterial Activity

The disc diffusion method was used to evaluate the antibacterial activity of the synthesized compounds against four bacterial strains viz; *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *S. aureus*. Each organism was cultured in nutrient broth at 37 °C for 24 h. Then 1 % broth culture containing approximately 106 colony forming units (CFU/mL) of test strain was added to nutrient agar medium at 45 °C and poured into sterile petri plates. The medium was allowed to solidify. 5 µL of the test compound (40 mg/mL in DMSO) was poured on 4 mm sterile paper discs and placed on nutrient agar plates. In each plate standard antibacterial drug (ampicillin) and metal complexes were added. The plates were incubated at 37°C for 24 h and the antibacterial activity was determined by

measuring the diameter of zones showing complete inhibition (mm) (Mohan *et al.*, 2015).

Radical Scavenging Activity

The percentage of free radical scavenging activity is shown in Fig-2. This assay is based on decrease in absorbance value of DPPH at 517 nm on addition of complex. The experiment involves diluting the working solution of the plant extracts and the ascorbic acid standard (700, 600, 500, 400, 300 and 200 $\mu\text{g}/\mu\text{L}$) in methanol. DPPH concentration was kept constant (2 mL, 0.004 %). To this varying concentration of plant extracts and standard were added. The mixture was shaken vigorously and kept in dark for 30 min at room temperature. Then the absorbance was measured at 517 nm in a spectrophotometer. The whole experiment was carried out using spectroscopic grade methanol solvent at 298 K. The radical scavenging activity has been measured by using the following Eq. 1.

$$\text{Suppression ratio (\%)} = [(A_0 - A_i)/A_0] \times 100\% \quad (1)$$

Where A_i =the absorbance in the presence of the ligand or its complexes, A_0 =the absorbance in the absence of the ligand or its plant extracts.

Results and Discussion

Phytochemical Analysis

The present study contributes valuable information of bioactive compounds in *S. asoca*. Qualitative analysis of plant different extract (bark, flower and leaves) was carried out for Alkaloids, Flavonoids, Glycosides, Saponins, Phenols, Steroids, Tannins and Triterpenoids. Methanol, ethanol and aqueous extract of bark, flower and leaves

has all the phytochemicals like flavonoids, glycosides, saponins, phenols, steroids, tannins and triterpenoids, except bark extract it has all phytochemical constituents except alkaloids, ethanol extract of flavonoids and also glycosides and tannins are absent in aqueous extract of the bark. In flower extract it has all phytochemical constituents except alkaloids are absent in all extract, flavonoids, glycosids and tannins are absent in aqueous extract and ethanol extract of the flower. In leaves extract it has all phytochemical constituents except alkaloids are absent in all extract, steroids are absent in methanolic extract, triterpinoids are absent in ethanolic extract and glycosides, tannins and phenols are absent in aqueous extract (Table-1). Which is similar to the reports of (Saha *et al.*, 2012; Nayak *et al.*, 2011). The plant bark, flower and leaves extracts were quantitatively analyzed for Flavonoids and Phenol (Table-2). Whereas, present our study reports of Steroids (Nayak *et al.*, 2011). Indicated that Steroids were absent in *S. asoca*. Several medicinal properties have been attributed to Steroids (Ghatak *et al.*, 2014 and Gayathri *et al.*, 2013) but surprisingly, Steroids were found in the present study. Flavonoids and Phenol are however reported in the present study which agrees with the findings of (Ghatak *et al.*, 2015).

Anti-Bacterial Studies

The antibacterial screening of the *S. asoca* different extracts (bark, flower and leaf) were performed against gram positive (*S. aureus*) and gram negative bacteria (*E.coli*, *P. aeruginosa* and *K. pneumoniae*) by the disk diffusion method. The activities of the compounds were compared with standard Ampicillin for antibacterial activity. The antibacterial properties of the imine base and its solvent extract evaluated and presenting in Fig-1, Table-3 and Graph-1, indicated that

the compounds are active in exhibiting antibacterial role like bark 0.4, 0.2, 0.5; flower 0.2, 0.4, 0.6 and leaf 0.6, 0.2, 0.2 in gram negative bacteria and bark 0.6, flower 0.4, leaf 0.3 in gram positive bacteria. The order of activity towards gram negative

bacteria is flower>bark>leaf and for gram positive bacteria the order is bark>flower>leaf. However reported in the present study which agrees with the findings of (Nayak *et al.*, 2011; Sainth *et al.*, 2009 and Sujatha *et al.*, 2013).

Table.1 Table Shows Preliminary Phytochemical Screening of Methanol, Ethanol and Aqueous Extract *Saraca asoca*

S. No	Phytoconstituents	Plant parts								
		Bark			Flower			Leaf		
		Met- OH	Et- OH	Aqueous	Met- OH	Et- OH	Aqueous	Met- OH	Et- OH	Aqueous
1.	Alkaloids	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
2.	Flavonoids	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve
3.	Glycosides	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve
4.	Saponins	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
5.	Phenols	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
6.	Steroids	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
7.	Tannins	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve
8.	Triterpenoids	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve

Note:-Et-OH- Ethanol, Met-OH- Methanol and -ve-Absent, +ve-Present

Table.2 Quantitative Analysis of the Methanol Extracts of *Saraca Asoca* for Estimation of Flavonoids and Phenols

S.No	Plant extract	Phytochemicals	Average Estimated value (mg/gm) (Mean±S.E)
1.	Flavonoids	Bark	4.72±0.74
		Flower	5.22±0.15
		Leaf	5.86±0.54
2.	Phenols	Bark	115.98±0.67
		Flower	125.32±0.63
		Leaf	146.37±0.64

* Phenols are expressed as Gallic acid equivalent (GAE) and Flavonoids are expressed as Quercetin equivalents (QE) in mg/100 gm

Table.3 Minimum Inhibition Zone (mm) Complexes (μg/ml)

Compound	Bacterial inhibition zone (mm) Gram (+)			Bacterial inhibition zone (mm) Gram (-)
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>
1. Bark	0.4	0.2	0.5	0.6
2. Flower	0.2	0.4	0.6	0.4
3. Leaf	0.6	0.2	0.2	0.3

Fig.1 Antimicrobial Activity of Bark, Flower and Leaves Extract of *S. asoca* (a) *E.coli*, (b) *P. aeruginosa*, (c) *K. pneumoniae* (Gram Negative) and (d) *S. aureus* (Gram Positive)
Ampicillin as Positive Control

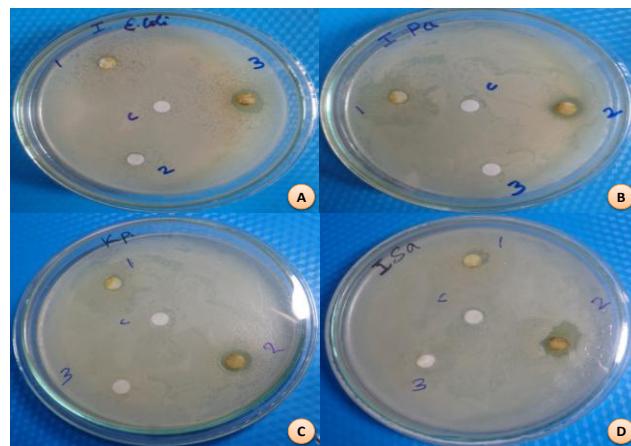
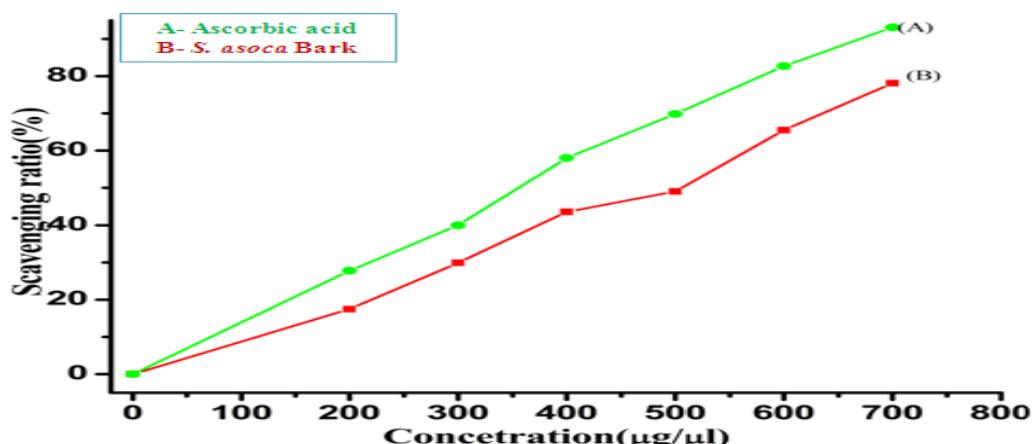
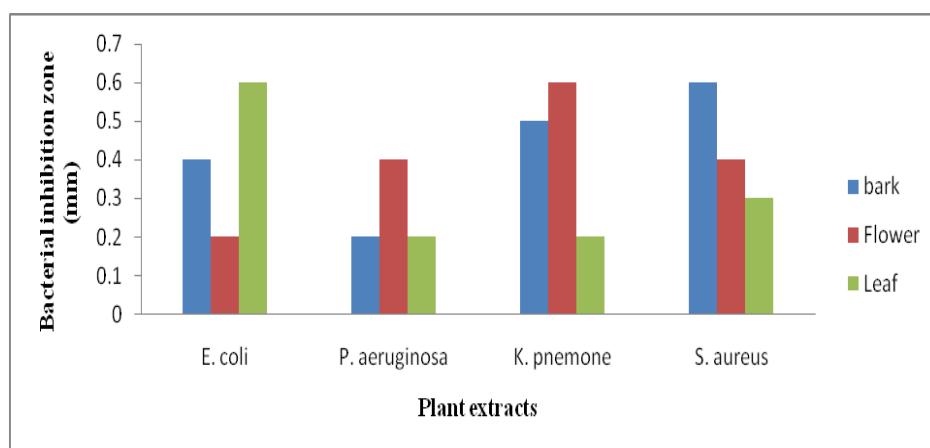


Fig.2 Radical-Scavenging Activity of the Bark Extract of *S. asoca* on DPPH Radicals (%)



Graph.1 Minimum Inhibition Zone (mm) Complexes ($\mu\text{g}/\text{ml}$)



Antioxidant Properties

The model of scavenging the stable DPPH radical is a widely used technique to screen antioxidant properties by spectrophotometer in a very short time period. When the reaction between antioxidant molecule and DPPH radical occurs, it results in decrease in absorbance at 517 nm. This is because the radical is scavenged by antioxidants through donation of hydrogen to form the reduced form (DPPH-H), and this property is also visually noticeable as the color changes from purple to yellow. The more rapidly the absorbance decreases, the more potent is the antioxidant compound. In the present study the antioxidant activity of bark extract of *S. asoca* was evaluated by scavenging stable DPPH radical (Fig:-2). The DPPH radical scavenging activities were found to be 28.16 % for ascorbic acid, 16.86 % for bark extract (B), at concentration of the 200 $\mu\text{g}/\mu\text{L}^{-1}$. Ascorbic acid exhibited higher DPPH scavenging activity than the compound at all concentrations. At the concentration of 700 $\mu\text{g}/\mu\text{L}^{-1}$ scavenging activities were found to be 95.08 and 78.12 % for Ascorbic acid and bark extract of *S. asoca* respectively. The metal scavenging activity which is the measure of antioxidant property at the concentration of above compounds at 200 $\mu\text{g}/\mu\text{L}^{-1}$ follows the order: Ascorbic acid > Bark extract of *S. asoca* while at higher concentration the same order is followed by bark extraction exchanged their position. However reported in the present study which agrees are similar with their antioxidative potential (Ghatak *et al.*, 2015; Panchawat *et al.*, 2010).

In conclusion, the Present study involving preliminary phytochemical studies of different extract (bark, flower and leaves) revealed the presence of all secondary metabolites different extract and some compounds were also estimated

quantitatively (Flavonoids and Phenols), methanol extraction is proved to be the best solvent system for the all parts *S. asoca*. Study confirms the antibacterial activity of bark, flower and leaf extract of *S. asoca* the extract found effective bacterial strain, the order of activity towards flower antibacterial activity higher then bark and leaf in gram negative bacteria, where as bark activity is more when compare to flower and leaf in gram positive bacteria. The antioxidant activity evaluated for the methanolic bark extraction of *S. asoca* has excellent compound activity to eliminate the hydroxyl radicals. These observations can lead to the development of new potent antioxidants. This is valuable information for preparation of drugs in pharmaceutical industry and stress the need for more intensive research since they play a great role in healthcare.

Acknowledgment

We are thankful to Department of Botany, Osmania University, Hyderabad for lab facilities and valuable suggestions and financial assistance is gratefully acknowledged.

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How to cite this article:

Mohan, Ch., Kistamma, S., Vani, P., Narshimha Reddy, A. 2016. Biological Activities of Different Parts of *Saraca asoca* an Endangered Valuable Medicinal Plant. *Int.J.Curr.Microbiol.App.Sci*. 5(3): 300-308. doi: <http://dx.doi.org/10.20546/ijcmas.2016.503.036>