Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
https://www.phytojournal.com
JPP 2023; 12(1): 44-51
Received: 15-10-2022
Accepted: 14-11-2022

Brijesh Shivhare
Discipline of Biochemistry, School of Sciences, IGNOU, New Delhi, Delhi, India

Ritu Rani
Department of Biochemistry, Maharshi Dayanand University, Rohtak, Haryana, India

Maneesha Pandey
Discipline of Biochemistry, School of Sciences, IGNOU, New Delhi, Delhi, India

Ramesh Kumar
Department of Biochemistry, Bundelkhand University, Jhansi, Uttar Pradesh, India

Corresponding Author:
Dr. Maneesha Pandey
Discipline of Biochemistry, School of Sciences, IGNOU, New Delhi, Delhi, India.

Antioxidant capacity and metabolic characterization of aqueous and ethanolic extract of *Saraca indica*

Brijesh Shivhare, Ritu Rani, Maneesha Pandey and Ramesh Kumar

DOI: https://dx.doi.org/10.22271/phyto.2023.v12.i1a.14547

Abstract
Saraca indica (Ashoka) is an important medicinal plant and the compounds present in this plant are very helpful in preventing various diseases. Further, it is an important drug used in many Ayurvedic formulations. Asoka is used in Ayurveda to treat a variety of conditions, including excessive uterine bleeding, gynaecological issues, internal piles, diabetes, dyspepsia, indigestion, burning sensation, blood disorders, fractures, tumours, bites, ulcerations, etc. Therefore, keeping in view of above beneficial effects, the aim of the present study was to investigate phytochemical screening, antioxidant capacity and metabolic characterization of aqueous and ethanolic extracts of *Saraca indica***.** Phytochemical evaluation was performed using standard protocol. The *in vitro* antioxidant activity, total phenolic content and total flavonoid content was determined. UV visible spectroscopy was used to recognize quantitative determination. The active metabolites of the plant *Saraca indica* have been studied using Fourier transform-infrared spectroscopy and gas chromatography-mass spectrometry. IC50 values of DPPH radical scavenging assay for aqueous and ethanolic extracts were 380µg/ml and 350µg/ml, respectively. IC50 values of $ABTS^{+}$ radical cation scavenging assay for aqueous and ethanolic extracts were 200µg/ml and 350µg/ml, respectively. Major functional groups and active metabolic compounds in the *S. indica* were identified by FT-IR and GCMS, respectively. *Saraca indica* is an excellent source of natural antioxidants and the antioxidant activity observed may be due to phenolic and flavonoid content in the aqueous and ethanolic extracts.

Keywords: *Saraca indica*, antioxidant potential, total phenolic content, total flavonoid content, FT-IR, GC-MS analysis

Introduction
In India, Ayurvedic science has a long history. A number of plants are utilized for therapeutic purposes, either whole or in specialized parts, such as dried fruits, bark, roots, leaves, and flowers. Secondary metabolites constitute the majority of natural products [1]. Evergreen *Saraca asoca* (Roxb.) Wilde or *Saraca indica*, often known as Sita Ashoka or Ashoka, is a member of the Caesalpiniaceae family. This plant is widely distributed throughout India, particularly in the Himalaya and the entire south area. It has cooling properties and is widely known for its medical usefulness. The body finds it highly beneficial to reduce organ heat that has accumulated as a result of weariness or hormonal imbalance. It aids in regulating blood composition and stabilizing blood circulation, ensuring that all body parts have the best possible supply to blood [2]. In addition, Ashok is a cardiac tonic that can be used as a supportive treatment for persons with hypertension, circulation issues, edema, congestive heart failure, etc. In the plant's leaves and bark, different phytoconstituents have been found. All plant parts, including the bark, leaves, and flowers, are considered to have medicinal value and are employed as therapeutic agents in the management of conditions such as diabetes, cancer, hemorrhagic dysentery, bleeding piles, uterine infections, and bacillary dysentery [3]. It was traditionally used as medication for a variety of conditions, including uterine fibroids, leucorrhea, piles, diarrhoea, and wound healing [4]. The bark, leaves, flowers, fruits, seeds, and roots of the *S indica* plant are all medicinally valuable. The *S. indica* tree has water-surface-colored bark that is dark brown and black. Women utilize Ashoka bark to treat uterine, menstrual, and menorrhagia problems. Its bark contains natural detoxifying characteristics that make it highly beneficial to maintain a healthy body free of toxins from the inside out and to improve skin complexion. The body can remain clear of toxins due to its natural cleansing properties. The flowers and leaves of this plant are used to cure fever, diarrhoea, diabetes, constipation, stomach ache, and acne while the bark of this plant exhibits antibacterial activity against a wide spectrum of pathogenic bacteria [5, 6].

The plant has lanceolate, oblong, and peripinnate leaves arranged in opposite to one another. Young leaves are red in colour; as they mature, it turns green [7].Alkaloids, steroids, flavonoids, tannins, saponins, terpenoids, polyphenolic, glycosides, and many carbohydrates have been found in the leaves of this plant, according to number of studies [8, 9]. This plant produces fractured, calyx petaloids, polygamous, apetalous, and deciduous flowers. It has been claimed that dried flower buds have antimicrobial properties [6]. In albino rats, *S. indica* flower aqueous suspension shows antiulcer activity [10]. Numerous uses for *S. indica* include the treatment of gynaecological diseases in women, all forms of abnormal vaginal discharge, uterine inertia, uterine discomfort, urinary calculi, dysuria, etc. Studies have found that seeds include fatty acids, steroids, flavonoids, and saracin [1, 8]. Primary and secondary compounds known as phytochemicals are found naturally in many therapeutic plants, leaves, and vegetables. Research has shown that they act as a defense mechanism to shield plants from a variety of ailments [11]. The purpose of this study is to qualitatively and quantitatively analyze the important phytochemicals found in the two different solvent extracts of the bark, leaves, flowers, and entire plant part of *S. indica*. The goal of the current inquiry is to identify which phytochemicals are found in the leaves of the Ashoka plant.

Materials and Methods

Sample collection, identification and extract preparation

The fresh plant parts such as leaves, bark, and flowers of *Saraca indica* were collected in March 2019 from a garden in Jhansi. The collected plant materials were brought to the laboratory on the same day. The plant was identified by Dr. Prem L. Uniyal, from the Department of Botany, University of Delhi New Delhi.

The samples were extracted using the Soxhlet method [12]. The Plant samples were pre-washed with tap water and then again washed with distilled water and left for air-drying at room temperature for 7-10 days. Finally, it was kept for oven-drying at 40°C to remove the residual moisture. The dried part of bark, leaves, and flowers were powdered using a mixer grinder and stored in an air-tight container for future use. Water and Ethanol (80%) were used for extraction using Soxhlet. About 15 gm of dry powder of each of the plant samples were used for extraction with aqueous and ethanol at room temperature using the Soxhlet apparatus and the extraction process was continued until the liquid was clear. The extract was then filtered out and concentrated to get a dry mass under vacuum using a rotatory vacuum evaporator. The sample was kept in an airtight container at 4º C for further use.

The percent extract yield (%) was calculated by using the formula.

$$\%\, Yield = \frac{\text{weight of dry extract}}{\text{weight taken for extraction}} \times 100$$

Phytochemical Analysis

All of the extracts, including the leaves, bark, flowers, and whole plant (mixture of leaves, bark, and flowers) were subjected to a thorough phytochemical examination as described elsewhere [13].

Quantitative estimation

Determination of Total Phenolic content

The Folin-Ciocalteau technique was used to determine the total phenolic content as described elsewhere [14]. Using Gallic acid as a reference control, the UV spectrophotometer was used to measure the absorbance at 765 nm. The amount of Gallic acid equivalents (mg GAE/g) was used to measure the total phenolic content.

Estimation of Total Flavonoid Content (TFC)

The determination of the total flavonoids content was done as previously mentioned [15].As a reference, the quercetin equivalent (mg QE/g) was used to calculate the flavonoid content. 500 µl of distilled water, 100 µl of 5% sodium nitrate, and 100 µl of different dilutions were combined and left to stand for 6 minutes. Following the addition of 150 µl of 10% aluminium chloride solution, the mixture was allowed to stand for an additional 5 minutes before 200 µl of 1 M sodium hydroxide solution was added. The absorbance was measured at 510 nm, the flavonoid content was calculated as mean±SD (n=3) and expressed as mg/gm of quercetin equivalent (QE) of dry extract.

DPPH radical scavenging activity

As previously mentioned, an estimate of the DPPH radical scavenging activity was performed [16]. 0.1 mM DPPH solution in 1 ml of methanol was thoroughly combined with various extract concentrations (0.5 ml each). The mixture was allowed to stand in the dark for 30 minutes. The multimode plate reader was used to measure the absorbance at 523 nm. As the standard and blank, identical amounts of DPPH and methanol were utilized. The scavenging activity was calculated using the following formula:

Scavenging activity (%) = (A control - A sample)/A control) x100

Where A sample is the absorbance of the test sample and A control is the absorbance of control.

ABTS radical scavenging assay

ABTS assay was carried out using the method described by Susheela [17]. The stock solutions were prepared using 7 mM ABTS solution and 2.45 mM potassium persulfate/ ammonium persulfate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12h at 30ºC in the dark. The solution was then diluted by mixing 1 mL ABTS solution with 60 mL methanol to obtain an absorbance of 0.706±0.001 at 734 nm using a UV spectrophotometer. Plant extracts (1 mL) were treated with 1 mL of the ABTS solution and incubated for 7 min. The absorbance was taken at 734 nm using the spectrophotometer. All the experiments were performed in triplicates.

The ABTS scavenging capacity of the extract was calculated as:

ABTS radical scavenging activity (%) = (A control - A sample)/A control) x100

Where A sample is the absorbance of the test sample and A control is the absorbance of control.

UV-Visible spectroscopy

UV-Visible spectroscopic analysis for the samples was performed by diluting one gram of the extracted powder with 10ml of the identical solvent [18]. The extracts were scanned in the wavelength extending from 200-800 nm using (Shimadzu UVd-1800 PC, Japan) and the individual peaks were noticed.

Fourier Transform Infrared (FT-IR) Spectrophotometry

FT-IR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to observe the

typical peaks and their functional groups. It is conceivably the most controlling tool for recognizing the kind of chemical bonds (functional groups) present in compounds. The plant constituents of dried powder sample of ethanolic extract were used for FT-IR investigation [19]. One hundred milligrams of the dried powder extract was condensed in KBr pellet in order to prepare translucent sample discs. In FTIR spectroscopy, powdered sample of the plant specimen was loaded with a scan range from 400 to 4000 cm^{-1} showing a resolution of 4 cm^{-1}.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS analysis was performed at Advanced Instrumentation Research Facility (AIRF), Jawaharlal Nehru University, New Delhi, India. Identification of phytoconstituents was based on Willey 8 and NIST 5.0 libraries attached to the GC-MS instrument. The following conditions used were: Rtx-5 MS column (30 m x 0.25 mm x 0.25 µm); helium (99.999%) carrier gas at a constant flow of 1.2 mL/min; 1 µl injection volume; injector split ratio of 1:10; injector temperature 260°C; electron impact mode at 70 eV; ion-source temperature 230°C. The oven temperature was programmed from 70°C (isothermal for 3 min), with an increase of 10°C/min to 250°C (isothermal for 5 min) and 15°C/min to 280°C (isothermal for 22 min). Helium was used as the carrier gas and the temperature programming was set with initial temperature 60°C for 2 min, ramp 10°C/min to 300°C, hold 6 min., 1 µl samples were injected with split less mode. Mass spectra was recorded over 35 - 650 amu range with electron impact ionization energy 70 eV. The total running time for sample was 49.98 min. Quantitative determinations were made by relating respective peak areas to TIC areas from the GC-MS. Retention time of extracted compounds were compared with stored components & results were tabulated.

Results and Discussion

Qualitative Phytochemical Analysis

Preliminary phytochemical screening was done for detecting the presence or absence of secondary metabolites in the aqueous and ethanolic extract. The screening was done for leaves, bark, flower and whole plant part (mixture of leaves, bark and flower) using two different solvents i.e. Aqueous and Ethanol (80%). The presence of flavonoids, carbohydrates, glycosides, saponins and steroids was observed in the aqueous extract of whole plant whereas carbohydrates, flavonoids, saponins, glycosides and steroids were present in the 80% ethanolic plant extract. The extracts were subjected to preliminary phytochemical analysis using standard chemical methods which mainly showed the presence of carbohydrates, flavonoids, phenols, glycosides, tannins, and saponins that were present in the bark, leaves, and flower of *Saraca indica* (Table 1).

Table1: Qualitative phytochemical analysis of aqueous and ethanolic extracts *Saraca indica*.

Phytochemical Tests	Aqueous extract				Ethanolic extract			
	Leaf	Bark	Flower	Whole plant	Leaf	Bark	Flower	Whole plant
Carbohydrates								
Benedict's test	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
Fehling's test	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
Iodine test	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
Flavonoids								
Alkaline reagent test	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
Saponins								
Foam test	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
Phenol & Tannin								
Ferric chloride test	- ve	- ve	- ve	- ve	+ ve	+ ve	+ ve	+ ve
Glycosides								
Liebermann's test	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
Salkowski's test	+ ve	+ ve	- ve	+ ve	+ ve	+ ve	- ve	+ ve
Keller-kilani test	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
Steroids								
Salkowski's test	+ ve	+ ve	- ve	+ ve	+ ve	+ ve	- ve	+ ve
Phenolic compounds								
Ferric chloride test	- ve	- ve	- ve	- ve	+ ve	+ ve	+ ve	+ ve

+ ve = presence- ve = absence

Quantitative phytochemical analysis

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The total phenolic content and flavonoid content was maximum in the ethanolic (125.32±.042 mg/g GAE and 8.33±0.24 mg/g QE) as well as aqueous leaf extract (175.98±0.29 mg/g GAE and 6.85±0.18 mg/g QE) as compared to bark, flower and whole plant extract. The second most phenolic content was observed in the ethanolic extract of bark followed by flower and whole plant extract.

Table 2: The total phenolic content (mg/g GAE of extract) and total flavonoid content (mg/g QE of extract) of aqueous and ethanolic extract of *Saraca indica* leaves, bark, flower, and whole plant.

Quantitative Estimation	Extraction Solvent	Plant parts			
		Leaves	Bark	Flower	Whole plant
TPC(mg/g GAE of extract)	Ethanol	125.32±.042	104.93±0.34	95.12±0.29	44.22±0.17
	Aqueous	175.98±0.29	70.42±0.21	54.56±0.18	35.64±0.08
TFC(mg/g QE of extract)	Ethanol	8.33±0.24	3.36±.31	4.87±0.29	4.94±0.16
	Aqueous	6.85±0.18	3.14±0.22	2.82±0.19	3.55±0.22

In vitro Antioxidant Activity

The ranges of the different extracts ability to scavenge free radicals at different concentrations are shown in Figure-1. The maximum amount of DPPH radical-scavenging activity was revealed to be in leaves (98.06%), which were followed by bark (84.12%), whole plant (70.63%) and flower (57.20%). Further, ABTS radical-scavenging activity was also maximum in leaves (95.95%) which was followed by bark (85.92%), whole plant (59%) and flower (72.43%). Thus, *S. indica* was shown to have scavenging abilities that were comparable to reference control i.e. Gallic acid (97.30%).

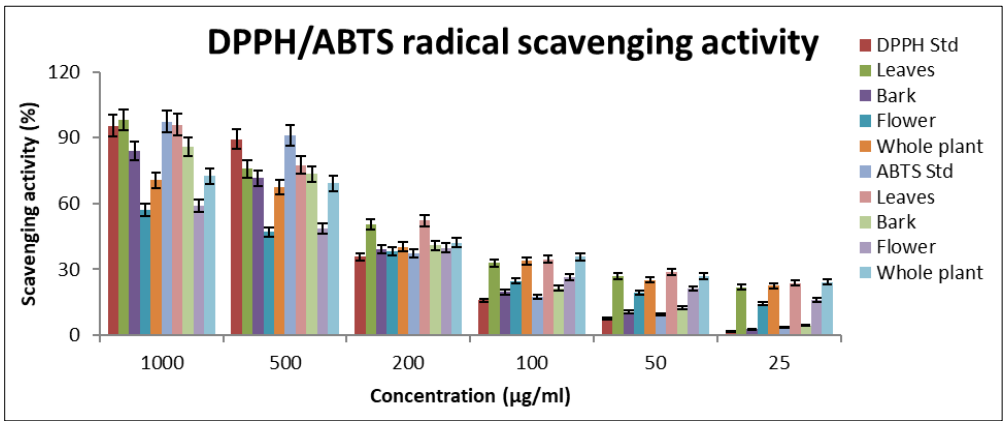

Fig 1: DPPH and ABTS radical scavenging activity of ethanolic extracts of *Saraca indica.* Values are expressed as the mean ± SD (N=3).

UV-Visible Spectroscopy

UV- visible spectroscopy interpretations of *S. indica* shows many major peaks. The UV spectrum demonstrates the peak value with the absorption value as mentioned in the Figure 2.

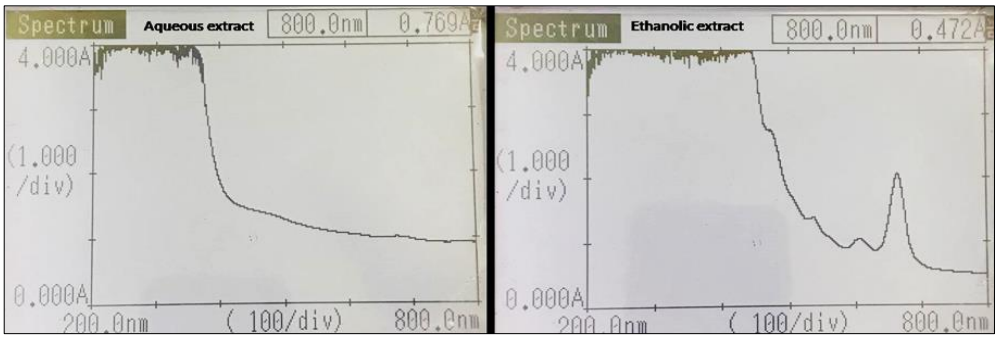

Fig 2: UV-visible spectrum analysis of aqueous and ethanolic extract of *Saraca indica*.

FT-IR and GC-MS

In order to identify potential metabolites for its excellent *in vitro* antioxidant activity, the metabolic composition of the *Saraca indica* was studied using FT-IR and GC-MS. Major functional groups such as C-Br, S=O, C-H, C-O, N-O, C=C, O-H, C-I, and N-H were detected using FT-IR on the ethanolic extract of *Saraca indica* (Table 3 and Figure 3). There were a total of 33 and 29 compounds found in the ethanolic and aqueous *Saraca indica* extract, respectively (Figure 4 & Table 4 and Figure 5 & Table 5).The different biological actions of several compounds are shown in (Table 6).

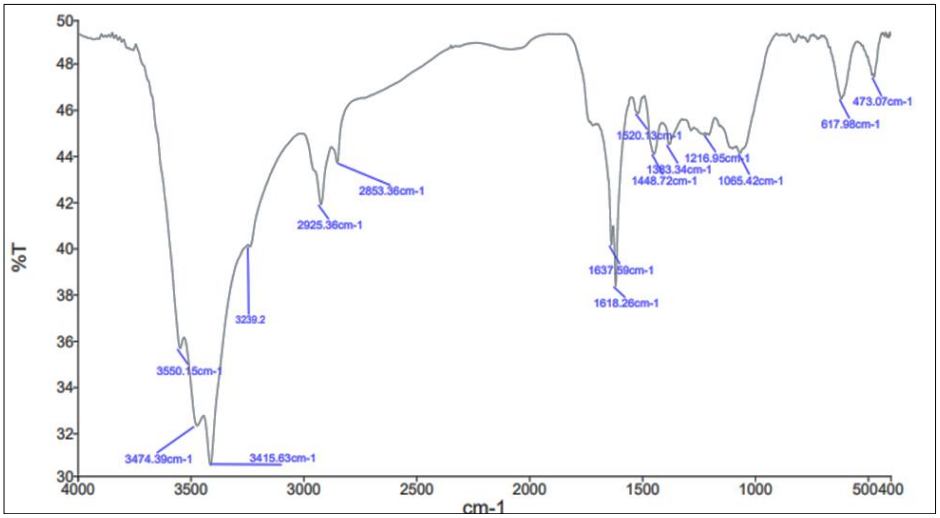

Fig 3: FT-IR spectrum of *Saraca indica* ethanolic extract.

Table 3: FT-IR analysis of *Saraca indica* ethanolic extract.

Peak No.	Absorption (cm^{-1})	% transmittance	Group	Compound class
1	3550.15	35.70	Strong O-H Stretching	Alcohol
2	3474.39	32.35	Medium N-H Stretching	Primary amine
3	3415.39	30.63	Medium N-H Stretching	Primary amine
4	2925.36	41.94	Strong N-H Stretching	Amine salt
5	2853.36	43.74	Medium C-H Stretching	Alkane
6	1637.59	40.17	Strong C=C Stretching	Alkene
7	1618.26	38.40	Strong C=C Stretching	α, β-Unsaturated ketone
8	1520.13	45.87	Strong N-O Stretching	Nitro compound
9	1448.72	44.12	MediumC-H Bending	Alkane
10	1383.34	44.53	Strong S=O Stretching	Sulfate
11	1216.95	44.96	Strong N-O Stretching	Alkyl aryl ether
12	1065.42	44.17	Strong C-O Stretching	Vinyl ether
13	617.98	46.50	Strong C-Br Stretching	Halo compound
14	473.07	47.45	Strong C-I Stretching	Halo compound

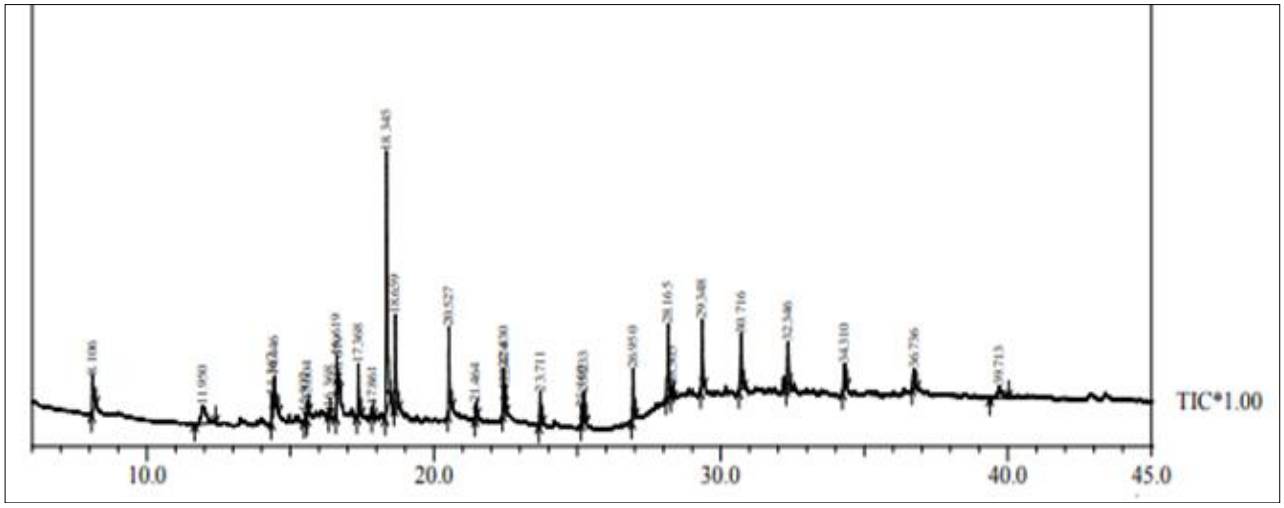

Fig 4: GC MS chromatogram of *Saraca indica* ethanolic extract.

Table 4: GC-MS analysis revealed the presence of phytochemical component in *Saraca indica* ethanolic extract.

S. No.	R. Time	Area%	Compound Name
1	15.633	39.71	Mome inositol
2	13.404	7.69	Phenol, 2,4-bis(1,1-dimethylethyl)-
3	28.299	6.16	Squalene
4	47.334	5.74	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, octadecyl ester
5	18.676	3.45	Oxalic acid, monoamide, N-(3,4-dimethylphenyl)-, heptyl ester
6	19.562	3.26	N-Heptadecanol-1
7	36.893	3.24	Stigmast-5-en-3-ol, (3.beta.)-
8	40.813	3.15	Betulin
9	32.86	2.9	Vitamin E
10	17.072	2.47	Neophytadiene
11	11.977	2.4	N-Tridecan-1-ol
12	29.341	2.14	Heneicosane
13	16.633	1.95	1-hexadecene
14	32.336	1.88	Tetratetracontane
15	14.406	1.74	N-Tridecan-1-ol
16	39.215	1.51	4,4,6a,6b,8a,11,12,14b-octamethyl-docosahydro-picene-3,13-
17	31.256	1.25	Beta.-tocopherol
18	20.521	1.12	1-octadecanol
19	32.185	1	Stigmast-5-en-3-ol, (3.beta.)-
20	19.783	0.96	2-hexadecen-1-ol, 3,7,11,15-tetramethyl-, [r-[r*,r*-(e)]]-
21	29.98	0.83	Delta.-tocopherol
22	38.087	0.78	12-hydroxyoleanan-3-yl acetate
23	26.941	0.75	2-methyloctacosane
24	17.38	0.64	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester
25	17.522	0.57	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
26	20.283	0.5	(Z)-Ethyl heptadec-9-enoate
27	18.355	0.49	1,2-benzenedicarboxylic acid, dibutyl ester
28	22.539	0.44	Hexadecanamide
29	24.203	0.32	1,2-benzenedicarboxylic acid

30	21.455	0.31	Oxalic acid, butyl 6-ethyloct-3-yl ester
31	27.458	0.29	Cyclononanone
32	20.227	0.24	1,8,11-heptadecatriene, (z,z)-
33	17.32	0.15	3,7,11,15-Tetramethyl-2-hexadecen-1-ol

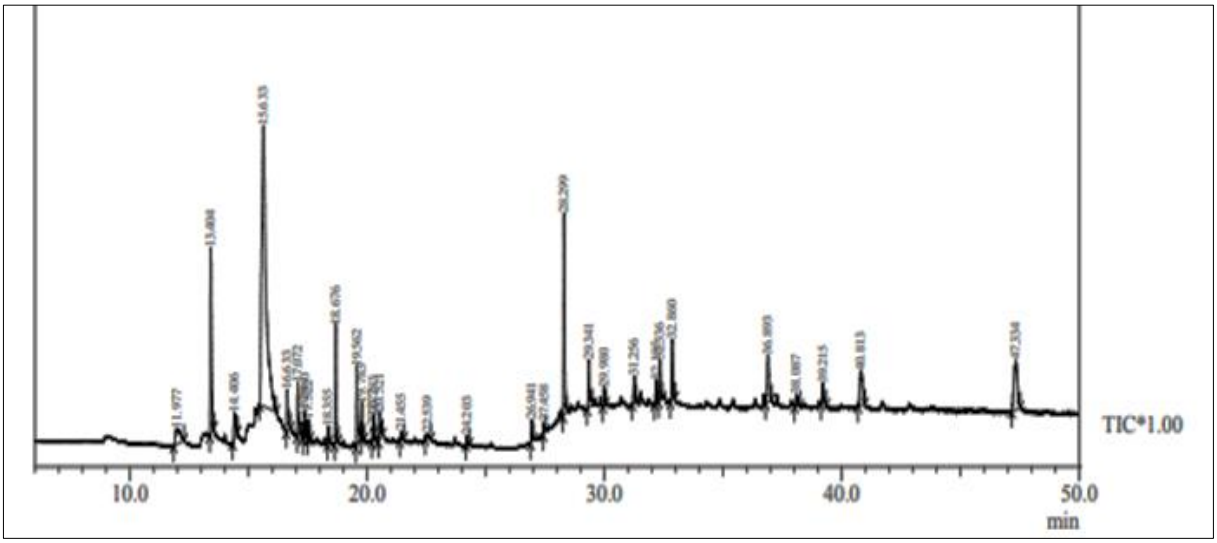

Fig5: GC MS chromatogram of *Saraca indica* aqueous extract

Table 5: GC-MS analysis revealed the presence of phytochemical component in *Saraca indica* aqueous extract.

S. No.	R. time	Area%	Compound name
1	18.345	24.26	Dibutyl phthalate
2	18.659	7.01	1-heneicosanol
3	28.165	5.91	Tetratetracontane
4	30.716	5.74	Tetracontane
5	11.95	5.54	1-octanol, 2-butyl-
6	20.527	5.17	N-tetracosanol-1
7	32.346	4.97	Hexatriacontane
8	29.348	4.76	Tetratriacontane
9	26.95	3.95	Tetratetracontane
10	17.368	3.7	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester
11	8.106	3.6	2-ethylhexyl acetate
12	34.31	3.06	Tetratetracontane
13	39.713	2.83	Tetratetracontane
14	16.619	2.69	N-heptadecanol-1
15	36.736	2.52	Tetracosane, 1-bromo-
16	14.446	2.48	Carbonic acid, undecyl vinyl ester
17	23.711	2.42	Heneicosane
18	22.43	1.71	Behenic alcohol
19	25.233	1.59	2-methyloctacosane
20	14.367	1.26	Formic acid, decyl ester
21	15.604	0.9	Sulfurous acid, 2-ethylhexyl nonyl ester
22	25.16	0.7	Cyclohexane, 1,4-bis(4-cyclohexylbutyl)-
23	21.464	0.67	Hexadecane
24	28.305	0.63	Squalene
25	17.861	0.47	7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
26	22.474	0.43	2-methylhexacosane
27	16.679	0.42	1-decanol, 2-hexyl-
28	15.507	0.35	1,6-methanonaphthalen-1(2h)-ol,octahydro-4,8a,9,9-tetramethyl-,
29	16.368	0.29	3,4,5,6-tetramethyloctane

Table 6: GC-MS analysis revealed the presence of phytochemical component in *Saraca indica* extracts and their biological activities.

Compound	R.Time	Area%	M.Formula	M Wt.	Group	Bioactivity
Mome inositol	15.633	39.71	$C_6H_{12}O_6$	180.156	Carbocyclic sugar	Anti-alopecic, anti-cirrhotic, anti-neuropathic, anti-diabetic
Dibutyl phthalate	18.345	24.26	$C_{16}H_{22}O_4$	278.34	Phthalate ester	Plasticizer, solvent for oil-soluble dyes, insecticides, antifoam agent, textile fiber lubricant, fragrance fixative, Antifouling antimicrobial
1-Heneicosanol	18.659	7.01	$C_{21}H_{44}O$	312.573	Fatty alcohol	Metabolite
Squalene	28.305	6.16	$C_{30}H_{50}$	410.73	Triterpenoid	Antimicrobial, Pesticide, antioxidant, anticancer, Neutralize different xenobiotics, anti-inflammatory, anti-atherosclerotic and anti-neoplastic
Tetratetracontane	28.165	5.91	$C_{44}H_{90}$	619.20	Alkane	Hypoglycaemic, Antioxidant activities
Tetracontane	30.716	5.74	$C_{40}H_{82}$	563.1	Alkane	Anti-inflammatory and Analgesic activity
1-Octanol, 2-butyl-	11.95	5.54	$C_{12}H_{26}O$	186.33	Fatty alcohol	Solvent and surfactant, cancer metabolism

2-Ethylhexyl acetate	8.106	3.6	$C_{10}H_{20}O_2$	172.26	Acetate ester	Antibacterial and anti-fungal, Cleaner and paint remover
n-Heptadecanol-1	19.562	3.26	$C_{17}H_{360}$	256.46	Alcohol	Antiarthritis, In treatment of skin diseases
Stigmast-5-en-3-ol, (3.beta.)-	36.890	3.24	$C_{29}H_{500}$	414.7	β-Sitosterol	Anti-inflammatory, Antipyretic, Antiulcer, Antiarthritic
Betulin	40.813	3.15	$C_{30}H_{50}O_2$	442.72	Triterpenoid	antiviral agent, an analgesic, an anti-inflammatory agent and an antineoplastic agent
Vitamin E(Alpha tocopherol)	32.860	2.90	$C_{29}H_{50}O_2$	430.71	Vitamin E compound	Antiaging, Analgesic, antidiabetic, Antileukemia, Anticancer, Hepatoprotective, Hypocholesterolemic, Antibronchitic, Anticoronary
Heneicosane	23.711	2.42	$C_{21}H_{44}$	296.6	Carbohydrates	Antibacterial, antimicrobial, Tetany, Anaemic, Hypercholesterolemic,
n- Tridecan-1-ol	11.977	2.14	$C_{13}H_{28}O$	200.36	Alcohol	Antibacterial, Natural mosquito control agent
Formic acid, decyl ester	14.367	1.26	$C_{11}H_{22}O_2$	186.30	Carboxylic acid	Food preservative, food and cosmetic additives, anti-pesticide, antibacterial.
1,2-Benzenedicarboxylic acid, Dibutyl ester	18.355	0.46	$C_{22}H_{34}O$	362.50	Phthalic acid	Plasticizer used in shower curtains, raincoats, food wraps, bowls, car interiors, vinyl fabrics.
Oxalic acid, butyl 6-ethyloct-3-yl ester	21.455	0.31	$C_{16}H_{30}O_4$	286.41	Ester	Antioxidant, Antimicrobial Larvicidal,bleaching agent

**Source: Dr. Duke's phytochemical and ethnobotanical database (online database)

Strong antioxidant activity is demonstrated by *S. indica*. The high concentrations of flavonoid and phenolic compounds in the ethanolic extract, which are known to exhibit antimicrobial, antiviral, antiulcerogenic, cytotoxic, antioxidant, antihepatotoxic, antipyretic, and anti-inflammatory activities, could be the cause of the ethanol extract's supremacy to the standard group [20]. Although active ingredients may have a significant impact on *S. indica* antioxidative activity, the polyphenolic components of the plant appear to be associated with its radical scavenging activity. By scavenging free radicals, chelating transition metals, mediating and inhibiting enzymes, among other processes, polyphenols operate as reducing agents and antioxidants [21]. According to research by Pal *et al.* [22], the ethanolic extracts of *S. indica* had total phenolic concentrations that are similar to the present values of 125.32±0.42 and 175.98±0.29 mg/g GAE, respectively. Similar experiments on *Achillea millefolium* were conducted by Keser *et al.* [23], who discovered that the aqueous extract of the leaves had a maximum flavonoid content of 6.85±0.18 mg/g QE, which is less than the present result of 8.33±0.24 mg QE/g in ethanolic extract of *S. indica*. according to Saha *et al.* [24], *S. indica* leaf extracts in aqueous and ethanol had maximal DPPH radical scavenging activities of 95.95% and 98.06%, respectively. Moreover, Nayak *et al.* [25] showed that the ethanolic extract of *S. indica* leaves had the highest levels of ABTS radical scavenging activity (97.30% inhibition), followed by bark, whole plant, and flower, and the highest levels of DPPH radical scavenging activity (98.06% inhibition). According to Kain *et al.* [26], the major functional groups found in the ethanolic extract of *S. indica* include halogen, alkane, alkene, alcohol, amine, and nitro compounds (Table 3). GC-MS found many substances that were reportedly to have antioxidant activity like Squalene, N-Heptadecanol-1, hexadecanamide1,2-benzenedicarboxylic acid, Oxalic acid, butyl 6-ethyloct-3-yl ester, betulin, dibutyl phthalate, 2-ethylhexyl acetate, tetradecanone, stigmasterol, heneicosane, neophytadiene, hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, alpha- tocopherol, carbonic acid, undecyl vinyl ester, 2-methyloctacosane, hexadecanone, vitamin E,n-tetracosanol-1. The evolution of modern drug research faces a new challenge with the use of Ashoka-derived molecules against diseases. Therefore, the production of a modern drug from *S. indica* should be addressed for the control of various diseases given the scenario of the globe shifting toward the use of nontoxic plant products with traditional medicine use.

Conclusion

Plants are a rich source of secondary metabolites with interesting pharmacological activities. The present study characterized the phytochemical profile of the *Saraca indica* extract using UV-Visible spectroscopy, FT-IR and GC-MS. The chromatogram shows the comparative concentration of different components getting eluted as a purpose of retention time. The heights of the different peaks indicate the relative concentration of the compounds present in the aqueous and ethanolic extract of *Saraca indica* extract. The mass spectrometer analyzed the compounds which were eluted at different time intervals to recognize the nature and structure of the compounds. These spectrums are finger print of the compound which can be identified from the NIST library. The identification of various bioactive compounds confirms the applications of *S. indica* extract.

Acknowledgment

The authors would like to acknowledge the Department of Biochemistry, Bundelkhand University, Jhansi and Discipline of Biochemistry, School of Sciences, IGNOU, New Delhi for providing the necessary facilities to carry out the study.

Conflict of interest

The authors declare no conflict of interest.

Abbreviations

TPC: Total phenolic content; TFC: Total flavonoid content; GAE: Gallic acid equivalent; QE: Quercetin equivalent; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

References

1. BorokerAA, PansareTA. Plant profile, phytochemistry and pharmacology of Ashoka (*Saraca asoca* (Roxb.) D.Wild)- A comprehensive Review. International Journal of Ayurvedic and Herbal Medicine. 2017;7(2):2524-2541.
2. Divya KR, Anjali AR, Rajesh T. Phytochemical screening of *Saraca asoca* (Roxb.), De. Wild. Journal of Pharmacognosy and Phytochemistry. 2017;6(3):518-521.
3. Franworth NR, MorisRW. Higher plants the sleeping giants of drug development. American Journal of Pharmatech Research, 1976;148:46-52.
4. Prathapan A, Nampoothiri SV, MiniS, Raghu KG. Antioxidant, antiglycation and inhibitory potential of *Saraca ashoka* flower against the enzyme linked to type 2 diabetes and LDL oxidation. European Review for Medical and Pharmacological Science.2012;16(1):57-65.
5. Ghatak A, Nair A, Chaturvedi P, Samant S, SoleyK, Kudale S,*et al*. Evalution of antioxidant activity, total phenolic content, total flavonids and LC-MS characterization of *Saraca asoca* (Roxb.) De. Wild.

International Journal of Advanced Research. 2005;3(5):318-327.
6. Seetharam YN, Sujeetha H, Jyotish-WaranG, Barad A, Sharamabasappa G, Parveen S. Antibacterial activity of *Saraca asoca* bark. India Journal of Pharmaceutical Sciences. 2003;65:658-659.
7. Pal TK, Bhattyacharya S, Dey A. Evaluation of antioxidant activity of flower extract (fresh and dried) of *Saraca indica* grown in west Bengal. International Journal of Current Microbiology and Applied Sciences. 2014;3(4):255-259.
8. Pradhan P, Joseph L, GuptaV, Chulet R, Arya H, Verma R, Bajpai A. *Saraca asoca* (Ashoka): A Review. Journal of Chemical and Pharmaceutical Research. 2009;1(1),62-71.
9. Mishra A, Kumar A, Rajbhar N, Kumar A. Phytochemical and pharmacological importance of *Saraca indica*. International Journal of Pharmaceutical and Chemical Science. 2013;2(2):1009-1013.
10. MaruthappanV, ShreeKS. Antiulcer activity of aqueous suspension of *Saraca indica* flower against gastric ulcers in albino rats, J. Pharm. Res, 2010;3(1):17-20.
11. Singh TH, Anantha K, KamalrajS, Kuriakose GC, Valayil JM, Jayabaskaran C. Phytomedicinal importance of *Saraca asoca* (Ashoka) an exciting past, an emerging present and a promising future, Current Science. 2015;109(10):1790-1801.
12. Nayak S, Sahoo AM, Chakraborti CK. Anthelmintic activity study of *Saraca indica* leaves extracts. Int J Appl Biol Pharm. 2011;2(2):37.
13. Singh V, Kumar R. Study of Phytochemical Analysis and Antioxidant Activity of *Allium sativum* of Bundelkhand Region. Int. J Life. Sci. Scienti. Res. 2017;3(6):1451-1458.
14. Kumar R, Sharma S, Devi L. Investigation of Total Phenolic, Flavonoid Contents and Antioxidant Activity from Extracts of Azadirachta indica of Bundelkhand Region. Int. J Life. Sci. Scienti. Res. 2018;4(4):1925-1933.
15. Sharma S, Verma O, Devi L. Kumar R. Antioxidant activity, total phenolic and flavonoid contents of *Aloe vera* leaves extract. J. Phytol. Res. 2021;34(1):49-56.
16. Kumar D, Sharma S, Kumar R. Free Radical Scavenging Activity of the Mixture of Different Medicinal Plants of Bundelkhand Region. World J Pharm. Res.2021;10(2):1008-1019.
17. Suseela V, Gopalakrishnan VK, Varghese S. *In vitro* Antioxidant Studies of Fruits of *Artemisia nilagirica* (Clarke) Pamp. Indian J. Pharm. Sci.2010 Sep;72(5):644.
18. Ibrahim KA. Synthesis and characterization of polyaniline and poly (aniline-co-o-nitroaniline) using vibrational spectroscopy. Arabian Journal of Chemistry 2017 May 1;10:S2668-74.
19. Meshref MN, Ibrahim MD, Huang R, Yang L, How ZT, Klamerth N, *et al*. Fourier transform infrared spectroscopy as a surrogate tool for the quantification of naphthenic acids in oil sands process water and groundwater. Science of the Total Environment 2020 Sep 10;734:139191.
20. Dubey CK, Meena AK, Mishra V, Singh P, Bansode FW, Singh RK. Antioxidant and Haematoprotective Activity of the *Saraca Indica* Stem Bark. Int J Pharm Sci Res 2015;6(4):1430-37.Doi: 10.13040/IJPSR.0975-8232.6(4):1430-37.
21. Sasmal S, Majumdar S, Gupta M, Mukherjee A,Mukherjee PK. Pharmacognostical, phytochemical and pharmacological evaluation for the antipyretic effect of the seeds of *Saraca asoca* Roxb, Asian Pacific Journal of Tropical Biomedicine. 2012;2(10):782-786.
22. Pal TK, Bhattacharyya S, Dey A. Evaluation of antioxidant activities of flower extract (fresh and dried) of *Saraca indica* grown in West Bengal. Int. J. Curr. Microbiol. Appl. Sci. 2014;3(4):251-259.
23. Keser S, Celik S, Turkoglu S, Yilmaz O, Turkogl I. Determination of antioxidant capacities of ethanol and water extracts of *Achillea millefolium* L.Asian J Chem. 2011;23:3172-6.
24. SahaJ, MukherjeeS, Gupta K, Gupta B. High performance thin-layer chromatographic analysis of antioxidants present in different parts of *Saraca asoca* (Roxb.) de Wilde. J. Pharm. Res. 2013, 1-6.
25. Nayak S, Sahoo AM, Chakraborti CK, Haque MN. Antibacterial activity study of *Saraca indica* leaves. Int. J. Pharm. Res. Dev. 2011;3(3):160-163.
26. Kain D, Kumar S, Vandana, Suryavanshi A, Arya A. Metabolic Characterization, Antioxidant Capacity, and *In vitro* Antiurolithiatic Potential of *Achillea millefolium* L. J Young Pharm. 2021;13(3):211-216.