RJC

RASĀYAN *J. Chem.*
Vol. 12 | No. 2 |616 - 624| April - June | 2019
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

PHYSICOCHEMICAL, PHYTOCHEMICAL, BIOLOGICAL AND CHROMATOGRAPHIC EVALUATION OF *Saraca Asoca* PLANT LEAVES

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ABSTRACT

The main objective of this work is to carry out a physicochemical, phytochemical, biological and chromatographic evaluation of *Saraca Asoca*. Different physico-chemical parameters, fluorescent test, ash analysis and extractive value of plant leaves have been carried out. The physico-chemical parameters like relative density, viscosity, surface tension and the refractive index of extract solution have been evaluated. Qualitative Phytochemical test has been done for both the extracts. The UV, IR and GC-MS of the extract are also reported. The extracts are tested for anti-malarial, anti-tuberclosis and anti-oxidant activity. Ether extract exhibited anti bacterial and anti TB activities.

Keywords: *Saraca asoca*, Extractive value, the biological activity of extracts, phytochemicals.

INTRODUCTION

Nowadays, there are various papers appear in which much more emphasis was given on Phytochemical and biological evaluation of plant extract.[1-5] Few papers also appear in which physicochemical, HPLC, GC-MS study has been reported. They are very important, since plants have been widely used from ancient times for the prevention, cure and treatment of disease particularly in the Indian subcontinent. Ashoka, commonly known as *asoca saraca* is used for various diseases. It belongs to the *Caesalpinaceae* family for the present study describes an analytical profile of *saraca asoca*[6]. Different Plant extracts contain several compounds. For example, from neem oil *(Azardirachta indica),* nimbin, the first bitter compound is isolated and then more than 135 compounds have been isolated from it.[7] The search for the biological activity of plant extract is mainly due to three reasons (i) prevalence of disease because of climatic change (ii) search of less expensive and more potent sources of drug and (iii) increased drug resistance power of microbes towards existing drugs. It is the well-known fact that most of the synthetic drugs developed have their beginning in herbal plant extract.[8]

EXPERIMENTAL

Material and Methods

All chemicals used for the present investigation were of AnalaR grade from sdfine chemicals Ltd. Double distilled water was used for the preparation of solution as well as aqueous extract. The plant leaves of *Saraca asoca* was collected from the Maulana Azad College campus. It was dried in shadow and ground to powder, which was extracted immediately using different solvents. The fluorescent tests and ash analysis are carried out. The relative density was calculated by using specific gravity bottle (10 mL). The viscosity of different solutions was determined using Ostwald's viscometer. Surface tension was

http://dx.doi.org/10.31788/RJC.2019.1222065

determined using stalagmometer and refractive index by Abbe's refractometer. The qualitative test for phytochemicals such as alkaloids, carbohydrate, proteins, amino acids, phenolic compounds and glycosides has been carried out as described in the literature. [9,10]

UV spectra were obtained on UV double beam spectrophotometer model ELICO 159. The FTIR instrument IRT3000, JASCO, having a serial no. B051061016 used to get IR and spectra were recorded using spectra manager. GC-MS was recorded from Savitribai Phule Pune University, Pune. The biological screening for antibacterial, anti- malarial and anti-tuberculosis activity has been done by disc diffusion method[11]. The anti oxidant activity was determined by the DPPH method.[12]

RESULTS AND DISCUSSION

Fluorescent Test

This is a qualitative test and can be used as a preliminary test. When the powder material is treated with acid, base and neutral reagents, it may produce fluorescent light. In the present investigation, fluorescent light is observed in n-butanol, conc. HCl, chloroform and in benzene. Hence in an organic solvent or when conc. HCl is used the powder produced fluorescence (Table-1)

The various constituents present in the plant material may exhibit a fluorescence phenomenon. UV light produces fluorescence in many compounds and some even in the visible range of day light. By treating with a different chemical reagent, the non fluorescent material can be converted to fluorescent. This is possible because of decomposition of compounds[13] chromatophores may be produced by chemical treatment.

Table-1: Florescent Test for Leave Powder

S. No.	Solution	Observation
1.	Powder (P) as such	Dark green
2.	P + n-butanol	Florescent green
3.	P + conc. HCl	Florescent green
4.	P + conc. HNO_3	Light yellow
5.	P + conc. $H2SO_4$	Dark brown
6.	P + chloroform	Florescent green
7.	P + ammonia	Reddish yellow
8.	P + toluene	Yellowish green
9.	P + glacial acetic acid	Yellow
10	P + 1N HCl	No change
11.	P + 1N NaOH	Reddish yellow
12.	P + 5% HCl	No change
13.	P + 5% NaOH	Reddish green
14.	P + benzene	Florescent green

Ash Analysis

The total ash content of the sample was found to be 6.6% out of which the water-soluble ash was 15% whereas acid insoluble ash was 45%. These values indicate that 6.6% of inorganic metal acid is present in the sample (Table-2). The acid insoluble ash contains silica material. The plant contains trace quantities of Arsenic (1ppm) and lead (5ppm).[14]

Table-2: Ash Value

S. No.	Ash	Percentage
1.	Total ash	6.6
2.	Water soluble	15
3.	Acid-insoluble	45

Extractive Values

Different solvents are used for the extraction of plant leaves. The amount extracted is different for different solvents due to various factors. Extractive values of plant leaves depend on: (i.)Types of solvent i.e polar non-polar etc., (ii) Solubility profile of constituents, (iii) Methods of extraction, (iv) species deviations, (v) cultivation season etc. In the present case, plant leaves of some Ashoka tree were extracted

with different solvents by using the same method in order to avoid the multiple variables. It is observed that more amount gets extracted in water (Table-3).

Table-3: Extractive Values of *Saraca asoca* Plant Leaves

S. No.	Extract	Percentage
1.	Water Extract	21.60
2.	Diethyl ether Extract	7.7

The extractive values of flowers of *saraca asoca* were reported to be highest in water (22% w/w) and lowest in chloroform (1.8% w/w) by earlier research.[15]

Physicochemical Properties

The physicochemical properties are very much important from an analytical point of view. Since viscosity, density, refractive index, surface tension etc in aqueous solution or any other solvent varies with nature and composition of solutes present in it. There are theories which explain variation in above properties, particularly if a single solute is present in the single solvent, binary solvent, ternary solvents, electrolyte/ non-electrolyte present in the solvent system. In the present case, plant extract is a mixture of several compounds, along with their composition and extract amount is not ascertained, therefore it is difficult to apply any theory to it, but the purpose is to find out co-relation between parameters and concentration so that some base for qualitative estimation can be established. A 30gm sample powder is extracted 300mL of water for 5 hours. The extract is filtered off. The density, viscosity, surface tension and refractive index of the filtrate were determined and recorded in (Table-4). It was then evaporated using rota- evaporator. The solid so obtained was used to prepare 100 ppm solution as a stock solution. This solution is used to prepare various dilutions and their physicochemical parameters were evaluated.

Table-4: Physical Parameters of Solution of Different Concentration

S. No.	Solution in ppm	Relative Density (dynes/cm^3)	Viscosity (Pascal sec)	Surface Tension (Newton/meter)	Refractive Index
1	5	1.0013	0.83694	57.4785	0.99625
2	10	1.0026	0.83798	47.8930	0.99495
3	20	0.9968	0.79841	43.0962	1.00063
4	40	0.9967	0.79830	54.0315	1.00087
5	60	0.9968	0.79841	49.8846	1.00073
6	80	0.9956	0.83210	51.1332	1.00199
7	100	0.9978	0.83393	51.2390	0.99976

At low concentration, the variation in physical properties is found to be irregular, but at higher concentration, the relative density, viscosity and surface tension increases with increase in concentration. The refractive index shows irregular trends over the entire concentration range, it may be due to nonuniformity of the extract solution. The physic-chemical parameters are most important for the authentication and quality evaluation of commercial plant/herbal drug samples Physic-chemical parameters play an important role and will assist is standardization and ultimately guarantee quality, purity and identification of samples.[16] There are different physicochemical parameters such as foreign matter, moisture contain, ash analysis, extractive values, foaming index, crude fiber index etc reported for leaves extract in alcoholic extracts of *saraca asoca.*[17]

Phytochemical Test

The aqueous extract of *saraca asoca* shows a positive test for carbohydrate and phenolic compounds etc. the carbohydrates and phenolic compounds were not detected in diethyl ether extract (Table-5). It is reported that the flowers of *saraca asoca* contain carbohydrate only[15].

Phytochemical screening, the acetone extract of Ashoka showed the presence of saponin , tannins, and flavonoids which inhibit pyrexia[18].The study has developed and optimized a convenient high throughput and reliable UPLC-Q-TOF-MS method to analyzed morphologically same parts of *s.asoca* which can be used for analysis and evaluation of complex herbal medicines[19]. In another study, Divya et al [20] reported the presence of carbohydrates, flavonoids, saponins, phenols etc in five different solvents. The methanolic

extract of *saraca asoca* bark reported to contains a substantial amount of flavonoids and reducing sugar [21]. The alcoholic extract of *saraca asoca* contains 57 mg of tannin per gram of sample[22].The flavonoids present in *Saraca asoca* exhibits preventive action against skin cancer[23].

Table-5: Qualitative Tests for *Saraca acosa* Plant Leaves

S. No.	Reagent	Water extract	DEE
1.	Detection of Alkaloids		
	Mayer's test	-ve	-ve
	Wagner's test	-ve	-ve
	Hager's test	+ve	+ve
2.	Detection of Carbohydrate		
	Molisch test	-ve	-ve
	Fehling's test	+ve	-ve
	Benedic test	+ve	-ve
3.	Detection of Proteins and Amino Acid		
	Biuret test	-ve	-ve
	Ninhydrin Test	-ve	-ve
4.	Detection of Phenolic Compound and Tannins		
	Ferric Chloride Test	+ve	-ve
	Gelatin test	-ve	-ve
	Lead Acetate Test	+ve	-ve
	Alkaline Reagent Test	+ve	-ve
5.	Detection of Glycosides		
	Borntrager's Test	-ve	-ve
	Legal's test	-ve	-ve

Spectral Analysis

The IR spectrum of an extract of *saraca asoca* was recorded (Fig.-1) Though it contains a mixture of compounds but still in order to find out various functional groups and a general finger print of samples, it will help. The various IR bands observed are represented in Table-6.

Table-6: IR Bands of *Saraca asoca* Plant Leaves Extract

Band (cm^{-1})	Intensity	Functional group
3347	Very Broad	O-H, N-H
2922	Sharp	Salts of primary amines, cyclic alkane
1603	Broad	C=C, C=N, N=O
1516	Sharp	C=C, C=N, N=O
1418	Broad	In the compound such as diethyl ketone, in which the methylene scissoring bond has been shifted to a lower frequency.
1074	Sharp	Sec, α-unsaturated, alicyclic five or six-membered rings.
932	Sharp	C-O, C-N
818	Broad	Fermi resonance bending band between C=O
770	Sharp	The lack of strong absorption bands in 650 cm^{-1} indicates a non-aromatic structure.
610	Very broad	The lack of strong absorption bands in 650 cm^{-1} indicates a non-aromatic structure.

The UV spectrum of water extract and diethyl ether extract shows a different pattern (Fig.-2 and 3). Water extract shows λmax at 208 nm and diethyl ether extract at 257 nm.

Chromatographic Study

The GC-MS chromatogram was run for 20 minutes. There are eleven peaks observed. The major peaks are at 6.652 min, 11.49 min and 14.99 min retention time. The expected compounds and presented in Table-7.

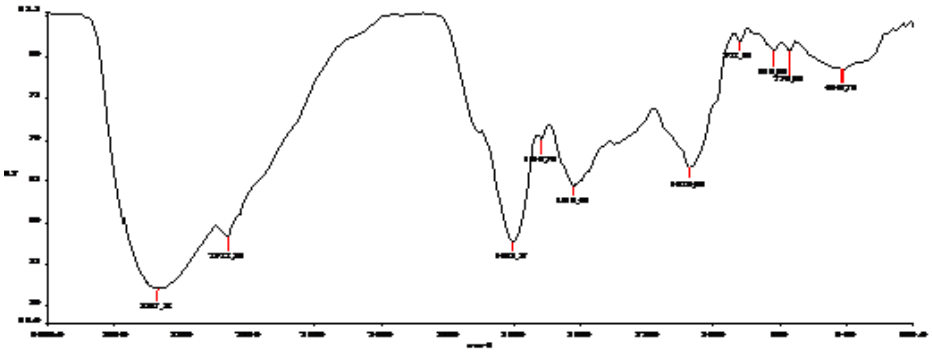


Fig.-1: IR Spectra of *Saraca asoca* Plant Leaves.

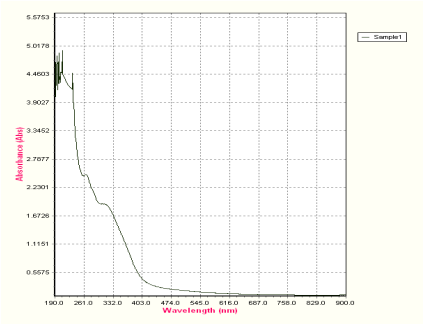


Fig.-2: UV-vis Spectra of Water Extract

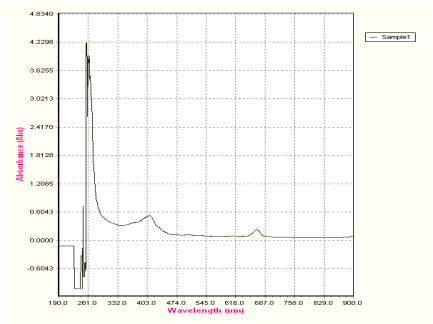
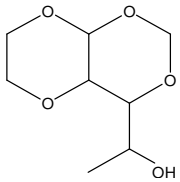
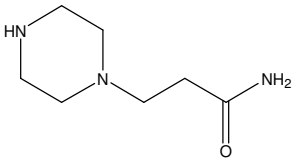
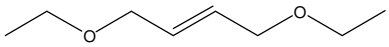
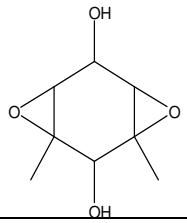
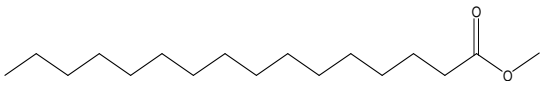
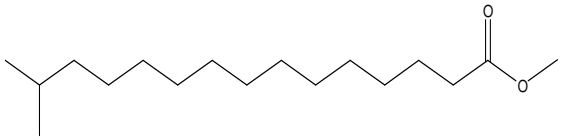
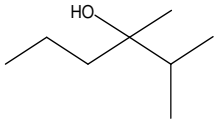
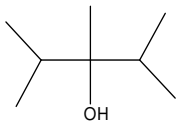
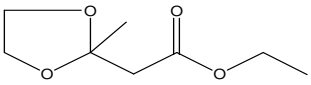


Fig.-3: UV-Vis Spectra of Ether Extract

Table-7: Expected Compounds of *Saraca asoca* from GC-MS Study

Retention Time	Compounds	Mol. Wt./ Name
6.652 min		MF: $C_8H_{14}O_5$ MW: 190 1,3,2,4-dimethylene-d-epirhamnitol
		MF: $C_7H_{15}N_3O$ MW: 157 3-(1-piperazimyl)propanamide
		MF: $C_8H_{16}O_2$ MW: 144 1,4-diethoxy-(2-butene)
		MF: $C_8H_{12}O_4$ MW: 172 1,3-dimethyl-4,8-dioxatricyclo[5,1,O,O(3,5)] octane-2,6-diol

11.49 min		MF: $C_{17}H_{34}O_2$ MW: 270 The methyl ester of hexadecanoic acid.
		MF: $C_{17}H_{34}O_2$ MW: 270 Methyl ester 14-methyl pentadecanoic acid
14.994 min		MF: $C_8H_{18}O$ MW: 130 2,3-dimethyl-3-hexanol
		MF: $C_8H_{18}O$ MW: 130 2,3,4-trimethyl-3-pentanol
		MF: $C_8H_{14}O_4$ MW: 174 Ethyl acetoacetate ethyleneaceta

Literature survey revealed that, the hydro distilled oils from the flowers of *saraca asoca* contains 28 compounds including E,E-α-farnesene and methyl salicylate as major components[24]. The leaves of *saraca asoca* contains number of compounds including catechin, epicatechin, epigallocathechin, galic acid etc.[25] *Saraca asoca* contains 0.048% w/w of catechin. It is flavonoids is utilized by naturopaths for the symptomatic treatments of several gastrointestinal respiratory and vascular disease[26]. *Saraca asoca* bark extract reveal two peaks in HPTLC corresponding to galic acid and epicatechin which results in antismutagenic and antigenotoxic properties of extact[27].

Biological Activity

The ether extract of the Ashoka has been screened for the antibacterial, antituberculosis and antimalarial activity. The extract was found to be active against *E.coli, B.Subtilus and S. Aurus* but inactive against S. Typhi. It has moderate sensitivity as anti-tuberculosis agent and does not exhibits any anti-malerial activity(Table-8). It is reported that the methanol axtract of *saraca indica* leaves shows strong in vitro anti-functional activity due to the presence of various phytomedicinal constituents[28]. The methanolic extract also exhibits antibacterial activity against gram positive and gram negative bacteria[29].The anti-bacterial properties varies with part of plants, solvent used for the extraction etc. the data obtained in such study leads to the discovery of new drug molecules. A qualitative structure activity relationship can be carried out[30].

The presence of alkaloids, glycosides, tannins and flavonoids in the ethanolic extract of *saraca indica* results into antihelmintic activity[31]. Malaria is caused by parasites i.e plasmodium species. It is the most

common disease of the world and particular in India. The malarial bacteria enter into body through a mosquito bite. There are different medicines available to cure malaria. But day by day drug resistance capacity of bacteria increasing hence some new plant materials with antibacterial activity searched through the world[32].

Table-8: Biological Activity of leaves of *Saraca asoca*

Compound	DEE	Water extract
E. Coli	Active	Inactive
B. Subtillis	Active	Inactive
S. Typhi	Inactive	Active
S. Aureus	Active	Inactive
Anti TB	Active	Inactive
Antimalerial	Inactive	Inactive

Anti-oxidant Activity

The anti-oxidant activity of Ashoka is determined by the DPPH method. These are represented in (Table-9 and 10). It is observed that, extracts have less percentage of inhibition compared to standard ascorbic acid, but increases with increase in concentration (Fig.-4). The IC_{50} value for ether extract was found to be 41.31µg/ml and for water extract 33.86 µg/ml. there are reports that concurrent administration of ethanolic extract of leaves of *saraca asoca* to albino rats, reducing the lipids alteration and induces antioxidant effect[33].

Table-9: Anti-oxidant Property of Ascorbic Acid (Standard)

S. No.	Concentration, µg/ml	Absorbance	% Inhibition	IC_{50} Value, µg/ml
1	5	0.491	40.48	14.97
2	10	0.453	45.09	
3	20	0.370	55.15	
4	30	0.335	59.39	
5	40	0.271	67.15	
6	50	0.264	68.00	

The methanolic extract of the bark of *saraca asoca* contains lignin glycosides which possess antioxidant potential[34].Approximately 90% of age-related disease are linked to activated oxygen[35]. The methanolic extract of *Saraca asoca* exhibits a high amount of gallic acid, quercetin, ellagic acid in flower and bark, due to which the extract exhibits high antioxidant activity with low IC_{50} value of 6.8 µg/mL and 6.6 µg/mL respectively[36]. The main ingredient and two famous ayurvedic formulations 'Ashokarishtam' and 'Asokagritham' is the bark of Ashoka. Thus two formulations are used in treating gyanic and irregular menstruation[37]. Apart from gynecological complications, *Saraca asoca* is used in the treatment of haemmorhagic dysentery, uterine pain, bacterial infections, cardiac and circulatory problem[38].

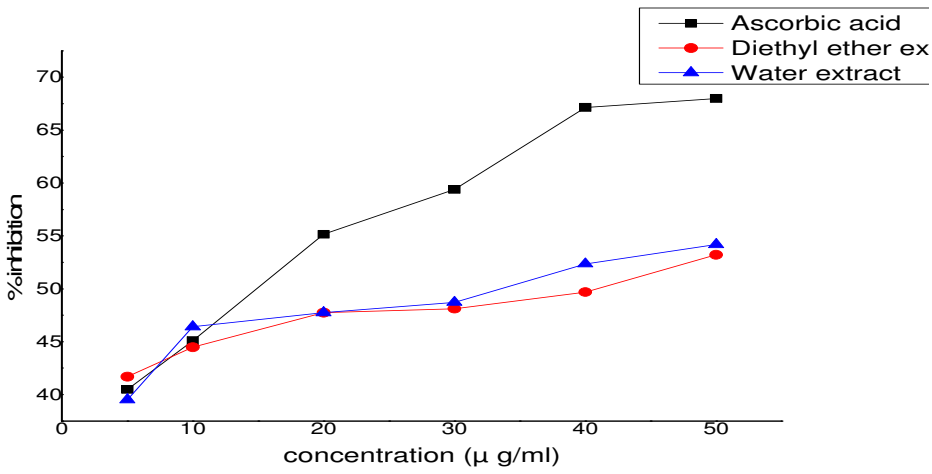


Fig.-4: Anti-oxidant Activity

Table-10: Anti-oxidant Activity of *Saraca asoca* Leaves Extract

Extracts	Concentration, µg/ml	Absorbance	% Inhibition	IC_{50} Value, µg/ml
Diethyl Ether	5	0.525	41.69	41.31
	10	0.536	44.48	
	20	0.544	47.75	
	30	0.540	48.12	
	40	0.546	49.69	
	50	0.552	53.21	
Water	5	0.532	39.51	33.86
	10	0.439	46.42	
	20	0.431	47.75	
	30	0.385	48.72	
	40	0.360	52.36	
	50	0.337	54.18	

CONCLUSION

In the present study Saraca Asoca leaves were successfully extracted using water and ether solvents. The extracts have shown different colors in solvents. The total ash content is found to be 6.6% out of which 15% is water soluble while 45% acid insoluble. The extractive values in an aqueous medium are 21.6% and that of diethyl ether 7.7%. The aqueous extract contains alkaloids, carbohydrates, phenolic compound and tannins. GC-MS study is also carried out and library search if also provided for different retention time. Ether extract is comparatively more active as compare to aqueous extract. Ether extract has shown activity against E. Coli, B Subtillis and S Aureus. it also possesses anti Tuberculosis activity.

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[RJC-2065/2018]