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Evaluation of Mast Cell Stabilizing, Anti-Inflammatory, and Anti-Oxidant Activity of Seed Extracts of *Saraca Asoka* (Roxb.), De. Wild

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Abstract

Saraca Asoka (Caesalpiniaceae) is a medicinal plant used traditionally for the treatment of various diseases. There is no scientific evidence for the anti-asthmatic activity of *Saraca Asoka*. Preliminary phytochemical screening of extract has revealed the presence various phytochemical components such as flavonoids, tannins, saponins, carbohydrates, phenols, glycosides, and fixed oils and fats. However, no alkaloids, proteins and amino acids were found in the extracts. The present study was evaluated for phytochemical screening, mast cell stabilizing, anti-inflammatory, and antioxidant activity of the methanolic extract of *Saraca Asoka*. In the present study, DPPH radical scavenging activity was highest in methanol extract (94.5 % ± 1.8%) of *Saraca Asoka* seeds. The *in vivo* anti-inflammatory activity was evaluated in rats using carrageenan-induced paw edema and *in vitro* antioxidant activity was performed by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) and ABTS. Quantitative estimation of total polyphenolic content of the (SA-ME) was estimated by Folin-Ciocalteu method. SA-ME (200 mg/kg body wt.) significantly decreased paw volume, after oral administration of SA-ME in carrageenan and formaldehyde injection. SA-ME also exhibit significant antioxidant activity. Total polyphenolic content was found to be (179 ± 0.27 mg/ml) and exhibited highest flavonoid content (8.42 ± 0.25 mg/ml). These results show that the methanol extract of *Saraca Asoka* seeds shows potential mast cell stabilizing, anti-inflammatory and anti-oxidant activity.

Keywords: Anti-inflammatory activity, Antioxidant, Mast cell stabilizer, *Saraca asoka*

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Introduction

Asthma is a chronic inflammatory disorder of the airways mainly associated with variable (usually reversible) airflow obstruction and enhanced bronchial hyper responsiveness to a variety of stimuli. During an asthmatic attack, the lining of the airways become swollen and muscles surrounding the airways become narrower. As a result, the inside of the airways become narrower, hence breathing becomes difficult.[1]

The prevalence of asthma worldwide is around 200 million with a mortality of around 0.2 million per year. Studies have indicated that asthma has increased by almost 7% during the last three decades in most countries including India. The estimated burden of asthma in India is more than 15 million.[2]

The current pharmacotherapy contains bronchodilators, anti-inflammatory agents, mast cell stabilizers, leukotriene modifiers, IgE antibody, etc. The limitations of current therapies are that, they may not produce complete cure and may not prevent all complications of bronchial asthma. Even though, these synthetic drugs are used, these are not completely safe especially for long term use and are associated with a number of serious side effects such as renal failure, liver failure, skeletal muscle tremor, hypokalemia, intense irritability, compromised immune system, and sustained high blood pressure. This has diverted the researchers toward the potential of medicinal plants and its herbal formulations claimed in the traditional systems of medicines like Ayurveda, these therapies can be successfully integrated with conventional therapy to provide maximal benefits to patients.[3]

Almost all parts such as bark, flowers, and seeds of *Saraca Asoka* are considered therapeutically valuable due to the presence of secondary metabolites such as alkaloids, terpenoids, flavonoids, steroids, glycosides, anthraquinones, phenolics, tannins, saponins, and other phytochemicals.[4]

Silver nanoparticles (AgNPs) using the extract of *Saraca Asoka* leaves have synthesized and evaluated and the extract of this leaves has been used as an antimicrobial agent.[5]

Asthma is an inflammatory disease of the lungs characterized by increased infiltration of leukocytes, especially eosinophil's, into the airways, and reduced respiratory function. The inflammation leads to bronchoconstriction, increased airway hyper-responsiveness, and mucus production.[6-10]

Pharmacognostic study, physiochemical analysis, toxicity assessment, and evaluated and the extracts of this seeds have been used as an antipyretic activity.[11]

In the present study was evaluated for phytochemical screening, mast cell stabilizing, anti-inflammatory, and antioxidant activity of the methanolic extract of *Saraca Asoka* for antiasthmatic potential has been carried out.

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Methods

Chemicals

All the chemicals used were of analytical grade, n-hexane, dichloromethane (DCM), ethyl acetate, and methanol (Merck life. Sci. Pvt. Ltd. India).

Plant Material

Seeds of *Saraca Asoka* were collected in august 2019, from Nanded localities, (Maharashtra, India), and the plant was authenticated by Botanical survey of India, Pune, Maharashtra, India. A voucher specimen (CPR2CG) was deposited in the herbarium for further use.

Extraction

Extraction was carried out with 300 g of the dried powdered seeds material of *Saraca Asoka*. The plant material was sequentially extracted with hexane, DCM, ethyl acetate, methanol, and water using Soxhlet apparatus. All the extracts were concentrated in rotary evaporator under reduced pressure.

Animals

Albino mice of either sex weighing (25–30 g) were housed under standard laboratory conditions. The animals had free access to food and water. Animal Ethical Committee of the Institute approved all the protocols of the study (Proposal no. SNIOP/CPCSEA/IAEC/CP-PL/12-2021).

Extraction of Plant Material

Extraction was carried out with 300 g of the dried powder of *Saraca Asoka* seeds. The plant material was successively extracted with different solvents such as hexane, DCM, ethyl acetate, methanol, and water. All the extracts were concentrated in rotary evaporator under reduced pressure.

Phytochemical Screening

The extracts were screened for various chemical constituents such as carbohydrates, alkaloids, tannins, flavonoids, glycosides, saponins, fats, proteins, and amino acids employing standard screening tests following conventional phytochemical screening protocols.[12-14]

Determination of Total Phenolic Content

Total phenolic content (TPC) was determined using Folin-Ciocalteu reagent.[15] The reaction mixture consists of solvent extract mixed with FC reagent, after 10 min incubation in dark, sodium carbonate (Na_2CO_3) was added to the mixture which was followed by 90 min incubation at 30°C. Phenolic content of these samples was determined spectrophotometrically using an Ultraviolet (UV) visible spectrophotometer at 760 nm. The standard curve was prepared using gallic acid (0.1 mg/mL) as standard.

Determination of Total Flavonoid Content

The total flavonoid content (TFC) was determined.[16] The plant extracts (500 µl) was added to 2% aluminum chloride ($AlCl_3$) solution in methanol (500 µl) and incubated at 30°C for 10 min. Readings were obtained at 368 nm in UV-visible spectrophotometer. The standard curve was prepared considering quercetin as standard compound.

0.3 mM DPPH solution (2 ml) was prepared in methanol, of which 0.5 mL of this solution was mixed with 100 µL of extracts. The mixture was kept in dark for incubation at 37˚C for 30 min. The absorbance was measured spectrophotometrically at 517 nm.[8-10]

The ability to scavenge DPPH radical was calculated using following formula:

$$\% \text{ Inhibition } = \frac{\text{Absor. of Control} - \text{Ab. Of sample}}{\text{Absorbance of control}} \times 100$$

ABTS Radical Scavenging Assay

Stock solutions included 7 mM ABTS solution and 2.4 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 12 h at 30°C in the dark. The solution was then diluted by mixing 1 mL ABTS solution with 60 ml methanol to obtain an OD of 0.706 ± 0.001 at 734 nm using the spectrophotometer.[16]

Plant extracts (1 mL) were allowed to react with 1 mL of the ABTS solution and the OD was taken at 734 nm after 7 min using the spectrophotometer. All the readings were taken in triplicates. The ABTS scavenging capacity of the extract was calculated as:

$$\text{ABTS radical scavenging activity } (\%) = \frac{\text{ABTS}_{\text{control}} - \text{ABTS}_{\text{sample}} \text{ X } 100}{\text{ABTS}_{\text{Control}}}$$

ABTS control is the absorbance of ABTS radical + methanol; ABTS sample is the absorbance of ABTS radical + sample extract/standard.

Evaluation of Acute Anti-Inflammatory Activity (Carrageenan-Induced Paw Edema in Rats)

During anti-inflammatory studies, paw edema was induced by injecting 0.1 ml of 1% (w/v) Carrageenan suspension into the sub planter region of the right hind paw of the rats.[21,22] The control group was orally administered saline (10 ml/kg) while the standard group was given Indomethacin (5 mg/kg) and test drug groups were given 300 mg/kg and 500 mg/kg of the test drug extract 1 h before Carrageenan injection. The measurement of paw edema was carried out by displacement technique using Plethysmometer to find out the circumference of paw edema immediately before and after at 1 h, 2 h, 3 h, and 4 h following the carrageenan injection.[23,24] The inhibitory activity was calculated according to the formula:

$$\% \text{ Inhibition } = \frac{(C_t - C_o) \text{ control} - (C_t - C_o) \text{ treated}}{(C_t - C_o) \text{ control}} \times 100$$

Where,

C_t is the paw circumference at time t.

C_o is the paw circumference before carrageenan injection

And

(C_t-C_o) is edema or change in paw size after time t.

Mast Cell Degranulation

Mice were divided into five groups (n = 6). A 3 days' treatment schedule was followed. Group-I received vehicle Tween-80 1%, (5 mL/kg, i.p.,). Group-II-IV were treated with Methanol extract of *Saraca asoca* (ME-SA) (100-300 mg/kg i.p.,), and Group-V received standard drug disodium chromoglycate (50 mg/kg. i.p.,). On day 4, each animal was injected with 10 mL/kg, 0.9% saline solution, into peritoneal cavity, by gentle massage, peritoneal fluid was collected after 5 min and transferred in to test tube containing 7–10 mL RPMI 1640 buffer medium (pH 7.2–7.4). This solution was then centrifuged at 400–500 rpm. Pellets of mast cells were washed with same buffer medium twice by centrifugation, discarding supernatant. The cell suspension from all the groups of rats was challenged with egg albumin (100 µg/mL) and incubated at 37°C for 10 min the cell suspension was stained with 1% toluidine blue and observed under microscope. Total 100 cells were counted from different visual areas. Percent protection against degranulation's was calculated.[23-25]

Statistical Analysis

The data were statistically analyzed using one-way ANOVA followed by Dunnett's *t*-test for individual comparison of groups with control. Results were expressed as Mean ± standard error of mean $P < 0.05$ was used to indicate statistical significance.[26]

Results and Discussion

Phytochemical Screening

Phytochemical classes characterized in different extracts of the seeds of *Saraca Asoka* are presented in Table 1.

Preliminary phytochemical screening of extract has revealed the presence of carbohydrates, flavonoids, polyphenols, tannins and saponins. However, no alkaloids, proteins, and amino acids were found in the extracts.

Estimation of Total Phenolic Content

TPC was determined using Folin-Ciocalteu reagent. From the analysis, it was observed that the methanol extract of seed showed highest phenolic content (179 ± 0.27 mg/ml) [Table 2]. The phenolic content decreased in the order of methanol > DCM> water > ethyl acetate > Hexane in the samples of *Saraca Asoka*. Hence, it was observed that methanol is a good solvent for the extraction of phenols and these results are in agreement with the study. Where methanolic extracts showed highest TPC. Furthermore, similar results were also observed in Mitragyna parvifolia leaf and bark extracts.

The phenolic compounds are very essential secondary metabolites in plant tissues which differ in their concentration during different stages in plant development; hence, phenolic compounds are considered to play very vital roles in plants such as nutrient uptake, photosynthesis, and protein synthesis usually most of the phenolic compounds are linked to other compounds such as esters, cellulose, proteins, and lignins.[18,19] Hence, these are very important compounds because their hydroxyl groups confer scavenging ability.

Estimation of Total Flavonoid Content

The methanolic extract of *Saraca Asoka* seeds (8.42 ± 0.25 mg/ml) exhibited highest flavonoid content [Table 2]. The results indicate that flavonoid content was mostly abundant in the seed of *Saraca Asoka*.[15] Flavonoids occur naturally in plants which not only have positive effect on human health but also possess antibacterial, antiviral and anti-inflammatory, anticancer, and anti-allergic activities.[20]

Flavonoid also shows strong antioxidant activity; hence, in our study, we observed a strong positive correlation between TFC and DPPH radical scavenging activity.

Estimation of DPPH Radical Scavenging Activity

Electron donation capacity of natural products can be easily measured by 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) purple-color solution. The method is based on scavenging of DPPH through the addition of a radical species or antioxidant that decolorizes the DPPH solution. The degree of color change is proportional to the concentration and potency of the antioxidants.[20] In the present study, DPPH radical scavenging activity was highest in methanolic extract (94.5% ± 1.8%), followed by hexane (82.06% ± 0.75%), DCM (80 ± 1.3), ethyl acetate (52.16% ± 0.86%), and water (42.06% ± 0.74%) [Figure 1]. Distilled water and ethyl acetate extracts showed less antioxidant activity as compared to other solvents. Higher phenol and

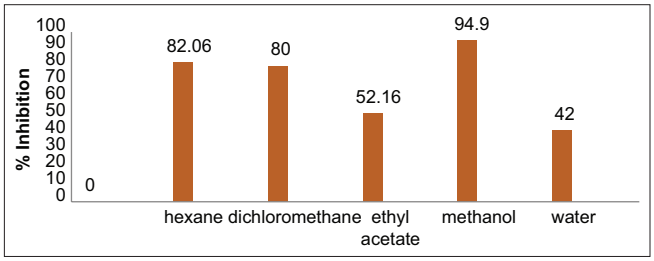

Figure 1: Percent 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity of seed extracts in *Saraca asoca*

Table 1: Preliminary phytochemical screening

Phytochemical tests	*Hexane*	*DCM*	*Ethyl acetate*	*Methanol*	*Water*
Flavonoids	Present	Present	Present	Present	Present
Tannins	Present	Present	Present	Present	Present
Alkaloids	Absent	Absent	Absent	Absent	Absent
Saponins	Present	Present	Present	Present	Present
Carbohydrates	Absent	Present	Present	Present	Present
Glycosides	Absent	Present	Present	Absent	Absent
Proteins and amino acids	Absent	Absent	Absent	Absent	Absent
Fixed oils and fats	Present	Present	Present	Present	Absent

DCM: Dichloromethane

flavonoid content in the plant tissue can also lead to increase in antioxidant activity.

ABTS Scavenging Activity

The ABTS radical cation is generated by the oxidation of ABTS with potassium persulfate, its reduction in the presence of hydrogen-donating antioxidants is measured spectrophotometrically at 734 nm.[20] The ABTS scavenging activity is highest in methanolic extract (91.9% ± 1.7%) and their after in ethyl acetate extract (88.42% ± 1.1%) [Figure 2]. The antioxidant activity was observed the lowest in water samples.

Anti-Inflammatory Effect using Carrageenan Induced paw Edema

The diameter and circumference of the paw edema was recorded by using the plethysmometer in different time periods, namely, at 1st h, 2nd h, 3rd h, and 4th h after inducing the Carrageenan suspension in the different groups. As shown in Table 3, the size of paw edema in case of control group grew substantially during almost the entire experiment while this increase was noticeably lower in case of all the other groups. The diameter of paw edema was the largest in case of the lower drug dose (300 mg/kg), followed by the higher drug dose of 100 mg/kg and comparatively lower in case of the standard group. After 4 h, the calculated inhibition w. r. t. the control was found to be 92.85% in the standard group, 80.95% in the 300 mg/kg group, and 66.66% in case of the 200 mg/kg group [Table 3].

Table 2: Total phenolic content and total flavonoid content

Extract	*Total phenolic content (GAE)*	*Total flavonoid content (quercetin)*
Hexane	36.12±0.16	1.21±0.15
Dichloromethane	154.22±0.12	3.12±0.18
Ethyl acetate	96.23±0.16	2.16±0.31
Methanol	179.12±0.27	8.42±0.25
Water	144.25±0.26	4.12±0.16

Values represent mean±SD. SD: Standard deviation, GAE: Gallic acid equivalent

Mast Cell Stabilization Study

The control group showed (73.50 ± 0.121) degranulation of mast cell while groups treated with different extracts (100–300 mg/kg, i.p.,) and disodium chromoglycate significantly ($P < 0.001$) protect degranulation of mast cells. ME-SA at dose (300 mg/kg) showed 56.24 and disodium chromoglycate 65.63 protection against degranulation as shown in Table 4.

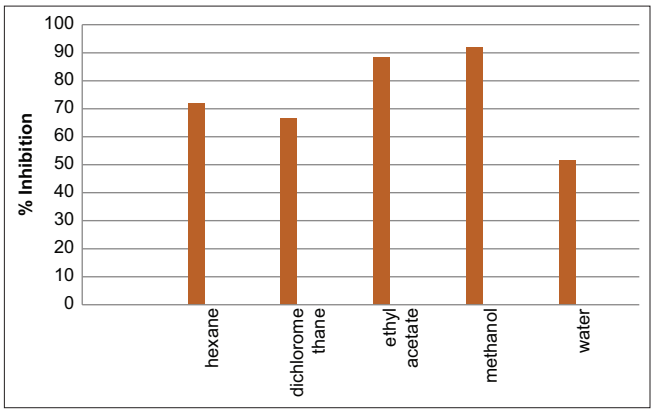

Figure 2: Percent ABTS radical scavenging activity of seed extracts of *Saraca asoca*

Table 3: Anti-inflammatory effect using carrageenan induced paw edema

Group	*Treatment and dose (mg/kg), p.o.*	*Paw edema volume (mL) (mean±SEM) (h)*					*Percentage inhibition^^*
		0	*1*	*2*	*3*	*4*	
1	Control 10 ml/kg	0.49±0.223	0.66±0.312	0.79±0.123	0.92±0.287	0.91±0.421	00.00
	Indomethacin						
2	5	0.51±0.315	0.53±0.412	0.56±0.156	0.61±0.324	0.54±0.126	92.85
	HE-SA						
3	100	0.50±0.356	0.63±0.425	0.75±0.325	0.89±0.412	0.86±0.362	14.28
4	200	0.51±0.116	0.59±0.369	0.64±0.254	0.78±0.481	0.77±0.318	38.09
5	300	0.52±0.378	0.58±0.325	0.64±0.462	0.74±0.158	0.73±0.459	50
	DCM-SA						
6	100	0.49±0.175	0.64±0.2248	0.72±0.456	0.88±0.142	0.86±0.475	11.90
7	200	0.51±0.146	0.63±0.451	0.76±0.452	0.89±0.389	0.85±0.165	19.04
8	300	0.51±0.241	0.61±0.152	0.74±0.352	0.86±0.125	0.80±0.165	30.95
	EAE-SA						
9	100	0.49±0.421	0.64±0.251	0.73±0.143	0.86±0.184	0.84±0.326	16.66
10	200	0.50±0.156	0.62±0.116	0.69±0.254	0.87±0.412	0.79±0.329	30.95
11	300	0.51±0.245	0.63±0.152	0.68±0.145	0.82±0.276	0.76±0.456	40.47
	ME-SA						
12	100	0.49±0.359	0.62±0.458	0.71±0.124	0.83±0.342	0.75±0.486	38.09
13	200	0.51±0.421	0.63±0.214	0.69±0.429	0.84±0.153	0.65±0.318	66.66
14	300	0.51±0.142	0.61±0.254	0.65±0.475	0.72±0.123	0.59±0.421	80.95
	WE-SA						
15	100	0.49±0.371	0.63±0.156	0.78±0.324	0.86±0.226	0.84±0.142	16.66
16	200	0.51±0.356	0.64±0.324	0.76±0.142	0.85±0.412	0.82±0.125	26.19
17	300	0.50±0.245	0.62±0.443	0.72±0.146	0.83±0.141	0.76±0.263	38.09

n=3; values are mean±SEM, ^^Compared with control after 4 h. Anti-inflammatory study shows that the methanolic extract of *Saraca Asoca* seeds more protective than other extracts.HE-SA: Hexane extract of *Saraca asoca*, DCM-SA: Dichloromethane extract *Saraca asoca*, EAE-SA: Ethyl acetate extract of *Saraca asoca*, ME-SA: Methanol extract of *Saraca asoca*, WE-SA: Water extract of *Saraca asoca*, SEM: Standard error of mean

Table 4: Effect of *Saraca asoca* seeds extracts administration on egg albumin induced degranulation of mast cell

Group	*Treatment and dose (mg/kg)*	*Percentage degranulation*	*Percentage protection*
	Control		
I	10	73.50±0.121	-
	Sodium cromoglycate		
II	50	25.26±0.321	65.63
	HE-SA		
III	100	68.12±0.169	7.31
IV	200	64.21±0.254	12.63
V	300	58.36±0.412	20.59
	DCM-SA		
VI	100	62.41±0.154	15.08
VII	200	56.84±0.161	22.66
VIII	300	52.58±0.214	28.46
	EAE-SA		
IX	100	64.23±0.236	12.61
X	200	56.23±0.246	23.49
XI	300	48.12±0.346	34.53
	ME-SA		
XII	100	45.48±0.125	38.12
XIII	200	38.63±0.284	47.44
XIV	300	32.16±0.154	56.24
	WE-SA		
XV	100	58.22±0.148	20.78
XVI	200	55.12±0.165	25.01
XVII	300	53.26±0.421	27.53

n=3; values are mean±SEM. Mast cell stabilizing study shows that the methanolic extract of *Saraca asoca* seeds more protective than other extracts. HE-SA: Hexane extract of *Saraca asoca*, DCM-SA: Dichloromethane extract *Saraca asoca*, EAE-SA: Ethyl acetate extract of *Saraca asoca*, ME-SA: Methanol extract of *Saraca asoca*, WE-SA: Water extract of *Saraca asoca*, SEm: Standard error of mean

Conclusion

In the present study, the successive extraction of *Saraca Asoka* seeds powder has been carried out in hexane, DCM, ethyl acetate, methanol, and water. Antioxidant study is carried out by estimation of DPPH radical scavenging activity and ABTS scavenging activity method. The methanol extract shows potent antioxidant activity. Mast cell stabilizing study shows that the methanolic extract of *Saraca Asoka* seeds more protective than other extracts. Furthermore, the methanolic extract shows good anti-inflammatory effect it may be due to presence flavonoids which is responsible for antiasthmatic potential.

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