RESEARCH ARTICLE

Pharmacological Study of the Anti-ulcer Efficacy of *Saraca asoca* Leaves Extract in Various Ulcer-inducing Models in Rats

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ABSTRACT

The ulcer is a common gastrointestinal disorder that is seen among many people. It is basically an inflamed break in the skin or the mucus membrane lining the alimentary tract. Ulceration occurs when there is a disturbance of the normal equilibrium caused by either enhanced aggression or diminished mucosal resistance. It may be due to the regular usage of drugs, irregular food habits, stress and so forth. In the present investigation anti-ulcer action of *Saraca asoca* leaf extract was determined in rats and results suggest that it possesses significant activity.

Keywords: Diminished, Practitioners, Equilibrium, Gastrointestinal, Recurrence

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INTRODUCTION

An ulcer is a break in the skin, epithelium, or mucous membrane that results from the expulsion of inflammatory necrotic tissue (Figure 1).[1] An ulcer prevents the afflicted organ from functioning normally.[2] There are many types of ulcers that are caused by different types of circumstances like gastric ulcers, duodenal ulcers, esophageal ulcers, bleeding ulcer, refractory ulcer, stress ulcer, pressure sores/decubitus ulcers, genital ulcers, skin ulcers.[3] The most common reason for ulceration is an imbalance between the amount of protection provided by the gastro-duodenal mucosal barrier and the pace at which gastric juice is secreted, as well as between the rates at which duodenal juices are able to neutralize stomach acid.[4] Typically occurring *Helicobactor pylori* infection of the stomach is linked to up to 60% of cases of peptic ulcers.[5,6] This infection may affect the D cells' ability to produce somatostatin, which over time might result in less gastrin-induced inhibition of acid generation, increased acid production, and decreased duodenal bicarbonate synthesis.[7-10]

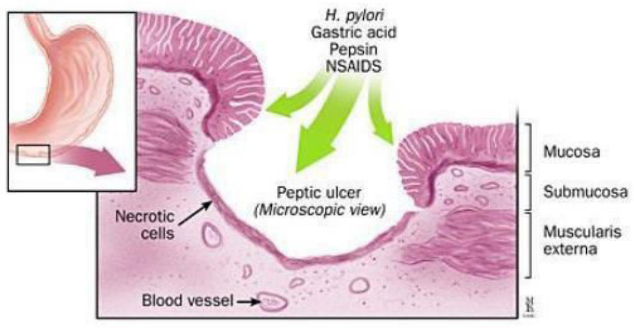

Figure 1: Pathogenesis of peptic ulcer diseases

Saraca asoca is a medium-sized tree and various parts of the plant is used in the treatment of various diseases as mentioned in traditional medicine. The plant contains saracin, saracadin and several other constituents.[11-15] So, far no any systematic study was conducted hence this work was taken.

MATERIAL AND METHOD

Collection and Authentication of Plant Material

The leaves of *S. asoca* were collected in March 2023 from the campus of Narainagroup of institutions, Panki, Kanpur, Uttar Pradesh India. The species was authenticated by Professor Navin K. Ambasht of Department of Botany Christ Church College, Kanpur, India.

Preparation of Extracts

The dried and powdered aerial parts *S. asoca* (1kg) and the dried residue; the yield of total extract (PTE) was 8.20%. The solid residues were collected carefully and stored in sealed glass bottles at 4°C for further experimental work. The dried extracts of the plants were previously subjected for preliminary phytochemical screening and used for estimation of total phenolic and flavonoid content, and *in-vitro* activity.[16]

Phyto-chemical Analysis

The extract obtained from the powdered leaves of *S. asoca* were subjected to phytochemical tests to determine the presence of secondary metabolites using standard procedures.

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Physico-chemical Parameter

Determination of foreign matter

Drug was examined for the absence of molds, insects, animal waste, and other contaminants including dirt, rocks, and other objects.[17] The drug sample under investigation was weighed at 100 g and thinly layered. Visual examination was used to identify the foreign material, which was then separated, weighed, and the amount of foreign matter present was estimated.[18]

Determination of Moisture Content (loss on drying)

In a tarred evaporating dish, 10 g of precisely measured medication was added. It was dried at 105°C for an hour before being weighed. The process repeated till constant weight.[19]

Determination of Total Ash

For its identification, 2 g of powdered material was put in an appropriate tarred silica crucible which had previously been ignited and measured. The medication was carefully weighed and spread out into a thin coating.[20] The material was heated incrementally, not exceeding 450°C, until it was carbon-free. It was then cooled in a desiccator, weighed, and the percentage of ash was estimated by comparing the variance in the empty weight of the melting pot's crucible with the total ash.[21]

Determination of Acid-insoluble Ash

The ash from the previous stage was heated in 25 cc of diluted hydrochloric acid for 5 minutes. On ash-free filter paper, the insoluble material was then gathered, warmed in hot water, and burnt to a constant weight. Ashes that are insoluble in acid were measured in relation to the air-dried medication.[22]

Determination of Total Ash

In 2 g of powdered substance was placed in a suitable tarred silica crucible that had already been lit and weighed in order to identify it. The medication was carefully weighed and spread out into a thin coating. Step by step, the material was heated to no more than 450°C until it was carbon-free.[23]

Qualitative Phytochemical Screening Test for Alkaloids

A little amount of the extract was filtered after being mixed with a few drops of weak hydrochloric acid. Mayer's reagent, Dandruff's reagent, Hager's reagent, and Wagner's reagent are a few examples of different alkaloidal reagents, were used to analyze the filtrate.[24]

Test for Flavonoids

Shinoda test: To the extract, A few magnesium turns were added, along with a few drops of strong hydrochloric acid. After 5 minutes of boiling, the mixture became crimson, indicating the existence of flavonoids.[25]

Test with sodium hydroxide: The extract in ethanol was mixed with a 10% solution of sodium hydroxide. Flavonoids are present as shown by the dark yellow hue.[26]

Test for Tannins and Phenolic Compounds

The extract was run in purified water. Thirds of the extract were then separated. The test extract was divided into three parts: a 10% sodium chloride solution, a 1% gelatin solution, and a gelatin salt reagent.[27] Each portion of the test extract received a different addition of solution. Tanning was demonstrated by either the later reagent or both the gelatin salt reagents are used to precipitate.[28]

A false-positive finding was discovered by precipitating sodium chloride solution. Test extracts were given a few drops of a mild ferric chloride solution (1% ferric chloride) to confirm positive results, which resulted in a black or green coloring. In order to detect white crystallites, the extract was mixed with a lead acetate solution.[29]

Test for Glycosides

Bontrager's test tiny amount of the extract was hydrolyzed in the presence of the hydrolysate and was then extracted with benzene after being exposed to hydrochloric acid for a few hours on a water bath. After applying a diluted solvent and solution to the benzene layer, a reddish-pink color was seen.[30]

Legal's test: Freshly produced sodium nitroprusside was added after the extract had been made alkaline using a few drops of 10% sodium hydroxide after being dissolved in pyridine. The production of a blue hue was then monitored.[31]

Test for Saponins

Foam test: The petroleum ether was used to extract a small amount of extract. After vigorously shaking the insoluble residue left over from extraction for 15 minutes, a small amount of water was added, and it was noticed to form honeycomb foam, which lasted for no less than 30 minutes.[32]

Test for Protein

Biuret test: An equal amount of 1% strong The extract is mixed with sodium hydroxide and a few drops of copper (II) sulfate; the emergence of a purple color indicates the presence of protein.[33]

Quantitative Phytochemical Screening DPPH ASSAY

When reduced by an antioxidant molecule, DPPH's 517 nm absorption peak in its radical state vanishes. The volume was increased to 4 mL with methanol by adding 1-mL of each extract at a variety of concentrations to 2 mL of newly manufactured 90 M DPPH methanolic solution. For one hour, the action mixture was set aside.[34] After 1-hour, a spectrophotometer was used to detect the absorbance at 517 nm. The excerpt was left out of the blank. The standard was DPPH. Calculating the reaction medium's percentage of inhibition in comparison to a blank. Using a formula,the radical scavenging capacity(RSC) was determined.[35]

$$\%RSC = \frac{(Absofcontrol)-(Absofsample)\times 100}{(Absofcontrol)}$$

Thin Layer Chromatography (TLC)

The extracts underwent thin-layer chromatography on TLC plates. Using a micropipette, spot the sample in a 1% solution using 2 to 5 g. different solvents, including butanol, acetone, and acetone-butanol (1:1). When a plate is exposed to UV light, black blotches are seen.

By using the Formula, the sample's Rf value was determined,

$$Rf = \frac{\text{Distance moved by solute from the origin}}{\text{Distance moved by solvent from the origin}}$$

For TLC profiling, the five consecutive extracts are employed. The extracts are filtered and concentrated before spotting to get rid of the solvent.[36]

RESULTS AND DISCUSSION

The experimental yield of n-Hexane of *S. asoca* leaves was found to be 11.90%. Water soluble extractive value showed the presence of sugar, acids and inorganic compounds and alcohol-soluble extractive values determined the presence of polar constituents. The physicochemical parameters total ash, acid insoluble ash and water soluble ash value were found to be 8.11, 4.02 and 2.12%, respectively (Table 2). The total ash value percentage showed the amount of mineral and earthy material present in the plant sample. The amount of acid-insoluble siliceous matter present in the plant sample was 4.02%.

Preliminary phytochemical results (Table 1) indicate the presence or absence of phytochemical constituents in n-Hexane extracts of the *Saraca* flower. Tannins, flavanoids, glycosides, saponins, phenols and anthraquinones were found to be present in n-Hexane extracts.

The Above results shows that the different concentration have different color and different absorbance which proves that higher the concentration higher the absorbance and the inhibition point is 800 µL which gives 2.62% yield and an absorbance is 1.560 (Table 3,4 and Figure 2)

Table 1: Table shows phytochemical screening of *S. asoca* leaves extracts

S.No	*Phytoconstituents*	*Test/Reagent*	*Results*
1-	Tannin	Ferric chloride	+
2-	Flavonoids	Sodium hydroxide Conc. H_2SO_4	+ +
3-	Glycosides	Liebermann's test Salkowski's test Keller Killani test	+ + +
5-	Saponins	Foam test	+
6-	Phenols	Ferric chloridre test	+
7-	Anthraquinones	Ferric chloride DiluteHCL	+ +

Table2: Extractive valuesof *S. asoca* leaves

S. No.	*Types of solvents*	*Percentage (%)w/w*
1-	Water soluble	2.12
2-	Moisture content	11.2
3-	Ash value	8.11
4-	Acid soluble	4.02

Table 3: Resultsshowstheabsorbanceindifferentconcentration

S. No.	*Solvents*	*Color*	*Concentration of sample (extract) (µL)*	*Absorbance*	*%Yield*
1-	Methanol	Yellowish	30	0.361	77.45
2-	Methanol	Pale yellow	150	0.366	77.13
3-	Methanol	Dark yellow	300	0.532	66.77
4-	Methanol	Bluishyellow	600	0.731	54.34
5-	Methanol	Light yellow	800	1.560	2.62

Table 4: Solvent and concentration of sample

S.No.	*Solvents*	*Color*	*Concentration of sample (DPPH)*	*Absorbance*
1-	Methanol	Purple	0.394 mg/mL	1.601

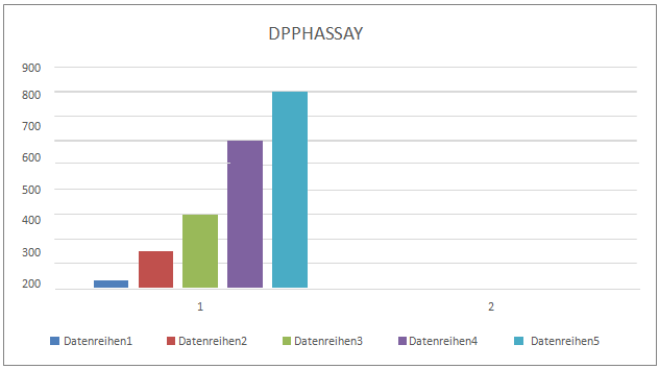
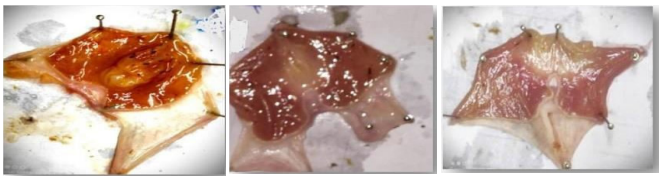


Figure 2: :Results showing absorbance at different concentration

Table 5: The above table shows the different RF values according to the polarity of the compound.

S.No.	*Mobile phase (ratio)*	*Sample*	*Rf value*
1.	Methanol: Dichloromethane (10:90)	RF1A	0.5
		RF2B	0.75
		RF3B	1
2.	n-Butanol: Acetic acid: H2O(4:1:5)	RF1A	0.75
		RF2B	1



Control group Standard group Treatment group

Figure 3: Ethanol induced ulcer model shows the results of different groups.

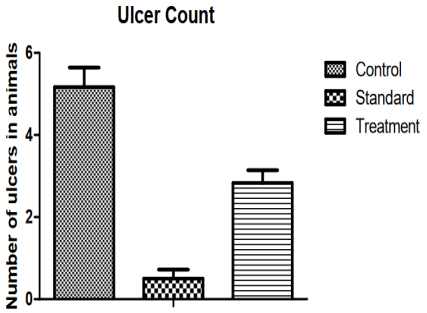


Figure 4: Statistical analysis shows the results of different concentrations of ethanol-induced ulcer model

Table 5 shows two different results in which we have taken the different mobile phases and the stationary phase is silica gel. The first mobile phase was taken are Methanol: Dichloromethane (10:90) in which we identified three compound and RF values of each compound is different which shows that RF_1A is more polar than RF_2B and RF_3B because RF1A travels slower than other compound and the same as the above-Butanol: Acetic

acid: H_2O (4:1:5) in which RF_1A are more polar than RF_2B, so we concluded that in Methanol: Dichloromethane isolated more compound than n-Butanol:Acetic acid:H_2O.

The *in-vivo* results of ethanol-inducing ulcers (Figures 3 and 4) show that there are three groups and every group shows a different result in we have taken the six animals in each group and we observed that the control group had more ulcers than the standard group and treatment group, which proves that the test drug which has been used is effective for the anti-ulcer activity.

Endomethacin Induced Gastric ulcers

The above figure shows the results of the control, treatment, and standard groups. The *in-vivo* results of indomethacin-inducing ulcers (Figures 5 and 6) show that there are three groups and every group shows different results in which we took the six animals in each group and we observed that the control group had more ulcers than the standard group and treatment group which proves that the test drug which has been used is effective for the anti-ulcer activity.

The histopathology result (Figure 7) shows that *H. pylori* present more in the control group in comparison to the standard group and test group which shows that the test drugs which have taken are effective for the anti-ulcer activity.

S. asoca locally known as the Ashoka tree is quite popular. For this study, information was gathered from 68 informants

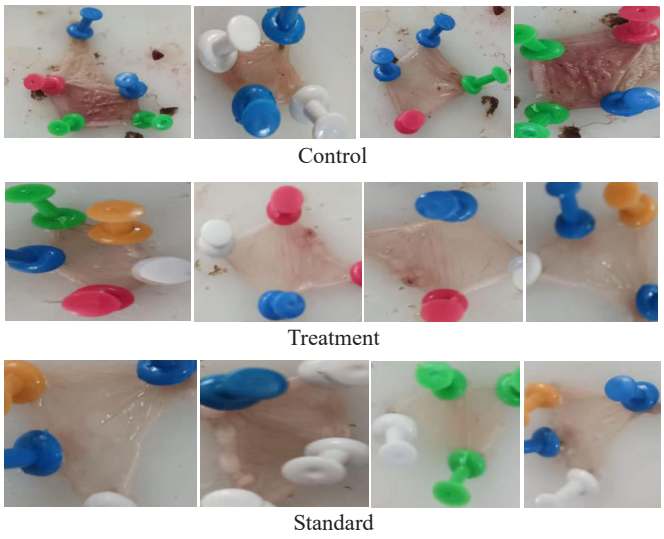


Figure 5: Results of control, treatment, and standard group

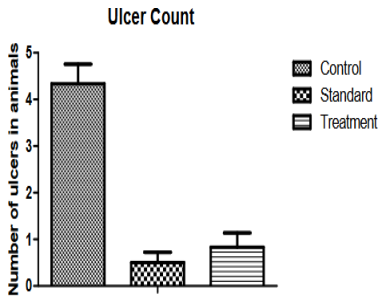


Figure 6: Statistical analysis shows the results of different concentrations of indomethacin induced ulcer

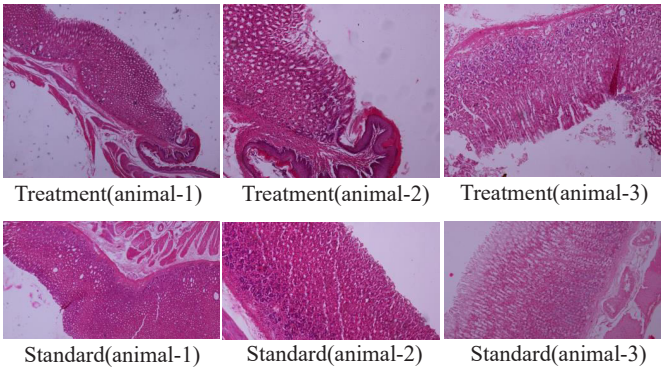


Figure 7: Histopathology of different testing group

using semi-structured questionnaires on the usage, taboos, traditional medicine, and conservation of the species. The plant is essential to the ceremonies and rituals, mythologies, meals, taboos, medicines, customs, and traditions of the meetei society. Without any type of standardization, folk medicine is used to treat a variety of ailments, including colds, coughs, influenza, easier deliveries, abortions, irregular periods, diarrhea, dysentery, cholera, and high blood pressure. Before any crude medication can be included in an herbal pharmacopeia, its physicochemical and pharmacognostic properties must be confirmed.

The extract was prepared using a soxhlet apparatus. The extract of *S. asoca* was prepared for the determination of antiulcer properties.

Then we did the *in-vitro* studies for the identification of chemical constituents that are responsible for the antiulcer activity like flavonoids, tannins, and saponins, these are the qualitative tests. Then we did the quantitative test like DPPH assay which shows the antioxidant activity and it proves that after the test *S. asoca* has antioxidant activity and we also did the protein estimation test to identify the protein in the extract.

CONCLUSION

In-vivo studies by using different ulcer-inducing models like ethanol-induced ulcer and indomethacin-induced ulcer and after completing the studies it has been proved that *S. asoca* extract have anti-ulcer activity. We have also done histopathological studies which showed that the control group has more ulcer than test drug so this study also proves that the *S. asoa* leaves have an anti-ulcer property in it.

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