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Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTI ULCER STUDIES OF THE LEAVES OF *SIDA SPINOSA*, LINN.

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ABSTRACT

The present study deals with preliminary photochemical screening and antiulcer evaluation of leaf of *Sida spinosa*, Linn (Malvaceae). The study also includes preparation of ethanolic extracts by continuous hot percolation with help of soxhlet apparatus. Different physiochemical parameters such as ash value, extractive value were determined. Fluorescence analysis of ethanolic leaf extract of *Sida spinosa* were noted under ordinary and UV light. It signifies there characteristics. Preliminary qualitative chemical test for ethanolic leaf extract shows the presence of phytosterols, fixed oils and fats, proteins, amino acid gum mucilage, and flavonoids and phenolic/ tannins. The ethanolic leaf extract of *sida spinosa* was explored for antiulcer effect by using pylorus ligation method in rats. At a dose 100 and 200 mg/kg showed protection effect of 70.85% and 73.42% respectively where as famotidine (reference drug) showed protection index of 72% at a dose of 20mg/kg.

Keywords: *Sida spinosa*, Physiochemical, Qualitative chemical tests.

INTRODUCTION

Natural products, mainly the plant derived constituents, have long been used as sources of drugs. Natural products are also of great interest in the process of drug discovery, due to their large diversity in nature, permitting the identification of lead molecules of greater interest for the development of new therapeutic agents.[1]Though the traditional Indian system of medicine has a long history of use of plant as drug, they lacked adequate scientific documentation, particularly in light of modern scientific knowledge[2] Leaves are reported to treat the pain, arthritis, asthma, bronchitis, burning sensation, haemorrhoids, intermittent fever and general debility, gonorrhea, gleet and scalding urine. Root is used as a tonic for diaphoretic. A decoction of it is said to be given as a demulcent in irritability of bladder, it also has aphrodisiac property [3-7].

Peptic ulcer disease is a serious gastrointestinal disorder. The formation of peptic ulcers depends on the presence of acid and peptic activity in gastric juice plus a breakdown in mucosal defenses. There are two major factors that can disrupt the mucosal resistance to injury: nonsteroidal anti-inflammatory drugs (NSAIDs) e.g. aspirin and *Helicobacter pylori* infection. As a matter of fact, many drugs were used to treat this disease but many of them cause adverse effects and recurrent infections frequently occur within a few weeks because of difficulty in eradication of *H. pylori*. This has been rationale for the development of new antiulcer drugs and search for novel molecule. Drugs of plants origin are gaining popularity and investigating for the various disorders including peptic ulcer[8]. Peptic ulcer is caused by a lack of equilibrium between the gastric aggressive factors and the mucosal defensive factors[9]. The present study is designed to explore the preliminary phytochemical, physiochemical and anti ulcer activity of *Sida spinosa*, Linn leaves.

MATERIAL AND METHODS

Plant Material

A large number of plant leaves of *Sida spinosa*, Linn where collected in the surroundings of Coimbatore and authenticated by botanical survey of India (BSI), Coimbatore, India. Leaves where washed with water and then dried in shade over two weeks and was coarsely powdered.

Analysis of physiochemical property in ethanolic leaf extract of *Sida spinosa*, Linn

The physiochemical parameters like the extractive values, ash content and florescence characteristics of powdered leaf where determined. The average percentage w/w of the ash content and the extractive values are determined. The fluorescence analysis was carried out according to the reported method [10-11]. The colour of the powdered leaf and its ethanolic extract were also studied under ordinary and ultraviolet light.

Extraction of plant leaf material

The leaves of *Sida spinosa* were dried in shade, and then the dried leaves were powdered to get a course powder. About 500gm powder transferred to Soxhlet apparatus and extracted with ethanol by continuous hot percolation method. The extract obtained was evaporated to remove excess solvent. A greenish black waxy residue obtained and it was stored in cool dry place for further evaluation of preliminary phytochemical contents.

Analysis of primary and secondary metabolites in the ethanolic leaf extract of *Sida spinosa*

The analysis for the presence of primary metabolites like proteins, carbohydrate, fixed oil and fats were done as per the standard procedure [11-12]. Similarly the secondary metabolites like alkaloids, flavonoids, phenolics and tannins, volatile oil, phytosterol, gum mucilage and lignin were also assessed in the leaf extracts of *Sida spinosa*.

Gastric ulcer induced by pylorus ligation[13]

Albino rats were housed in individual cages and fasted (water allowed) for 72 hours prior to pyloric ligation, care being taken to avoid coprophagy. Under light chloroform anaesthesia the abdomen is opened by a small midline incision below the xiphoid process; pyloric portion of the stomach is slightly lifted out, and ligated avoiding traction to the pylorus or damage to its blood supply. The stomach is replaced carefully, and the abdominal wall closed by interrupted sutures. The drugs are administered orally two hours prior to pyloric ligation. They are deprived of both food and water during the postoperative period, and are sacrificed at the end of six hours after operation. Stomach is dissected out and the contents are drained into a tube and this is subjected to gastric juice was collected for performing gastric secretion study and analysis for pH and for free and total acidity. The stomach is then cut open along the greater curvature and the inner surface is examined for any ulceration. The mean ulcer size was calculated by dividing the total length (in mm) of ulcers for all the animals divided by total number of animals

Gastric secretion study

The gastric juice was collected and centrifuged and it volume and pH were measured.

Total Acidity

A volume of 2 ml. Diluted gastric juice was titrated with 0.1 M sodium hydroxide run from a micro burette using phenolphthalein as indicator and the acidity was expressed as mg. HC1/150 gm body weight of rat.

Free Acidity

It is determined in similar manner using topfer's reagent as indicator and sodium Hydroxide was run until canary yellow colour was observed.

Statistical Analysis

The results are expressed as mean S.E.M and subjected to student t test by comparing with the control.

RESULT AND DISCUSSION

The powdered leaf of *Sida spinosa* was subjected to preliminary physicochemical and phytochemical analyses which were found to be very promising. The determination of Ash value gave an idea of earthy matter (or) the inorganic composition and other impurities present along with the drug. The extractive values determined are useful for the determination of exhausted (or) adulterated drug. The physiochemical values determined are tabulated in Table No 1.

Table 1: Physicochemical standard values for *Sida spinosa*, Linn.

S. No	Physicochemical Constants	Average Values %w/w Leaves
	Total ash	10.52
	Acid insoluble ash	1.25
	Water soluble ash	5.12
	Water insoluble ash	3.25
	Sulphated ash	9.35
	Alcohol ash	2.32
	Water soluble extractive	7.95
	Chloroform extractive	3.25
	Loss on drying (Desiccators)	0.32
	Loss on drying	0.45

The fluorescence characteristic studied under ordinary and UV light showed the visibility of varying colours which are tabulated accordingly in Table No 2.

Table 2: Fluorescence analysis of leaf extract of *Sida spinosa*, Linn.

S. No.	Particulars of the treatment	Under ordinary light	Under UV light (366nm)
	Powder as such	Green	Dark Green
	Powder + 1N NaOH (aqueous)	Green	Blackish Green
	Powder + 1N NaOH (alcoholic)	Brown	Dark Green
	Powder + 1N HCl	Dark Green	Yellowish Green
	Powder + 50% HNO_3	Brown	Emerald Green
	Powder + 50% H_2SO_4	Brown	Emerald Green
	Powder + Methanol	Pale Green	Green
	Powder + Ammonia	Brownish Green	Blackish Green
	Powder + Iodine	Brown	Greenish Brown
	Powder +$FeCl_3$	Brown	Dark Green

The preliminary phytochemical screening revealed the presence of phytosterols, fixed oils and fats, tannins and phenols, protein, amino acid and flavonoids in the ethanolic extract of *Sida spinosa* and is tabulated appropriately in Table No 3.

Table 3: Phytochemical analysis of the extract of leaves of *Sida spinosa*, Linn.

S. No.	Name of the test	Procedure	Observation	Alcoholic Extract
	Alkaloids	Drug +		
		Dragondroffs reagent	Orange Colour	--
		Mayers reagent	White ppt.	--
		Hager's reagent	Yellow ppt.	--
	Carbohydrate	Drug +		
		Molishs reagent + Conc. H_2SO_4	Purple Colour	--
		Fehling's solution A&B	Brick red Colour	--
	Glycosides	Anthrone + H_2SO_4 + Heat	Purple/green	--
	Phytosterols	Libermann test	Bluish green	+
		Salkowski test	Red & fluorescent	+
		Noller's test	Pink colour	+
	Saponins	Drug + water + shaking	Formation of honey comb like froth	--
	Fixed oil and Fats	Spot test	Stains appear after drying	+
	Tannin & phenols	Drug + $Fecl_3$	Intense colour	+
		Drug + lead acetate + water	White ppt.	+
	Proteins	Biuret test	Violet colour	+
		Xanthoprotein test	Orange colour	+
		Millon's reagent test	White ppt.	+
		Lead acetate test	White ppt.	+
	Gums & mucilage	Drug + water	Thickening of substance	--
	Flavonoids	Shinodaw's test	Red colour	+
		Zn-HCl acid reduction test	Magenta colour	+
	Volatile oils			--
	Lignin			--

The ethanolic leaf extract of *sida spinosa* showed significant anti-ulcer effect against ulcer induced by pylorus ligation method was a dose dependent manner. In this model at a dose 100 and 200 mg/kg showed protection effect of 70.85% and 73.42% respectively where as famotidine showed protection index of 72% at a dose of 20mg/kg (Table No 4)

Table 4: Effect of *Sida spinosa* Linn extract on Pylorus ligated Albino Rats

S. No.	Groups	Volume of gastric juice	pH	Total acidity	Free acidity	Ulcer Index
1	Control	1.6 ± 0.08	1.2 ± 0.04	93 ± 5.8	73 ± 4.1	35.4 ± 3.2
	Sida spinosa extract (100mg/kg)	0.6 ± 0.02*	4.4 ± 0.11*	30 ± 2.9*	18 ± 1.3*	10.6 ± 3.1*
	Sida spinosa extract (200mg/kg)	0.53 ± 0.06*	4.6 ± 0.17*	29 ± 3.2*	19 ± 1.8*	9.7 ± 2.8*
	Famotidine (20mg/kg)	0.5 ± 0.03*	4.2 ± 0.07*	26 ± 1.6*	18 ± 1.3*	10.2 ± 1.3*

Results are expressed as mean SEM, n=6,* p<0.001 Vs respective control by student t test

The histopathological sections of the drug treated group *sida spinosa* extract 100mg/kg had Shown a reduction in ulcer focus and a hyperplastic gastric mucosa with regenerating mucosal epithelium when compared to control group. (Fig 1, 2, and 3).

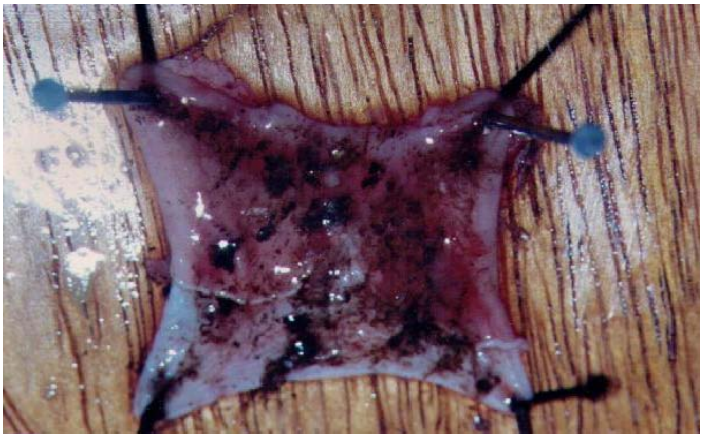

Fig. 1: Control group

Fig. 2: Ethanolic *Sida spinosa* extract 100mg/kg group

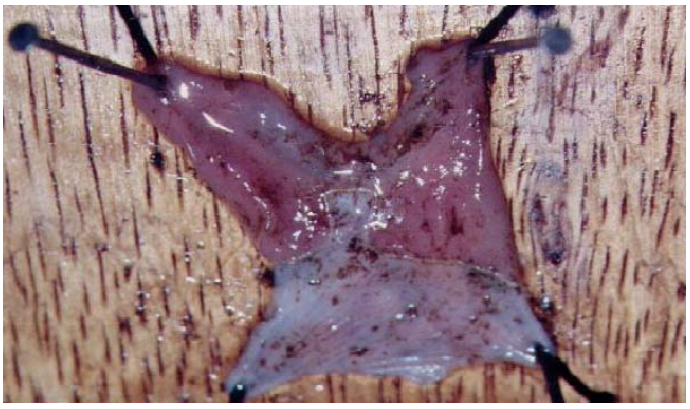

Fig. 3: Famotidine 20mg/kg treated group

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