Advances in Bioresearch
Adv. Biores., Vol 16 (4) July 2025: 479-497
©2025 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.16.4.479497

Advances in Bioresearch

ORIGINAL ARTICLE

Analyzing the Pharmacognostical, Phytochemical and Anti-hyperuricemic Activity of Root of *Sida cordifolia linn* in Rat Hyperuricemia Model

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ABSTRACT
The main objective of the present work was to study the pharmacognostical and phytochemical evaluation of Sida cordifolia extract for the treatment of hyperuricemia. The enzyme assay was done by using xanthine oxidase (XO). The XO inhibitory activity in vitro was performed by using different doses of root extract and the degree of XO inhibition was expressed as IC_{50}. The anti hyperuricemic activity of Sida cordifolia was tested in the potassium oxonate-induced hyperuricemic rats with oral treatment of 100 mg and 200 mg/kg doses. Physico-chemical properties revealed total ash (10.00w/w), acid insoluble ash (1.60w/w), water-soluble ash (1.22w/w), water-soluble extractive (10.00w/w), petroleum ether soluble extractive (2.00w/w) and ethanol soluble extractive (9.00w/w). The loss on drying was found to be (6.12w/w). In preliminary phytochemical investigation revealed the presence of carbohydrates, alkaloids, flavonoids, glycosides, phenol, steroid, tannins and saponins. In HPTLC two peaks (Rf-0.30) for vasicine and (Rf- 0.61) vasicinone were obtained. In the DPPH assay, the ethanol extract of S.cordifolia displayed the highest radical scavenging activity, by effectively reducing DPPH radical with an IC_{50} value of 20.42± 0.58 g/ml and NOS assay IC_{50} value of 174.12± 0.53. The ethanolic extract of Sida cordifolia has moderate activity of XO inhibition with IC_{50} 92.51 ug/ml. Sida cordifolia extract in enzyme kinetic analysis caused a decrease in the Vmax and inhibited XOD activity. Furthermore, all doses of Sida cordifolia root extracts were able to considerably reduce serum uric acid levels in hyperuricemic rats. In comparison to allopurinol 90.3%, residual XOD activity in liver homogenates of Sida cordifolia extract (200 mg/kg) considerably decreased the generation of uric acid in the liver homogenates by suppression of XOD activity by 76.0%. The present study confirms the antihyperuricemic activity of Sida cordifolia root extracts.
Keywords: *Sida cordifolia linn, Ethanolic Extract, total flavonoid, phenolic content, xanthine oxidase, Antihyperuricemic*

Received 19.03.2025 Revised 11.06.2025 Accepted 27.07.2025

How to cite this article:
Dhananjay B. D and Snigdha D. M. Analyzing the Pharmacognostical, Phytochemical and Anti-hyperuricemic Activity of Root of *Sida cordifolia linn* in Rat Hyperuricemia Model. Adv. Biores., Vol 16 (4) July 2025: 479-497

INTRODUCTION

Hyperuricemia is defined as an unusually high uric acid level in the blood. Uric acid occurs mostly as urate, the ion form [1-2], in bodily fluid pH settings. Hyperuricemia is characterized as serum uric acid values greater than 6 mg/dL for females, 7 mg/dL for men, and 5.5 mg/dL for kids (under 18 years old).It has been reported that about 10% of adults will be affected at some point in their lives [4,5].

Not only is hyperuricemia a known risk factor for gout, but it has also been closely connected to other disorders such as hypertension and kidney failure [6]. According to the findings of the prospective study, men with gout had a higher risk of nonfatal myocardial infraction than men without gout, and the increased mortality risk among men without preexisting coronary heart disease (CHD) is primarily due to an increased risk of cardiovascular disease (CVD) death [7].

There are currently very few pharmacotherapeutic drugs available for the treatment of hyperuricemia and gout. Natural materials, primarily of plant origin, have long been utilized in traditional medicine to treat gout and hyperuricemia [8,9]. Bala (*Sida cordifolia* Linn.) of the Malvaceae family is found

throughout India's hotter regions and is rather abundant in states such as Uttar Pradesh, West Bengal, Karnataka, Andhra Pradesh, Tamil Nadu, Rajasthan, and Kerala. It is a common weed found in garbage palaces [10]. Bala is also known as Bariyara in Hindi, Kharethi in Bengali, Chikana in Malyalam, Baladana in Gujarati, Simaka in Punjabi, and Country mallow in English [11].

Because the leaves contain modest amounts of both ephedrine and pseudoephedrine [12], and the roots and seeds contain alkaloid ephedrine, vasicinol, vasicinone, and N-methyl tryptophan [13-15], the entire plant is utilized as a therapeutic herb [16,17]. Because of ephedrine, several ayurvedic preparations of this plant are used in the Indian subcontinent for asthma, weight loss, increased energy [18], chronic dysentery, and gonorrhea [19,20]. Its leaves have recently been reported to have cardiovascular effects [21], analgesic, antiinflammatory [22], and hypoglycemic activities [23].

The current work is concerned with the preliminary phytochemical screening and extensive pharmacognostical analysis of the root of *Sida cordifolia*, which involves macro and microscopic examinations, as well as the measurement of the extract's physicochemical properties. A pharmacological evaluation study was designed to evaluate the antihyperuricemic potency of the ethanol extract of *Sida cordifolia* root to allopurinol and to investigate the ethanol extract's XO inhibitory effect.

MATERIAL AND METHODS

Drug and chemicals

Allopurinol was obtained from Ifars pharmaceutical laboratories. Potassium oxonate, potassium dihydrogen phosphate, Xanthine, Xanthine oxidase and dimethyl sulfoxide (DMSO) were purchased from sigma chemicals Co. The UA kit was purchased from sclavo diagnostic. All chemicals and reagents used in this study were of analytical grade.

Plant material:

The sample of *Sida cordifolia linn* plant was collected in the month of August from Ashvi Bk, Sangamner, Maharashtra. The plant was identified and authenticated by the taxonomist of Botanical Survey of India Pune and a voucher specimen (BSI/WRC/Tech./22) was deposited at BSI, Pune.

Macroscopic and Organoleptic studies:

The macroscopic examination of a medicinal plant aided in the rapid identification of plant material and also plays an important part in drug standardization. The fresh root was exposed to macroscopic investigations that included organoleptic characteristics such as color, odor, appearance, taste and texture.

Microscopic evaluation

i) Microscopy of root:

The root of *Sida cordifolia* was harvested, fixed for 24 hours in FAA solution (Formalin-Aceto-Alcohol: Formalin, Acetic acid each 5 ml, in 90 ml of 70% ethanol), dehydrated with a graded series of tertiary-butyl alcohol, and cast in paraffin blocks for qualitative microscopic inspection. The paraffin-embedded specimens were then cut into sections using a rotary microtome, and the sections were then de-waxed. These sections were safranin-stained, and a compound microscope with projections of 10X and 40X was used to study them [24].

ii) Microscopy of powder:

The dried root of *Sida cordifolia* is ground using an electric grinder, passed through sieve No. 60, and then submitted to microscopic investigations to analyze the presence or absence of various types of tissues or structures. Powder microscopy was carried out in accordance with the procedures of Kokate [25] and Khandelwal [26].

Physicochemical parameters:

Physiochemical values mainly percentage of extractive values and ash values were determined according to the standard methods [27, 28]. The quality control methods of medicinal plant materials are done as per WHO guidelines [29, 30].

a. Determination of ash values:

To estimate the ash content of the drug, approximately 3 g of powder became dispersed in a pre-ignited and weighed silica crucible. After that, the crucible was gently burnt to eliminate all of the carbon. After cooling, the crucible was weighed to decide the whole ash content, and the ash was analyzed to decide the acid-insoluble and water-soluble ash. The proportion of overall ash becomes calculated by the usage of the air-dried pattern as a general.

b. Determination of extractive values:

Given the drug's complexity and chemical nature, extractive values were decided using 3 distinctive solvents: petroleum ether, ethanol, and water. Approximately five g of powdered material was dealt with to non-stop Soxhlet extraction with 100 ml of petroleum ether and ethanol as solvents, while water

extraction was executed with the use of the maceration technique. The determination of a crude drug's extractive values is beneficial in the evaluation procedure in any place where chemical factor assessment is applicable. The extracts are focused in a rota-vaporizer and dried in a vacuum desiccator after extraction. The extraction values are then computed as a percent w/w of the solvent-soluble extractive in comparison to the air-dried drug.

c. Determination of moisture content:

The moisture content was calculated with the use of the loss of weight on drying (LOD) technique. 5gm of the medicine (powdered root sample) changed into taken for this reason and saved in an oven at 105^{o}C until a consistent weight became obtained. As a comparison to the air-dried material, the amount of moisture present inside the sample was estimated.

d. Fluorescence analysis:

When exposed to ultraviolet radiation, crude medications exhibit their own distinctive fluorescence, which is depending on their chemical ingredients. This study is beneficial for detecting adulterants during the crude drug evaluation process. In the current investigation, one gram of crude drug was placed in a watch glass and fluorescently analyzed as such and after treatment with several reagents.

Preliminary phytochemical screening:

Plants are thought to be bioreactors or biosynthetic labs because they manufacture a diverse spectrum of therapeutically relevant chemicals in the form of secondary metabolites. Thus, for identifying plant ingredients and establishing a chemical profile of a crude medicine for adequate evaluation, a systematic preliminary phytochemical screening of plant material is required. For preliminary screening, phytochemical extracts were treated to a conventional technique for detecting several phytoconstituents [31].

Estimation of Flavonoids

The total flavonoid concentration was calculated using the aluminium chloride colorimetric technique and Quercetin as a standard [32]. 0.1ml of $AlCl_3$ (10%), 0.1ml Na-K-tartarate, and 2.8ml distilled water were added progressively to aliquots of extract solutions (1g/ml). The solution mixture was shaken vigorously. After 30 minutes of incubation, the absorbance at 415nm was measured. A standard calibration curve at 415nm was created using known concentrations of Quercetin (20, 40, 60, 80, and 100 g/ml). The flavonoid concentrations in the test samples were determined using the calibration plot and reported as mg Quercetin equivalent / g of sample.

Total Phenolic Content

In a volumetric flask, standard Gallic acid (10mg) was dissolved in 100ml distilled water (100g/ml of stock solution). Pipette 0.5 to 2.5ml aliquots from the aforementioned stock solution into a 25ml volumetric flask. Then, to each volumetric flask, 10ml of distilled water and 1.5ml of Folin-Ciocalteu reagent, diluted according to the label specifications, were added. After 5 minutes, 4ml of 1M Sodium carbonate was added, followed by 25ml of distilled water. Simultaneously, the plant extract (0.5ml of 1:10mg/ml) in methanol was combined separately with the above reagents. After 30 minutes, the absorbance at 765nm was measured, and the calibration curve for the standard was shown as absorbance against concentration. The amount of Phenolic content was calculated using this graph [33].

Thin layer Chromatography:

TLC is a chromatographic method used to separate mixtures of chemicals. TLC is carried out on a sheet of aluminum foil coated with a thin layer of adsorbent silica gel, which is available commercially as 60 F254 (Merck). Samples prepared in various solvents were spotted as a single spot onto the TLC plate using capillary tubes [34]. TLC plates were initially examined in a UV chamber, and Rf values were determined.

Pharmacological evaluation

Experimental animal

Male Wistar rats (150-180 g) were used in this investigation, and they were obtained from the animal house at Ashvin Ayurvedic College and Hospital Manchi Hill Sangamner. The rats were divided into groups of six (n = 6), and the animals were housed in isolated plastic cages in the animals' room for a week under a regulated condition of temperature 252EC and humidity 3015% with an A12:12 light: day cycle. The animals were fed regular food and given unlimited water. The study was approved by Institutional Animal Ethics Committee (IAEC), ashvin ayurvedic college and hospital manchi hill sangamner, Maharashtra, India (ARAC/AH/01/2021). Animals were divided in to control group, standard group and extracts treated groups.

Antioxidative assays

DPPH radical scavenging assay

2,2-Diphenyl-1-picrylhydrazyl hydrate (DPPH) radical scavenging assay was performed as previously described [35]. Plant extracts or ascorbic acid (as a positive control) was dissolved in water to obtain the

final concentrations of 10, 100, 200,400, 800 and 1000 mg/ml. Each solution (20 mL) was added to a 96-well microliter plate containing 180 mL of 0.1mM DPPH in methanol. After 30 min incubation in the dark at room temperature, the absorbance at 517nm was measured using a micro plate reader (Infinited VR M200, Tecan, Switzerland). The DPPH radical scavenging activity percentage was calculated using Equation (1) as follows:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{(Absorbance control - Absorbance sample)}}{\text{(Absorbance control)}} \times 100$$

The DPPH radical-scavenging activity (%) was plotted against the plant extract or ascorbic acid concentration (mg/mL) to determine the concentration to decrease DPPH radical-scavenging by 50% (called IC_{50}).

2, 2-Azinobis 3-ethyl-benzothiazoline-6-sulphonic acid (ABTS) assay

For ABTS assay, the procedure followed a previously described method [35] with some modifications. The stock solutions containing 7mM ABTS and 2.45mM potassium sulfate were prepared, and the working solution was prepared by mixing the two stock solutions in equal quantities for 12–16 h in the dark at room temperature. The solution was then diluted by mixing 1mL of ABTS solution with 24mL of methanol to obtain an absorbance of 1.100 ± 0.020 units at 734nm using a microplate reader (InfinitedVR M200, Tecan, Switzerland). Fresh ABTS solution was freshly prepared for each assay. The sample (10 mL) was mixed with 200 mL of ABTS radical action solution in 96-well plates. The absorbance was determined at 734 nm using a microplate reader (InfinitedVR M200, Tecan, Switzerland).All determinations were carried out in triplicate. Trolox was used as a standard. The results are expressed as the mg Trolox equivalent antioxidant capacity (TEAC)/g extract.

***In vitro* XO inhibitory activity**

The xanthine oxidase (XO) inhibitory effect of *Sida cordifolia* extract was assessed spectrophotometrically at 290 nm according to Sunarni [36] and Yumita [37] with minor changes. The mixture assay consists of 0.9 mL of 0.05 M sodium phosphate buffer (pH 7.5 at 25°C), 1 ml of *Sida cordifolia* extract (100 µg mLG1 in DMSO) and 0.1 mL of XO enzyme solution (0.1unit mLG1 in phosphate buffer, pH 7.5) was prepared in cold buffer directly before using. After a 15 min pre-incubation at 25°C, after that the reaction was allowed to start by addition of 2000 µL of freshly prepared solution of substrate (0.15 mM xanthine solution). Next, a further incubation process was achieved for the reaction mixture at 25°C for 30 min. After addition of 1 mL of 1N HCl solution into assay mixture for stopping the reaction, the absorbance was recorded at wave length 290 nm by using UV/Vis spectrophotometer (Chrom Tech, USA) against the blank which is prepared in the same procedure but with replacement of enzyme solution by phosphate buffer. The positive control solution was prepared by using allopurinol (100µg/ml) in DMSO. The inhibitory activity against the XO was stated as the percentage of inhibition (%)

$$\text{XO inhibition (\%)} = \{1-\alpha/\beta\} \times 100$$

Where, α represents the activity of XO in absence of the tested substance (*Sida cordifolia* powder) and β is the activity of XO with presence of *Sida cordifolia* powder. Different concentrations of both *Sida cordifolia* powder and allopurinol (1, 2, 3, 4, 5, 10, 25, 50 and 100 µg/ ml) were used for evaluation of XO inhibitory activities and then the dose-response logarithmic curve was applied to determine the median maximum inhibitory concentration IC_{50}.

Enzyme kinetic analysis of *Sida cordifolia* extract on XOD inhibition

Determination of type of XOD inhibition by plant extract was performed by Lineweaver–Burk plot analysis [38]. Three concentrations of *Sida cordifolia* extract (125, 250 and 500 mg/ml) assessed in different concentrations of xanthine as a substrate (15, 30, 60 and 120 mm).

The enzyme reaction was performed as described above. The inhibitory constant (Ki) for XOD inhibition by plant extract was determined by a non-least squares regression of the observed data following the equation using Solver Add-in equipped with Microsoft Excel 2010:

$$V = \frac{Vmax \,.S}{Km + S\,(1 + I/Ki)}$$

where v and Vmax represent the initial and maximum velocities of the uric acid formation, respectively (mmol/min), Km represents the Michaelis constant (mm), and S and I represent the substrate (mm) and inhibitor concentrations (mg/ml), respectively. For the non-linear optimization, the generalized reduced gradient (GRG) algorithm of the Solver add-in implemented in Microsoft Excel 2013 was employed.

***In vitro* effects of plant extract on plasma uric acid levels in potassium oxonate induced-hyperuricemic rat**

Hyperuricemic rat model was prepared by giving potassium oxonate as uricase inhibitor. The experiment was performed according to the Liu's method [39] with minor modification in potassium oxonate dose. Rats were divided randomly into normal control, hyperuricemic control, standard (allopurinol), and sample groups (n=6 each). Allopurinol and extract of *Sida cordifolia* were prepared in suspension dosage form using 0.3% carboxymethylcellulose sodium (CMC-Na). The *Sida cordifolia* extract at dose of 100 mg/kg body weight (bw) and 200 mg/kg bw was given orally to rats. As a standard, allopurinol was administered in the same manner at a dose of 10 mg/kgbw. The volume of the suspension which was administered based on body weight of rat. The rats were fasted 1 day before being used in the experiment. Water was withdrawn from the animals 1hr prior to drug administration. The animals were transferred to the laboratory at least 1hr before the potassium oxonate-induced hyperuricemia experiment. Before potassium oxonate administration, blood was collected for determining serum UA level on 0 h. Briefly, rats were injected intraperitoneally with potassium oxonate (250 mg/kg bw suspended in CMC-Na 0.3%) to increase the serum UA level, except normal control group, 1 hr before the drug administration. Blood samples were collected from rats by tail vein bleeding in duration 1-3rd h for obtaining UA level on 1st, 2nd, and 3rd h after drug administration. The blood was allowed to clot for 30 minutes at room temperature and then centrifuged at 10.000 × g for 7 minutes to obtain the serum. The serum was stored at -20°C before UA level determination. Serum UA level was determined by enzymatic-colorimetric method, using a standard diagnostic kit, according to manufacturer's instructions.

Effects of plant extract on residual activity of XOD in liver homogenates ex vivo

Residual activities of XOD in the excised livers were determined by measuring uric acid concentrations generated from xanthine. Briefly, the livers were homogenized in five volumes of cold 80mM sodium pyrophosphate buffer, pH 7.4, and centrifuged at 3000 g for 10 min at 4 ^{0}C. After the lipid layer was removed, the remaining part was further centrifuged at 10,000 g for 60 min at 4 ^{0}C, and the obtained supernatants were used to measure the residual XOD activity.

A portion (100 ml) of each liver homogenate and 800 ml of 80 mm sodium pyrophosphate buffer (pH 7.4) were mixed and incubated at 25 ^{0}C for 10 min. Then, 500 mL of 120 mm xanthine solution was added, mixed and incubated for 30 min. The reaction was terminated after 0 and 30 min by adding 100 mL of 1N HCL. Thereafter, the collected samples were centrifuged at 3000 g for 10 min, and uric acid concentrations were measured by HPLC. The total protein concentration in the liver homogenate was determined using Protein Assay Bradford Reagent (Wako Pure Chemicals, Osaka, Japan). The residual XOD activity was determined and expressed as nmole of uric acid formed/min/mg protein.

Statistical Analysis

Results are represented as Mean ± SEM. The test extract, standard and control were analyzed with the help of one-way analysis of variance (ANOVA) followed by Dunnett's Test. P values < 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

A systematic approach is required in pharmacognostic research to confirm and determine the identity, purity, and quality of a crude medication. This comprehensive and rigorous pharmacognostic investigation will provide useful information for future research.

Macroscopic and Organoleptic Studies: The root is thick and wood. The surface of the root is irregularly fissured; the fissures are shallow. The thickness of the periderm varies along the circumference as shown in Fig.2.

Fig.1: *Sida cordifolia* plant **Fig.2: Part used- Root** **Fig.3: Powdered drug**

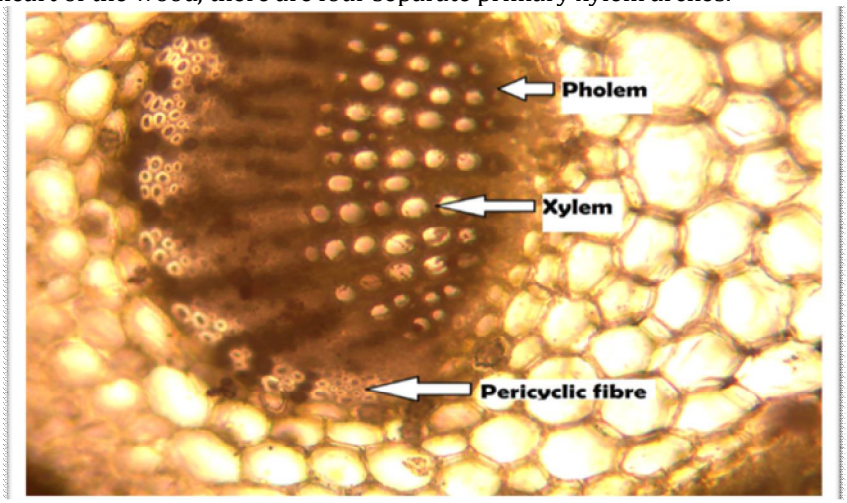
Microscopic Studies:

Microscopy of root:

T.S of root (Fig.4.A.) shows single layered epidermis covered by cuticle; cortex is made up of group of parenchymatus cells. Cortex narrow and is comprising of 3-4 rows of tangentially elongated cells.

Within the cortex, calcium oxalate crystals and minute starch grains are common. Secondary phloem is found in conical strands and is made up of 5-6 tangential bands of thick walled bast fibres alternating with thin-walled phloem elements. Crystal clusters can be found in some phloem parenchyma cells. Almost all phloem ray cells contain calcium oxalate crystal crystals.

Secondary xylem is consisting of vessels, parenchyma, fibers, and medullary rays. Vessels can be found in groups of three or alone, and their size and shape vary. The vessels are surrounded by xylem parenchyma, which contains starch granules and thick-walled plentiful fibers. Medullary rays are composed of numerous, uni- or bi-serriate, radially elongated cells, the majority of which contain calcium oxalate crystals. At the heart of the wood, there are four separate primary xylem arches.



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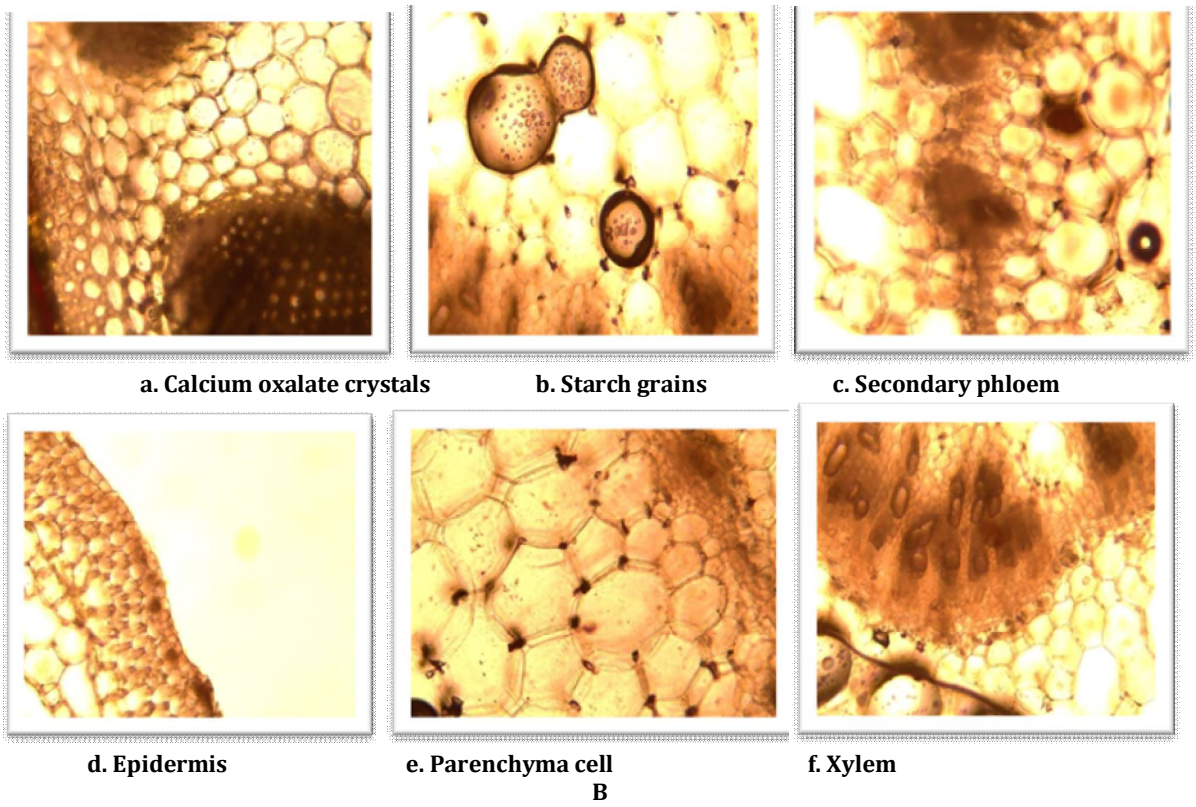


Fig. 4 A-T.S. of *Sida cordifolia* root, B-shows a. Calcium oxalate crystals, b. Starch grains, c. Secondary phloem d. Epidermis, e. Parenchyma cell and f. Xylem

Powder characteristics:

The organoleptic evaluation of powder revealed the following characteristics. The powder is light green color with characteristic odor and taste. On microscopic examination, the powder showed covering trichome unicellular, Prismatic Calcium oxalate crystals, Starch grains, Rhomboidal calcium oxalate crystals, stellate trichome and Xylem fibers.

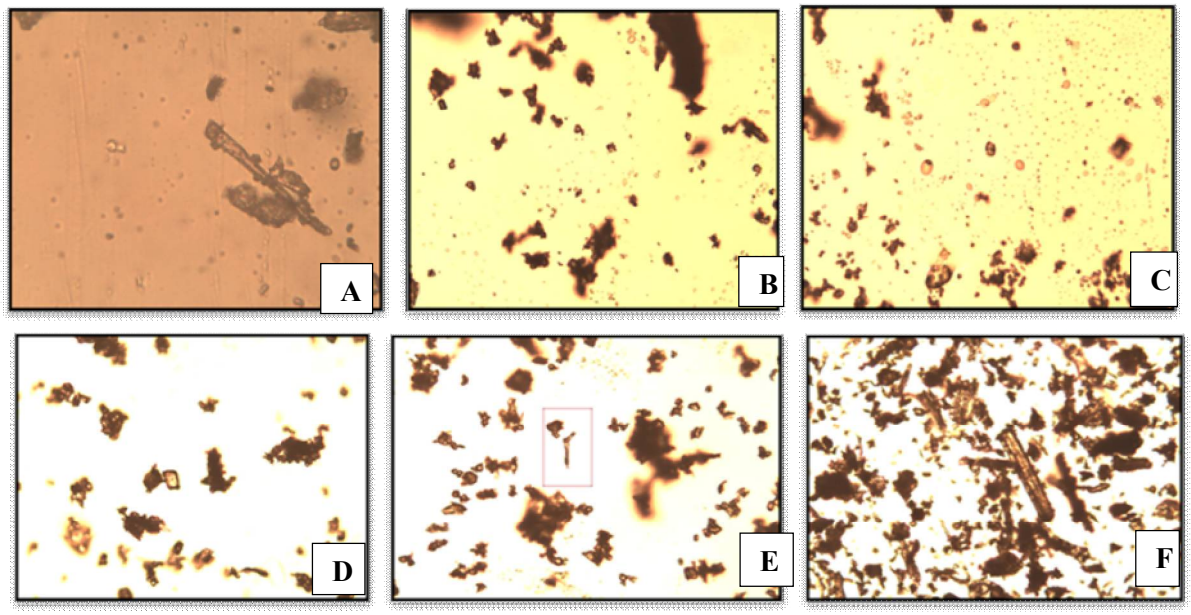


Fig.5 Powder characteristics of *Sida cordifolia* root

A.Covering trichome unicellular B.Prismatic Calcium oxalate crystals
C.Starch grains D.Rhomboidal calcium oxalate crystals
E. Stellate trichome X400 F. Xylem fibers

Proximate analysis:
Various physicochemical parameters such as ash levels, extractive values, moisture content, and fluorescence were tested and the findings are provided (Tables 1-3).

Table no.1: Physiochemical parameter of root of *Sida cordifolia*

Sr.No.	Physiochemical parameter	%W/W
1	Total Ash	10±0.62
2	Water soluble ash	1.22±0.12
3	Acid insoluble ash	1.60±0.34
4	Loss on drying	6.12±0.19

Table no.2: Different Solvent extractive values of *Sida cordifolia* root by %(w/w)

Extract	Method of extraction	Colour	Consistency	Percentage yield
Pet ether extract	Soxhlet	Light yellow brown	Slight sticky	2.00w/w
Ethanol extract		Brown	Sticky	9.00w/w
Water extract	Maceration	Greenish brown	Sticky	10.00w/w

S.cordifolia root powder and the various extracts fluorescence analysis are shown in tables 3 and 4.

Table 3: fluorescence analysis of *Sida cordifolia* root

Treatment	Day light	UV light	
		254nm	366nm
Powder	Faint yellow	Yellowish	White
Powder + water	Pale green	Pale green	Green
Powder + 1N HCl	Dark Yellow	Black	Dark Yellow
Powder+ HNO_3	White	Faint yellow	Faint yellow
Powder+1N NaOH	Dark brown	green	Faint yellow
Powder+ 1N KOH	Whitish yellow	Dark Yellow	Faint yellow
Powder+H_2SO_4	White	Brown	Faint yellow
Powder + Ethanol	Faint yellow	White	Yellow
Powder + Acetone	White	Faint yellow	White
Powder + Methanol	Faint yellow	Yellowish	White

Table 4: Fluorescence analysis of various extracts of the root of Sida cordifolia

Sr.no	Extracts	Daylight	UV light	
			short 254nm	long365nm
1	Hexane	Pale green	Yellowish green	Pale green
2	Ethyl Acetate	Greenish black	Dark green	Greenish black
3	Ethanol	Dark green	Green	Green
4	Aqueous	Brown	Pale brown	Pale yellow

In *S. cordifolia* powder and extracts there was no characteristic fluorescence seen.

Preliminary phytochemical screening:

Table no.8: phytochemical analysis of root of *Sida cordifolia* extract

Chemical constituents	Name of test	Pet. Ether extract	Ethanol extract	Water extract
Alkaloids	Dragondorff's test	+	+	-
Amino acid	Millon's test	-	-	-
Carbohydrate	Molish test	-	-	+
Flavonoids	Shinoda test	+	+	+
Glycoside	Killer-Killianitest	-	+	-
Protein	Biuret test	-	-	-
Phenols	Ferric chloride test	-	+	-
Saponines	Froth formation test	-	+	+
Steroids	Libermann-Burchard test	+	-	-
Tannins	Ferric chloride test	+	-	-

- Absent ; + Present

Preliminary phytochemical screening: Extracts obtained by continuous Soxhlet were subjected to be subjected for standard qualitative phytochemical tests to identify the presence of chemical constituents (viz., alkaloids, glycosides, tannins, flavonoids, sterols, fats, oils, phenols and saponins) present in them. (Table 8)

Estimation of Flavonoids:

The estimation of Flavonoids was carried out by the colorimetric method

Table-9: Total Flavonoid content of *Sida cordifolia* root extract

Concentration	Absorbance 415nm
10	0.19
20	0.37
30	0.52
40	0.62
50	0.75
Ethanol extract	0.51

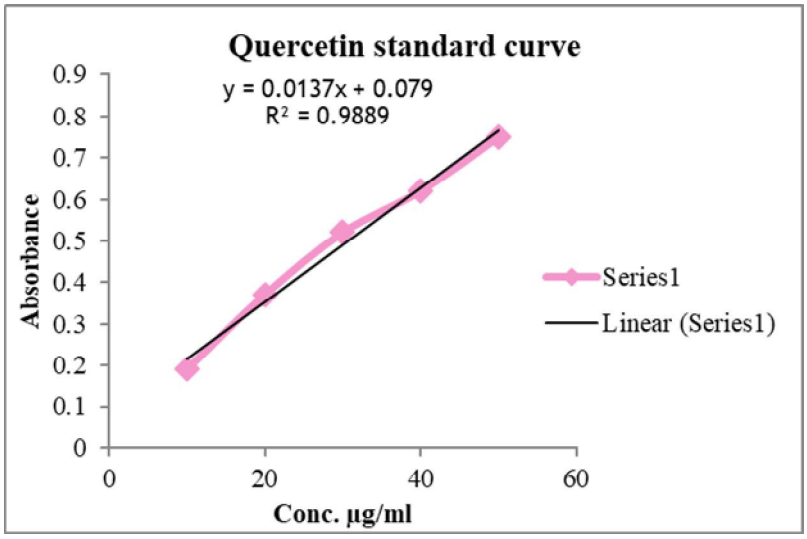


Fig. 6: Calibration curve for the standard Quercetin

The concentration of Flavonoid present in Ethanol extract was found to be 26.8µg equivalent to Quercetin in 1 mg (2.68%).

Estimation of Phenol

The estimation of Phenols was carried out by the colorimetric method

Table 10: Total Phenolic content of *Sida cordifolia* root extract

Concentration	Absorbance 415nm
50	0.075
100	0.108
150	0.165
250	0.255
350	0.327
Ethanol extract	0.229

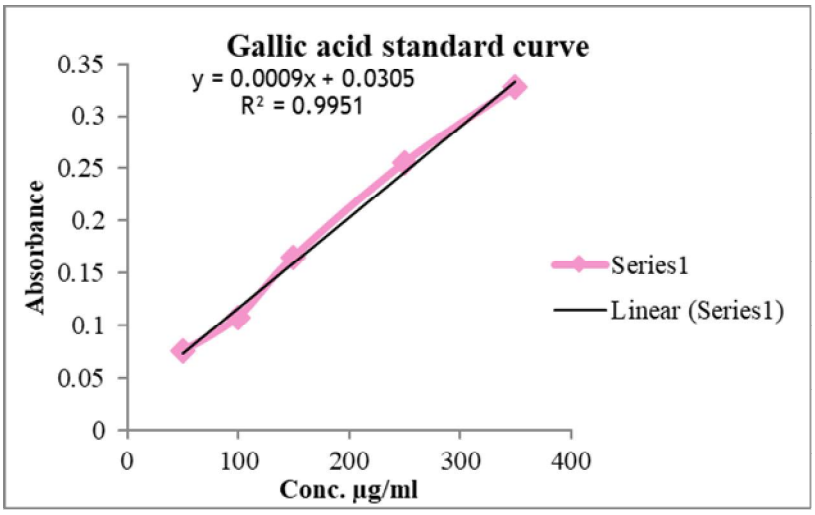


Fig. 7: Calibration curve for the standard Gallic acid

The concentration of Phenols present in Ethanol extract was found to be 182.02µg equivalent to Gallic acid in 1 mg (18.2%).

Estimation of heavy metals

Heavy metal contamination of medicinal plant materials can result in chronic or severe toxicity. As a result, it has become vital to ensure the heavy metal content of all beginning materials, as well as the presence of other necessary inorganic elements. The presence of heavy metals was determined by elemental analysis, and the results are shown in the table below.

Table 5: Content of heavy metals in powder of *S.cordifolia*

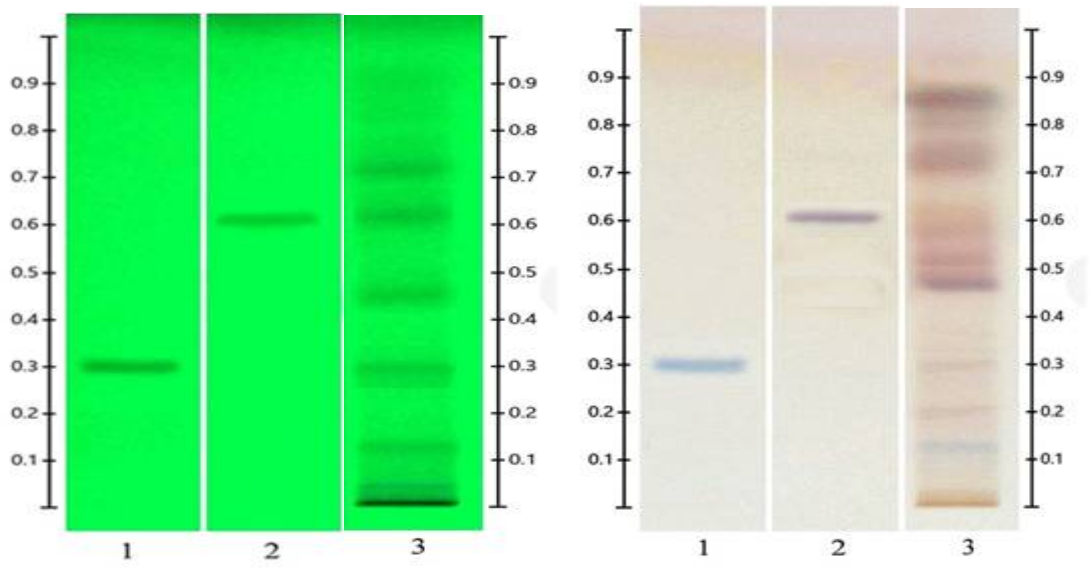
S.no	Inorganic elements	Content (µg/mg)
1	Cadmium	0.026
2	Arsenic	0.000
3	Mercury	0.000
4	Lead	0.000

HPTLC fingerprinting of *Sida cordifolia* extracts

HPTLC fingerprint development showed 2 spots at Rf 0.3 (for vasicine) and 0.61 (for vasicinone) were observed in the chromatogram of the ethanolic extract of root of *S. cordifolia* along with other components. HPTLC fingerprint was developed for identification of marker compounds band in ethanolic extract in comparison with reference standard band (Fig. 8).

Table11:Rf values of spots obtained in TLC

Extracts	Solvent system	no. of Spots	Rf value (uv)
Ethanol	Toluene: Chloroform: Ethanol (28.5: 57: 14.5)	7	0.14 0.21 0.30 0.48 0.61 0.74 0.86



A B

Fig. 8 Developed HPTLC plate photograph of ethanolic extracts of S. cordifolia root with marker compounds. A 254 nm before derivatization. B Visible mode after derivatization
(1: vasicine, 2: vasicinone and 3: ethanolic extract)

The linearity of the calibration curve of vasicine and vasicinone are shown in fig.9 and fog.10

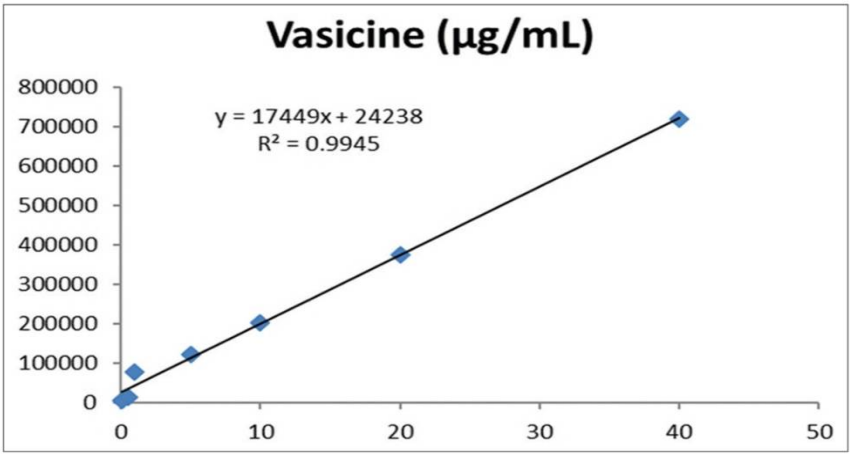


Fig. 9 Calibration plot for vasicine

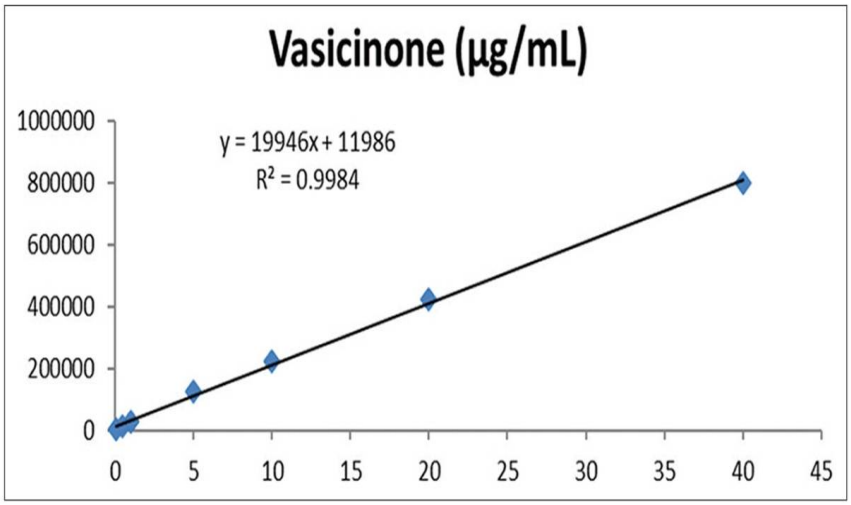


Fig. 10 Calibration plot for vasicinone

In the HPLC system, twenty microliters of each adequately diluted sample solution were injected in triplicate. Each had its peak areas measured. The linear regression equation produced from the calibration curves was used to calculate the amount of vasicine and vasicinone in the sample extracts. The chromatograms of the extracts revealed two peaks at Rt 5 and 8.7 (Figure 12), along with additional components. There was no interference from other components in the extract in the analysis of vasicine and vasicinone. The chromatogram shows these components at dramatically differing Rt values.

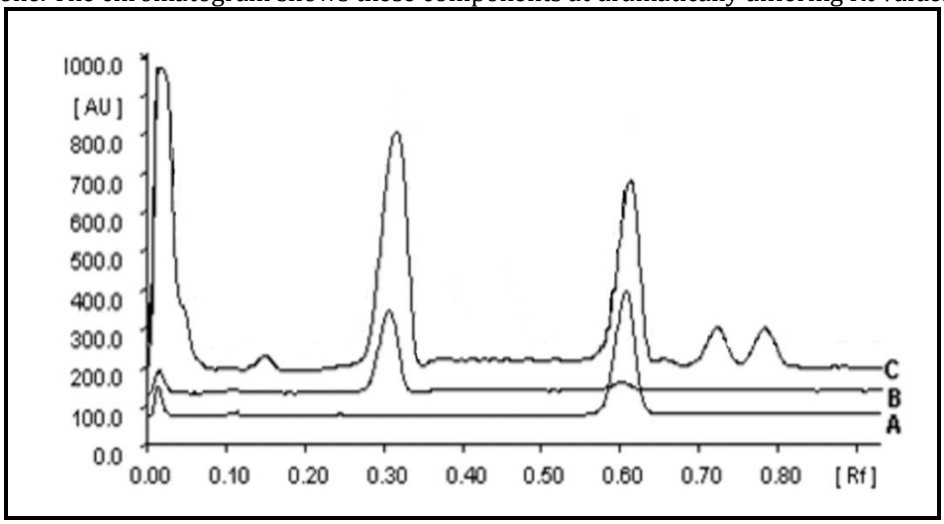


Fig 11. HPTLC of standard along with extract of *Sida cordifolia* (A) Vasicinone standard, (B) Vasicine standard (C) *Sida cordifolia* extract at 298 nm.

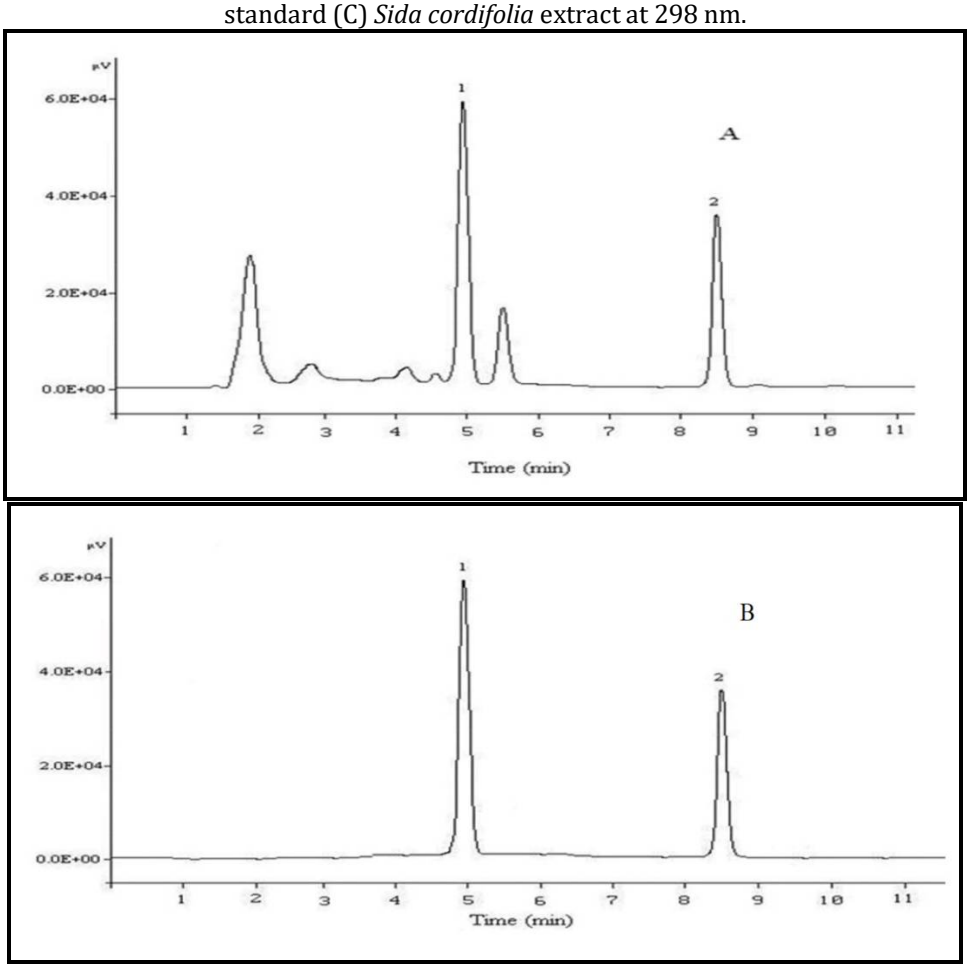


Fig.12 HPLC chromatogram of *S. cordifolia* (A) and Reference standard (B); Peak 1 is of vasicine (Rt: 5.0), peak 2 is of vasicinone (Rt: 8.7), and unknown peaks are of other components present in the extract.

Pharmacological Activity

Antioxidant activity of *S. cordifolia*

Phytochemicals like anthocyanins, polyphenols, ellagitannins and carotenoids mainly shown by antioxidant activity. These phytochemicals are also beneficial in the prevention of chronic illnesses. These phytochemicals' antioxidant capacity has been demonstrated not only in vitro but also in vivo human research. Because S. cordifolia has secondary metabolites such as phenols and flavonoids, it is being researched for anti-oxidant properties.

DPPH scavenging activity

Table 11: DPPH free radical Scavenging activity of *S.cordifolia* extract

Conc (µg/ml)	Ethanol extract % inhibition	Ascorbic acid % inhibition
10	28.12±0.54	45.12±0.52
50	46.58±0.45	55.65±0.71
100	59.12±0.89	68.36±0..65
200	65.42±0.68	76.18±0.55
300	72.78±0.38	84.89±0.32
500	81.20±0.51	90.26±0.26
IC_{50} (µg/ml)	50.42±0.58	20.93±0.48

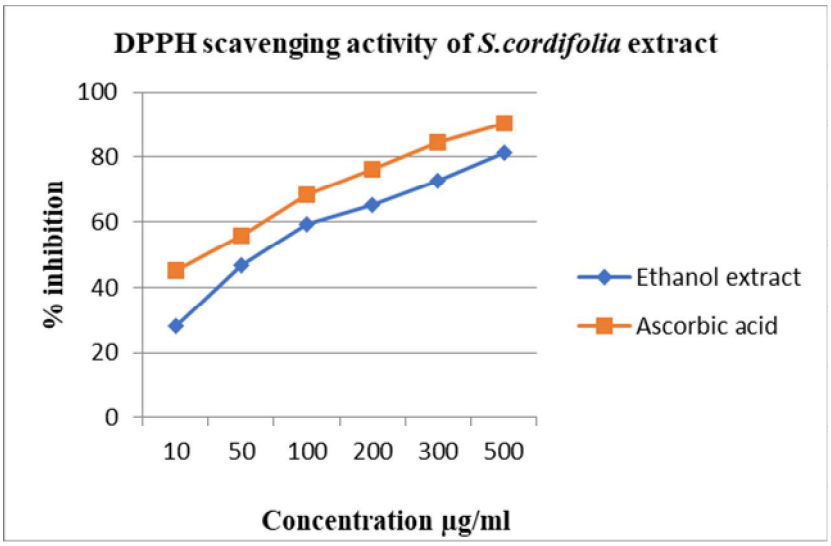


Fig 9: DPPH free radical scavenging activity of *S.cordifolia* extracts

Nitric Oxide (NO) scavenging activity

Table 12: Nitric Oxide scavenging activity of *S.cordifolia* extract

Conc (µg/ml)	Ethanol extract % inhibition	Ascorbic acid % inhibition
10	20.04±0.62	35.78±0.84
50	29.36±0.45	43.18±0.37
100	42.39±0.36	52.64±0.57
200	50.04±0.48	60.22±0.75
300	61.28±0.72	71.32±0.69
500	67.12±0.49	79.22±0.48
IC_{50} (µg/ml)	175.12±0.53	112.90±0.59

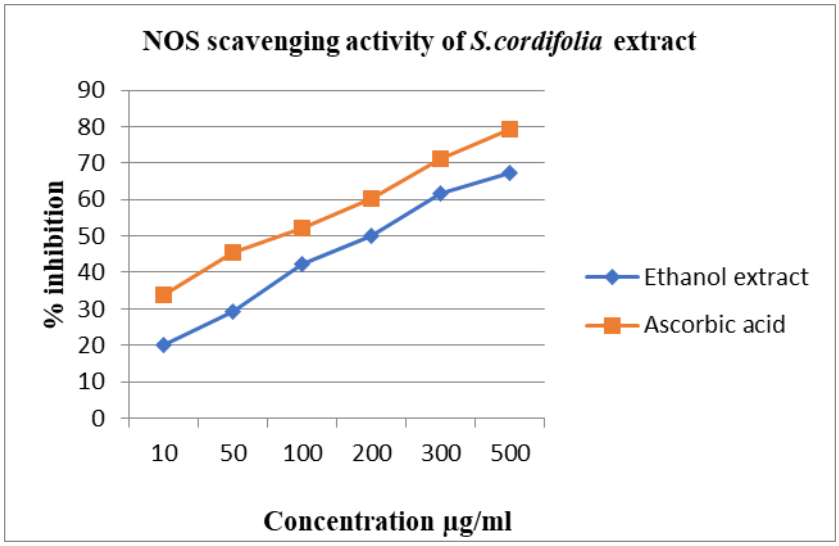


Fig.10: NO radical scavenging activity of *S.cordifolia* extract.

The percentage of inhibition in the *S.cordifolia* extract for both assays was exhibited in relation to ascorbic acid and was shown to increase gradually with concentration increase. In the DPPH and NOS assays, the standard IC_{50} value for ascorbic acid was determined to be 20.93 g/ml and 112.90 g/ml, respectively.

In the DPPH assay, ethanol extract displayed the highest radical scavenging activity, strongly suppressing DPPH radical with IC_{50} Value of 50.42+ 0.58 g/ml and in the NOS assay with IC_{50} Value of 175.12+ 0.53 g/ml. This suggests that DPPH is a better assay than NOS for assessing *S.cordifolia* antioxidant capacity.

Effect of *S.cordifolia* extracts on in vitro xanthine oxidase (XOD) activity

In the table 13 shows the inhibitory effects of *S.cordifolia* extract and allopurinol on bovine milk xanthine oxidase at varied doses. At a dose of 100 g/ml, each inhibited XO by more than 50%. At the maximum dose of 100 g/ml, *S.cordifolia* inhibited XO by 68%, while the conventional XO inhibitor, allopurinol, inhibited XO by 95% at the same concentration.

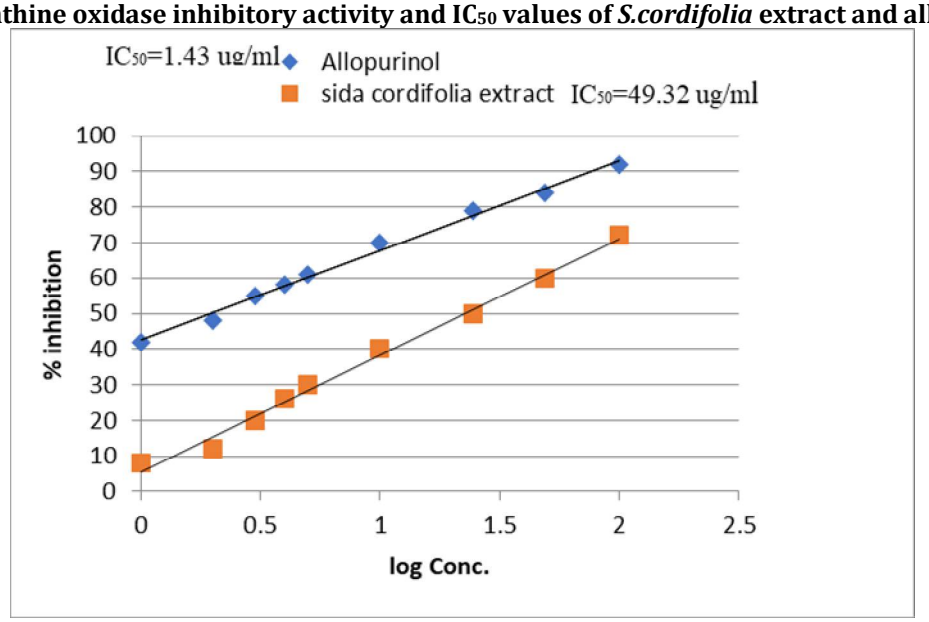
The inhibitory activity of xanthine oxidase for both *S. cordifolia* extract and allopurinol was also represented as an IC_{50}, which denotes the quantity of the standard drug or sample examined necessary for 50% inhibition of xanthine oxidase activity under the identical experimental conditions.

The IC_{50} values for allopurinol and *S.cordifolia* extract were calculated using the program GraphPad Prism V 6.05 (GraphPad Prism Software, Inc., USA) and corresponded to 1,834 g/ml and 95.243 g/ml, respectively.

Table 13: Xanthine oxidase inhibitory activity of *S.cordifolia* and allopurinol at different concentrations

Concentration (µg/ml)	XO inhibitory activity (%)	
	Allopurinol	*S.cordifolia* extract
1	42±1.2	10±1.2
2	52±1.6	12±0.8
3	56±0.6	16±1.1
4	62±0.4	19±0.2
5	68±2.0	22±0.3
10	72±2.1	38±1.4
25	81±1.1	45±1.8
50	90±0.6	68±2.2
100	95±1.5	68±2.2

Fig.11: Xanthine oxidase inhibitory activity and IC_{50} values of *S.cordifolia* extract and allopurinol



Enzyme kinetic analysis of *S.cordifolia* extract on XOD inhibition in vitro

With the help of Lineweaver-Bruk analysis the presence of *S.cordifolia* caused a decrease in the Vmax of XOD compared to the control with very little change in the Km of xanthine, indicating a typical reversible, noncompetitive inhibition of enzymatic reaction (Figure 12). The *S.cordifolia* extract has a Ki value of 376.3 mg/mL.

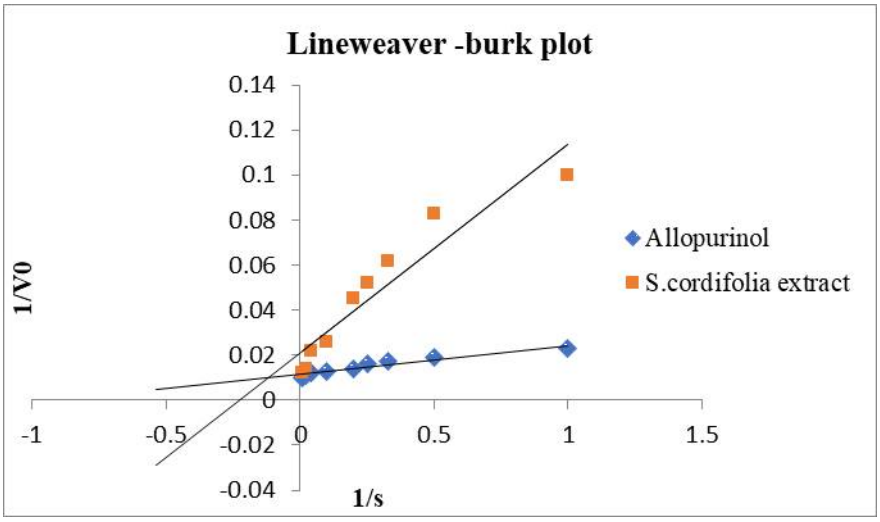


Fig.12: Lineweaver–Burk plots for Enzyme kinetic analysis of xanthine oxidase activity inhibited by *S.cordifolia* extract.

***In vivo* effects of *S.cordifolia* extract on plasma uric acid levels in potassium oxonate-induced hyperuricemic rat**

The antihyperuricemic activity of *S.cordifolia* root extract on hyperuricemic rats using potassium oxonate were shown in Table 14 and Fig. 13. Potassium oxonate is a uricase inhibitor that can cause hyperuricemia in rats. The rat produced by potassium oxonate can be used as a hyperuricemia animal model to test samples that impact blood UA levels, as well as to test potential treatment medicines in particular illnesses linked with elevated UA levels. Treatment with potassium oxonate causes hyperuricemia in rats, as evidenced by a significant increase in UA levels as compared to normal rats.

At 0 h, the UA levels in all treatment groups were not significantly different from one other or from normal control rats. Each animal in the normal control group, which received only vehicle (CMC-Na), had the same serum UA level. Oral pre-treatment with allopurinol at a dose of 10 mg/kg bw resulted in a substantial reduction in UA level in hyperuricemia rats at the 1-3rd hr following treatment (p0.05). The UA level in the allopurinol group was not different from the normal control group after 1-3 hours of oral therapy.

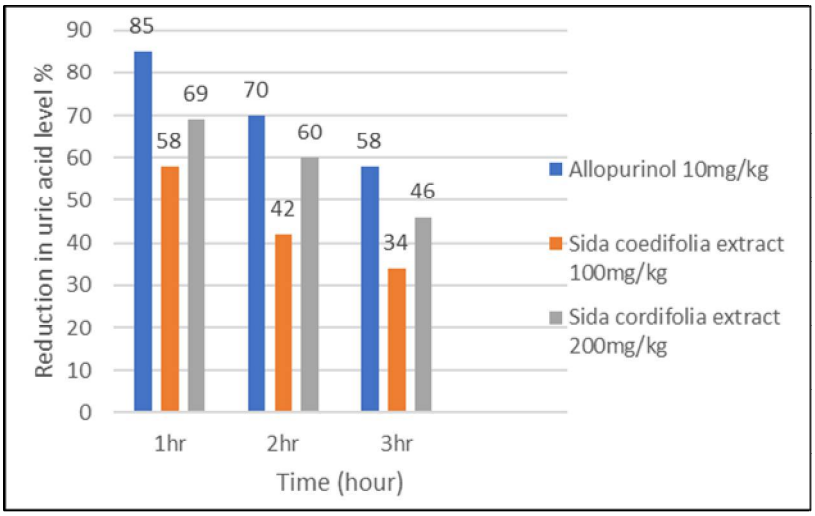
This finding showed that allopurinol treatment resulted in complete recovery and could reduce serum UA levels to normal levels. The profile UA level in the hyperuricemic group remained elevated three hours after sample administration, whereas the serum UA level in the extract group in the first and second hours were significantly different from the hyperuricemic group (p0.05), with only the first hour showing no difference with the normal control. These results showed that the extract 400mg/kg exhibited potential antihyperuricemic activity when compared to allopurinol, although it took longer to recover too normal.

Table 14: Effect of *S.cordifolia* extract on serum uric acid level

Group	Dose (mg/kg)	Serum uric acid level (mg/dl			
		0h	1h	2h	3h
Normal control	-	2.64±0.32	2.41±0.38ry	2.33±0.26r	2.45±0.38
Hyperuricemic control	-	2.55±0.24	3.85±0.27cz	4.01±0.37cz	3.36±0.23cz
Allopurinol	10	2.46±0.36	1.82±0.40br	1.93±0.16r	2.20±0.51r
Sida cordifolia extract	200	2.74±0.36	2.78±0.19rz	3.30±0.28crz	3.21±0.28a
Sida cordifolia extract	400	2.52±0.20	2.63±0.16rz	3.23±0.23crz	3.09±0.65

Values are expressed as Mean±SEM for 6 rats.
a, b, c: Compared to normal control (p<0.1, 0.05, 0.01), p, q, r: Compared to hyperuricemic control (p<0.1, 0.05, 0.01), x, y, z: Compared to allopurinol group (p<0.1, 0.05, 0.01),

Fig. 13: Reduction in serum uric acid level after *S. cordifolia* extract treatment in hyperuricemia rat



Effects of *S.cordifolia* extract on residual XOD activity in liver homogenates ex vivo

Treatments with allopurinol (10 mg/kg) and *S.cordifolia* extract (200 mg/kg) significantly reduced uric acid formation in liver homogenates ex vivo by 89.09% and 68.18%, respectively, compared to the untreated hyperuricemic group (p 0.01 and p 0.05, respectively) (Table 15).Treatment with *S.cordifolia* extract (100mg/kg) reduced uric acid generation by decreasing XOD activity in liver homogenates by approximately 43.09%; however, these findings were not statistically different from the untreated hyperuricemic group.

Table 15: Residual xanthine oxidase activity (XOD) in liver extracted from rat treated with *S.cordifolia* extract.

Groups	XOD activity (nano mole/min/mg protein)	Inhibition (%)
Normal control	0.98±0.07	-
Hyperuricemic control	1.84±0.15	-
Allopurinol	0.12±0.001**	89.09
S.cordifolia extract	1.10±0.20	43.63
S.cordifolia extract	0.44±0.12*	68.18

Data are mean ± SEM (n=6). * p < 0.05 compared with the untreated hyperuricemic group. **p < 0.01 compared with the untreated hyperuricemic group.

DISCUSSION

The macroscopic and microscopic characteristics of *S.cordifolia* were investigated. Powder microscopy aids in the identification of minute fragments and adulterants, which are some of the roots distinguishing characteristics. A physicochemical study was carried out to determine the confirmation and quality of the crude medication. The ash value is a significant metric in determining low-grade products and exhausted medications. A high ash value suggests substitution, contamination, or adulteration. The overall ash value was determined to be 10%w/w. The acid insoluble ash was found to be 3.32% and the water-soluble ash was determined to be 4.90%.

The extractive value is useful for predicting the type of elements. Water solubility, alcohol solubility (ethanol), and pet ether solubility were determined to be 18.00%, 15.40%, and 2.00%, respectively. The amount of volatile stuff present is determined by the loss on drying, which was found to be 1.12%.There was no detectable fluorescence in either the powder or the extracts.

The content of Flavonoid in Ethanol extract was determined to be 31.4g equivalent to Quercetin in 1 mg (3.14%), while the concentration of Phenols in Ethanol extract was 229.53g equivalent to Gallic acid in 1 mg (22.9%).

The level of lead 0.027ug/ml in the heavy metal analysis of *S. cordifolia* extract is within the normal range. Phytochemical investigations show that the root of *Sida cordifolia* is high in a variety of phytoconstituents. Alkaloids, flavonoids, phenols, saponins, steroid, and tannin were found in the ethanol extract. TLC analysis revealed that the ethanolic extract had two spots. In the chromatogram of the sample extract, two spots at Rf 0.30 (for vasicine) and 0.61 (for vasicinone) were seen along with other components in HPTLC.

In the DPPH assay, ethanol extract displayed the highest radical scavenging activity, strongly suppressing DPPH radical with IC50 Value of 20.42+ 0.58 g/ml and in the NOS assay with IC50 Value of 174.12+ 0.53 g/ml. This suggests that DPPH is a better assay than NOS for assessing *S.cordifolia* antioxidant capacity.

In vitro xanthine oxidase (XOD) inhibitor activity of *S.cordifolia* extract was determined to be 68% when compared to Allopurinol 95%, and the extract's IC_{50} value is 94.32ug/ml when compared to Allopurinol 1.52ug/ml.

A probable mechanism is that any item may reduce uric acid levels in the blood by reducing the action of the xanthine oxide enzyme, which inhibits uric acid synthesis. The *S.cordifolia* extract inhibited xanthine oxidase activity in vitro significantly. Many bioactive elements, such as polyphenols, have been found in *S.cordifolia* extract. Previous research has shown that these compounds have a high inhibitory action against xanthine oxidase. Stigmasterol is essential in the treatment of hyperuricemia. The fundamental mechanism is that they can reduce the levels of IL-17, TNF-, and IL-1 in hyperuricemia rats by reducing liver XOD activity. Lupeol is primarily responsible for the extract's urate-lowering action.

Lineweaver-Burk analysis of *S.cordifolia* extract on XOD inhibition revealed that the presence of *S.cordifolia* caused a decrease in the Vmax of XOD compared to the control with very little change in the Km of xanthine, indicating a typical reversible, noncompetitive inhibition of enzymatic reaction.

The antihyperuricemic activity of *S.cordifolia* extract on hyperuricemic rats using potassium oxonate was found to significantly reduce uric acid levels in the blood, and the extract 200mg/kg had potential antihyperuricemic activity as compared to allopurinol, but required a longer time to return to normal.

In comparison to allopurinol, residual XOD activity in liver homogenates of *S.cordifolia* extract (200 mg/kg) considerably decreased the generation of uric acid in the liver homogenates by suppression of XOD activity by 76.0% as compare to allopurinol 90.3%.

CONCLUSION

This study demonstrated for the first time the antioxidative and antihyperuricemic effects of *Sida cordifolia* extract in vivo and in vitro. The extract significantly lowered uric acid levels in the blood of potassium oxonate-induced hyperuricemic rats through xanthine oxidase inhibitory activity. In particular, in vivo antihyperuricemic effect of *Sida cordifolia* extract was explained by a mechanism of XOD inhibition in the liver, which was identified to be a noncompetitive type of inhibition. The results obtained in this study suggest that *Sida cordifolia* can be used as a natural remedy for the treatment of hyperuricemia.

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