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# *ASSESSMENT OF PHARMACOGNOSTICAL, PHYSIOCHEMICAL, AND IN-VITRO ANTI-INFLAMMATORY POTENTIAL OF S. CORDATA*

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*Anti-inflammatory, Sida cordata, Protein denaturation, Pharmacognostical investigation, Physicochemical evaluation.*

***ABSTRACT***
**Background:** The current study aimed to investigate the pharmacognostical, physiochemical, and anti-inflammatory potential of *Sida cordata*. In this study, macroscopic, microscopic, and physicochemical analyses were performed. **Methodology**: This medicinal plant, commonly used in traditional medicine, was evaluated using the in vitro albumin denaturation method. The study aimed to assess the plant's ability to inhibit protein denaturation, a critical factor in the inflammatory process. The water extract and acetic acid extract of *Sida cordata* were tested at various concentrations (10, 50, 100, 150, 200, 250 µg/ml), and the results indicated a dose-dependent inhibition of albumin denaturation. **Result & Discussion:** The rate of inhibition of egg albumin denaturation for water extract (WE) is 86.55±0.63, and acetic acid extract (AAE) is 82.18±1.43. The maximum inhibition observed was compared with the standard anti-inflammatory drug (diclofenac sodium). **Conclusion:** These findings suggest that the water and acetic acid extracts of *Sida cordata* possess significant anti-inflammatory activity, supporting its traditional use as a remedy for inflammatory conditions.

## *INTRODUCTION*

Inflammations are complicated biological responses to harmful stimuli such as pathogens, irritants, or injury. It plays a vital role in the pathogenesis of various long-term diseases, including cardiovascular diseases, arthritis, and cancer [1, 2]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are generally used to manage inflammatory conditions, but their long-term use is associated with adverse effects such as gastrointestinal ulcers and renal damage [3, 4]. Therefore, there is a growing interest in identifying natural anti-inflammatory & anti-diabetic agents with fewer side effects [5, 6]. The investigation of ethno-botanical resources offers a viable strategy for discovering novel anti-inflammatory agents, thereby directing our research toward *Sida cordata*, a plant species with a rich ethnopharmacological history. *Sida cordata*, a plant of the "Malvaceae" family commonly known as "Bala", has been traditionally used in Ayurvedic medicine to treat conditions such as fever, muscle pain, and inflammation. Several cultures have claimed its anti-inflammatory, anti-diabetic, analgesic, flavonoid, and saponin properties, which have been attributed to its medicinal properties. Several bioactive compounds, including alkaloids, flavonoids, and saponins, have been identified in *Sida cordata*

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[7-9]. Pharmacognostical studies play a crucial role in plant identification. Detailed macroscopic, microscopic, and physicochemical analyses will be of enormous significance in evaluating plant materials used in herbal medicines to ensure reliable outcomes and maintain their purity and efficacy [10].

The albumin denaturation method is an established in vitro model for evaluating anti-inflammatory activity. In this method, the ability of constituents to prevent the denaturation of albumin, a protein involved in inflammatory responses, is used as an indicator of anti-inflammatory activity [11–13]. The current study aims to determine the pharmacognostical, physiochemical, and in vitro anti-inflammatory properties of *Sida cordata* using these methods and to compare its activity with that of the standard anti-inflammatory drug, diclofenac sodium [8, 14].

## *MATERIAL AND METHODS*

### Plant collection and authentication

The whole plant parts of *Sida cordata* were collected from Chhuikhadan, Rajnandgao district, Chhattisgarh state and identified by Dr Vinay Rajan, HoD and scientist-E (Ref No: S.Bh.V.S/M.CHH.K/PRASH./2023-24/789 Dated-08/02/2024) at Botanical survey of India, Allahabad, Uttar Pradesh, India [15, 16].

### Chemicals and Reagents

Bovine serum albumin (BSA), 3,5-dinitrosalicylic acid(DNSA), Diclofenac Sodium (standard anti-inflammatory drug, used as standard drug), dimethyl sulfoxide (DMSO), phosphate-buffered saline (PBS), and other reagents were of analytical grade [13,18,19].

## Pharmacognostical evaluation

### Morphological & microscopic evaluation

In this examination, the plant material was assessed by its color, tests, size, shape, odor, and special features such as touch and texture. For the microscopic analysis, T.S. (transverse section) were prepared and stained. The microscopic analysis was performed [17].

### Physiochemical analysis

Physiochemical parameters, such as ash value and extractive values, have been determined using the specified method and the WHO standards on quality control methods for medicinal plant materials [17].

### Extraction

The plant material was washed, air-dried, and powdered. A 500g sample of the dried powder was subjected to ultrasound–assisted extraction (UAE) with distilled water and acetic acid for 2 hours. The extracts were filtered (using Whatman No. 1), and the solvent was separated under low pressure (using a rotary evaporator). The concentrated extracts were kept at 4 °C until further use [17].

### Evaluation of anti-inflammatory property by the protein denaturation (Egg Albumin method)

The anti-inflammatory property of the extracts was calculated. The extracts were used for this assay, including 0.2ml of egg albumin, 2.8 ml of PBS (phosphate-buffered saline at pH 6.4, and 0.6 ml of plant extract at various concentrations dissolved in 0.2% DMSO. The concentration range of the extracts in the total reaction solution is 10-50 µg/mL. The samples were incubated for 10 min at 37°C. After the incubation period, the solutions were heated in a water bath for 20 min to denature the egg albumin. Sample absorbance was measured at 660 nm when it was cool. Diclofenac sodium (standard drug) and egg albumin (0.2ml), DMSO (0.6ml of 0.2%), and PBS (2.8ml, pH 6.4) were used as a control for the study [18, 19, 20]. The % inhibitions of protein denaturation were measured by using the given formula:

$$\%\ \boldsymbol{Inhibition} = \frac{\boldsymbol{Ac - As}}{\boldsymbol{Ac}} \times \mathbf{100}$$

*Where, As = absorbance of sample, Ac = absorbance of control.*

### Statistical Analysis

All the experiments were conducted in a set of three. The data were reported as mean + standard deviation (SD). One-way analysis of variance (ANOVA) was used for statistical analysis. A p-value below 0.05 was considered statistically significant. The values are expressed as mean ± SD (n = 3) [21, 22].

## *RESULTS AND DISCUSSION*

### Macroscopic Study

For macroscopic observation, fresh young leaves and aerial parts were used. The macro–morphological features of the plant parts were observed under a magnifying lens and simple microscopy. The plant is prostrate or semi-prostrate, up to 80 cm tall. Profusely branched at the base, all parts contain stellate and simple trichomes [17]. Fresh *Sida cordata* leaves are dark green, with a slight, characteristic odor and a tasteless flavor. The shape of the leaf is Ovate or lanceolate, length is 1-5.5 cm, and breadth is 1-5 cm. Apex acuminated, margin is crenate to serrate, base is

cordate or rounded, venation is reticulate pinnate, surface is pubescent, petiole is patiolate, 1-4 cm long, and stipule is stipulate. Fresh *Sida cordata* stems are dark green in color, the odor is slight and characteristic, and the taste is tasteless.

**Figure 1: Plant *Sida cordata*, dried *Sida cordata*, dried crude powder *Sida cordata***

The shape of the stem is cylindrical; size is 0.4 to 0.5 cm in diameter with variable length, surface is pubescent, and the fracture is short. Fresh *Sida cordata* roots are yellowish-brown, odorless, and tasteless. The stem is cylindrical, 0.3 to 0.8 cm in diameter, with variable length; the surface is rough due to rootlets, and the fracture is irregular. Flowers are axillary, solitary with a 1.5-2.5 cm long pedicel. Calyxes are fused in the middle, and corollas are pale yellow. The staminal column is 2-3 mm long and is simple-hairy. Fruits are depressed globular, pubescent at the top, beaked, 5 mericarps, dehiscent, and smooth. Seeds are brown, 2 mm long, and glabrous [17].

**Microscopic Study**

Fresh leaves and the aerial parts of both plants were studied transversely and longitudinally, using surface preparation and sectioning. The different parts of the plant, such as leaves, stems, and midribs, were studied according to the method described in [17].

**Microscopy of a leaf**

The leaf is Dorsiventral. In the lamina region, the upper Epidermis is single-layered, rectangular parenchymatous cells, containing a cuticle layer, anisocytic stomata, and stellate trichomes. Mesophyll-It consists of one to two-layered vertically elongated palisade cells below the upper epidermis. Between the palisade layer and the lower epidermis, thin-walled, loosely arranged parenchymatous cells are present. The lower epidermis is Similar to the upper epidermis & stomata are more numerous. In the midrib region, the upper epidermis is similar to the lamina portion. Collenchyma is a few-layered collenchymatous cell present below the upper epidermis and above the lower epidermis. Vascular bundle is a closed collateral type, single arc-shaped, with xylem present above phloem. Pericyclic fibers are present below and above the vascular bundle. Cortical parenchyma is the remaining area in the midrib, filled with parenchyma cells compactly arranged. Lower epidermis is similar to upper epidermis. Sometimes, vascular strands are present in the lamina [17].

**Microscopy of the stem**

Epidermis is single-layered, rectangular, tangentially elongated parenchymatous cells with a cuticle layer. Few uniseriate, unicell- ular covering trichomes are present. Hypodermis is below the epidermal layer. 2-3 layers of collenchyma cells are present. Cortex is composed of several layers of thin-walled cellulose parenchyma cells. Pericyclic fibers are present in groups forming two discontinuous layers. The vascular bundle is a closed collateral type. Phloem contains parenchyma sieve tubes & companion cells. Xylem is well developed, consisting of xylem parenchyma, vessels & fibers, and uniseriate, modular rays. Pith is larger, thin-walled, parenchyma cells are present, starch grains & needle-shaped calcium oxalate crystals are present in the pith [17].

**Microscopy of Root**

Cork consists of several layers of polygonal, compact lignified cells with brown content. Cortex is composed of several layers of thin-walled, polygonal parenchymal cells. A group of pericyclic fibers is present in the form of several discontinuous rings. Rosette-type calcium oxalate crystals are present throughout the cortex. The vascular bundle is a closed collateral type. Phloems consist of phloem parenchyma, sieve tubes, and companion cells. Xylem consists of xylem parenchyma, vessels, fibers, and uniseriate to multiseriate medullary rays. Starch grains are scattered in the cortex and xylem portion [17]

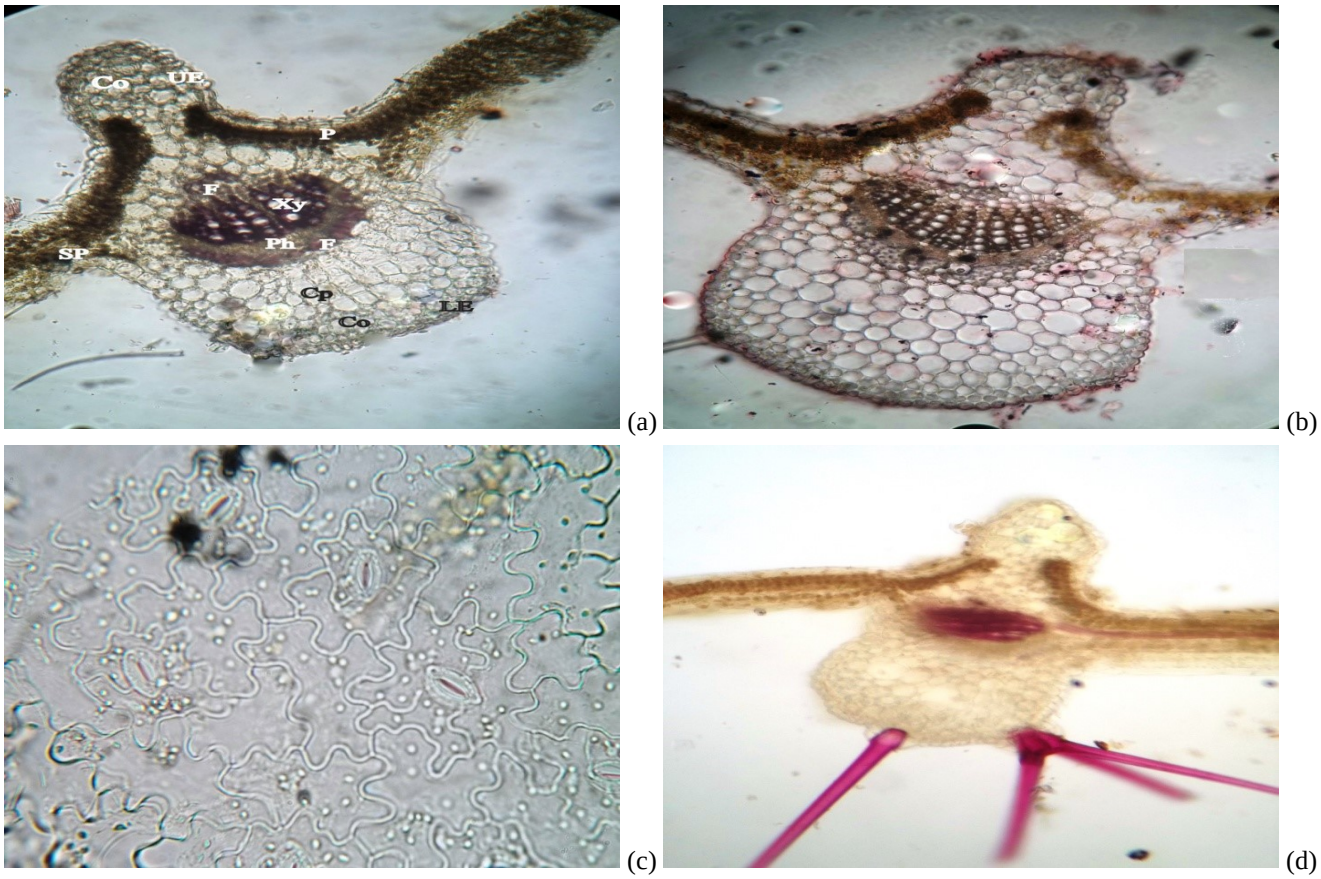


**Figure 2: (a)Co - Collenchyma, Cp – Cortical Parenchyma, F-Fiber, LE-Lower Epidermis, P-Palisade Cell, Ph-Phloem, SP-Spongy Parenchyma, T-Trichome, UE- Upper Epidermis Xy-Xylem. (b) T.S. of leaf *Sida cordata*. (c)Surface of the leaf showing Cuticle. (d) T.S. of leaf of *Sida cordata* showing stellate trichomes.**

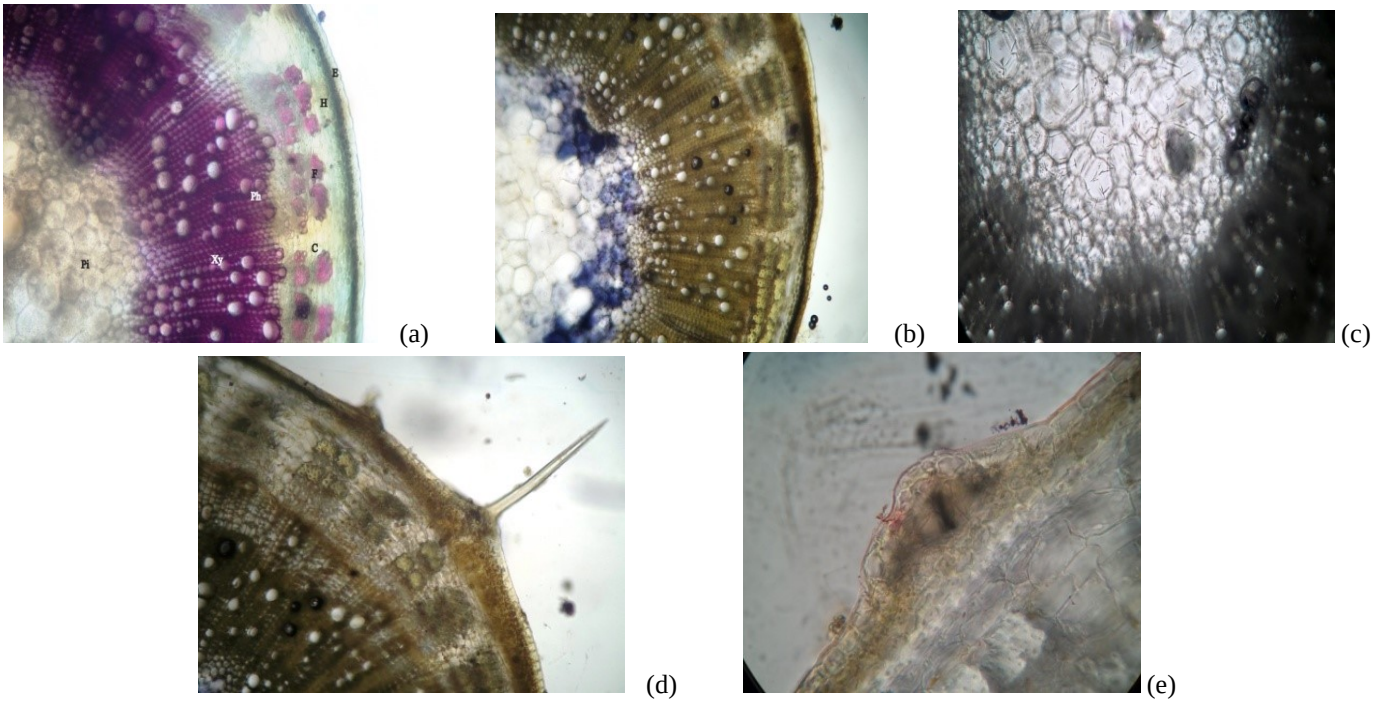


**Figure 3: (a) T.S. of Stem of *Sida cordata* E –Epidermis, H-Hypodermis, F-Fiber, C-Cortex, Ph-Phloem, Xy-Xylem, Pi-Pith. (b) T.S. of stem Showing Starch. (c) Surface of the stem Showing Calcium oxalate Crystal. (d) T.S. of the stem showing the Trichome. (e) Surface of the stem Showing Cuticle**

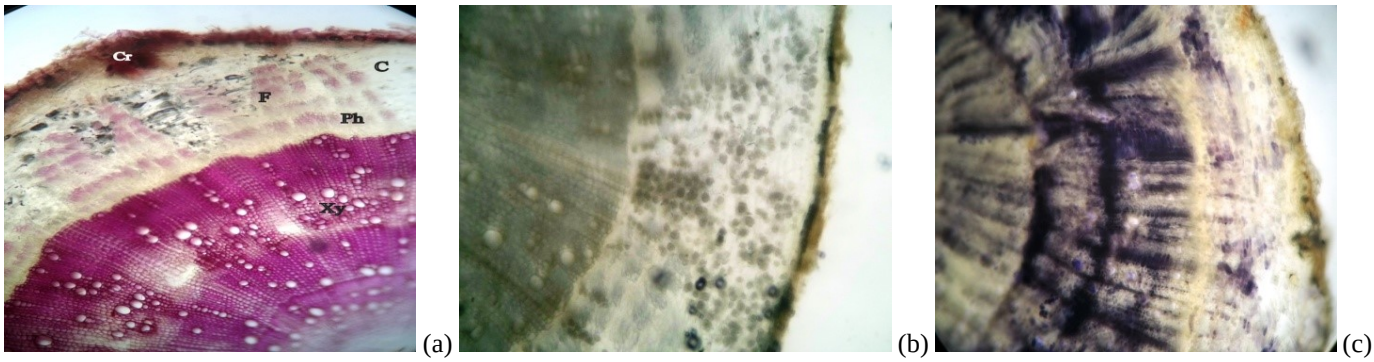


**Figure 4: (a) T.S. of Stem of *Sida cordata*, C-Cork, F-Fiber, Cr-Cortex, Ph-Phloem, Xy-Xylem. (b) Trans section of the stem showing Ca oxalate Crystal. (c) Surface of the stem showing Starch**

**Powder Microscopy**

Microscopical studies of powdered drug of the whole plant have been performed as per Practical Pharmacognosy-Techniques & Experiments by Khandelwal KR and Practical Pharmacognosy by Kokate CK. Epidermis is thin, wavy-walled, and parenchyma cells with irregular shapes. Stomata are anisocytic stomata. Mesophyll is a portion of the Spongy parenchyma with vascular strands. Parenchyma is thin-walled parenchyma cells. Trichome is uniseriate, unicellular covering trichomes. Xylem vessels are fragments of lignified spiral vessels. Starch is a simple starch grain [17]

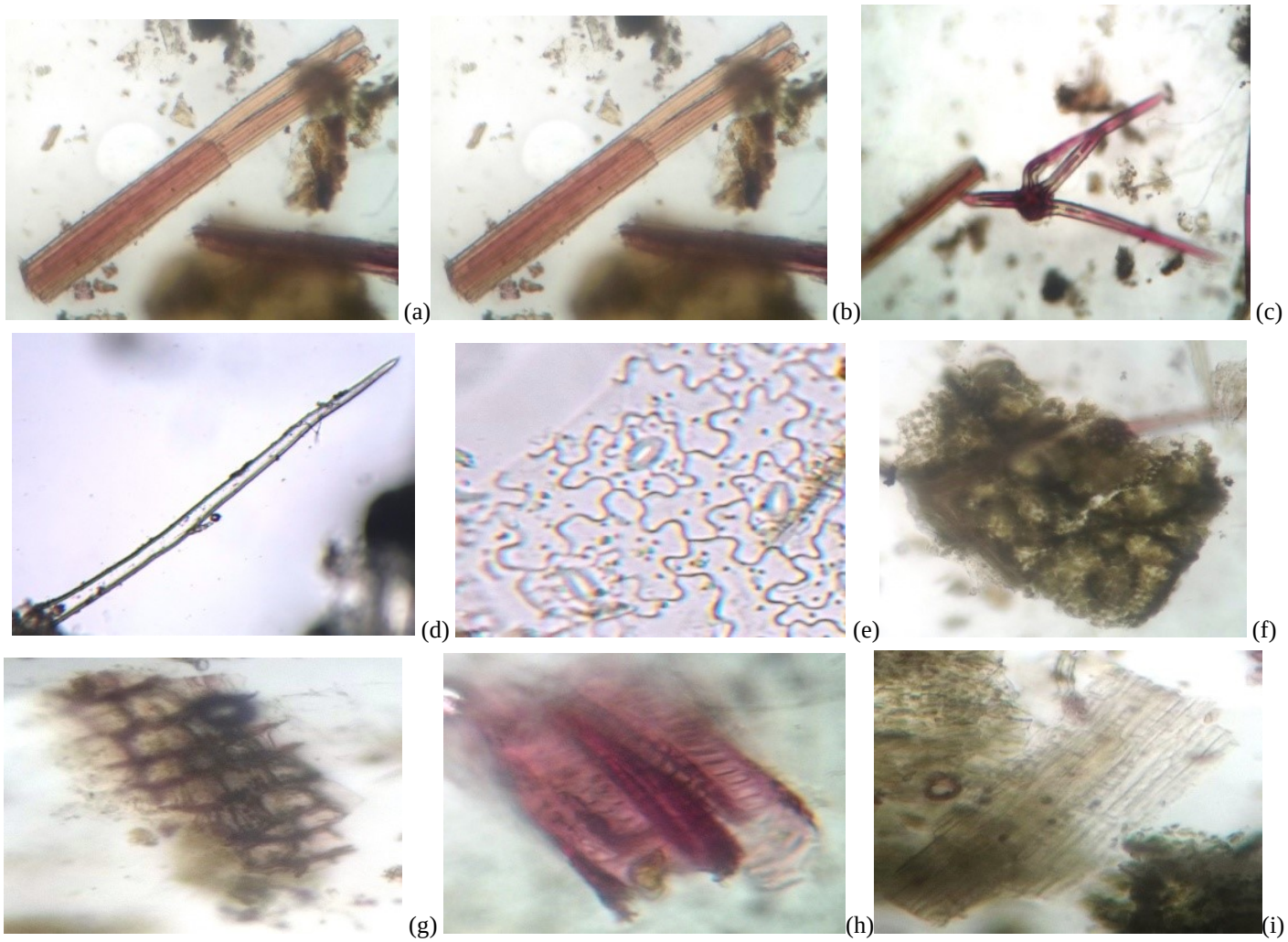


**Figure 5: (a) Fiber (b) Parenchyma (c) Stellate (d) Trichome (e) Stomata (f) Mesophyll (g) Cork (h) Vessel (i) Epidermis**

**Physiochemical Properties**

The values of several physiochemical characteristics tested, including the extractive of the plant S. Cordata. The plant's dry powder lost 2.24±0.35% after drying. The total ash value of powder of the whole plant was 8.73±0.25%, including water soluble ash and acid insoluble ash, which were 2.43±0.31% and 3.16±0.21% respectively. Also, the sulfated ash, nitrated ash, and carbonated ash were 16.33±0.34%, 14.57±0.41%, and 16.97±28% respectively. The maximum extractive value was found in water (12.34%), whereas the minimum extractive value was found in chloroform (1.04%) [17].

**Table 1: Physicochemical evaluation of S. Cordata**

| No | Parameters | % value |
|---|---|---|
| **1** | Loss on drying | 2.24±0.35 |
| **2** | Total ash | 8.73±0.25 |
| **3** | Water-soluble ash | 2.43±0.31 |
| **4** | Acid-insoluble ash | 3.16±0.21 |
| **5** | Sulphated ash | 16.33±0.34 |
| **6** | Nitrated ash | 14.57±0.41 |
| **7** | Carbonated ash | 16.97±0.28 |
| **8** | Chloroform extractive value | 1.04 % |
| **9** | Ethyl acetate extractive value | 1.33% |
| **10** | Ethanol extractive value | 6.34% |
| **11** | Acetic Acid extractive value | 6.66% |
| **12** | Methanol extractive value | 6.51% |
| **13** | Water extractive value | 12.34% |

**In-Vitro Anti-Inflammatory Evaluation**

The potential anti-inflammatory properties of the water and acetic acid extracts of *Sida cordata* were evaluated in vitro using a protein inhibition and denaturation assay [20, 24, 25]. However, the study showed a direct correlation between increases in the percentage of inhibition and increases in the concentration of plant extracts [22, 24]. The maximum inhibition rates were determined for the water extract, the acetic extract, and the standard drug, diclofenac sodium, at a concentration of 250 µg/ml; the results (Figure 6) were 86.55±0.63, 82.18±1.43, and 88.76±0.52, respectively. Based on the results, it appear that the water extract ($IC_{50}$ value- 51.21±5.82) has better (P>0.05 vs AAE) anti-inflammatory properties then the acetic acid extract ($IC_{50}$ value- 68.57±2.47) and is similar (P<0.05 vs standard drug) effective to the standard drug (showed in table 2), which means that this plant has good anti-inflammatory properties [25, 26]. As per the % inhibition linearity, the IC50 values of *Sida cordata* extracts and diclofenac sodium, the standard drug, are shown in Table 2 [27, 28]. A one-way ANOVA (Dunnett's test) is used to compare the groups.

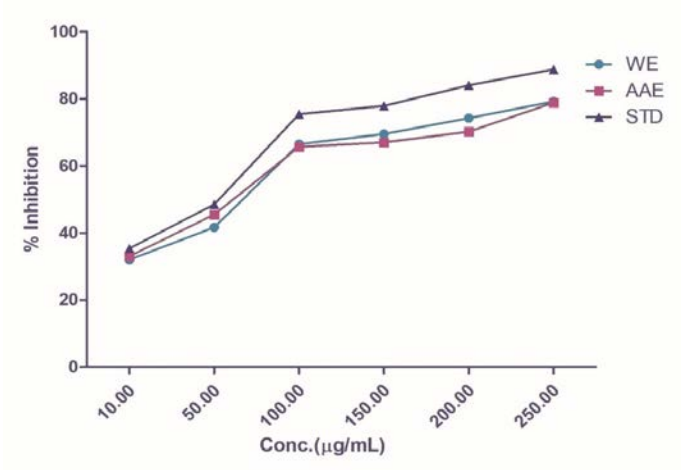


**Figure 6: The inhibition of protein denaturation. All values are presented as mean±SEM (n=3).**

**Table 2: $IC_{50}$ values of *Sida cordata* extracts and diclofenac sodium, where *P<0.05 vs STD, #P<0.05 vs WE.**

| SN | Test Drug | $IC_{50}$ value |
|---|---|---|
| **1** | Water Extract (WE) | 51.21±5.82 |
| **2** | Acetic Acid Extract (AAE) | 68.57±2.47*# |
| **3** | Diclofenac Sodium (STD) | 43.15±2.40 |

The albumin denaturation method is widely recognized as a reliable in vitro assay for evaluating the anti-inflammatory potential of extracts. Denaturation of proteins, particularly albumin, plays a key role in the inflammatory process by releasing inflammatory mediators. Thus, preventing protein denaturation is a helpful marker for anti-inflammatory activity [29]. In this study, *Sida cordata* exhibits significant anti-inflammatory activity, as evidenced by its ability to inhibit albumin denaturation in a dose-dependent manner. The maximum inhibitions noted at 250 µg/ml were compared with those of diclofenac sodium, a commonly used NSAID. This suggested that *Sida cordata* may have a mechanism of action similar to that of NSAIDs, possibly through inhibition of the cyclooxygenase (COX) enzyme or other inflammatory pathways. Because previous studies have shown that polyphenolic compounds inhibit inflammation by inhibiting the COX enzyme, and this plant contains polyphenols, its anti-inflammatory action may occur through the COX enzyme [8, 9, 30, 33]. The active compounds in *Sida cordata*, including alkaloids, flavonoids, glycosides, and saponins, are known to possess anti-inflammatory properties. These compounds may interact with the albumin protein structure, preventing its denaturation and, consequently, modulating the inflammatory response. The results of this study are consistent with previous

reports demonstrating the anti-inflammatory potential of *Sida cordata* across various experimental models [8, 9]. While the current study provides evidence of the anti-inflammatory activity of the plant extracts, further investigations are needed to elucidate the specific mechanisms involved. In vivo studies and the isolation of active compounds from *Sida cordata* would help in understanding the plant's pharmacodynamic and therapeutic potential [31, 32].

## *CONCLUSION*

The present study demonstrates that pharmacognostical analysis of the whole plant of *Sida cordata* was conducted to standardize and establish the plant identity. Macroscopic and microscopic characteristics of the plant were studied. Physicochemical parameters were determined, including ash and extractive values. Also, this plant exhibits significant in vitro anti-inflammatory activity, as evidenced by its ability to inhibit albumin denaturation. The results of the anti-inflammatory activity showed that the water extract had greater activity than the acetic acid extract and was comparable to that of diclofenac sodium. These findings support the addition of details regarding its identification and standardization for future research, including its traditional use in treating inflammatory conditions, and suggest its potential as a source of natural agents that reduce inflammation. Further studies, including in vivo testing and isolation of active constituents, are necessary to validate these results further and explore the clinical applicability of *Sida cordata* in the management of various types of inflammation.

## *FINANCIAL ASSISTANCE*

NIL

## *CONFLICT OF INTEREST*

The authors declare no conflict of interest.

## *AUTHOR CONTRIBUTION*

Devendra Kumar Sahu designed and interpreted the experiment's design and outcomes in relation to the work and performed the entire practical work. Alok Singh Thakur contributed to drafting the manuscript. The final manuscript was read and approved by all authors.

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