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In vitro evaluation of biological activities of aqueous extract of *Sida cordifolia*

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ABSTRACT

Introduction: Herbs are making a comeback, and the herbal renaissance is taking place all over the world. Medicinal plants continue to play an essential role in developing and emerging countries. Considering the chemical and therapeutic elements obtained in natural form from plants and plant extracts, they play an essential role in modern medicine.
Materials and Methods: The present study aims to evaluate the phytochemicals, antimicrobial and anti-inflammatory activity of the aqueous extract of *Sida cardifolia*.
Results: The phytochemical examination revealed the presence of alkaloids, steroids, coumarins, and glycosides in the root's aqueous extract. The antimicrobial activity against the Gram-negative organisms (that cause urinary and secondary infections) was performed. The aqueous extract showed maximum activity against *Pseudomonas* sp with a zone of inhibition of 28 mm and an almost similar zone size was measured against antibiotics Ciprofloxacin. The anti-inflammatory tests were also performed and the extract exhibited an appreciable presence of anti-inflammatory metabolites.
Conclusion: The test extracts inhibited protein (albumin) denaturation in a concentration-dependent manner, according to the current data. Secondary metabolites comprise a vast number of functional moieties that interact to create a broad range of biological functions. Many modern drugs are based on plants and plant-based compounds, and they are currently being used to treat various ailments.

1. Introduction

Sida cordifolia Linn. (*S. cordifolia*) is commonly known as 'bala' and is widely used in Ayurveda. It grows well through the plains of India, especially, in damp climates. It is also used in the traditional medicine systems in China, Brazil, and other countries for a wide range of illnesses.Bala has traditionally been used as an anti-rheumatic, antipyretic, analgesic, anti-asthmatic, laxative, diuretic, hypoglycemic, nasal decongestant, and pain relief in sciatica.[1] The root of *S. cordifolia* has been recently reported as a potential remedy to reduce the severity of Parkinsonism.[2] Other plant parts, including the leaves, stems, and seeds are also employed in traditional medicine for several medicinal purposes.[3]

Sida cordifolia grows well through the plains of India, especially, in damp climates. The shrub grows up to 0.75 – 1.5 meters in height. The root and the stem are stout and strong. The leaves are heart-shaped, 2.5-7 cm long, and 2.5-5 cm broad with 7-9 veins. The flowers are small, yellow or white, solitary, and axillaries. The fruits are moong-sized, 6-8 mm in diameter. The seeds are called Bijabanda in Ayurveda, are greyish black in color and smooth. The plant flowers from August to December and fruiting occurs from October to January.[4]

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It has a long history of use by Ayurveda and rural area, particularly for medicinal properties. It is in use as folk medicine in India since time immemorial. The herb is tonic, astringent, emollient, aphrodisiac, and beneficial in the treatment of respiratory system problems, according to Ayurveda. The bark is said to be cooling. It can help with blood, throat, and urinary system problems, piles, phthisis, and insanity, among other things.[5] The herb has anti-inflammatory, analgesic, and tonic properties. *Sida cordifolia* oils are used to the sore muscles and sore joints in rheumatism and arthritis with the crushed leaves can be carried out a cataplasm to alleviate local pains and because of its astringent value for the cure of external wounds or imperfections of the skin. Root juice is also used to promote the healing of wounds. Besides, it shows antiplaque and antifungal activities.

Sida cordifolia, with its ephedrine and pseudoephedrine, has gained a lot of interest and is now marketed. *Sida cordifolia* is used as a weight reduction product due to its hypoglycemic (blood sugar decreasing) effect, which may aid to minimise fat accumulation with fat cells.[6] *S. Cordifolia* appears to have anti-inflammatory qualities as well as the ability to boost pain tolerance. The anti-inflammatory efficacy of *Sida cordifolia* aerial and root extracts in ethyl acetate and alcohol extracts was dose-dependent. An antimicrobial evaluation has been done by using different extracts of *Sida cordifolia* against bacteria by the disc diffusion method. Dimethyl sulphoxide (DMSO), the solvent control, showed no effect against the tested bacteria and fungi.[7] Jaiswal investigated the use of botanicals in wound healing. Tissue healing is an important process that serves as the foundation for various surgical manipulations. It can be aided by a variety of herbal remedies.[8] Plants and their extracts have enormous promise for treating many types of wounds. The phytomedicines for wound healing are not only inexpensive, but also allegedly safe. However, there is a need for scientific validation, standardization, and safety evaluation of plants of the traditional medicine before these could be recommended for healing of the wounds. Pharmacological screening of botanicals is necessary for viewing new chemical entities in normal subjects, which is designed to search for novel drug actions at an early stage of drug development.

Drugs can control hazardous germs, resulting in the formation of different drug-resistant bacteria and frightening clinical conditions in the treatment of illnesses. Several novel antibiotics have been developed by the pharmaceutical industry; yet, microorganism resistance to these medications has grown. Bacteria, in general, have the genetic potential to transfer and acquire resistance to synthetic medicines used as therapeutic agents.[9] As a result, new infection-fighting tactics to manage microbial infections are required. This study aimed to evaluate the antimicrobial activity of *S. cordifolia* used in Ayurveda and traditional medicinal systems for the treatment of manifestations caused by a microorganism. In the present study, the phytochemical analysis of the aqueous extract along with its antibacterial and anti-inflammatory studies was performed.

Fig. 1: Sida cordifolia

1.1. Taxonomic hierarchy

1. Kingdom: Plantae
2. Subkingdom: Tracheobionta
3. Super division: Spermatophyta
4. Division: Magnoliophyta
5. Class: Magnoliopsida
6. Subclass: Dilleniidae
7. Superorder: Malvanae
8. Order: Malvales
9. Family: Malvaceae
10. Subfamily: Malvoideae
11. Genus: Sida L.
12. Species: Sida cordifolia L.

2. Materials and Methods

2.1. Collection and Identification of Sample

The root of *Sida cardifolia* was collected from Cochin Arya Vaidya Sala store, Ernakulam, Kerala. The root of the plant was washed thoroughly, cleaned and shade dried properly. The dried roots were grinded and stored in an airtight container for further use.

2.2. Extraction of aqueous extract of Sida cardifolia

About 20 g of powder of each tested plant material was soaked in 100 ml of distilled water in a round bottom

flask and heated for 30 min at 90°C, before the overnight incubation. After incubation, the soaked powder in the aqueous extract was filtered using Whatman filter paper. The extract was then stored at 4°C for further studies.

2.3. Evaluation of organoleptic characters

The organoleptic characters of the sample were evaluated based on the method described by Siddiqui et al., 1995.[10] Organoleptic evaluation refers to the formulation of colour, odour, and texture.

2.4. Phytochemical screening

The phytochemical analysis was carried out on aqueous extract of the root of *Sida cardifolia* using the standard procedure described by Sofowora, 1993;[11] Trease and Evans, 1989;[12] Harborne, 1973.[13] The presence of tannins, alkaloids, saponins, glycosides, Steroid, terpenoids, coumarins, and flavanoids were carried out.

Test for tannins: To 2ml of test solution add a few drops of 5% ferric chloride solution. The formation of blue–green colour indicated the presence of tannins.

Test for Saponins: 10ml of distilled water was added to the sample and mixed vigorously. The appearance of frothing which lasts more than 5 minutes indicated the presence of saponins.

Test for flavonoids: 2ml of 2% Sodium hydroxide was added to the test solution; a concentrated yellow colour was produced which decolourises after the addition of 2 drops of acid, indicating the presence of flavonoids.

Test for Steroids: To 5ml of the solution 2ml of chloroform and concentrated sulphuric acid was added. No red colour in the chloroform layer indicates the presence of steroids.

Test for Glycosides Liebermann's test: Crude extract was mixed with each of 2ml of glacial acetic acid and drops of ferric chloride. The mixture was cooled in ice. Carefully concentrated sulphuric acid was added. A brown ring was observed which indicated the presence of a steroidal nucleus, i.e., glycine portion of glycoside.

Test for alkaloids: A fraction of extract was treated with 3-5drops of Wagner's reagent [1.27g of iodine and 2g of potassium iodide in 100ml of water] and observed for the formation of a reddish-brown precipitate (or colouration) which indicates the presence of alkaloids.

Test for coumarins: 3 mL of 10% sodium hydroxide was added to 2 mL aqueous plant extract and yellow colour was observed in positive results.

Test for Terpenoids: 2.0 ml of chloroform was added with the 5 ml aqueous plant extract and evaporated on the water bath and then boiled with 3 ml of H_2SO_4 concentrated. A reddish-brown colour formed which showed the entity of terpenoids.

2.5. Antimicrobial assay

Test pathogens

The bacterial cultures used were Pseudomonas sp., Escherichia coli, and Klebsiella sp.

2.5.1. Agar-well diffusion method

The antibacterial activity of the extract was tested against the selected Gram-negative bacterial strains using agar well diffusion method.[14] The standard inoculums suspension (10^6CFU/ml) was streaked over the surface of the Sterile Muller Hinton Agar plates using a sterile cotton swab to ensure confluent growth of the organisms. The wells of 6 mm size were cut in the agar plates with the help of sterile cork borer and the wells were loaded with various concentrations of extract (50μl, 100μl and 150μl). The positive and negative controls were also used. All the plates were incubated at 37ºC for 24-48 hours. The zone of inhibition of bacterial growth was measured in millimeters and recorded. After incubation, the plates were observed for the formation of a zone of inhibition and the zone sizes were measured.

2.5.2. Disk diffusion test

The various antibiotic disc was used to study their action on the selected bacterial pathogens. The antibiotic disc used were Ampicillin, Amoxycillin, Gentamycin, Streptomycin, and Ciprofloxacin (30 μg each). The standard inoculums suspension (10^6CFU/ml) was streaked over the surface of the Sterile Muller Hinton Agar plates using a sterile cotton swab to ensure confluent growth of the organisms. The disc was then dispensed onto the respective plates. All the plates were incubated at 37ºC for 24 hours. The zone of inhibition of bacterial growth was measured in millimeters and recorded. After incubation, the plates were observed for the formation of a zone of inhibition and the zone sizes were measured.

2.6. Anti-inflammatory bioassay in-vitro

The anti-inflammatory test was done as described by Gunathilake et al., 2018[15] with slight modifications. The reaction mixture consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate-buffered saline (pH 6.4), and 2 ml of varying concentrations of the test extract, by which the concentrations were 100, 500μl. A similar volume of distilled water served as a control. Then the mixtures were incubated at 37°C ± 2°C in a biological oxygen demand incubator for 15 min and then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm. Diclofenac sodium was used as reference drugs respectively and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

% inhibition = 100 × (1- [A_2/ A_1]).

Where, A_2 = absorbance of the test sample, and A_1 = absorbance of control.

3. Results

3.1. Collection and Identification of sample

The *Sida cardifolia* plant Cochin Arya Vaidya Sala, Ernakulam and were shade dried, grinded, and stored in an airtight container.

Fig. 2: *Sida cardifolia* (root)

3.2. Evaluation of organoleptic characters

Organoleptic characters were macroscopically investigated and were tabulated (Table 1).

Table 1: Organoleptic characters of *Sida cordifolia* root

S. No	Physical state	Property
1	Colour	Dark Brown
2	Odour	Non aromatic
3	Texture	Hard

3.3. Phytochemical screening

Qualitative phytochemical analysis was performed and the results are tabulated. (Table 2).

Table 2: Preliminary phytochemical analysis (Figure 3)

S. No.	Test	Result
1	Test for tannins	-
2	Test for Flavanoids	-
3	Test for alkaloids	+
4	Test for Steroids	+
5	Test for Coumarins	+
6	Test for Glycosides	+
7	Test for Terpenoids	-
8	Test for Saponins	-

+ Positive, - negative

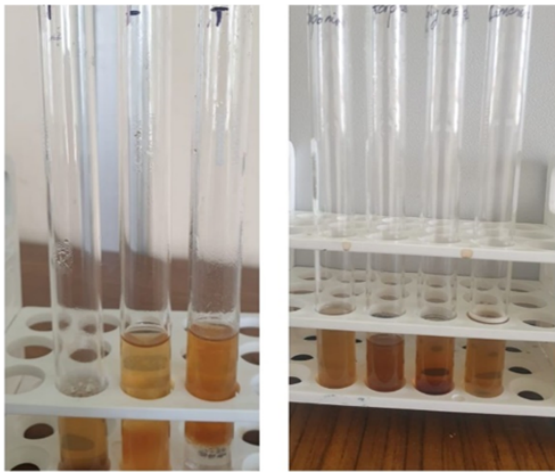

Fig. 3: Phytochemical studies

3.4. Antimicrobial assay

3.4.1. Well diffusion assay

The aqueous extract showed excellent antibacterial activity against selected Gram-negative organisms which are the main causative agents of urinary tract infections and secondary infections. The maximum zone of inhibition of 33 mm (100 μl) was measured for Pseudomonas sp compared to Klebsiella sp. which showed a zone of inhibition of 13 mm (100 μl) (Table 3, Figure 4).

Table 3: Antibacterial activity [zone of inhibition (mm)] of the extract

S. No.	Bacterial Strain	Zone of Inhibition (mm)	
		50 μl	100 μl
1	Escherichia coli	R	23 mm
2	Klebsiella sp	R	13 mm
3	Pseudomonas sp	24 mm	28 mm

3.4.2. Disk diffusion test

The zone of inhibition of various antibiotic disks against selected pathogens was measured and recorded.

3.5. Anti-inflammatory Bioassay In-vitro

The in-vitro anti-inflammatory test was performed for the test. In the present study, the In-vitro anti-inflammatory effect of *Sida cardifolia* against the denaturation of egg albumin was performed. The present findings exhibited a concentration-dependent inhibition of protein (albumin) denaturation by the test extracts.

4. Discussion

Plants and plant extracts have an important role in modern medicine as their chemical and medicinal constituents are found in natural form. Many contemporary medications that

Table 4: Antibiotic susceptibility [zone of inhibition (mm)] of the antibiotic disk

S.No.	Test organisms	GEN	AMP	CIP	AMX	S
1	*Escherichia coli*	14 mm	7 mm	26 mm	10 mm	14 mm
2	*Klebsiella sp*	12 mm	8 mm	22 mm	13 mm	7 mm
3	*Pseudomonas sp*	13 mm	R	28 mm	R	R

GEN- Gentamycin, AMP – Ampicillin, CIP – Ciprofloxacin, AMX – Amoxicillin, S – Streptomycin

Table 5: Percentage of inhibition rate of protein denaturation

S.No.	Concentration of extract (μl)	Percentage inhibition
1.	100	55%
2.	500	66%

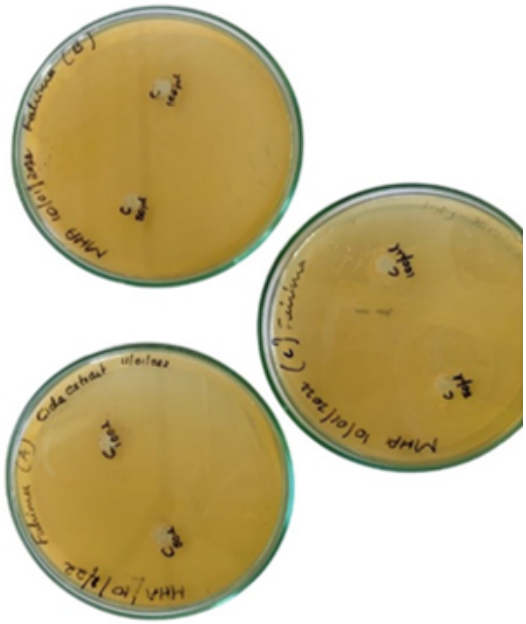

Fig. 4: Well diffusion assay

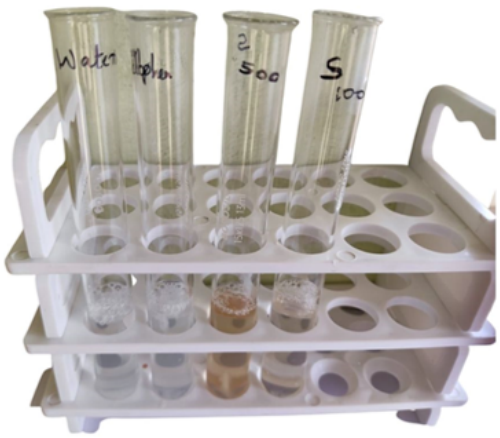

Fig. 5: Anti-inflammatory test

are now in use for various ailments are based on plants and plant-based products. Several Sida species, generally known as "Bala," have been shown to have analgesic, anti-inflammatory, hypoglycemic, and hepatoprotective properties. The present study was performed to obtain aqueous extracts of *Sida cardifolia* root, to assess the organoleptic and qualitative characters, and to check its antibacterial activity and anti-inflammatory activity.

The aqueous extraction of the root was done. Similar extractions were performed by Aswathy and Sushma, 2019[16] and they reported that among the extracts tested maximum result was observed in water and alcohol extract of Sida cordifolia, L. followed by chloroform, petroleum ether, and benzene. Acetone extract of Sida cordifolia produced the poorest results. The factors influencing solvent selection include the amount of phytochemicals to be extracted, the rate of extraction, the diversity of different compounds extracted, the diversity of inhibitory compounds extracted, the ease of subsequent extraction handling, the toxicity of the solvent in the bioassay process, and the potential health hazard of the extract. Based on the qualitative phytochemical analysis carried out in this study the presence of alkaloids, steroids, coumarins, and glycosides were detected in the aqueous extract of the root. They are normally produced by plants as an evolutionary adaptation to harsh environments or in response to attacks by other organisms.[17] However, substances generated as secondary metabolites have been identified to accidentally give antimicrobial protection to people. Numerous plants extract components as well as phytochemicals that present antioxidant / free radical scavenging properties.[18,19] Secondary metabolites associated with medicinal plants provide a protection mechanism against predation through quite a number of microbes, insects as well as herbivores.[20] Secondary metabolites are a big reservoir of structural moieties that interact to demonstrate a wide range of biological functions.

The phytochemical analysis was performed wherein the presence of alkaloids, steroids, coumarins, glycosides were detected in the aqueous extract of the root. Secondary metabolites are a big reservoir of structural moieties that

interact to demonstrate a wide range of biological functions.

In the current study, the antimicrobial activity against the Gram-negative organisms (that causes urinary and secondary infections) were performed. The aqueous extract showed maximum activity against Pseudomonas sp with a zone of inhibition of 28 mm and almost similar zone size was measured against antibiotics Ciprofloxacin. The action of various antibiotics was also studied against the selected pathogens. The aqueous extract showed maximum activity against Pseudomonas sp with a zone of inhibition of 28 mm and an almost similar zone size was measured against antibiotics Ciprofloxacin. The action of various antibiotics was also studied against the selected pathogens. The Phytochemical screening of the extracts of Sida cordifolia revealed that these compounds have significant application against human pathogens, including those that cause enteric infections.[21] According to a study conducted by Mahesh and Satish, 2008,[22] the Methanol leaf extracts of Acacia nilotica, Sida cordifolia, Tinospora cordifolia, Withania somnifera, and Ziziphus mauritiana were examined against some bacterial species, by disc diffusion method, which displayed significant antimicrobial activity. Recently in a study conducted by Mradu et al., 2020, they reported that the aqueous extract could be directly responsible for its antimicrobial, anti-leucorrhoeal, and astringent properties. The rejuvenating action of Bala (Sida cordifolia Linn.) extends to the nervous, circulatory, and urinary systems. It has a diuretic effect, is useful in urinary problems, and is also used in inflammations, bleeding disorders being cooling, and astringent.

Sida cordifolia[23] had potent anti-inflammatory compounds and was tested using standard methods. In the present study, the In-vitro anti-inflammatory effect of *Sida cardifolia* against the denaturation of egg albumin was performed. The test extracts inhibited protein (albumin) denaturation in a concentration-dependent manner, according to the current data. Protein denaturation has an unexpected process that includes changes in electrostatic hydrogen, hydrophobic, and disulfide bonding.[24] Protein denaturation results in the generation of autoantigens in inflammatory disorders such as rheumatoid arthritis, cancer, and diabetes. As a result, inflammatory activity can be reduced by inhibiting protein denaturation.[25] The effect of F. racemosa extracts at high concentrations on albumin denaturation was much greater than that of reference medicines. The present findings exhibited a concentration-dependent inhibition of protein (albumin) denaturation by the test extracts. Thus studies should be persued to analyse the quantitative estimation of detected phytochemicals. Further test can be performed to study the exact mechanism of action for its pharmacological properties over its antimicrobial and anti-inflammatory effects.

Previously, the effect of different plant parts on protein denaturation has been evaluated by many scientists, for example, Semecarpus anacardium bark on bovine albumin, an ethanolic extract of Wedelia trilobataon bovine albumin, Albucas etosaon egg albumen.[15] Hence, by inhibition of protein denaturation, inflammatory activity can also be inhibited.

By this study, it was confirmed that the selected plant species is a potent source of useful drugs. However, further studies are required in this direction for its comprehensive analysis including qualitative or semi qualitative analysis, characterizing its chemical structure, and assess its biological activities

5. Conclusion

Medicinal plants are recognized to be an indispensable source of natural secondary metabolites with potential therapeutic effects. In recent years the natural metabolites from herbal components have attained much attention from their chemical counterpart because of their minimal side effects and low cost. In the present study, *Sida cardifolia* plant roots were collected and the aqueous extract was taken for further studies.

Plants and plant-based products are bases of many modern pharmaceuticals that are currently in use for various diseases. The plant-based bio-active compounds have an effective dosage response with minimal side effects when compared to the synthetic compounds. The presence of phytochemicals (secondary metabolites) is responsible for their therapeutic effects. It also expresses hope for the creation of many more unique therapeutic agents or templates from such plants, which may one day be used to produce synthetically better therapeutic compounds. It is very necessary to introduce new and biologically safe and active drugs for an eco-friendly lifestyle. Phytochemicals found present in the Sida cordifolia, L. indicate their potential as a source of principles that may supply novel medicines.

6. Source of Funding

None.

7. Conflict of Interest

None.

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