Comparative Analysis of three Sida Species: Antimicrobial, Phytochemical and Antioxidant Properties

Dr. P. Mohan[1], V. C. Mounika[2], P. Kavya Sree[3], B.M. Jyoshna[4], Dr. P. Sandya Priya[5]
[1, 5]. Lecturer, Dept. of Biotechnology, S. V. Arts College, TTD, Tirupati, Andhra Pradesh, India
[2,4] Student, Dept. of Biotechnology, S. V. Arts College, TTD, Tirupati, Andhra Pradesh, India
[3]. Student Dept. of Biotechnology, Krishna Teja College, Tirupati, Andhra Pradesh, India

***Abstract*—In the present study the methanol and chloroform leaf extracts of *Sida acuta*, *Sida spinosa* and *Sida cordifolia* were screened for the presence of phytochemical components and tested for antibacterial activity against *Bacillus, Streptococcus* and *Escherichia coli*. Plant extracts were obtained using methanol and chloroform through Soxhlet extraction. Solubility tests, qualitative and quantitative phytochemical analysis were conducted. Result revealed the presence of alkaloids, terpenoids, phenols, saponins, tannins, steroids, carbohydrates and proteins. The study indicated that the phytochemical constituents of three species of *Sida* are different. It was concluded that *Sida* plants have a significant antimicrobial activity and highlighting their potential for developing natural antimicrobial agents.**

***Index Terms*—Antimicrobial Activity, Disc diffusion, Sida acuta, Sida cordifolia, Sida spinosa, Solubility Test.**

I. INTRODUCTION

Sida acuta, Sida spinosa, and Sida cordifolia belong to the Malvaceae family (Jain A et al.,2011), and are traditionally used to treat various ailments, including fever, skin diseases, diarrhoea and respiratory disorders (Karou SD et al., 2007 & Sharma D et al., 2023).

Plants used in the present study

Sida acuta

Also known as "Broom Weed", is a perennial herb or shrub found in subtropical regions (Nagarajan et al.,2022). It's rich in phytochemicals like alkaloids, saponins, and flavonoids (Ezeabara et al., 2008). Traditionally, it's known for its antimalarial, anti-inflammatory, and anticancer properties (Banzouzi JT et al., 2002 & Nieto-Camacho A et al., 2016).

Sida spinosa

Also known as "Prickly Sida" or "Nagabala" "Prickly Mallow (Khilari et al., 2018),, is a perennial plant commonly found in India (Sharma D et al.,2023). It's traditionally used to treat various conditions, including gonorrhea, respiratory issues, fever, and wounds (Deshavath et al.,2012,). The plant contains phytochemicals like alkaloids, flavonoids, and saponins, and has shown antibacterial, antifungal, antidiabetic, and antioxidant properties (Selvadurai et al., 2011).

Sida cordifolia

Also known as "Bala" in Ayurveda, is a perennial subshrub native to tropical America but found in tropical regions worldwide (Sharma AK et al., 2015). It's used in Ayurveda anssd Siddha medicine to treat respiratory and urinary disorders (Jain A et al.,20111& Shetu HJ et al., 2019). The plant contains phytochemicals like alkaloids and flavonoids (Ghosal S et al., 1975 & Ranjani Sivapalan S.2015) and has shown anti-inflammatory, anti-ulcer, anticancer, antibacterial and antioxidant properties (Kumar S et al.,2006 & Subramanya MD et al., 2015).

Sida plants are widely accessible and have therapeutic and antioxidant properties, making them important for research. (Auddy B,. et al., 2003) With their traditional use in Ayurveda and Siddha medicine, further pharmacological studies could lead to new breakthroughs. Comparing Sida plants helps understand their chemical and pharmacological properties, enabling the development of new medicines. The most beneficial plant can be selected to formulate effective treatments (Singh S et al., 2009).

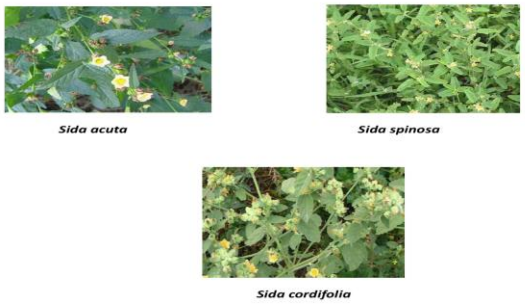

Fig- 1: Plants used in the present study

Target Microorganisms

Escherichia coli

Escherichia coli (E. coli) is a gram-negative, rod-shaped bacterium that can live with or without oxygen (Basavaraju M et al., 2022). It naturally inhabits the intestines of warm-blooded animals, including humans. Most strains are harmless and even beneficial, aiding digestion and producing Vitamin K2.

Staphylococcus aureus

Staphylococcus aureus (S. aureus*)* is a gram-positive, spherical bacterium that forms grape-like clusters, measures about 0.8-1 diameter and are non-motile, non-sporing *(R. Ananthanarayan et al., 2005*). It's typically harmless but can cause serious infections like bloodstream infections, pneumonia, and food poisoning if it enters the body through cuts, wounds, or medical procedures.

Bacillus sp.

Bacillus sp. is a gram-positive, aerobic, rod-shaped bacterium *(R. Ananthanarayan et al.,2005*). Commonly found in soil, dust, water, and air. While it can cause foodborne illnesses, it's also used industrially for probiotics and bioremediation *(Bharati KA. et al., 2016)*

II. MATERIALS AND METHODS

A. Collection of plant material

Sida acuta and Sida cordifolia plants were collected from Padmavathi Mahila University, Tirupati, Andhra Pradesh and Sida spinosa plant was collected from the dry lands behind Lakshmi Nagar, Renigunta Road, Tirupati, Andhra Pradesh, according to their morphological and physical attributes.

B. Preparation of leaf extracts

The leaves of *S. acuta, S. cordifolia* and *S. spinosa* were washed, shade-dried for 3 days, powdered using a mortar and pestle, and finely sieved to obtain fine particles

i. Preparation of Aqueous leaf extracts

10g of plant powder was mixed with 100ml of sterile distilled water, heated at 60°C for 5 minutes, cooled, filtered through paper and Whatman No. 1 filter paper, and then refrigerated at 4°C.

ii. Preparation of Methanol leaf extracts

5g of plant powder was extracted using a Soxhlet apparatus (Bhatnagar & Gopala Krishna, 2013), with 150ml of methanol at 64.7°C for 4 hours. The extract was collected, evaporated, and the dried contents were scraped, sealed, and stored for further analysis.

iii. Preparation of Chloroform leaf extracts

5g of plant powder was extracted with 150ml of chloroform in a Soxhlet apparatus at 61.2°C for 4 hours. The extract was collected, evaporated, cooled, and the dried contents were scraped and sealed for future analysis.

C. Anti-microbial activity assay by disc diffusion method

Antimicrobial efficacy was assessed using the disc diffusion method. 20 ml of sterile Muller Hinton Agar was poured into sterile Petri plates and allowed to solidify. Bacterial cultures were swabbed on plates, sterile filter paper discs containing the plant extract were then placed on the inoculated plates. Control discs containing only the solvent were also included and plates were incubated at 37°C for 24 hours. If the extract has anti-microbial properties, it will inhibit microbial growth, forming a clear zone around the disc. After incubation the diameter of the inhibition zone was measured in millimeters which evaluate the anti-microbial effect of that particular extract.

D. Phytochemical analysis

Preliminary phytochemical screening of the extract was conducted using standard procedures outlined by Brain and Turner Brain, *(K.R and Turner, T.D. 1975*) and Evans *(Evans, W. C. 1996*). The extracts underwent qualitative analysis to detect the presence of various phytochemical constituents, including Alkaloids, flavonoids, steroids, terpenoids, anthraquinones, phenols, saponins, tannins, carbohydrates, proteins and amino acids, oils and resins, following the methods *(Harborne JB. et al., 1973 & Shaikh, et al., 2020)*

Test for Alkaloids

For alkaloid detection,

Mayer's reagent: 1ml of leaf extract was mixed with 1ml of Mayer's reagent; a yellow precipitate indicated a positive result.

Wagner's reagent:1ml of extract was treated with 1ml of Wagner's reagent (iodine solution). A reddish-brown precipitate confirms the presence of Alkaloids.

Test for Flavonoids

Lead acetate test: A yellow precipitate formed upon adding lead acetate to the extract indicated the presence of flavonoids.

H_2SO_4: An orange color formed with H_2SO_4 indicated the presence of flavonoids.

Test for steroids

A violet-to-blue-green ring formed after adding acetic anhydride and sulfuric acid to the extract, indicating steroids.

Test for Terpenoids

Salkowski's test: A brown ring at the interface after adding chloroform and sulfuric acid indicated the presence of terpenoids.

Test for Anthraquinones

Borntrager's test: The extract turned pink after treatment with HCl, chloroform, and ammonia, indicating anthraquinones.

Test for Phenols

Ferric chloride: A bluish-black color formed with ferric chloride, indicating phenolic compounds.

Test of Saponins

The extract was shaken with 5ml of distilled water.

Test of Tannins

A small amount of plant extract was combined with water and gently heated in a water bath. After filteration, a few drops of ferric chloride solution were added to the clear filtrate. The formation of a dark green coloration confirmed the presence of tannins.

Test of carbohydrates

The extract was dissolved in 5ml of distilled water and filtered. “The filtrate was subjected to carbohydrate detection by treating it with concentrated H_2SO_4 and ninhydrin”. A violet ring indicated the presence of carbohydrates.

Test for Protein and Amino Acid

Biuret test: A violet color formed after adding NaOH and copper sulphate, indicating protein.

Ninhydrin test: About 0.5 mg of extract was treated with two drops of freshly prepared 0.2% ninhydrin solution and then heated. The appearance of pink to purple colour indicates the presence of proteins, peptides, or amino acids.

Test for Oils and Resins

A portion of the extract was applied to filter paper. The appearance of a translucent or and greasy spot suggested the presence of oils and resins.

Solubility test

A solubility test determines a compounds ability to dissolve in a solvent usually in liquids at equilibrium temperature *(Majeed F et al., 2022*) Common solvents used include water, ethanol (99%), methanol (99%), chloroform (99%), acetone (99%), petroleum ether (99%), di-methyl-sulfoxide (DMSO) (99%), 1N diluted HCl, 1N diluted NaOH and oils such as olive oil (100%) and coconut oil (100%). This test is used for compounds insoluble in water *(Silva RL et al., 2006).*

E. Quantitative Test for Phytochemical constituents

The Phyto chemical constituents quantified in all three plant species.

i. Determination of total Alkaloid content

1mg of plant extract was dissolved in DMSO, mixed with 1ml of 2N HCl, filtered, and transferred to a separating funnel. Subsequently, 5ml of bromocresol green solution and 5ml of phosphate buffer were added. The mixture was vigorously shaken with multiple aliquots of chloroform (1-4ml). The chloroform layers were collected and pooled into a 10ml volumetric flask, and the final volume was adjusted with chloroform. A series of standard atropine solutions (20,40,60,80, and100ug/ml) were prepared using same protocol. Absorbance was measured at 470nm using a UV-Vis spectrophotometer. The alkaloid content was expressed as milligrams of atropine equivalent per gram of extract (mg AE/g) *(Shamsa F et al., 2008)*

ii. Determination of total Flavonoid content

The total flavonoid content was estimated using aluminum chloride colorimetry, starting with 1ml of plant extract mixed with 4ml of distilled water. To these, 0.3ml of 5% sodium nitrate solution was added, and after 5min 0.3ml of 10% aluminum chloride solution was introduced. Following another 5-minutes incubation, 2ml of 1M sodium hydroxide was added, and the final volume was made up to 10ml with distilled water. Quercetin standards (20-100 µg/ml) were prepared similarly. The absorbance of both

standard and test solutions was recorded ant 510nm against a reagent blank using a UV-Visible spectrophotometer. Flavonoid content was calculated and expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g), *(Sulaiman CT et al.,2012).*

iii. Determination of total tannins content

Tannin content was estimated using the Folin-Ciocalteu method, mixing 0.1 ml of plant extract mixed with10ml volumetric flask containing 7.5ml of distilled water,0.5ml of folin-ciocalteu phenol reagent, and 1ml of 35% sodium carbonate solution. The final volume was adjusted to 10ml with distilled water. The mixture was thoroughly was shaken and incubated at 30 C for 30minutes.Standard solutions of gallic acid (20,40,60,80, and 100ug/ml) were prepared as reference standards. Absorbance was measured at 725nm using a UV-Vis spectrophotometer, with a blank as reference. The tannin content was expressed in a term of mg of gallic acid equivalents per gm of extract (mg GAE/g extract*) (Aswal BS et al., 1984*).

iv. Determination of total Phenolic Content:

The concentration of phenolic compounds in the plant extract was determined spectrophotometrically using the Folin-Ciocalteu method. The reaction mixture included1 ml of the extract and 9ml of distilled water, to which 1ml of Folin-Ciocalteu reagent was added. The solution was mixed thoroughly and allowed to react for 5minutes, followed by the addition of 10 ml of 7 % Na2CO3 solution. The volume was adjusted to 25ml with distilled water. A calibration curve was generated using standard gallic acid solutions (20-100 µg/ml). After Incubating the reaction mixture for 90 min at 30 C, absorbance was recorded at 550nm using a UV- Visible spectrophotometer. Total phenolic content was calculated and expressed as mg of gallic acid equivalents per gram of extract (mg GAE/g extract) *(Ali S and Mustafa M. 2017).*

F. Antioxidant Activity: Hydrogen Peroxide Scavenging Assay

Antioxidant potential of plant extracts is commonly evaluated through in vitro assays that measured their capacity to neutralize free radicals and reduce oxidative stress. “Among the widely used methods are the DPPH(2,2-Diphenyl-1-picrylhydrazyl) assay, ABTS (2,2’-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) assay”, and hydrogen peroxide(H2O2) scavenging assay. These tests assess antioxidant activity by tracking the reduction of a coloured reagent or inhibition of radical-mediated reactions.

The hydrogen peroxide scavenging assay specifically evaluates the ability of a sample to neutralize H2O2, a reactive oxygen species known to contribute to cellular damage. This method, the sample is incubated with hydrogen peroxide, and the decreasing H2O2 concentration is measured-typically via UV spectrophotometry. The percentage of scavenged H2O2 reflects the antioxidant strength of the test sample.

A 2mm hydrogen peroxide solution was prepared using 0.2M phosphate buffer (pH 7.4). To make the buffer, 50ml of 0.2M potassium dihydrogen phosphate was mixed with 39.1ml of 0.2M sodium hydroxide in a200ml volumetric flask. The mixture was then diluted to the final volume with distilled water to obtain a buffer solution at pH 7.4 an equal volume (50ml) of this buffer was mixed with 50ml of hydrogen peroxide solution and left at room temperature for 5minutes to allow free radical formation. To evaluate the antioxidant activity, 100ul of plant extract at various concentrations (50, 100, 150, 200, 250 and 200 ug/ml in distilled water) was added to 600 ul of the prepared hydrogen peroxide solution. The mixture was incubated for 30 minutes in the dark. “Absorbance was then measured at 239nm using a UV-Vis spectrophotometer”. A standard reference solution of 800 ug/ml was prepared by dissolving to mg of Ascorbic acid in 2.5ml of distilled water. From these, serial dilutions matching the test concentrations (50 to 300ug/ml) were prepared. Distilled water was used as a blank. All measurements were carried out in triplicate.

Scavenging activity was calculated using the formula:

H2O2 Scavenging% = (A Blank - A Sample) / A Blank × 100

The percentage inhibition values were plotted against concentration to determine the IC_{50} values.

III. RESULTS AND DISCUSSION

Anti-microbial Activity of plant extracts

Anti-microbial assay showed that *Sida cordifolia* exhibited anti-microbial activity against *E. coli* and *S. aureus* from the aqueous extract. *Sida acuta* showed notable activity against *Bacillus* in the aqueous extract.

Sida spinosa displayed relatively limited activity against *Bacillus* in aqueous extracts.

Qualitative Analysis of Phytochemicals

The following were the results of the qualitative phytochemial analysis from both chloroform extracts and methanolic extracts.

S.No	Phyto constituent	*Sida spinosa*		*Sida cordifolia*		*Sida acuta*	
		Chloroform extract	Methanolic extract	Chloroform extract	Methanolic extract	Chloroform extract	Methanolic extract
1.	Alkaloids	+	+	+	_	+	_
2.	Flavonoids	_	_	_	_	_	_
3.	Steroids	+	_	_	_	_	_
4.	Terpenoids	_	+	_	_	_	_
5.	Anthraquinones	_	_	_	_	_	_
6.	Phenols	_	+	_	_	+	_
7.	Saponins	_	_	_	_	_	_
8.	Tannins	+	_	_	_		_
9.	Carbohydrates	+	_	_	_	+	_
10.	Proteins/ Amino acids	+	+	_	_	+	_
11.	Oils/resins	_	_	_	_	_	_

Table 1: Qualitative Analysis of the Phytoconstituents

Solubility Test

Solubility test was performed on the components which were immiscible in the water. Methanolic components and chloroform components of Sida Cordifolia and Sida Acuta were immiscible in the water. When tested with different solvents, both the components were completely miscible in 99% chloroform and partially soluble in 99% Acetone. When the concentration of the solvent was decreased, the components were shown to be immiscible.

Quantification of Phytochemicals

The total content of alkaloids, flavonoids, phenols and tannins of *S. acuta, S. cordifolia, S. spinosa* were represented in the following table. Alkaloid contents in the *Sida Spinosa* were least at the 1.40 mg/ml, Flavonoid contents of *Sida cordifolia* was higher 0.47 mg/ml. The phenolic components are high in *Sida acuta* with 0.89 mg/ml. The Tannins were seen higher in the *Sida Spinosa* with 1.30 mg/ml.

S. No	Phyto Constituent mg/ml	*Sida Spinosa*	*S. cordifolia*	*S. acuta*
1.	Alkaloids	1.40	2.0	2.0
2.	Flavonoids	0.39	0.47	0.39
3.	Phenols	0.70	0.70	0.89
4.	Tannins	1.30	0.60	0.96

Table- 2: Quantitative analysis of Phytochemicals

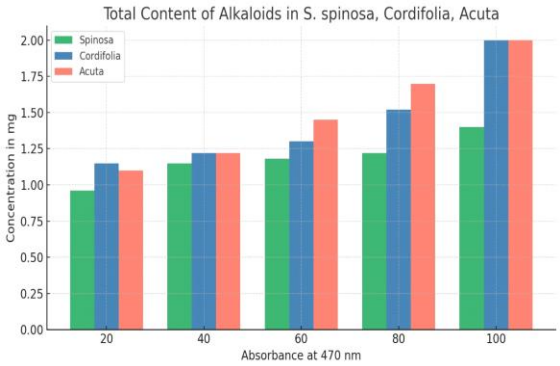

Figure- 2: The total content of Alkaloids in *S. acuta, S. cordifolia, S. spinosa*

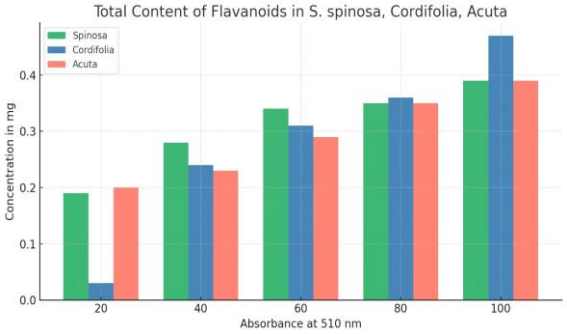

Figure- 3: The total content of Flavonoids in *S. acuta, S. cordifolia, S. spinose*

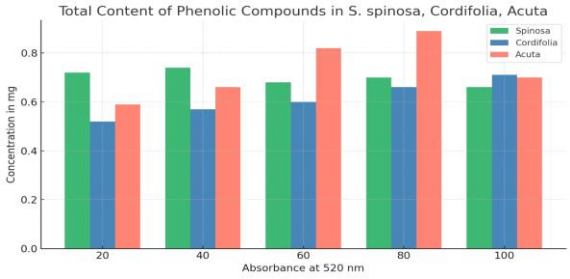

Figure-4: The total Content of Phenols in *S. acuta, S. cordifolia, S. spinosa*

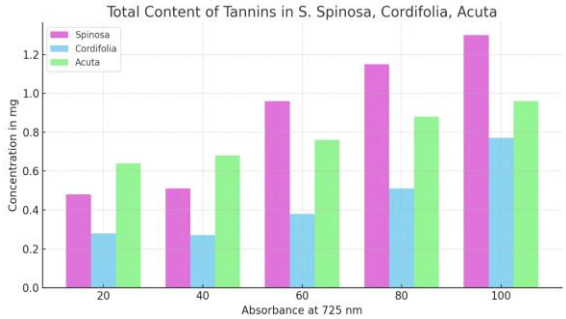

Figure- 5: The total content of Tannins in *S. acuta, S. cordifolia, S. spinosa*

Anti-oxidant activity of H2O2 Scavenging:

The Aqueous extract of *Sida spinosa* was evaluated for its antioxidant potential using the hydrogen peroxide (H_2O_2) scavenging assay. At a concentration of 100 mg/ml, the extract exhibited 48.2% scavenging activity. This indicates a moderate ability of the extract to neutralize hydrogen peroxide and reduce oxidative stress.

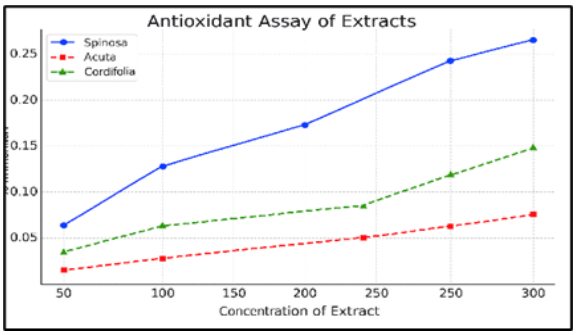

Figure- 6: The Antioxidant Assay of S. Acuta, S. Cordifolia, S. Spinosa

IV. CONCLUSION

Medicinal plants are crucial in preventing various diseases. The anti-inflammatory, anticancer, antiviral, antibacterial and antifungal activities of the medicinal plants were due to presence of secondary metabolites. The present research focused on the anti-microbial properties, qualitative and quantitative Phyto chemical analysis and antioxidant abilities of the plants *Sida acuta, Sida spinosa, and Sida cordifolia*. The selected three medicinal plants are the source of the phytochemical mixtures of alkaloids, terpenoids, phenols, carbohydrates, and proteins/amino acids. The extracted bioactive compounds varied in the three species. Of the 2 different solvents used methanolic extract of S. spinosa showed maximum number of phytochemical constituents. S. acuta, S. cordifolia, and S. spinosa showed varying antimicrobial and antioxidant properties, with S. acuta having extensive potential, S. cordifolia strong potential, and S. spinosa exhibiting moderate antimicrobial and strong antioxidant activities. The research supports the scientific acceptance of traditional medicine, gives a base for new plant-based medicines, and helps select suitable plants for particular treatments by using a comparative method on the *Sida* genus. So, before using natural compounds in medicine, it is necessary to keep researching their safety, standardize how they are made, learn how they function, develop formulas for them and conduct clinical research.

REFERENCES

[1] Jain A, Choubey S, Singour PK, Rajak H, Pawar RS. (Apr 2011). Sida cordifolia (Linn) -- An overview. J Appl Pharm Sci.;1(2):23--31. Available from: https://imsear.searo.who.int/handle/123456789/150743

[2] Karou SD, Nadembega WMC, Ilboudo DP, Ouermi D, Gbeassor M, De Souza C, Simpore J. (2007) Sida acuta Burm. f.: a medicinal plant with numerous potencies. Afr J Biotechnol.;6(25):2953-2959.

[3] Sharma D, Mishra R, Saluja MS. (2023) A brief overview of the ethnomedical, pharmacological, and phytochemical uses of *Sida spinosa*. Int J Med Pharm Drug Res.;7(5):15--9. Available from: https://aipublications.com/ijmpd/detail/a-brief-overview-of-the-ethnomedical-pharmacological-and-phytochemical-uses-of-sida-spinosa/

[4] Nagarajan, Srinivasan & Murali, R. (2022). Article Info An overview of the traditional importance, phytochemistry, and pharmacological properties of Sida acuta Burm.f. Annals of Phytomedicine 11(2): 245-254, 2022 Annals of Phytomedicine: An International Journal http://www.ukaazpublications.com/publications/index.php. Annals of Phytomedicine: An International Journal. 11. 10.54085/ap.2022.11.2.27.

[5] Ezeabara, Chinelo & Egenti, Miracle. (2018). Phytochemical and antimicrobial investigations on various parts of Sida acuta Burm. f. Journal of

Ayurvedic and Herbal Medicine. 4. 71-75. 10.31254/jahm.2018.4206.

[6] Banzouzi JT, Fall D, Bories C, et al.(2002). Studies on medicinal plants of Ivory Coast: investigation of *Sida acuta* for in vitro antiplasmodial activities and identification of an active constituent. *J Ethnopharmacol*.;81(3):305--9.

[7] Nieto-Camacho A, Ramírez-Apan T, Hernández-Ortega S, Álvarez-Toledano C, Gómez E, Chans GM. (2016) Anti-hyperglycaemic, antioxidant, and anti-inflammatory activities of extracts and metabolites from *Sida acuta* and *Sida rhombifolia*. *Quím Nova*. 2016;40(2):215--21. https://doi.org/10.21577/0100-4042.20160182

[8] Khilari, Shobha & Pawar, Dr & Bhosle, dr. pooja. (2018). Pharmacognostical and Phytochemical Evaluation of Root of Sida Spinosa Linn. The Global Journal of Pharmaceutical Research. 7. 176-181. 10.20959/wjpr201812-12461.

[9] Deshavath, Narendra & Bastipati, Suresh & Priyanka, Mohana & Yamini, Karanam & Malothu, Ramesh. (2012). Formulation and evaluation of herbal ointment consisting of *sida spinosa* leaves extracts. 2. 40-42.

[10] Selvadurai, s & Senthamarai, Rathinam & Sri, Tarinee & Vijaya Kirubha, T. & Krishnan, Vasuki. (2011). Anitimicrobial activity of ethanolic extract of the whole plant of Sida Spinosa Linn. (Malvaceae). J. Nat. Prod. Plant Resour. 1.

[11] Sharma AK. (Dec 2015) Medicinal properties of Bala (Sida cordifolia Linn. and its species). Int J Ayur Pharma Res. 13;1(2):1--9. Available from: https://ijapr.in/index.php/ijapr/article/view/40

[12] Shetu HJ, Nur D, Akter F, Zahin N, Dash PR. (2019). Pharmacological and phytochemical importance of Sida cordifolia: A review. Int J Life Sci Rev.;5(4):60--73. Available from: https://ijlsr.com/bft-article/pharmacological-and-phytochemical-importance-of-sida-cordifolia-a-review/

[13] Ghosal S, Chauhan RBPS, Mehta R. (1975) Alkaloids of *Sida cordifolia*. *Phytochemistry*.;14(5):830--2.

[14] Ranjani Sivapalan S. (2015). Phytochemical study on medicinal plant -- Sida cordifolia Linn. Int J Multidiscipline Res Dev.;2(1):216--220. Available from: https://www.allsubjectjournal.com/archives/2015/vol2/issue1/2-1-53

[15] Kumar S, Prasad S, Prasad S, et al. (2006) Evaluation of the antioxidant activity of Sida cordifolia. *Phytotherapy Research*.;20(12):1036--41. doi: 10.1002/ptr.2029.

[16] Subramanya MD, Pai SR, Upadhya V, Ankad GM, Bhagwat SS, Hegde HV. (2015) Total polyphenolic contents and in vitro antioxidant properties of eight Sida species from Western Ghats, India. J Ayurveda Integer Med.;6(1):24--28. doi: 10.4103/0975-9476.146544.

[17] Auddy B, Hazra B, Mitra A, et al. (2003) Screening of antioxidant activity of three Indian medicinal plants traditionally used for the management of neurodegenerative diseases. *J Ethnopharmacol*.;84(2--3):131--8.

[18] Singh S, Singh M. (2009) Effect of temperature, light and pH on germination of twelve weed species. *Indian Journal of Weed Science*.;41(3&4):113--26.

[19] Basavaraju M, Gunashree BS. (11 Nov 2022). Escherichia coli: An Overview of Main Characteristics. In: Starčič Erjavec M, editor. Escherichia coli - Old and New Insights. London: IntechOpen;. p. 1--24. Doi: 10.5772/intechopen.105508. Available from: https://www.intechopen.com/chapters/84764

[20] R. Ananthanarayan, C.K. Jayaram Paniker(2005). Staphylococcus (Textbook of Microbiolgy, fifth edition (Pg. No. 177-185).

[21] R.Ananthanarayan, C.K. Jayaram Paniker Bacillus (textbook of microbiology, fifth edition (Pg.No. 222).

[22] Bharati KA. (Oct 2016). Identification of Indian Sida through mericarp. *Pharmacognosy Journal*.;8(5):490--6. doi: 10.5530/pj.2016.5.14.

[23] Brain, K.R and Turner, T.D. (1975) The Practical Evaluation of Phytopharmaceuticals 9780856080128 https://books.google.co.in/books?id=jXZxQgAACAAJ. Wright-Scientechnica

[24] Evans, W. C. (1996). Trease and Evans' Pharmacognosy (14th ed.). W.B. Saunders Company Ltd

[25] Harborne JB. (1973). *Phytochemical methods: a guide to modern techniques of plant analysis*. 2nd ed. London: Chapman & Hall.

[26] Shaikh, Junaid & Patil, Matsyagandha. (2020). Qualitative tests for preliminary phytochemical screening: An overview. International Journal of Chemical Studies. 8. 603-608. 10.22271/chemi.2020.v8.i2i.8834.

[27] Majeed F, Anjana JC. (2022) In vitro evaluation of biological activities of aqueous extract of Sida cordifolia. Indian J Microbiol Res.;9(2):124--130. doi: 10.18231/j.ijmr.2022.022.

[28] Silva RL, Melo GB, Melo VA, Antoniolli AR, Michellone PR, Zucoloto S, et al. (2006). Effect of the aqueous extract of Sida cordifolia on liver regeneration after partial hepatectomy. Acta Cir Bras.Silva RL, Melo GB, Melo VA, Antoniolli AR, Michellone PR, Zucoloto S, et al. et al. Effect of the aqueous extract of Sida cordifolia on liver regeneration after partial hepatectomy. Acta Cir Bras.

[29] Shamsa F, Monsef H, Ghamooshi R, Verdian-Rizi M. (2008). Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. *Thai J Pharm Sci*.;32(1):17--20.

[30] Sulaiman CT, Balachandran I. (2012) Total phenolics and total flavonoids in selected Indian medicinal plants. *Indian J Pharm Sci*.;74(4):258--60.

[31] Aswal BS, Bhakuni DS, Goel AK, Kar K, Mehrotra BN, Mukherjee KC. (1984). Screening of Indian plants for biological activity: Part X. Indian J Exp Biol.

[32] Ali S and Mustafa M. (2017) Synthesis and characterization of zinc oxide nanoparticles using zinc acetate dihydrate and sodium hydroxide. Academia.edu; Available from: https://www.academia.edu/34116182/Synthesis_and_Characterization_of_Zinc_Oxide_Nanoparticles_using_Zinc_Acetate_Dihydrate_and_Sodium_Hydroxide