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THERAPEUTIC POTENTIAL AND CHARACTERIZATION OF *SENNA ALATA*: AN ETHANO-MEDICINAL PLANT

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Keywords:

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ABSTRACT: *Senna alata* is an effective novel pharmaceutical medicinal plant. The hot extraction method was used to prepare methanol and ethanol extracts of leaves. The extracts were further evaluated to study Antimicrobial potential, phytochemical analysis, FTIR, and Antioxidant activity. The FTIR analysis showed various functional groups that served as secondary metabolites and responsible for antimicrobial activity. The antimicrobial activity of the extract may be due to the presence of secondary metabolites like alkaloids, terpenoids, phenols, saponins, flavonoids, glycosides and tannins. The *Senna alata* leaves were evaluated for antimicrobial activity against clinically important bacterial strains such as *E. coli, B. cereus, B. subtilis, S. aureus, P. aeruginosa, P. fluorescens,* and fungi strain namely *A. niger* by agar well diffusion method. The ethanolic extract of the leaves of *Senna alata* showed the highest antioxidant activity. *S. alata* has a great antimicrobial ability which needs to be intensively discussed further.

INTRODUCTION: Plants have played an important role in human lives. Since, ancient times, about 80% of the world's population depends solely on traditional remedies for their health care needs. The 7000-8000 plant species are used today because of the presence of some biologically active and naturally occurring phytochemicals present in them, which protect them from pollution, stress, and drought, as well as pathogenic microorganisms. The herbal medicines are safer and more effective [1]. In tracking down new antimicrobial compounds from plants, the world's researchers still have the thrust.

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Senna alata, a traditional medicinal plant used for this study, has been very successful in many human ailments and is involved in the therapeutic of several forms of skin infections, intestinal parasitosis, syphilis and haemorrhage [2]. *Senna alata* (previously named *Cassia alata*) is a medicinal plant of the Leguminosae family. It has many common names such as Candle bush, Emperor Candlestick, Christmas candle, Acapulo, Ringworm bush and Calabra bush.

It is a shrub with usually an average height of between 1 and 5 meters and has horizontally spread branches. Its leaves are par pinnate of between 30 to 60 cm long and consisting of 8 to 20 pairs of leaflets. Each leaflet is oblong or elliptic-oblong and rounded at both ends. Its flowers are dense in auxiliary racemes, about 20 to 50 cm long and 3 to 4 cm broad. The inflorescence looks like a yellow candle. The plant fruits are thick, flattened with wings and glabrous pods [3].

Plants are considered to be an essential source of secondary metabolites, primarily phenolic compounds such as fistucacidin, bioflavonoids, triflavonioids, rhein glucosides, sennosi de A and B, chrysophenol and physionom isolated from leaves Kaempferol, tetramer of leucopelargonidine with free glycol complex, bianthraquinone glycoside, rhein, fistulin, alkaloids, flowers triterpenes and bark oxyanthraquinone and dihydroxyanthraquinone from the bark [4]. *S. alata* has a high therapeutic value as an antipyretic, analgesic, antioxidant and anti-inflammatory, hypoglycaemic. It has been used in the treatment of various disorders such as leucoderma, rheumatism, skin diseases, tuberculosis, eye and liver ailments [4, 5]. The bioactivity of the alcoholic extracts of *S. alata* leaves has been reported to have fungistatic activity against some mycotic organisms such as *T. mentagrophytes, Microsporum gypseum* and *Epidermophyton floccosum* [5].

The detection of a particular compound is typically accomplished by combining many physiochemical methods such as ultraviolet spectroscopy (UV), gas chromatography-mass spectrometry (GC-MS), circular dichroism spectroscopy (CD), thin layer chromatography, optical rotation, Fourier Transform Infrared (FTIR) spectroscopy, nuclear magnetic resonance spectroscopy (NMR) and liquid chromatography [6].

Polyphenolic compounds tend to be active free radical scavengers and their ability to serve as antioxidants depends primarily on their chemical structure, their capacity to donate/accept electrons, thus delocalizing the unpaired electron inside the aromatic structure and the polyphenols are commonly categorized into two categories: flavonoids and phenolic acids [7].

MATERIAL AND METHODS:

Collection of Plant Material: The fresh, disease-free leaves of *S. alata were* collected from different locations of Nashik city, Maharashtra, India. The authentification of the plant material was confirmed in Dept. of Botany K. R. T. Arts, B. H. Commerce and A.M. College, Nashik. The plant material was first washed under running tap water and then in sterilized water. The leaves were shade dried in a dark room. The dried leaves were then homogenized using mortal and pestle into a fine powder and stored in an airtight glass container at 4 ºC until future use.

Preparation of Plant Extracts: The twenty-five gram of dried powder of *S. alata* leaves was taken into a cotton thimble separately and extracted with 250 ml of solvents of Methanol and Ethanol using soxhlet apparatus for 6-8 h. The solvent was then evaporated to dryness using a rotary vacuum evaporator to obtain only the dried extract. The final quantity of the extracts was measured, and double dilution method was used for the preparation of concentrations of the extract. The extracts were dissolved in DMSO to make final concentrations of 200 mg/ml.[8]

Pathogenic Organisms: The cultures of pathogenic microorganisms *viz Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Aspergillus niger, Pseudomonas aeruginosa,* and *Pseudomonas fluorescens* for antimicrobial activity were obtained from Dept. of Microbiology, K. R. T. Arts, B. H. Commerce, and A. M. College, Nashik, Dist. Nashik, Maharashtra, India. The cultures were sub-cultured and stored in the refrigerator at 4 ºC until further use.

Preparation of Inoculum: The inoculum of each isolate was prepared using McFarland Specifications. According to CLSI recommendations, the inoculum size was established using the $BaSO_4$ and sulfuric acid turbidity standard equal to 0.5 McFarland, which contained $2x\ 10^8$ CFU/ml. The loopful of microbial culture was suspended in Mueller Hinton broth and incubated at 37 ºC for 24 h and after incubation; dilutions of inoculums were prepared to obtain the equivalent optical density [9]. The fungal isolate was grown on Sabouraud dextrose agar (SDA) at 25 ºC until sporulation of fungal spore occurred. The spores were harvested and scraped using a glass rod. The harvested spores were standardized to an OD of 0.1 and 1 microliter of standardized fungal spore suspension was evenly spread on SDA medium using spreader [10].

Antimicrobial Activity of *S. alata:* The antimicrobial assay was performed using the technique of agar well diffusion as per the CLSI guidelines. The sterile Mueller-Hinton agar plates were prepared, and the inoculum was spread evenly on the agar medium surface using sterile cotton swab

aseptically. The 6 mm cork borer was made to sterilize with alcohol and was further used to prepare wells on the seeded Muller-Hinton agar plates for bacteria and for fungal isolates SDA (Sabouraud Dextrose Agar) medium was used. The double dilution technique was used for the preparation of concentrations of the extracts and in each well 25 µl of the extracts was added. The plates were then placed in the refrigerator for 30 min. for the diffusion of the extract into the agar plates. The plates were incubated at 37 °C for 24 h the plates containing fungal culture were incubated at 25 °C for 36 h. The inhibition zones (mm) were measured. The entire procedure was carried out in triplicate and in sterile conditions in Laminar Air Flow [11].

Determination of MIC and MBC of the *S. alata* Extract: The Minimum Inhibitory Concentration Assay is a technique used to determine the extract's lowest concentrations, which is needed to kill the bacteria. The MIC of the methanol and ethanol leaf extracts was determined for each test organism in triplicate. The concentrations of the extracts were prepared in the range of 1.625, 3.125, 6.25, 12.5, 25, 50, 100, and 200 mg/ml. The 1 ml of the extracts at double strength concentrations were added to test tubes containing 1ml of Mueller Hinton(MH) broth for the bacteria and potato dextrose broth for the fungi.

A loopful of the test organisms previously diluted to 0.5 McFarland turbidity standards for bacterial isolates and 10^6 spores/ml for fungal isolates was introduced to the respective tubes. The procedure was repeated using the standard antimicrobials Streptomycin and Griseofulvin instead of the extracts. A tube containing Muller Hinton broth only was seeded with the test organisms to serve as a negative control. Tubes containing bacterial cultures were then incubated at 37 °C for 24 h while tubes containing fungal spore cultures were incubated at 25 °C for 36 h. After incubation, 0.2% phenyl tetrazolium salt was added to the tubes to observe the colour of the tubes, and MIC value was noted. To determine minimum bactericidal (MBC) and minimum fungicidal concentrations (MFC), a loopful of broth which did not show any growth in MIC determination was taken, and streaks it on sterile Mueller Hinton agar plates for bacterial cultures and for Fungal cultures SDA plates were used. To serve as a control, Mueller Hinton agar and SDA culture medium were streaked with the respective test organisms. Plates were then incubated at 37 °C for 24 h for bacterial cultures and for fungi were incubated at 25 °C for 48 h. After incubation, the concentration at which no visible growth was seen was recorded as the MBC and MFC, respectively [12].

Phytochemical Analysis of *S. alata* Extracts: *S. alata* leaf extracts were screened for alkaloids, saponins, tannins, phenols, flavonoids, terpenoids, steroids, cardiac glycosides, and reducing sugars as per standard protocol.

Alkaloids: To the 10ml of methanol, 200 mg of the plant extract was added; it was boiled and filtered after cooling. To that filtrate 1% HCL was added, followed by a few drops of Dragendorff reagent.

Saponins (Frothing test): 5 ml of the distilled water and 0.5 mg of the plant extract were shaken vigorously for 2 min in the test tube.

Tannins: To the 200 mg of plant extract, 10 ml of distilled water is added, the mixture is boiled and filtered after cooling. Few drops of $FeCl_3$ are added to the filtrate.

Polyphenols: To 5 ml of the extract, 1ml of $FeCl_3$ (1%) and 1ml of K_3 (Fe (CN)$_6$ (1%) were added.

Flavonoids: 5 ml of dilute ammonia, followed by few drops of concentrated H_2SO_4, was added to the plant extract.

Terpenoids (Salkowski test): 2 ml of $CHCl_3$ and 3 ml of concentrated sulphuric acid (H_2SO_4) were added to 200 mg of the plant extract.

Steroids (Liebermann-Burchard Reaction): 10 ml of chloroform and acetic anhydride was added in the ratio 1:1 to 200 mg of the plant extract.

Cardiac Glycosides: 1 ml of glacial acetic acid containing a few drops of $FeCl_3$was added to 2 mg of the plant extract. The above mixture is treated with concentrated sulphuric acid (H_2SO_4).

Reducing Sugars: Few drops of Fehling's solution A and B were added to the plant extract.

The phytochemical tests were confirmed using standard procedures with characteristic color changes and precipitation reactions [8, 13].

FTIR Analysis of *S. alata* Extracts: The methanolic and ethanolic extracts of leaves were centrifuged at 3000 rpm for 10 min and then centrifuged samples were filtered using Whatman No.1 filter paper. The filtrate was then diluted with the respective solvents with a ratio of 1:10. The FTIR analysis of the sample was done using the Shimadzu IR spectrophotometer system to identify the signature peaks and their functional groups [14].

Antioxidant Activity of *S. alata* Extracts DPPH Assay: The 1, 1 – diphenyl – 2 – picryl - hydrazyl (DPPH) scavenging assay was used for the determination of antioxidant activity of methanolic and ethanolic extracts of leaves of *S. alata*. The absolute ethanol was used in preparation of sample and 0.1 ml of sample was added in same volume of DPPH solution. After 30 min of incubation in the dark at room temperature, the absorbance was taken at 520nm using a spectrophotometer. Ascorbic acid was used as standard control. All tests were run in triplicate and averaged [15].

The percentage scavenging was calculated using the following formula,

DPPH scavenging activity (%) = ($A°_{520}$ control - $A°_{520}$ sample / $A°_{520}$ control) ×100

RESULTS AND DISCUSSION:

Collection of Plant Material: The healthy and fresh leaves of *S.alata* were collected from different locations of Nashik District, Maharashtra.

FIG. 1: LEAVES OF *S. ALATA*

Preparation of Plant Extract: The methanolic and ethanolic extracts of leaves were prepared and used for further procedure.

FIG. 2: SOXHLET EXTRACTION OF *S. ALATA*

Antimicrobial Activity of *S. alata* Extracts: Leaf extracts of *S. alata* with different solvent systems showed antimicrobial properties against different Gram-positive and Gram-negative bacteria, including different fungus [16].

The methanolic and ethanolic extracts of leaves of *S. alata* possess secondary metabolites which showed antimicrobial activity against pathogenic microorganism. The highest zone of inhibition of methanolic extracts was shown against *A. niger* with a zone of inhibition 22 mm followed by *P. aeruginosa* (21 mm), *E. coli* (19 mm), *S. aureus* (18 mm), *B. subtilis* (18 mm), *P. fluorescence* (17 mm), *B. cereus* (15 mm).

The highest zone of inhibition of ethanolic extract of leaves of *S. alata* was observed against *A. niger* with zone of inhibition 20 mm, followed by *B. subtilis* (19 mm), *S. aureus* (19 mm), *E. coli (*18 mm), *B. cereus* (17 mm), *P. aeruginosa and P. fluorescence* (14 mm).

The Streptomycin and Griseofulvin were taken as a positive control, and methanol and ethanol served as a negative control for the respective extract [17;] the results are summarized in **Table 1.**

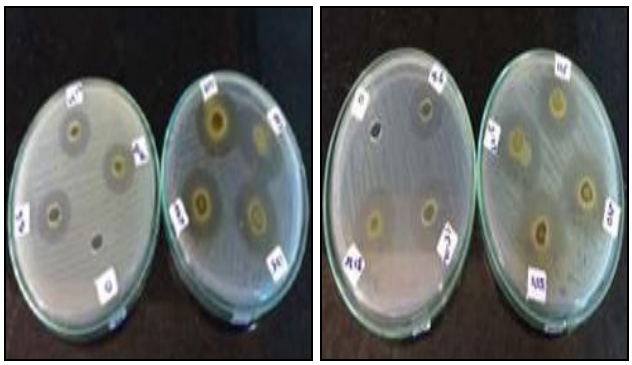

FIG. 3: ANTIMICROBIAL ACTIVITY OF EXTRACTS OF *S. ALATA*

TABLE 1: ANTIMICROBIAL ACTIVITY OF EXTRACTS OF *S. ALATA* AGAINST PATHOGENIC MICROORGANISM

Test m-Microorganism	Methanolic Extract	Ethanolic Extract	Positive Control		Negative Control
	Zone of Inhibition (mm)	Zone of Inhibition (mm)	Streptomycin	Griseofulvin	DMSO
E. coli	19±1.35	18±19.0	22±0.75	ND	0
P. aeruginosa	21.10±0.84	15.40±0.6	21.7±0.87	ND	0
B. subtilis	18.0±0.25	19.43±0.12	20.0±0.25	ND	0
B. cereus	15.20±0.73	17.10±0.37	20.0±0.74	ND	0
S. aureus	18.25±0.18	19.0±0.75	21.60±0.67	ND	0
P. fluorescence	17.14±0.95	14.0±0.45	19.0±1.50	ND	0
A. niger	22.0±0.55	20.2±0.37	ND	23.24±1.20	0

ND: Not Determined

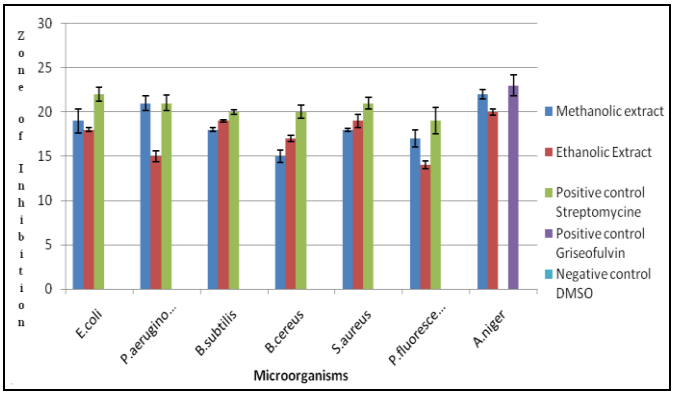

FIG. 4: ANTIMICROBIAL ACTIVITY OF EXTRACTS OF *S. ALATA*

Determination of MIC and MBC Values of the Extract: The MIC value of methanolic extract and ethanolic extracts for *A. niger* was determined for the concentrations to range from 1.625 to 200 mg/ml. The results are summarized in **Table 2**. The MIC value for a methanolic extract for *A. niger* was 12.5 mg/ml and for an ethanolic extract for *A. niger* was 6.25 mg/ml. The MBC values of the extracts were determined by observing growth on cultural media plates. The MBC value for the extracts was as shown in **Table 3.** The MBC value for the methanolic extract for *A. niger* was found to be 10.42 mg/ml and 4.46 mg/ml respectively [11].

FIG. 5: MIC VALUES OF EXTRACT

TABLE 2: MIC VALUES OF EXTRACTS OF *S. ALATA*

Extracts	MIC	
	Concentrations of the extracts(mg/ml)	Turbidity
Methanolic (*A. niger*)	200	-
	100	-
	50	-
	25	-
	12.5	-
	6.25	+
	3.125	+
	1.625	+
Ethanolic (*A. niger*)	200	-
	100	-
	50	-
	25	-
	12.5	-
	6.25	-
	3.125	+
	1.625	+

+: presence, - : absence

TABLE 3: MBC VALUES OF EXTRACTS OF *S. ALATA*

Extracts	MBC	
	Concentrations of the Extracts(mg/ml)	Growth
Methanolic (*A. niger*)	12.5	-
	11.36	-
	10.42	-
	9.61	+
	8.93	+
	8.33	+
	7.81	+
	7.35	+
	6.94	+
	6.58	+
	6.25	+
Ethanolic (*A. niger*)	6.25	-
	5.86	-
	5.21	-
	4.81	-
	4.46	-
	4.17	+
	3.91	+
	3.68	+
	3.47	+
	3.29	+
	3.125	+

+: presence, - : absence

Phytochemical Analysis of *S. alata Extracts*: The Phytochemical screening of extracts of *S. alata* was carried for the presence of alkaloids, flavonoids, carbohydrate, protein, saponins, terpenoids, tannins, anthraquinones, and cardiac glycosides. The phytochemical constituents of extracts differed considerably depending on the solvent used for the extraction of bioactive components, and the results are presented in **Table 4.** Methanolic extract showed the presence of alkaloids, flavonoids, glycosides, phenols, steroids, and tannins. The ethanolic extract showed the presence of secondary metabolites such as alkaloids, flavonoids, glycol-sides, phenols, saponins, steroids, terpenes and tannins. Such findings showed that the presence of various secondary metabolites in different extracts may be attributed to the solubility of phytochemical compounds in different solvents and are the key factors are responsible for the antimicrobial activity of plant extracts [2, 12].

TABLE 4: PHYTOCHEMICAL ANALYSIS OF *S. ALATA* EXTRACTS

S. no.	Phytochemical Components	Methano Lextract	Ethanol Extract
1.	Alkaloids	+	+
2.	Tannins	+	++
3.	Terpenoids	_	+
4.	Cardiac Glycosides	+	+
5.	Steroids	+	+
6.	Flavonoids	++	+
7.	Saponins	_	+
8.	Polyphenol	+	+
9.	Reducing sugar	+	+

FTIR Analysis of *S. alata* Extracts: The Fourier Transform Infrared Spectroscopy (FTIR) identifies chemical bonds in a molecule by producing an infrared absorption spectrum. The bioactive compounds of *S. alata* of methanolic and ethanolic plant extracts were identified by using FTIR spectrophotometer [1].

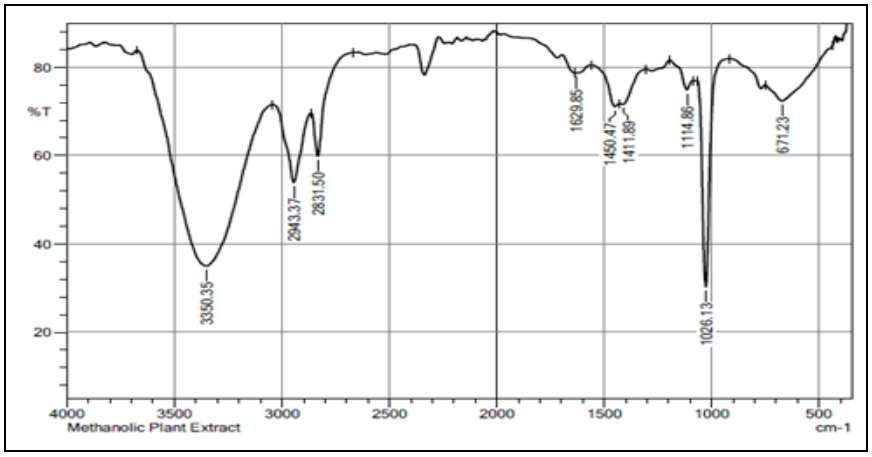

FIG. 6: FTIR ANALYSIS OF METHANOLIC EXTRACT OF *S. ALATA*

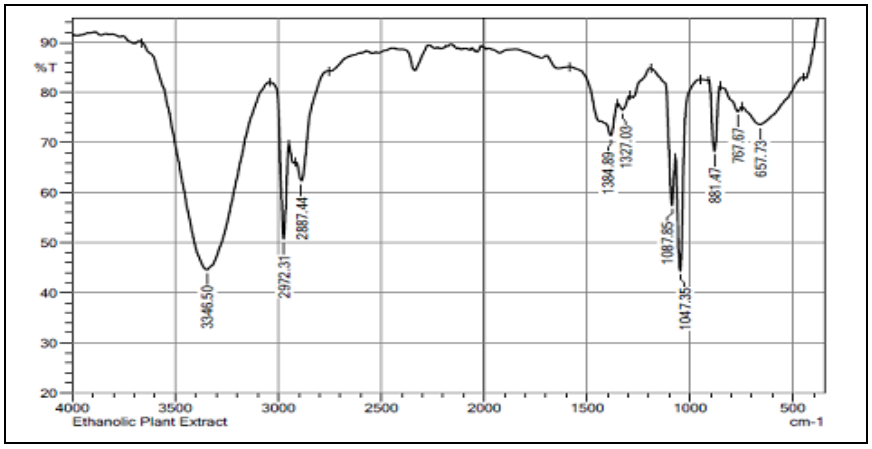

FIG. 7: FTIR ANALYSIS OF ETHANOLIC EXTRACT OF *S. ALATA*

The FTIR analysis of a methanolic extract of leaves of *S.alata* as shown in **Fig. 6** showed total 9 peaks. The IR spectrum band showed an absorption band at wavelength of 671.23cm^{-1}, 1026.13 cm^{-1}, 1114.86cm^{-1},629.85cm-1, 2831.50cm^{-1}, 2943. 37cm^{-1} and 3350. 35cm^{-1} indicated the presence of

aromatic C-Cl isothiocyanate, sulfones, aromatic, isocynates, alkanes, alkanes, and carboxylic acid functional groups respectively. The FTIR analysis of ethanolic extract of leaves of *S.alata* as shown in **Fig. 7** showed total 10 peaks. The IR spectrum band showed an absorption band at the wavelength of 657.73 cm^{-1}, 881.47cm^{-1}, 1047.37cm^{-1}, 1384.89cm-2887.44cm^{-1}, 2972.31cm^{1} and 3346.50 cm^{-1} indicate the presence of C-I, C-Cl halogen compound (Chloro compound), N-H primary and secondary amines, C-N stretching aliphatic amines, N=O bend nitro group, H-C=O stretching aldehydes, C-H alkanes, N-H stretching amines and amides, functional groups respectively [13].

Anti-oxidant Activity of *S. alata* Extracts: The antioxidant activity of *S. alata* leaf extracts was calculated using the capacity of the secondary metabolites to reduce the radical scavenging function free DPPH by pairing of an odd electron with hydrogen which turns the purple color of DPPH into yellow.

The percentage of antioxidant activity of extracts was increased with an increase in the extract concentration. The methanolic extracts of leaves showed the highest scavenging activity at a concentration of 200 mg/ml, while the lowest was found at 12.5 mg/ml. The ethanolic extract showed the highest scavenging activity at a concentration of 200 mg as compared to methanolic extract; this may be due to flavonoids, phenols, alkaloids and tannins in the leaves of the plant. Compared with the standard ascorbic acid, the wide range of antioxidant activity shown by extracts may be due to the wide variety of bioactive compounds [14].

TABLE 5: SCAVENGING ACTIVITY OF *S. ALATA* EXTRACTS

Extracts	Conc. (mg/ml) %	Scavenging Activity	Scavenging Activity of Ascorbic Acid
Methanolic	200	25.92	75.63
	100	23.57	54.29
	50	20.18	38.29
	25	16.07	33.47
	12.5	13.84	20.98
Ethanolic	200	45.92	75.63
	100	22.57	54.29
	50	17.18	38.29
	25	14.07	33.47
	12.5	10.84	20.98

CONCLUSION: The antimicrobial activity methanolic and ethanolic leaf extracts of *S. alata* were determined. Both the extracts showed highest zone of inhibition against *a .niger*. The MIC and MBC values are also determined for *A. niger*. The phytochemical analysis showed the presence of various secondary metabolites, which are responsible for the antimicrobial properties of the extracts. The FTIR evaluation of the extracts was carried out to determine the presence of functional gp. The ethanolic extract showed the highest antioxidant activity as compared to methanolic extract. Now a day's drug resistance acquired by microorganisms causes a major threat to the public so more focus needs to be paid in using medicinal plants with antimicrobial properties. The present study indicates that the plant can be used to treat GIT, UTI, and wound infections as well as to treat fungal infections. Further work to be done to identify the active component of *S. alata* used in the preparation of the drug.

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CONFLICTS OF INTEREST: Nil

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