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PHYTOCHEMICAL CHARACTERIZATION AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF *Aerva lanata* L.

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ABSTRACT

In the present study was to investigate the physicochemical analysis, phytochemical screening, histochemical, UV-Visible analysis and antimicrobial activity of *Aerva lanata* L. flower extract. The phytochemical screening *Aerva lanata*L. flower showed that the presence of tannin, saponins, terpenoids, flavonoids, alkaloids, triterpenoids, protein, anthroquinones, polyphenol and glycosides whereas phlopatannins was absent in methanol and aqueous extracts. Steroids present only methanol extract and carbohydrate present only aqueous extracts. Quantitative analysis revealed that the plant has of phenol(332mg/gm),flavonoids(170mg/gm), tannin (85mg/gm) and terpenoids (73.20mg/gm) were presented The histochemical analysis further confirmed in the presence of flavonoids, tanninand saponin. The fluorescence behavior of leaves powder proved by this study. The result of UV-VIS spectroscopic analysis confirms the presence of phenolic compounds in the *Aerva lanata*L. flower extract. The results reveal that extract of *Aerva lanata*L. flower were significantly effective against bacteria species *E. coli*and fungi species *C. albicans*

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INTRODUCTION

Research regarding medicinal plant is a highlighted issue today. Medicinal plants are the nature's gift of human being to make disease free healthy life. It plays a vital role to preserve our health. Plants synthesize an array of chemical compounds that are not involved in their primary metabolism. These 'secondary compounds' instead serve a variety of ecological functions, ultimately to enhance the plant's survival during stress. In addition, these compounds may be responsible for the beneficial effects of fruits and vegetables on an array of health related measures (Dahanukar, 2000).Medicinal plants are the nature's gift to human being to make disease free healthy life. It plays a vital role to preserve our health. India is one of the most medico-culturally diverse countries in the world where the medicinal plant sector is part of a time-honoured tradition that is respected even today. Hence, the main traditional systems of medicine include Ayurveda, Unani and Siddha (Kotnis *et al.*, 2004).

Plant and plant products play a wide range of biological properties.Use ofplants as a source of medicine has been inheritedand is an important component of the health caresystem. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. Keeping in view, the present study was carried out to investigate the phytochemical analysis of *Aerva lanata*flowers.

MATERIALS AND METHODS

Collection of plant materials

The flowers of *Aerva lanata* L. were collected in December 2017 from Thanjavur, Tamil Nadu, India. The *A. lanata*L. flowers were washed several times with distilled water to remove the traces of impurities from the flowers. Flowers was spread out in a plain paper and shade dried at room temperature for about 10 days and makes a fine powder using grinder mixture. The powder materials were used for further studies.

Preparation of plant extract:

2 gram of the powder of *A. lanata*L. flowers were transferred into different conical flask (250ml). The conical flask containing 50ml of different solution (methanol and water). The conical flask containing *A. lanataL.* flowers were shakeit well for 30 minutes by free hand. After 24 hrs, of the extract were filtered using whatman filter paper No.1 and filtrateused for further analysis.

Phytochemical screening

Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973 and 1984).

Quantitative analysis of phytochemicals

Total phenols estimated by the method of Edeoga *et al.*, (2005) Flavonoid determine by the method of Bohm and Kocipai-Abyazan (1994) Tannin determination by method of Van-Burden and Robinson (1981) Total terpenoid content in the leaf extracts were assessed by standard method (Ferguson, 1956).

Qualitative analysis of Vitamins (Pearson, 1976; Patel, 2005). Determination of Fluorescence behavior of plant powder (Rao *et al.*, 2011)

Histochemical tests

A small quantity of dried and finely powdered leaves sample wasplaced on a grease free microscopic slide and treated with specific chemicals and reagentsandwaited for 1-2 minutes.Apositive test for histochemicals was indicated by the appearance of the appropriate colour change afterapplication of the reagent. Using a light microscope to observeand record any colour changes. The leaf powder treated with diluted ammonia and H_2SO_4 gave yellow colour indicates flavonoids. Plant powder treated with ferric chloride to give Dark blue to black indicates the presence of tannin. Plant powder treated with H_2SO_4 (few drops) to give yellow colour indicates the presence of Saponin.

UV-Visible analysis

The extracts were examined under visible UV-Visible spectrum. The sample is dissolved in same solvent. The extracts were scanned in the wavelength ranging from 340-960 nm using Systronic Spectrophotometer. These solutions were scanned in turn at intervals of 10 nm and the characteristic peaks were detected. The peak value of the UV-Visible was recorded.

Determination of antimicrobial activity

The antimicrobial activity was performed by disc diffusion method. Antibiogram was done by disc diffusion method (NCCLS, 1993; Awoyinka *et al.*, 2007) using plant extracts. Petri plates were prepared by pouring 30 ml of NA /PDA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab is dipped into a standardized bacterial/ fungi test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plate. Briefly, inoculums containing bacteria specie were spread on Nutrient agar plates and fungus strains were spread on potato dextrose agar. Using sterile

forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (50µl) were laid down on the surface of inoculated agar plate. The plates were incubated at 37ºC for 24 h for the bacteria and at room temperature (30±1) for 24-48 hr. for yeasts strains. Each sample was tested in triplicate. The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the samples were measured using a millimeter scale.

RESULTS AND DISCUSSION

In the present study was carried out on the *Aerva lanata*L. flower revealed the presence of medicinally active constituents. The phytochemical characters of the *Aerva lanata*L. flower investigated and summarized in Table-1 and Plate 2 and 3.The phytochemical screening *Aerva lanata*L. flower showed that the presence of tannin, saponins,terpenoids,flavonoids, alkaloids,triterpenoids,protein, anthroquinones,polyphenolandglycosideswhereas phlopatanninswas absentin methanol and aqueous extracts. Steroids present only methanol extract and carbohydratepresent only aqueous extracts.

Reena Ganesan *et al*., (2013) aimed to carry out preliminary phytochemical of six different solvents extracts from leaf and leaf derived callus of *Sebastiania chamaelea*. The preliminary phytochemical analysis reflects the presence of phenolic compounds, carbohydrate, alkaloids, phytosterols, fats and oils, terpenoids. The result highlights among two extracts, leaf extract show negligible activity than callus extracts

Kumar *et al*., (2013) investigated the preliminary phytochemical screening of the leaves of the plant *Lasia spinosa* (Lour) Thwaites. The phytochemical screening showed that the methanol and aqueous extracts contained alkaloid, the carbohydrates and the phenolic compounds were present in all of the solvent extract except petroleum ether extract. The chloroform, ethyl acetate and the aqueous extract contained glycosides whereas the saponins present in methanol and aqueous extract. The ethyl acetate extract contain only the flavonoids.

Quantitative analysis

Quantitative analysis revealed that the *Aerva lanata*L. flower has flavonoids, saponin, tannin and tannin. Significant amount of phenol(332mg/gm),flavonoids(170mg/gm), tannin (85mg/gm) and terpenoids (73.20mg/gm) were presented(Table 2). The above phytoconstituents were tested as per the standard methods.

The secondary metabolites formed also are an important trait for our food plants (taste, colour, scent, etc.) and ornamental plants. Moreover, numerous plant secondary metabolites such as flavonoids, alkaloids, tannins, saponins, steroids, anthocyanins, terpenoids, rotenoids etc. have found commercial application as drug, dye, flavour, fragrance, insecticide, etc. Such fine chemicals are extracted and purified from plant materials (Das *et al.*, 2010).

Vitamins

Vitamins are organic substances that are essential in tiny amounts for growth and activity of the body. Theyare obtained naturally from plant and animal foods. Organic in this definition refers to the chemistry andmolecules of vitamins. The word organic means that the molecules of the substance contain the elementcarbon. The term also means that vitamins can be destroyed and become unable to perform their functions inour bodies. Too much heat, certain kinds of light and even oxygen can destroy some vitamins. Vitamins work with other substances in the body like enzymes and minerals. Together they perform suchfunctions as strengthening bones, healing wounds, keeping the skin healthy, building cells, and helping toresist infections. The amountsof vitamins ingested from food are measured in micrograms or milligrams (Okwu, 2004).The vitamins of the *Aerva lanata* L. flower investigated and summarized in Table-3.

Histochemical analysis of leaves powder of *Aerva lanata* L.flower

Histochemistry is the branch of histology dealing with theidentification of chemical components of cells and tissues,it is a powerful tool for localization of trace quantities ofsubstances present in biological tissues. Histochemicaltechniques have been employed to characterize structureand development, and to study time course of depositionand distribution of major phytocompounds (Krishnan *et al.*, 2001). In the present study,*Aerva lanata*L. flower were treated with specific chemicals and reagents. The *Aerva lanata*L. flower powder treated with diluted ammonia and H_2SO_4 gave yellow colour indicates flavonoids, treated with $FeCl_3$gave green colour indicates tannin and treated withconcentrated H_2SO_4gave yellow colour indicates saponin ((Table 4 and Plate 4**)**. This results further confirmed the presence of phytochemicals.

Fluorescence behavior of *Aerva lanata* L. flower powder

Fluorescence analysis of entire leaves of *Aerva lanata*L. has been carried out in daylight and under UV light. Fluorescence analysis of leaf powder of *Aerva lanata*L. was carried out by the

treatment of different chemical reagents such as $AlCl_3$, H_2SO_4, HCl, NH_3, HNO_3, CH_3OH and NaOH. The powders were observed in normal daylight and under short (245 nm) and long UV light (365 nm) and the results were presented in Table 5 and Plate 6.

In the fluorescence analysis, the plant parts or crude drugs may be examined as such or in their powdered form or in solution or as extracts. Although, in most of the cases the actual substances responsible for the fluorescence properties has not been identified, the merits of simplicity and rapidity of the process makes it a valuable analytical tool in the identification of plant samples and crude drugs (Denston, 1946).

Ultraviolet/visible (UV/VIS) spectroscopy

UV-Visible spectrophotometry technique is simple, rapid, moderately specific and applicable tosmall quantities of compounds.UV-visible spectroscopy can be performed for qualitative analysis and for identification of certainclasses of compounds in both pure and biological mixtures. Preferentially, UV-visible spectroscopycan be used for quantitative analysis because aromatic molecules are powerful chromophores in theUV range. Natural compounds can be determined by using UV-visible spectroscopy. Phenoliccompounds including anthocyanins, tannins, polymer dyes, and phenols form complexes with ironthat have been detected by the ultraviolet/visible (UV-Vis) spectroscopy (Kemp, 1991).

The UV-VIS profile (Fig.1) of the *Aerva lanata*L. flower extract was studied at a wavelength range of 340 to 940 nm. Three major bands wererecorded at 380, 430 and 530 nm. The result confirms the occurrence of peaks at 340-940 nm reveals that the absorption bands are due tothe presence of flavonoids, phenol and its derivatives (Liu *et al.*, 2006). The result of UV-VISspectroscopic analysis confirms the presence of phenolic compounds in the extract of *Aerva lanata* L. flower.

Antimicrobial activity

Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Emergence of pathogenic microorganisms that are resistant/multi-resistant to major class of antibiotics has increased in recent years due to indiscriminate use of synthetic antimicrobial drugs. In addition, high cost and adverse side effects are commonly associated with popular synthetic antibiotics, such as hypersensitivity, allergic reactions, and immunosuppressant and are major burning global issues in treating infectious diseases (Karaman *et al*., 2003).

This situation forced scientists to search for new antimicrobial substances with plant origin. Plant extract of *Aerva lanata*L. flower was screened against *Escherichia coli* species of bacteria and *Candida albicans* species of fungi were evaluated using the standard agar disc diffusion method. The disc diffusion method is used to detect the antimicrobial activity of plant extract. The solidified Nutrient agar plates were swapped with the test organism and the samples were impregnated. After the incubation the zone was measured. The antimicrobial activity of plant extracts was detected by the indication of zone around the disc. The *in vitro* antimicrobial activity of the *Aerva lanata*L. flower extract against these bacteria and fungi were qualitatively assessed by the presence of inhibition zones represented in the photographic plate 7. The inhibitory activities in culture media of the microbes reported in Table 6 were comparable with standard antimicrobiotic viz. chloramphenicol and fluconazole.

Table.1 Qualitative analysis of Phytochemicals in *Aerva lanata* L.flower

S.No	Test analysis	Methanol extract	Aqueousextract
1	Tannin	+	+
2	Phlobatannin	-	-
3	Saponin	++	+
4	Flavanoids	++	+
5	Steroids	+	-
6	Terpenoids	++	+
7	Triterpnoids	+	++
8	Alkaloid	+	+
9	Carbohydrate	-	++
10	Protein	+	++
11	Anthroquinone	+	++
12	Polyphenol	+	+
13	Glycoside	+	+

(-) Indicates Absence; (+) Indicates Presence;(++) Moderately present

Table 2 Quantitative phytochemical analysis of *Aerva lanata* L.flower extract

S.No	Secondary Metabolites	Result (mg/gm)
1	Phenol	332±23.24
2	Flavonoids	170.6±7.04
3	Tannin	85.00±0.70
4	Terpenoids	73.20±4.90

Values are expressed as mean ± SD for triplicates

Table 3 Qualitative analysis of vitamins in *Aerva lanata* L.flower

S.No	Vitamins	Observation
1	Vitamin A	-
2	Vitamin C	+
3	Vitamin D	-
4	Vitamin E	+

(+) Presence (-) Absence

Table 4 Histochemical analysis of leaves powder of*Aerva lanata* L. flower

S.No	Charecterisation	Observation	Result
1	Tannin	Green	++
2	Flavonoids	Yellow	++
3	Saponin	Yellow	+
4	Steroids	Green	+
5	Terpenoids	Orange	++
6	Alkaloid	Reddish brown	+
7	Glycoside	Brown	++
8	Polyphenol	Blue green	++

Note: (+) Presence; (++) present with high intensity of the colour

Table 5 Fluorescence behavior of *Aerva lanata* L. flower powder

S.No	Test	Visible Light	Short UV	Long UV
1	Plant powder	Green	Green	Black
2	Plant powder treated with water	Green	Green	Black
3	Plant powder treated with Hexane	Green	Green	Black
4	Plant powder treated with Chloroform	Green	Green	Black
5	Plant powder treated with Methanol	Green	Green	Black
6	Plant powder treated with Acetone	Greenn	Green	Black
7	Plant powder treated with 1N NaOH (water)	Green	Green	Black
8	Plant powder treated with 1N HCl	Green	Green	Black
9	Plant powder treated with sulphuric acid with an equal amount of water	Brown	Brown	Black
10	Plant powder treated with Nitric acid dilute with an equal amount of water	Yellow	Green	Black

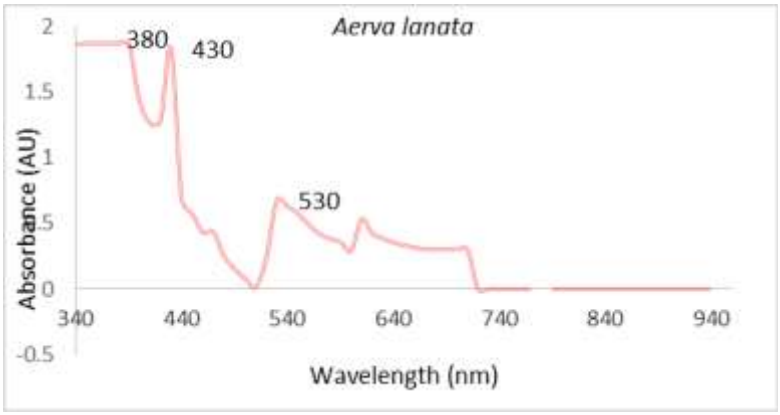

Table: 6Antimicrobial activities of *Aerva lanata* L.flower

Microbial Organism	50µl	100 µl	150 µl	Standard
Escherichia coli (mm)	9.75±0.68	10.25±0.71	11.75±0.82	13.00±0.91
Candida albicans (mm)	4.75±0.33	6.00±0.42	6.25±0.43	10.75±0.75

Values were expressed as Mean ± SD.
Bacterial standard - Chloramphenicol
Fungal standard - Fluconazole

Plate: 7 Antimicrobial activities of *Aerva lanata* L.flower

CONCLUSION

Present investigation reported, *Aerva lanata* L. flower extract is warehouse of chemo-diversity which will be useful in screening for medicines like steroids, alkaloids, phenolics, flavonoids and some other chemicals. The results are encouraging but scientific scrutiny is absolutely necessary before being put in practice. This study is the first scientific report that provides convincing phytochemicals and antimicrobialevidence for the relevance of *Aerva lanata*L. flower thus providing scientific validity to its uses for infectious diseases by the local populace of south India.

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