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A STUDY ON PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *Tridax procumbens* L.

S. Viniya and K Tamilselvi*

PG and Research Department of Botany, Kunthavai Naacchiyaar Govt.Arts College For Women (Autonomous) Thanjavur – 613 007, Tamil Nadu

ABSTRACT

The medicinal value of the chosen plant *Tridax procumbens* leaves has not been extensively worked out. Therefore, the present study was to investigate the phytochemical screening, histochemical, UV-Visible analysis and antimicrobial activity of *Tridax procumbens* leaves extract. The phytochemical screening *Tridax procumbens* leaves showed that the presence of tannin, saponins, flavonoids, terpenoids, triterpenoids steroids, glycosides, anthroquinones, polyphenol and protein whereas phlopatannins was absent in methanol and aqueous extracts. Alkaloids present only aqueous extract. Carbohydrate present only methanol extract. Quantitative analysis revealed that the plant has phenol (480mg/gm), flavonoids (370mg/gm), tannin (216mg/gm) and terpenoids (20mg/gm) were presented. The histochemical analysis further confirmed in the presence of flavonoids, tannin and 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 *Tridax procumbens* leaves extract. The results reveal that extract of *Tridax procumbens* leaves were significantly effective against bacteria species *E. coli* and fungi species *C. albicans*

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Corresponding author
Mrs. K Tamilselvi,
Assistant Professor
PG and Research Department Of Botany, Kunthavai Naacchiyaar Govt.Arts College For Women, (Autonomous) Thanjavur – 613 007, Tamilnadu

INTRODUCTION

Green plants synthesise and preserve a variety of biochemical products, many of which are extractable and used as chemical feed stocks or as raw material for various scientific investigations. Many secondary metabolites of plant are commercially important and find use in a number of pharmaceutical compounds. However, a sustained supply of the source material often becomes difficult due to the factors like environmental changes, cultural practices, diverse geographical distribution, labour cost, selection of the superior plant stock and over exploitation by pharmaceutical industry (Ashis, 2003). Plants are the basic source of knowledge of modern medicine. The basic molecular and active structures for synthetic fields are provided by rich natural sources. This made worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural product in health care most of the drugs derived from plants were developed because of their use in traditional medicine. According to WHO, It was found that there were 35000 to 70000 species of plant which are used as medicaments. Actually the medicinal plants are the plants which have property to cure disease. They have special chemical properties by the virtue of which they can be used as conventional drugs. It was observed that there were 150 compounds identified in plants. Keeping in view, the present study to investigate the phytochemical analysis of *Tridax procumbens* leaves.

MATERIALS AND METHODS

Collection of plant materials

The leaves of *Tridax procumbens* were collected in December 2017 from Thanjavur, Tamil Nadu, India. The *Tridax procumbens* leaves were washed several times with distilled water to remove the traces of impurities from the leaves. Leaves 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 *Tridax procumbens* leaves were transferred in to different conical flask (250ml). The conical flask containing 50ml of different solution (methanol and water). The conical flask containing *Tridax procumbens* leaves were shake it well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using whatman filter paper No.1 and filtrate used 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).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 was placed on a grease free microscopic slide and treated with specific chemicals and reagents and waited for 1-2 minutes. A positive test for histochemicals was indicated by the appearance of the appropriate colour change after application of the reagent. Using a light microscope to observe and 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 *Tridax procumbens* leaves revealed the presence of medicinally active constituents. The phytochemical characters of the *Tridax procumbens* leaves investigated and summarized in Table-1 and Plate 2 and 3. The phytochemical screening *Tridax procumbens* leaves showed that the presence of tannin, saponins, flavonoids, terpenoids, triterpenoids steroids, glycosides, anthroquinones, polyphenol and protein whereas phlopatannins was absent in methanol and aqueous extracts. Alkaloids present only aqueous extract. Carbohydrate present only methanol extract.

Hassain *et al.,* (2011) screened phytochemical constituents from methanol leaf extract of *Bombax malabaricum*. Various organic 11 solvent extracts of *Pedalium murex* were subjected to preliminary phytochemical screenings by Thamizh mozhi et al. (2011). Selected 53 traditionally used medicinal plants from western region of India for their qualitative phytochemical screenings, total phenol and flavonoids contents. Pascaline *et al.,* (2011) screened phytochemical constituents of some medicinal plants used by the Nandis of South Nandi District, Kenya.

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.

Table.1 Qualitative analysis of Phytochemicals in *Tridax procumbens* leaves

S.No	Test analysis	Methanol extract	Aqueous extract
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

Quantitative analysis

Quantitative analysis revealed that the *Tridax procumbens* leaves has flavonoids, saponin, tannin and terpenoids. Significant amount of phenol (520mg/gm), flavonoids (101mg/gm) and terpenoids (10mg/gm) were presented (Table 2). The above phytoconstituents were tested as per the standard methods.

Table 2 Quantitative phytochemical analysis of *Tridax procumbens* leaves extract

S.No	Secondary Metabolites	Result (mg/gm)
1	Phenol	520±36.40
2	Flavonoids	101±7.07
3	Terpenoids	10±0.70

Values are expressed as mean ± SD for triplicates

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

Vitamin D is important in bone formation. Most vitamin D is made when sunshine hits the skin. Too much sun can contribute to skin cancer, and using a sunscreen of SPF 15 or more will block vitamin D formation. Milk and margarine are both fortified with vitamin D. Those over the age of 65 only make about half as much vitamin D as children from the same amount of light exposure, so it is recommended to take a supplement for these people to get enough vitamin D. A vitamin D deficiency can cause an older disease called rickets, and it is cured by cod-liver-oil, which has a high concentration of vitamin D. Vitamin D is stored in the liver and as little as 5 times the Daily Value can produce unhealthy weight loss, vomiting, and calcium deposits in the lungs and kidneys (Clark, 2008). The vitamins of the *Tridax procumbens* leaves investigated and summarized in Table-3.

Table 3 Qualitative analysis of vitamins in *Tridax procumbens* leaf

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

(+) Presence (-) Absence

Histochemical analysis of leaves powder of *Tridax procumbens* leaves

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues, it is a powerful tool for localization of trace quantities of substances present in biological tissues. Histochemical techniques have been employed to characterize structure and development, and to study time course of deposition and distribution of major phytocompounds (Krishnan *et al.*, 2001). In the present study, *Tridax procumbens* leaves were treated with specific chemicals and reagents. The *Tridax procumbens* leaves 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 with concentrated H_2SO_4 gave yellow colour indicates saponin (Table 4 and Plate 5**)**. This results further confirmed the presence of phytochemicals.

Table 4 Histochemical analysis of leaves powder of *Tridax procumbens* leaves

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

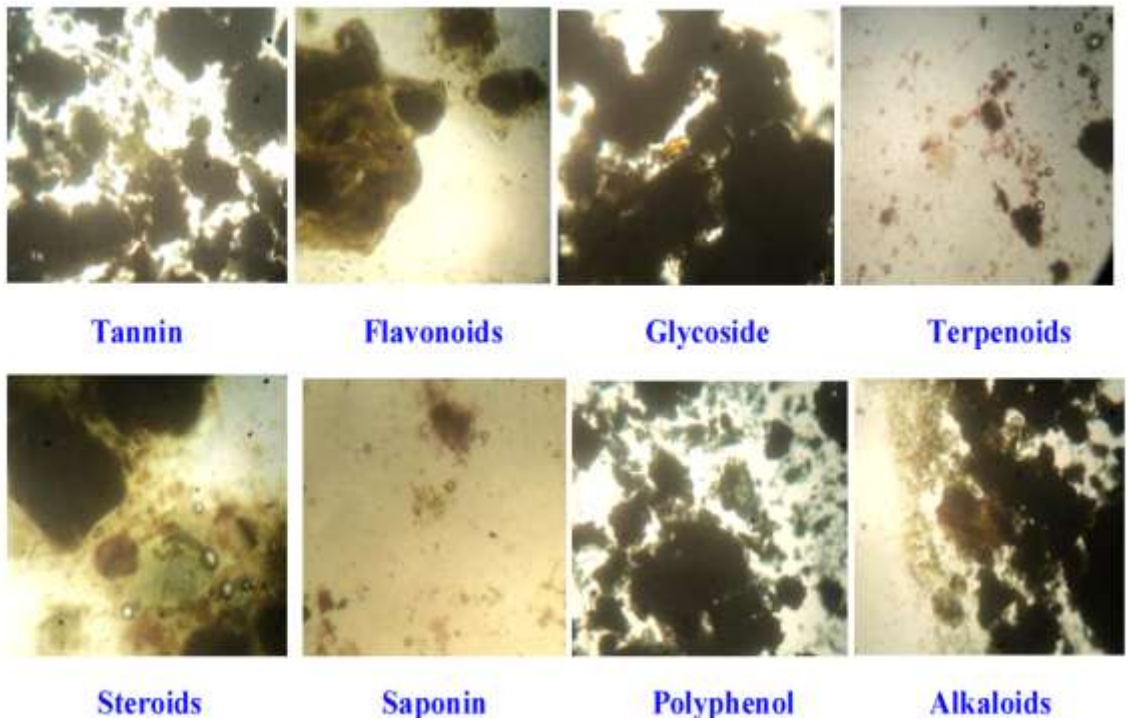

Plate.5: Histochemical analysis of leaves powder of *Tridax procumbens* leaves

Fluorescence behavior of *Tridax procumbens* leaves powder

Fluorescence analysis of entire leaves of *Tridax procumbens* has been carried out in daylight and under UV light. Fluorescence analysis of leaf powder of *Abutilon indicum* 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.

Table.5: Fluorescence behavior of *Tridax procumbens* leaves 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	Dark green	Black
6	Plant powder treated with Acetone	Green	Green	Black
7	Plant powder treated with 1N NaOH (water)	Green	Dark green	Black
8	Plant powder treated with 1N HCl	Brownish green	Green	Black
9	Plant powder treated with sulphuric acid with an equal amount of water	Green	Green	Black
10	Plant powder treated with Nitric acid dilute with an equal amount of water	Yellow	Light green	Black

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 to small quantities of compounds. UV-visible spectroscopy can be performed for qualitative analysis and for identification of certain classes of compounds in both pure and biological mixtures. Preferentially, UV-visible spectroscopy can be used for quantitative analysis because aromatic molecules are powerful chromophores in the UV range. Natural compounds can be determined by using UV-visible spectroscopy. Phenolic compounds including anthocyanins, tannins, polymer dyes, and phenols form complexes with iron that have been detected by the ultraviolet/visible (UV-Vis) spectroscopy (Kemp, 1991).

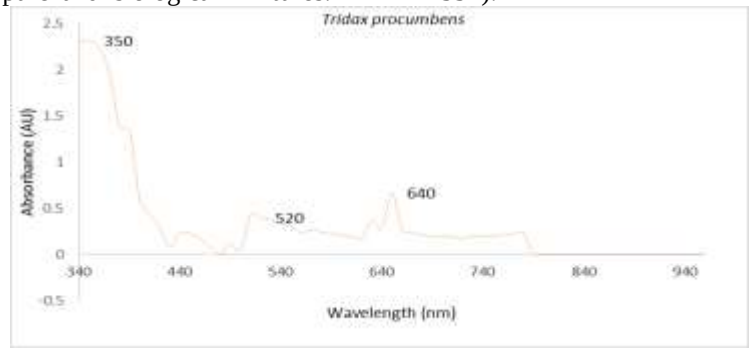

Fig.1: UV-Visible spectrum analysis of *Tridax procumbens* leaves

The UV-VIS profile (Fig.1) of the *Tridax procumbens* leaves extract was studied at a wavelength range of 340 to 940 nm. Three major bands were recorded at 340, 480 and 660 nm. The result confirms the occurrence of peaks at 340-940 nm reveals that the absorption bands are due to the presence of flavonoids, phenol and its derivatives (Liu *et al.*, 2006). The result of UV-VIS spectroscopic analysis confirms the presence of phenolic compounds in the extract of *Tridax procumbens* leaves.

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 *Tridax procumbens* leaves 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 *Tridax procumbens* leaves 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 and 7 were comparable with standard antimicrobiotic viz. chloramphenicol and fluconazole.

Table.6: Antibacterial activities of *Tridax procumbens* leaves

Microbial Organism	20 ml of inhibition concentrations			
	50µl	100 µl	150 µl	Standard
Escherichia coli (mm)	6.75±0.47	10.25±0.71	12.50±0.87	11.75±0.82
Candida albicans (mm)	9.25±0.64	12.25±0.85	13.00±0.91	12.00±0.84

Values were expressed as Mean ± SD.

Bacterial standard – Chloramphenicol ; Fungal standard

BACTERIA **FUNGUS**

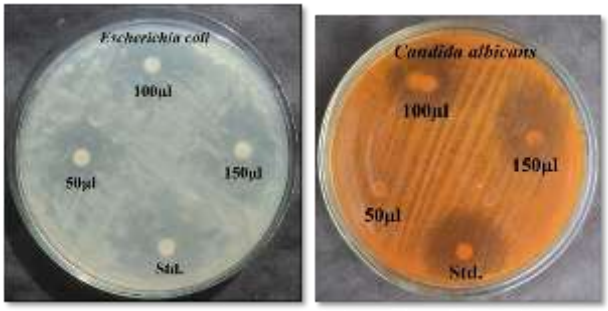

Plate.7: Antibacterial activities of *Tridax procumbens* leaves

CONCLUSION

Present investigation reported, *Tridax procumbens* leaves 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 antimicrobial evidence for the relevance of *Tridax procumbens* leaves thus providing scientific validity to its traditional consumption by the local populace of south India.

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