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Research Article

PHYTOCHEMICAL SCREENING, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF LEAF EXTRACTS OF *TRIDAX PROCUMBENS*

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ABSTRACT

Medicinal herbs have been used comprehensively against various diseases over a long phase of time. Nature has provided abundant plant wealth source, which possess various medicinal values. The essential values of some medicinal plants have been known longer, but a large number of them remain unexplored. It is quite important to investigate the uses and to conduct experimental studies to describe their curative properties. *Tridax procumbens* a medicinal herb, commonly known as "coat buttons" has been used in medicine since times immemorial. The Present study deals with Phytochemical Screening, Mineral, Antioxidant and Antimicrobial Activity of Leaf Extracts of *Tridax procumbens.* The results of the phytochemical analysis showed the presence of vital secondary metabolites in ethanol extract than aqueous extract which play a role in plant disease resistant mechanism. Ethanol extract of *Tridax procumbens leaves* showed massive antimicrobial and antioxidant activity. High content of sodium and potassium in mineral analysis showed the role in metabolism. It is hoped that the important Phytoconstituents, Minerals, antioxidants and antimicrobial properties analyzed in *Tridax procumbens* leaves will open new avenues in medical field in the treatment of various diseases.

Keywords: *Tridax procumbens,* Ethanol, Minerals, antioxidants, Antimicrobial activities.

INTRODUCTION

Medicinal plants have been used as a commendable source for centuries as an alternative remedy for treating human diseases because they contain numerous active constituents of therapeutic value. The improvement of microbial resistance to antibiotics has led the researches to scrutinize the alternative sources for the treatment of resistant strains. Presently the world population relies on plant based medicines and serves as first line of defense in maintaining health and fighting many diseases**.** (Trease and Evans., 1989)

Use of plants as a source of medicine has been inherited and is an important component of the health care system in India. The use of herbal medicine is progressively growing with roughly 40 per cent of population reporting use of herb to treat medical illnesses. In western world also, the use of herbal medicines is steadily growing with approximately 40 per cent of population reporting use of herb to treat medical illnesses within the past year. Public, academic and government interest in traditional medicines is growing exponentially due to the increased incidence of the adverse drug reactions and economic burden of the modern system of medicine. (Rabi *et al*., 2008)

Medicinal plants synthesize substances that are useful for the maintenance of health in humans and other animals. These include chemical substances called secondary metabolites that play a vital role in defense mechanism against various microorganisms and insects. (Nirmaladevi *et al*., 2008)

Medicinal plants possess antioxidant properties that protect the plant cells against the production of reactive oxygen species from cellular damage. Phenolic compounds present in the medicinal plants play a vital role in scavenging free radical species and protects human body against diseases (Kahkonen *et al*., 1999)

Traditional knowledge about medicinal plants has continuously nonstop the search for new cures. Supplementation of herbal antioxidants is indispensable to suppress the oxidative stress in a healthier way. (Varalakshmi *et al., 2017*)

Tridax procumbens Linn is a wide spread weed and a Flowering plant that contains various medicinal values. The plant has been established for the treatment of wound healing, dysentery, epilepsy, hypertension, hepatotoxicity, hemorrhage and metabolic syndrome (Susila *et al*., 2002). Traditionally, *Tridax procumbens* has been in use in India as anticoagulant, antifungal and insect repellent. Its leaf extracts were known to treat infectious skin diseases in folk medicines. It is a well-known ayurvedic medicine for liver disorders or hepato-protective nature besides gastritis and heart burn. (Wani *et al*., 2010). In view of dearth of information, the present study has been undertaken to investigate the Phytochemical, Mineral, Antioxidant and Antimicrobial effects of *Tridax procumbens* Linn (Leaves).

MATERIALS AND METHODS

The fresh parts of *Tridax procumbens* were collected from the local field of Lalgudi, Tiruchirapalli. The materials were washed with distilled water and air dried in the shade for two weeks. The plant materials were authenticated by the Botanists, St.Joseph's College, Tiruchirapalli.

Extraction of plant material

The dried leaves were grounded to coarse powder. 500 mg of powder was successively extracted with solvents like Ethanol and Aqueous extract. The extraction process was carried out using soxhlet apparatus for 36 hours.

1. Phytochemical studies

The crude product of *Tridax procumbens* obtained in soxhlet extraction technique was subjected to qualitative evaluation for the presence of Phytochemicals (Obadoni *et al*., 2001).

2. Antioxidant Assay

2.1: Superoxide dismutase (SOD)

The leaves (0.5 g) were ground with 3.0 ml of potassium phosphate buffer, centrifuged and used the supernatant. The assay mixture contained 1.2 ml sodium pyrophosphate buffer, 0.1 ml phenazine methosulphate (PMS), 0.3 ml nitro blue tetrazolium (NBT), 0.2 ml enzyme preparation and water in a total volume of 2.8 ml. The reaction was initiated by the addition of 0.2 ml of NADH, incubated at 30°C for 90 seconds and arrested by the addition of 1.0 ml glacial acetic acid. The reaction mixture was then shaken with 4.0 ml n-butanol, allowed to stand for 10 minutes and centrifuged. The intensity of the chromogen in the butanol layer was measured at 560 nm (Kakkar *et al.,* 1984).

2.2: Catalase (CAT)

To 3.0 ml of H_2O_2-phosphate buffer, added 40 µl of enzyme extract (20% homogenate in phosphate buffer) and mixed thoroughly. The time essential for a reduction in absorbance by 0.05 units was documented at 240 nm (Luck, 1974).

2.3: Peroxidase (POD)

Enzyme extract (0.1 ml) (20% homogenate in phosphate buffer) was added to 3.0 ml pyrogallol solution, and adjusted the spectrophotometer to read zero at 430 nm. Added 0.5 ml of H_2O_2 and the change in absorbance was recorded every 30 seconds up to 3 minutes. (Reddy *et al*., 1995).

2.4: Glutathione S-transferase (GST)

Homogenized 0.5 g of the leaf sample with 5.0 ml of phosphate buffer centrifuged and used the supernatant for the assay. The reaction mixture in a total volume of 2.9 ml contained 0.1 ml GSH, 0.1 ml CDNB and phosphate buffer. The reaction was initiated by adding 0.1 ml of enzyme extract and observed the readings for every 15 seconds at 340 nm for a minimum of 3 minutes. (Habig *et al*., 1974).

2.5: Polyphenol oxidase (PPO)

The plant tissue (0.5 g) was homogenized in extraction medium (2.0 ml) containing HCl, sorbitol and NaCl. To 2.5 ml of phosphate buffer in the test cuvette, added 0.3 ml of catechol solution and set the spectrophotometer at 495 nm. Then added 0.2 ml of enzyme extract and recorded the change in absorbance for every 30 seconds upto 5 minutes. (Esterbauer *et al*., 1977).

2.6: Glutathione Reductase

The activity was measured by the method of Carlberg *et al*., (1975)**.** Plant sample (0.5 g) was homogenized with 2.5mM of Phosphate buffer (pH 7.5), 1mM EDTA, 0.7mM oxidized glutathione and 0.1mM NADPH and the content were made up to 3 ml with water. The change in optical density was monitored after adding enzyme sample at 340nm for 3 minutes at 30 seconds intervals.

2.7: Determination of ascorbic acid

The ascorbic acid was determined according to Cakmak and Marschner (1992) with some modification. The leaf extracts (0.5ml of 1:10 g/ml (100mg/ml) in ethanol was separately mixed with 5 ml of 5%meta-phosphoric acid, and centrifuged at 4000 rpm for 30 minutes. The reaction mixture contained 0.2 ml aliquot of the 4000 rpm supernatant, 0.5 ml 150 mM phosphate buffer (pH 7.4) containing 5mM EDTA, 0.1 ml (10 mM) DTT (1,4-dithiothreitol) and 0.1 ml(0.5%, w/v) N-ethylmaleimide (NEM) to remove excess DTT. The color was developed after addition of the following reagents in the reaction mixture: 0.4 ml (10%) trichloroacetic acid (TCA), 0.4 ml (44%) ortho-phosphoric acid, 0.4 ml (4%) 2, 2'-bipyridine in 70%ethyl alcohol, and 0.2 ml (3%) $FeCl_3$. The mixture was then incubated at 40°C for 40 minutes, and the absorbance was measured at 525 nm. Ascorbic acid was used as a standard in the range of 0 to100 µg /ml.

2.8: Determination of carotenoids

Total carotenoids were determined by the method of Jensen (1978). One gram sample was extracted with 100 ml of 80% ethanol solution and centrifuged at 4000 rpm for 30 minutes. The supernatant was concentrated to dryness. The residue was dissolved in 15 ml of diethyl ether and after addition of 15 ml of 10%methanolic KOH the mixture was washed with 5% ice-cold saline water to remove alkali. The free ether extract was dried over anhydrous sodium sulphate for 2 h. The ether extracts were filtered and its absorbance was measured at 450 nm by using ether as blank.

3: Determination of the free radical scavenging activities

The *in vitro* free radical scavenging activities of *Tridax* leaves were evaluated by DPPH, hydrogen peroxide, nitric oxide, and ferrous ion radical scavenging assays.

3.1: DPPH radical scavenging assay

DPPH (2,2-diphenyl-1-picryl-hydrazyl) free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol. DPPH radical scavenging activity was measured according to the method of Mensor *et al*., (2001). A methanolic solution of 0.3 mM DPPH (0.5ml) was added to equal volume of sample homogenate (20% homogenate was prepared in Tris EDTA buffer, pH 7.2) and allowed to react at room temperature. DPPH in methanol without plant extract served as positive control. After 30 minutes, the mixture was centrifuged and the absorbance of the supernatant was measured at 518 nm and converted in to percentage radical scavenging activity.

3.2: Hydrogen peroxide radical scavenging assay

The hydrogen peroxide scavenging activity was determined according to the method described by Ruch *et al*., (1989). A solution of H_2O_2 (4mM) was prepared in phosphate buffer (pH 7.4). The concentration was determined spectrophotometrically from its absorption at 230 nm which was determined after 10 minutes against a blank solution containing phosphate buffer without H_2O_2.

3.3: Nitric oxide scavenging assay

Nitric oxide scavenging activity of *Tridax* leaves were determined by the method described by Green *et al*., (1982). 3 ml of reaction mixture containing sodium nitroprusside in PBS and the plant extract was incubated at 25°C for 150 minutes. Controls without test compound were kept in an identical manner. After incubation, 0.5ml of Griess reagent was added. The absorbance of the chromophore formed was read at 546nm.The percentage inhibition of nitric oxide generation was measured by comparing the absorbance values of control and those of test compounds (plant extract).

3.4: Ferrous ion radical scavenging activity

The ferrous ion radical scavenging activity was determined by the method described by Dasmaltchi *et al*., (2008). The reaction mixture contained 1.0 ml of various concentrations of the extract, 0.1 ml of 2 mM $FeCl_2$ and 3.7 ml of methanol. The control contained all the reaction reagents except sample. The reactions were initiated by the addition of 0.2 ml of 5 mM ferrozine. After 10 minutes at room temperature, the absorbance of the mixture was determined at 562 nm against a blank. A lower absorbance of the reaction mixture indicated a higher Fe^{2+} chelating ability.

4: Analysis of Minerals

The mineral content present in the *Tridax* ethanolic leaf extract was detected and analyzed by Atomic absorption Spectroscopy.

Procedure

The Leaf Sample was Dried and grounded in to fine powder. Concentrated Nitric acid was added to the powder and left over night. The content was heated to dryness and the Ash was collected. To the Ash 5ml of Hydrochloric acid was added and Sample was calibrated.

5: ANTIMICROBIAL activity of *Tridax procumbens*

The antimicrobial activity of *Tridax* ethanolic leaf extract were tested in four different types of pathogenic bacteria such as *Bacillus subtilis, Bacillus megaterium, Escherichia coli, and Staphylococcus aureus* which were cultured on agar plates supplemented with different concentrations of plant extract by Agar well diffusion method. Pure Kanamycin (50µl) as a standard antibiotic for comparison of the results was taken. Disc Diffusion method were adapted to study the antibacterial activity of the plant extracts in triplicates and compared with the standard antibiotic.

RESULTS AND DISCUSSION

Table I: Phytoconstituents present in *Tridax* leaves of Ethanolic extract and aqueous extract

Phytoconstituents	*Tridax(leaves)*	
	Ethanol Extract	Aqueous Extract
Alkaloids	+	-
Glycosides	+	+
Phenol	+	-
Flavonoids	+	-
Steroids	+	-
Tannins	+	-
Saponins	+	-

Table I Shows the level of phytochemical constituents present in Ethanolic and Aqueous Extracts of *Tridax procumbens* .The Ethanol extract possess more constituents when compared with aqueous extracts. These bioactive agents were shown to inhibit pathogenic microorganisms. (Benjamin *et al*., 1981)

Table II: Antioxidant Analysis in *tridax* leaves

Antioxidants	*Tridax* leaves
SOD	17.09 ± 1.54
CAT	155 ± 2.90
POD	5.89 ± 0.53
GST	0.9 ± 0.002
PPO	1.9 ± 0.06
Glutathione Reductase	78 ± 0.78
Carotenoids(mg/100ml)	14.6 ± 0.40
Ascorbic acid(mg/100ml)	2.0 ± 0.30

The values are Mean ± SD of triplicates
SOD = expressed as U/mg of protein
CAT = expressed as µM of H_2O_2consumed/min/mg
POD = change in OD/min/mg of protein µM of H_2O_2consumed/min/mg protein
GST = n moles CDNB conjugated min/mg protein
PPO= activity was expressed as change in absorbance at 412 nm per minute/g fresh weight of tissue
Glutathione Reductase = n moles of NADPH oxidized/min/mg protein

Table II shows the activities of antioxidants in *Tridax* ethanolic leaf extract. From the results obtained, it was clear that the leaves possess considerably higher activities of all the enzymic antioxidants analyzed. The ethanolic Extract of leaf of *Tridax* possessed high percentage of radical scavenging activity, due to their hydroxyl groups. The antioxidant activity may be due to the hydrogen donating ability and the free radical scavenging activity of secondary metabolites present in the Plants. They can react with active oxygen radicals, such as hydroxyl radicals (Hussain *et al*., 1987) superoxide

anion radicals (Afanselv *et al*., 1989) and lipidperoxyl radicals and inhibit the lipid peroxidation at an early stage.

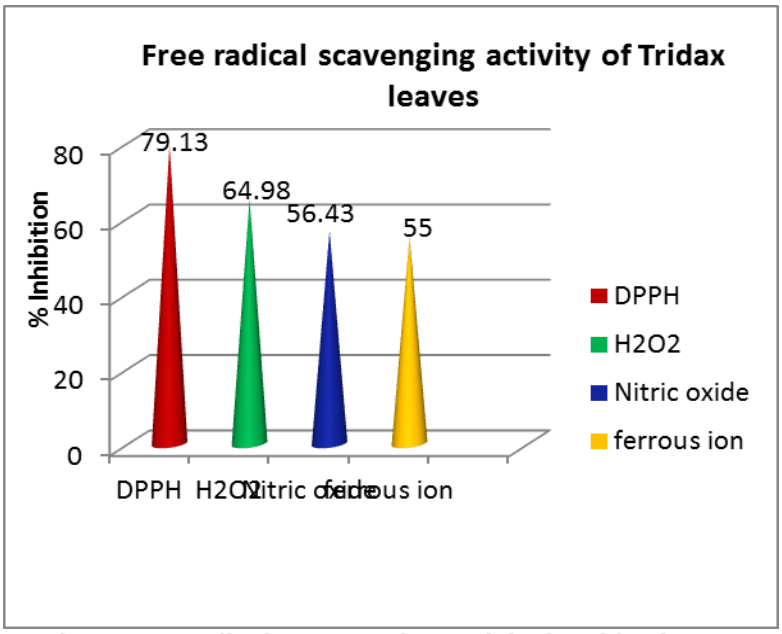

Fig. I: Free radicals scavenging activity in *tridax leaves*

Free radicals and reactive oxygen species may cause deleterious effects in humans. The effects may cause Heart dysfunction, cancer, Arthritis etc. Plant sources act as natural antioxidants and play a role in scavenging free radicals from damage. Figure I show the free radical scavenging activity of *Tridax* leaves. The result confirms that *Tridax* leaves have potential antioxidant activity which may be due to the presence of more phytoconstituents which prevents the formation of lipid peroxides thus disrupting membrane organization**.** (Okawa *et al*., 1970)

Table III: Mineral Analysis

Table III shows the level of minerals in ethanolic extract of *Tridax* leaves. The values represents mean values of triplicates.*Tridax* showed enormous and rich amount of potassium and sodium which play an important role in metabolism, acid and water balance. (Ujowundu *et al.*, 2008)

Table III: Mineral Analysis in *Tridax* leaves

Minerals	(mg/100g)
Magnesium	2.45
Calcium	15.00
Iron	20.86
Potassium	55.00
Sulphur	5.45
Zinc	15.00
Manganese	10.56
Boron	6.78
Sodium	40.98

Table IV: Antimicrobial activity of Ethanolic Extracts of *Tridax*

s.no	Concentration in mg	*Staphylococcus aureus*	*Bacillus Subtilis*	*E.coli*	*Bacillus megaterium*	Kanamycin
1	0.25	2.8mm	1.8 mm	1.6 mm	2.5 mm	2.5 mm
2	0.50	3.9 mm	1.9 mm	0.8 mm	2.0 mm	3.8 mm
3	1.00	5.5 mm	2.4 mm	1.5 mm	0.5 mm	5.2 mm

Table IV shows the antimicrobial effect of *Tridax procumbens* in ethanolic leaf extract. The activities were determined in different concentrations such as 0.25 and 0.50 and 1.00mg .The antimicrobial activity were studied against *Staphylococcus, Bacillus Subtilis, E.coli* and *Bacillus megaterium* **.**The maximum zone of inhibition of 5.5mm for *Tridax* was observed in 1mg concentration against *Staphylococcus*. Jayashree and Maneemegalai (2008) observed that ethanolic and methanolic extracts of leaves of *Tridax* procumbens possessed significant antibacterial activity against microorganisms (*E.coli, Bacillus subtilis, Staphyllococcus aureus, klebsiella pneumonia* and *Pseudomonas aeruginosa*).The minimum inhibition zone (0.5mm) was observed in same concentration against *Bacillus megaterium* for *Tridax*. Plants rich in tannins have antibacterial potential due to their basic character that allows them to react with proteins to form stable water soluble compounds thereby killing the bacteria by directly damaging its cell membrane (Mohamed Shamshihabudeen*etal*.,2010).

CONCLUSION

The investigation of the study reveals the presence of many important phytoconstituents and vital minerals in leaf extracts of *Tridax procumbens* which can provide various useful biological activities. *Tridax procumbens* leaves acquire good antioxidant role and thus act as an efficient free radical scavenger implicating its use in drug and food industries. The plant has significant anti microbiological activity, which clearly depicts and proves the efficacy of using *Tridax* as a Therapeutic drug.

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