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Phytochemical characterization and antioxidant activity of leaf extract of *Tridax procumbens* L.

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Abstract
Traditional medicine is an important resource for the development of potentially useful new chemotherapeutic agents. The essential values and uses of some plants have been worked out and published, but many of them remain unexplored to date. In this study, acetone extract of leaves of *Tridax procumbens* was subjected to Phytochemical Screening, GC-MS study and Antioxidant activity. The results of the phytochemical analysis showed the presence of vital secondary metabolites in acetone extract which play a role in plant disease resistant mechanism. Among the 15 compounds obtained from GCMS study, the major constituents were Dodecane, 2,6,10-trimethly-, alkane compound with a tune of, Hexadecanol, Tridecanol, Fatty acid ester and Triterpenoid. This extract showed massive antioxidant activity. Inhibitory activity was increasing with increasing concentration with maximum 64.95% which indicates the presence of strong antioxidant activity. The range of inhibitory percentage of extract of *T. procumbens* was between 30 -65 with IC50 value of 200µg.

Keywords: phytochemical, *Tridax Procumbens*, acetone extract, antioxidant activity

Introduction
The evaluation of plant is based on phytochemical and pharmacological approaches leading to the drug discovery through natural product screening. Presently the world population relies on plant based medicines and serves as first line of defense in maintaining health and fighting many diseases [1]. 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 [2].
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 [3]. The health promiting benefits of antioxidants of plants are thought to be resulted from their potential effects against the reactive oxygen species.
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 [4]. *Tridax procumbens* Linn. commonly known as coat button, is a noxious and wide spread invasive weed that contains various medicinal values. Traditionally, *T. procumbens* has been in use in India for the treatment of various ailments [5, 12]. The literature survry reveals that solvent extracts of *T. procumbens* L. plant possesses potent antioxidant property but information on acetone extract of *T. procumbens* L. is lacking. In view of dearth of information, the present study has been undertaken to investigate the Phytochemical, and Antioxidant effects of *Tridax procumbens* Linn (Leaves).

Materials and Methods
Plant Sample
The plant sample was collected by uprooting the whole plant from the fields of a local village "Mittinangkuppom, Vaniyambadi. The authentication of the plant was done by a Dr. Abdul Nazer, Associate Professor of Botany, New College, Chennai, Tamilnadu and the voucher specimen was deposited in the museum of Islamiah College (Autonomous), Vaniyambadi.

Preparation of the sample
The leaves of the plants are cleaned, disinfected and dried. All dried samples were crushed using a grinder. 20g of crushed samples of leaves was placed in Soxhlet thimble. 200ml of solvent was used, which was added in Round bottom flask. The heater is set at 55°C and Round bottom flask was placed in the heating mantle. All soxhlet extraction was carried out till clear solvent is observed during reflux. For acetone distillation 6 hours distillation was found to be sufficient.
The acetone extract was concentrated using Vaccum Rotary Evaporator and the residue was dried in Petridish and kept in refregirator until further use.

Phytochemical Screening
Test for flavonoids
In a test tube containing 0.5 ml of extract, 5-10 drops of dilute HCl and Zn Cl or magnesium were added the solution was boiled for a few minutes. Presence of reddish pink or dirty brown color confirms flavonoid.

Test for saponins
In a test tube containing 0.5ml of aqueous extract, a drop of sodium bicarbonate was added, shaken vigorously.

Appearance of froth confirms saponins.

Test for steroids
To 2ml of chloroform extract, 1ml of concentrated $H_2 SO_4$ was added carefully along the sides of the test tube. Appearance of red color in the chloroform layer confirms steroids.

Test for tannin and phenolic compound
Ferric chloride test
To the extract added ferric chloride. Greenish black color confirms a positive result.

Antioxidant property / radical scavenging assay
DPPH (2, 2-diphenyl-1-picrylhydrazyl) was arranged from Sigma-Aldrich (USA). Ethanol was arranged from VWR Chemicals. Free radical scavenging assay (Free radical scavenging activity) of cold pressed seed oil was measured in terms of radical scavenging ability using the stable free radical DPPH. Different concentrations (10µl, 20µl, 30 µl, 40µl & 50µl) of sample were taken and tested for optimum reaction. 50µl of samples was found to be optimal for reaction with 3ml of 0.08% Solution of DPPH along with 10 ml of Ethanol. The tubes were incubated at 25ºC for 60 minutes. The absorbance value was recorded at 510 nm using PerkinElmer LAMBDA™ 25 UV/Vis Spectrophotometers. The same procedure was followed for control without the sample [13].
DPPH Scavenging ability (%) = [A control – A sample/ A control] × 100

GC-MS analysis
GC–MS analysis of methonolic extract was carried out on a GC Clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 mm x 0.25 mm x 0.25 µm df, composed of 5% Diphenyl / 95% Dimethyl poly siloxane), operating in the electron impact mode a70 ev. Helium (99.999%) was used as the carrier gas at a constant flow of 1 ml/min and an injection volume of 2µl was employed (split ratio of 10:1), injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 550 Da.

Identification of components
Interpretation of mass spectrum was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular formula and structure of the components of the test material were ascertained.

Statistical analysis
The results are presented as means ±SD and are processed statistically by analysis of variance (ANOVA).

Results
Several plants are used traditionally as medicinal agent for internal and tropical application. The very common plant used for injury is *Tridax procumbens* L as extract. However, the scientific validation on its medicinal value may help to develop better drugs formulations. In this study, pharmacological evaluation of anti-oxidant activity of Acetone extract of leaves of *Tridax procumbens*. L was carried out to investigate that the *T. procumbens* have been used in traditional medicines to determine their potential sources of novel antimicrobial compounds.
The findings of the present study of phytochemical screening of *T. procumbens* revealed the presence of phenols, flavonoids, saponins, sterols, terpenoids, quinines and tannins in the acetone extract of leaves investigated.
GC-MS analysis is one of the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols, acids esters etc. The GCMS analysis of *T. procumbens* leaves revealed the presence of 15 compounds that contribute to the medicinal quality of the plant and have been identified after comparision with NIST Library. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration(peak area %) are presented in Table 2. The GC-MS chromatogram of the compounds detected was shown in Figure- 1. Among the 15 compounds, the major constituents were Dodecane, 2,6,10-trimethly-, alkane compound with a tune of (14.27%), Hexadecanol (6.6%), Tridecanol (4.89%), Fatty acid ester (2.88%) and Triterpenoid (2.66%). Other compounds along with minor cosntituents wer also reported.
The crude acetone extract of leaves of *T. procumbens* was tested for the antioxidant activity for the various concentrations of crude extracts ranged from 50 to 400 µg/ml by DPPH method and the standard ascorbic acid was used. Inhibitory activity was increasing with increasing concentration with maximum 64.95% which indicates the presence of strong antioxidant activity. The range of inhibitory percentage of extract of *T. procumbens* was between 30 -65 with IC50 value of 200µg.

Table 1: Preliminary Screening of Phytochemicals of Leaf Extract

Phytochemicals	*Tridax procumbens*
Alkaloids	+
Flavonoids	+
Saponin	-
Tannin	+
Quinon	+
Steroid/Terpenoid	+
Phenols	+

Table 2: Chemical components and activities in the leaf extract of *T. procumbens*

RT	Compound	Mol. Formula	Mol. Wt	%	Type	Activity
2.51	Dodecane,2,6,10-trimethyl-	$C_{15}H_{32}$	212	14.27	Alkane compound	No activity reported
3.96	Octane, 2,4,6-trimethyl-	$C_{11}H_{24}$	156	1.01	Alkane compound	No activity reported
5.39	Hexane, 3,3-dimethyl-	C_8H_{18}	114	1.1	Alkane compound	No activity reported
8.05	Benzoic acid, 4-ethoxy-ethyl ester	$C_{11}H_{14}O_3$	194	4.22	Aromatic acid ester	Antimicrobial and Preservative
11.9	1-Hexadecanol	$C_{16}H_{34}O$	242	6.61	Alcoholic compound	Antimicrobial
11.9	1-Tridecanol	$C_{13}H_{28}O$	200	4.89	Alcoholic compound	Antimicrobial
13.17	(R)-(-)-(Z)-14-Methyl-8-hexadecen-1 ol	$C_{17}H_{34}O$	254	1.62	Alcoholic compound	No activity reported
13.07	Acetic acid, octadecyl ester	$C_{20}H_{40}O_2$	312	1.96	Ester compound	Antimicrobial
4.07	Oleyl Alcohol	$C_{18}H_{36}O$	268	1.16	Alcoholic compound	Antimicrobial
15.07	Pentanoic acid, 10-undecenyl ester	$C_{16}H_{30}O_2$	254	2.88	Fatty acid ester	No activity reported
19.5	Oxirane, [(hexadecyloxy)methyl]-	$C_{19}H_{38}O_2$	298	1.93	Oxirane compound	No activity reported
26.0	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281	0.84	Amino compound	Antimicrobial and Anti-inflammatory
29.5	gamma.-Sitosterol	$C_{29}H_{50}O$	414.7	0.81	Steroid compound	No activity reported
30.7	2-Piperidinone, N-[4-bromo-n-butyl]-	$C_9H_{16}BrNO$	233	1.45	Alkaloid	Antimicrobial, Anti-inflammatory and Antioxidant
32.23	Betulin	$C_{30}H_{50}O_2$	442	2.6	Triterpenoid	Antibacterial, Antioxidant, Antitumor, Cancer preventive,

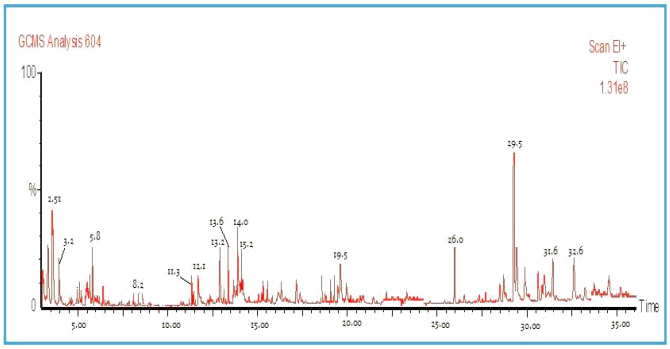

Fig 1: GC-MS Chromatogram obtained from the leaf extract of *T. procumbens*

Table 4: Antioxidant activity of leaf extract of *T. procumbens*

Concentration	Extract of *T. procumbens*		Standard Ascorbic acid	
	Absorbance	%Inhibition	Absorbance	% Inhibition
50 µg/ml	0.42	29.15	0.2	70.26
100 µg/ml	0.38	34.09	0.22	68.09
150 µg/ml	0.3	43.96	0.24	65.91
200 µg/ml	0.25	50.14	0.27	62.65
250 µg/ml	0.22	53.84	0.29	60.48
300 µg/ml	0.19	57.54	0.32	57.22
350 µg/ml	0.16	61.25	0.39	49.61
400 µg/ml	0.13	64.95	0.43	45.26
IC50		200 µg/ml		350µg/ml

Discussion

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities [14].

The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites [15] possessing pharmacological properties such as antiapoptosis, antiageing, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [16]. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds [17].

The present study clearly indicated the presence of some of the important medicinal compounds of *Tridax procumbens* studied. Eight secondary metabolites from the aqueous and methanolic leaf extract of *T. procumbens* Linn were calculated [18]. Presence of eight phytochemicals as alkaloids, tannin, saponin, steroid, phlobatannin, terpenoids, flavonoids and cardiac glycosides form the methanolic extract of leaves of *T. procumbens* Linn was reported [19].

Further, detailed investigation of the active compounds of the plant for the extract mechanism of action will contribute greatly to the development new pharmaceuticals.

The DPPH assay is based on the measurement of the relative inhibition of the extract at various concentrations. Chemicals which are able to change the colour of DPPH free radical from purple to yellow can be considered as antioxidants and therefore, a radical scavenger [20]. The results of the DPPH radical scavenging activity of *T. procumbens* showed that they possess very high percentage antioxidant activity, 65 % at a concentration of 400 µg/ml. DPPH is a relatively stable Nitrogen centered free radical that easily accepts an electron or hydrogen, it react with suitable reducing agents as a results of which the electrons become paired off and the solution losses colour depending on the number of electrons take up [21]. The results show that the extracts may have hydrogen donors thus scavenging the free radical DPPH, with high absorbance percentage at 400µg/ml which was observed to be higher than ever those of the standards (ascorbic) used. This result shows that there is a relationship between the phenol content of medicinal plants and antioxidant activity. This finding supports earlier reports that the plant metabolites like flavonoids, tannins, catechins and other phenolic compounds possesses antioxidant activity [22] and have played a preventive role in the development of cancer, heart and age related diseases. They also bee reported to be chemo-preventive agents by lowering cholesterol and repairing damage cells [3]. Similarly study showed that among the eight extracts and standard tested for antioxidant activity using the DPPH method, the crude methanolic extracts of green tea, black tea exhibited best antioxidant [23].

GC-MS method used for the analysis of the obtained extracts can be an interesting tool for testing the amount of some active principles in herbs used in cosmetics, drugs,

pharmaceutical or food industry. It is evident from the Table 4 that all fractions have a complex chemical composition. Some of the GC-MS peaks remained unidentified, because of lack of authentic samples and library data of corresponding compounds.

Compounds having anti-inflammatory, antibacterial, antifungal, skin conditioning properties, cancer preventive, immunostimulant, chemo preventive, lipoxygenase-inhibitor have been identified. The plant is extensively used traditionally as a diuretic in India.

The investigation concluded that the stronger extraction capacity of acetone could have been produced number of active constituents responsible for many biological activities. So that those might be utilized for the development of traditional medicines and further investigation needs to elute novel active compounds from the medicinal plants which may create a new way to treat many incurable diseases.

Conclusion

The increasing interest gained by antioxidants is due to the health benefits provided mainly by natural sourced low molecular weight antioxidants. This involves in preventing the occurrence of oxidative-stress related diseases, caused by the attack of free radicals. From this study, it may be concluded that the biological potentialities shown by the selected herbal compounds would pave the way for further investigation on herbal products to screen the compounds responsible for the significant antioxidant and other pharmacological activities.

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