

## PHYTOCHEMICAL ASSAY AND STUDY OF ANTIOXIDANT ACTIVITY OF PLANT EXTRACTS OBTAINED FROM TRIDAX PROCUMBENS EMPLOYING RADICAL SCAVENGING METHOD



### Chemistry

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### ABSTRACT

The chemical composition of plant extracts obtained from *Tridax procumbens* have been determined by spectrophotometer. This method is highly accurate, clean and economical. These phytochemicals showed remarkable antioxidant activity when compared with ascorbic acid. The DPPH radical scavenging activity for aqueous and organic extracts of this plant is significant from the therapeutic point of view.

### KEYWORDS

antioxidant activity, spectrophotometer, radical scavenging

#### Introduction :

The man used herbs as therapeutic agents and medicines, which could be easily available in nature. The nature has provided abundant plant wealth for all living creatures, which possess medicinal values. Some plants with specific biological activities have been studied and the results have been published, however some are unexplored till date. Therefore it is necessary to explore their uses and to conduct pharmacological studies of them to know about their medicinal properties.

The *Tridax procumbens* extracts have been used for various pharmacological effects, antimicrobial activity against both gram positive and gram negative bacteria and stimulate wound healing. From the leaves of the plant, flavones, glycosides, monosaccharide, polysaccharide and asteraceae have been isolated. The Western state of Nigeria uses the leaf of *Tridax procumbens* as treatment to reduce blood pressure. Recently, the aqueous extracts of *Tridax procumbens* are used to regulate blood pressure and heart rate in rats and to elucidate its mechanism of action<sup>1</sup>.

Flowers of *Tridax procumbens* have been extensively used in Ayurvedic system of medicine for various ailments and is dispensed for *Bhringraj* and is used for liver disorder. The plant is native of tropical Africa, Asia, Australia and India. The *Tridax procumbens* is found along road sides, waste grounds, rail road, river bank, dikes, meadows and dunes. The *Tridax procumbens* is very important for their spreading stems and abundant seed production. The leaves of *Tridax procumbens* are simple, opposite, acute, inflorescence capitulum. There are two type of flowers *Tridax procumbens* i.e. ray florets and disk florets<sup>2</sup>.

Events of numerous pathological including inflammation processes are associated with ROS generation i.e. generation of reactive oxidative species and consequently the induction of several chain reactions like lipid per-oxidation and others. The ROS generate either by cellular metabolism like Glycolysis. Some reactive oxidative species are extremely reactive and therefore interact with some vital macro molecules including lipids, nucleic acid and proteins<sup>3</sup>.

*Tridax procumbens* exhibits some properties like antiseptic, insecticidal and hair growth promoting. Also more commonly, the use of plant leaves are to check haemorrhage from cuts, wounds and bruises. Since it has remarkable influence on wound healing and the present study is to evaluate its haemostatic activity<sup>4</sup>.

For the development of new chemotherapeutic agents, medicinal plants are considered as an important source of potentially useful structure. For that the first step is the biological and phytochemical screening of plant extracts and or extract from which traditional preparation used in popular medicine. The strategies for the

preparation involves collection of test crude extracts based on a combination ethnopharmacology and daily healers practices. The several preparation of medicinal plant species are used as antiarrhoeal agents. However, intestinal Amibiasis is one of the current disease and which have diarrhoea as a symptoms in tropical regions. Also for the treatment of amobiasis, traditional preparations from the medicinal plant are still used for that purpose<sup>5</sup>.

The importance of medicinal plant is well known to us, the treasure house of potential drug is the kingdom plant and recently there has been an increasing awareness about the importance of medicinal plant. Drugs from the plants are easily available, less expensive, safe and efficient and rarely has side effect. For medicinal use, the plants which have been selected is most obvious choice of examining the current search for therapeutically effective new drug such as anticancer drugs, antimicrobial drugs<sup>6</sup>.

Antioxidant functions are associated with lowered DNA damage, diminished lipid per-oxidation, or inhibited malignant transformation in vitro; further, they are associated epidemiologically with lowered incidence of certain types of cancer and degenerative diseases such as ischemic heart disease and cataracts. There is a wide variety of potential per-oxidant challenges that cells and tissues as well as constituents of body fluids (e.g. lipoproteins) may encounter as a normal attribute of aerobic life. To maintain a steady state, there is a matching system of antioxidant defence that uses various strategies, enzymatic and non-enzymatic<sup>7</sup>.

Plants are a source of compounds with antioxidant activity such as phenolic acids, flavonoids including anthocyanins and tannins, vitamins and carotenoids that may be used as pharmacologically active products. Widespread empirical use of wild plants demands for accurate and reliable information on their phytochemicals and antioxidant activity, as well as on the potential benefits and prospective products, such as nutraceuticals and phytomedicines. This is the case of three species that have long been used in the Iberian Peninsula as important medicinal plants and less often as food or food additives.

A general consensus has been reached during last few years that diet has a major role in the development of chronic diseases, such as cancer, coronary heart disease, obesity, diabetes type 2, hypertension and cataract. This consensus suggests that a predominantly plant-based diet rich in fruits and vegetables, pulses and minimally processed starchy staple foods reduces the risk for development of these diseases significantly. This is a safe principle that promises to provide for improved public health. However, these general recommendations avoid the issue of which dietary plants to eat. A large and remaining challenge, therefore, is to identify the most beneficial dietary plants<sup>8</sup>. Since diabetes is a global problem, it is an important task to identify and improve the information regarding the medicines used for

curing this ailment<sup>9</sup>.

**Materials and Methods :**

**Methods of Extraction :**

The modernisation of herbal medicine has also raised quite a more than a few eyebrows in matters related to safety and quality of herbal medicine. In other words, the standardisation and quality aspect of herbal medicine becomes a high profile issue. Extraction methods involve separation of medicinally active fractions of plant tissue from inactive/inert components by using selective solvents and extraction technology. Solvents diffuse into the solid plant tissues and solubilised compounds of similar polarity. Quality of plant extract depends on plant material, choice of solvents and the extraction methods.

**Collection of plants:**

*Tridax procumbens* was collected from Bund garden, Pune in Maharashtra state, India. Fresh plants were used for extraction and isolation of their phytochemicals. Phytochemicals and extracts were stored in the refrigerator and used whenever needed.

**Extraction:**

**Aqueous extraction:**

The 25g of crushed whole plant material was soaked in 25 mL, 50 mL, and 100 mL each in distilled water for 24 hrs. The extract was filtered by using muslin cloth. The final volume were corrected to viz. 25 mL, 50 mL, and 100 mL by washing residue with distilled water and used for all experimental purposes.

**Isolation of Phytochemicals:**

The phytochemicals were isolated by taking 35g of material and it was grinded and homogenized by methanol: water mixture (4:1). Then it was filtered and the filtrate was evaporated. 2M H<sub>2</sub>SO<sub>4</sub> was added to the residue and it was extracted with chloroform to yield terpenoids. Aqueous acid layer was basified with NH<sub>4</sub>OH and then extracted with chloroform-methanol (3:1). This extract afforded most of the alkaloids whereas remaining aqueous basic layer was evaporated and extracted with methanol to furnish the quaternary alkaloids. During purification of quaternary alkaloids, tannins were separated.

**Dilution and concentration:**

Dilution and concentrations were prepared using literature procedure (33).

**Dilution of Water Extracts:**

25 mL, 50 mL, and 100 mL of these dilutions were prepared by washing residue with distilled water and these dilutions were used for evaluation of all the activity.

**Concentration of isolated phytochemicals:**

50µg/mL, 100µg/mL, 200µg/mL and 500µg/mL concentration were prepared using respective solvent for DPPH radical scavenging activity.

**DPPH radical scavenging activity:**

Free radical scavenging activity of different extracts of leaves and flowers of *Tridax procumbens* Linn. plant were measured by 1, 1-diphenyl-2-picryl hydrazyl (DPPH). For this purpose, 0.1 mM solution of DPPH in ethanol was prepared. This solution (1 ml) was added to 3 ml. of different extracts in Methanol at different concentration (50,100, 200, 500ppm). Here, only those extracts are used which are miscible in Methanol. The mixture was shaken vigorously and allowed to stand at room temp for 30 min. and the absorbance was measured at 517 nm. with spectrophotometer.

Reference standard compound used was ascorbic acid and experiment was done in triplicate. The value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using Log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity. The percent DPPH scavenging effect was calculated by using following equation:

$$\text{DPPH scavenging effect (\%)} \text{ or Percent inhibition} = \frac{A_0 - A_1}{A_0} \times 100.$$

Where, A<sub>0</sub> is the Absorbance of control reaction and A<sub>1</sub> is the Absorbance in presence of test or standard sample.

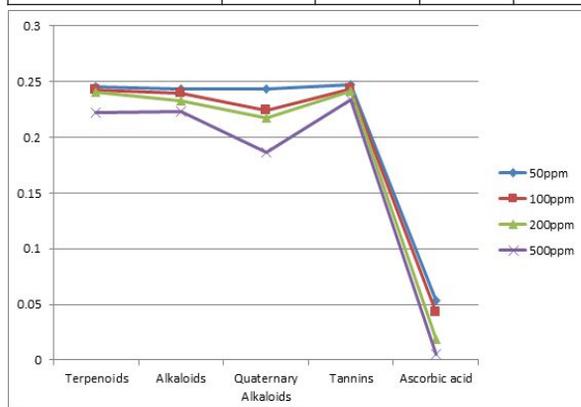
**Results and Discussion:**

The present protocol provides a simple, efficient and accurate determination of various phytochemicals without employing separation of them. This is highly economical and clean method for the screening of phytochemicals. The actual separation of individual compounds may be carried out if required. In this case separation is not essential since the desired information regarding the composition of phytochemicals in the plant extracts and their biological activity is feasible.

DPPH radical scavenging activity is one of the most widely used method for screening antioxidant activity of *Tridax procumbens* plant phytochemicals and aqueous extracts. **Table 1** shows the absorption due to various compounds present in the plant extract and **Table 2** shows the antioxidant activities of phytochemicals with standard ascorbic acid. Generally, the antioxidant properties of these aqueous extracts were found to be concentration dependent. In these results quaternary alkaloids showed better percentage of inhibition as compared to other isolated phytochemicals at all concentration that is 50ppm, 100ppm, 200 ppm and 500ppm. The absorbance and antioxidant property of the aqueous extracts are shown in **Table 3** and **Table 4** respectively. The aqueous extracts showed remarkable antioxidant activity as evidenced by DPPH scavenging method.

**Table-1**  
**Absorbance of different isolated phytochemicals with Standard ascorbic acid at 517nm.**

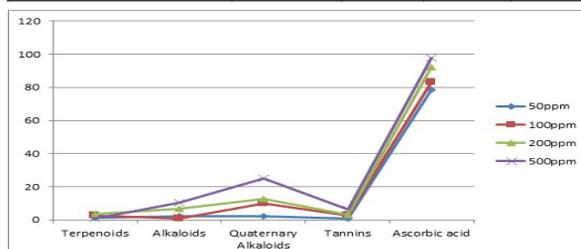
Phytochemical of plants	Absorbance			
	50ppm	100ppm	200ppm	500ppm
Terpenoids	0.2457	0.2423	0.2401	0.2221
Alkaloids	0.2434	0.2398	0.2324	0.2232
Quaternary Alkaloids	0.2432	0.2241	0.2171	0.1863
Tannins	0.2470	0.2431	0.2410	0.2333
Ascorbic acid	0.0530	0.0425	0.0190	0.0054



**Fig 1 - Absorbance of different isolated phytochemicals with Standard ascorbic acid at 517nm.**

**Table-2**  
**% inhibition of different isolated phytochemicals with Standard ascorbic acid.**

Phytochemical of plants	% of Inhibition			
	50ppm	100ppm	200ppm	500ppm
Terpenoids	1.40	2.76	3.65	0.910
Alkaloids	2.32	0.94	6.74	10.43
Quaternary Alkaloids	2.40	10.07	12.88	25.24
Tannins	0.88	2.44	3.29	6.38
Ascorbic acid	78.73	82.94	92.37	97.83



**Fig 2 - % inhibition of different isolated phytochemicals with Standard ascorbic acid.****Table-3 : Absorbance of different aqueous extracts at 517 nm**

Extraction of Plant	Concentration in ml	Absorbance
Aqueous extract	25	0.2287
	50	0.2386
	100	0.2422

**Table-4 : % inhibition of different aqueous extract.**

Extraction of Plant	Concentration in ml	% Inhibition
Aqueous extract	25	7.33
	50	3.32
	100	1.86

**Conclusion:**

Radical scavenging activity of aqueous and organic extracts of *Tridax procumbens* is better in comparison with the activity exhibited by standard ascorbic acid. The method used for activity determination is simple, fast and economical and environmental friendly.

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