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# INVITRO ANTI-OXIDANT AND ANTI-INFLAMMATORY PROPERTY OF ETHANOL EXTRACT OF *TRIDAX PROCUMBENS* LEAVES

**Sedhuraman S.*, Balaji A., Amerthiya D., Kavya G., Meena C., Lironisha L., Kannikaparameswari N.**

Department of Biochemistry, Dr. N.G.P Arts and Science College, Coimbatore-641048.

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***Corresponding Author**
**Sedhuraman S.**
Department of Biochemistry,
Dr. N.G.P Arts and Science
College, Coimbatore-641048.

## ABSTRACT

The present investigation was aimed to evaluate the in vitro anti-oxidant and anti-inflammatory property of leaves of *Tridax procumbens*.L by DPPH assay and membrane stabilization assay. Action was observed in dose dependent manner. The result shows that in DPPH assay 250 µg/ml of chosen extract, *Tridax procumbens* Ethanol Extract (TPEE) showed maximum protection 45% and standard drug provided 69% protection. Similarly in membrane stabilization assay the selected extract at concentration of 500 µg/ml showed maximum results 78% and standard drug Diclofenac sodium provided 58% protection. In additional, phytochemical analysis of *Tridax procumbens* showed the presence of alkaloid, flavonoid, tannin, saponin, amino acid and sugars. It reveals that the phytochemical constituents are responsible for anti-oxidant and anti-inflammatory property. The future work will be determination of anti-oxidant and anti-inflammatory activities by in vivo methods.

**KEYWORDS:** *Tridax procumbens*; in vitro anti-oxidant; DPPH assay; in vitro anti-inflammatory property; membrane stabilisation assay method.

## INTRODUCTION

Plants play a very prospective role, as it is one of the most important component of the biodiversity. They act as centralized key in maintaining the balance and stability of earth's environment. Our universe is composed of about 5, 00,000 species of plants.[1]

With 'Herbal Renaissance' happening all over the globe, medicinal herbs are staging a phenomenal comeback. Ethno botanical information estimates that more than 6000 plant

species forming about 40% of the higher plant diversity, are used in its codified and folk healthcare traditions.[2] As sources of biologically active molecules and blue prints for the modified derivatives with enhanced activity and or reduced toxicity, plant-derived drugs form an important segment of pharmacopoeia.[3]

It has been estimated that herbal medicines serve about 80% of the world's population health need for millions of people in rural areas of developing countries and more than 65% of the global population use traditional medicine as basic healthcare.[4] WHO estimated that approximately one fourth of the 500 million prescriptions written in US each year contain a mention of leafy plant extracts or active ingredients obtained from Plant substance.[5] According to one estimate 20,000 to 35,000 species of plants of plants are used as medicines, pharmaceuticals, cosmetics and nutraceuticals by different ethnic groups the entire world over.[6]

The use of medicinal herbs for curing disease has been documented in history of all civilization. It was concluded that plats contain active principles, which are responsible for the curative action of the herbs. The isolated active constituent of medicinal herbs and after testing some found to be therapeutically active.[7] It is necessary to convert ethno-medicine practises into organized system either following through scientific extractive evaluations and/or on Ayurveda systemic approaches. In recent, herbal medicines and extracts have gained interest for its affordability, low pricing, no side effects, solutions for chronic diseases and disorders, time tested remedies (folklore), preventive approaches, etc.[8]

Oxygen is an indispensable part of aerobic life. However under certain circumstances it can seriously affect our well-being through the formation of reactive oxygen species, representing both free radicals and non-free radicals. Molecules containing unpaired electrons are known as free radicals that cause tissue collapse by means of DNA, Protein and lipid damage.[9] Free radicals contribute more than one hundred disorders in humans.[10] Catalase and Hydroperoxidase enzymes functions as natural antioxidants in human body. Due to depletion of natural antioxidants in different maladies, consuming antioxidants as free radical scavengers may be necessary.[10,11,12,13]

Recently there has been an upsurge of interest in therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury.[14] It has been mentioned that antioxidant activity of plants might be due to their phenolic compounds.[15] An easy rapid and

sensitive method for antioxidant screening of plant extract is free radical scavenging assay using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) stable radicle spectrophotometrically. In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases.[16]

Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as: the increase of vascular permeability, increase of protein denaturation and membrane alteration.[17] Lysosomal enzymes released during inflammation produce a variety of disorders where the extracellular activity of these enzymes is said to be related to acute or chronic inflammation. Stabilisation of lysosomal membrane is important in limiting the inflammatory response by inhibiting the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation or damage upon extracellular release or by stabilizing the lysosomal membrane.[18]

HRBC or erythrocyte membrane is analogous to lysosomal membrane and its stabilisation implies that the extract may as wee stabilise the lysosomal membrane. Stabilisation of human red blood cell membrane (HRBC) by hypotonicity induced membrane lysis can be taken as an *in-vitro* measure of anti-inflammatory activity of drugs or plant extracts. The management of Inflammation related diseases is a real issue in rural community; the population in these areas uses many alternative drugs such as substances produced from medicinal plants.

*Tridax procumbens* is a species of flowering plant in the daisy family (Asteraceae).[19] It is found in tropical and subtropical areas of the world growing with natural crops, along roadsides pastures, fallow land and waste areas.[20] It has herbaceous, semi-prostate habit, and can grow anywhere from 15-40 cm in height. The leaves are elongated, opposite, ovate and serrated margins, hirsute on the abaxial and adaxial sides.[21] It has been known by several names such as Coat buttons, Mexican daisy in English, Thata poodu, Vettukaya-Thalai in Tamil, Jayanti veda in Sanskrit, Ghamra in Hindi, Dagadi pala in Marathi, Herbe caille in French.[22]

Reports from tribal areas in India, states that the leaf juice can be used to cure fresh wounds, to stop bleeding and as a hair tonic. Despite these known benefits, it is still listed in the United States as a noxious weed and regulated under the Noxious Weed Act.[23,24,25] The aim of the present study is to identify the antioxidant potential & anti-inflammatory activity of

ethanolic extract of *Tridax procumbens* through invitro methods such as DPPH assay and membrane stabilisation assay.

## MATERIALS AND METHODS

### Collection of plant materials

The plant *Tridax procumbens*.L was collected from the local areas of Coimbatore district during the month of January 2019 and the leaves was kept in sterile bags and then taken to laboratory for further purposes. Further identified by botanical survey of India (southern circle), Coimbatore.

### Processing of plant sample

The leaves were washed with water thoroughly to remove all the soil particles. The leaves were shade dried in room temperature for about 7 days. Then the dried leaves were grinded to coarse powder using an electrical blender and then sieved through a mesh to get a fine powder which is stored in a sterile airtight container.

### Solvent extraction

The dried powdered sample of the leaves and roots were weighed 30g and dissolved in 150ml of ethanol in a soxhlet apparatus by continuous heat exposure for 48 hours till the solution becomes clear and then the extract were collected in a tube. The extract was concentrated under reduced pressure 22-26 mmHg at 45ºC and the residue obtained was stored at 4ºC for further use.

### Antioxidant activity (Free radical scavenging activity) determination

The stable 1, 1-diphenyl-2picrylhydrazyl radical (DPPH) was used for determination of free radicals-scavenging activity of the extracts.[26] Different concentrations of each herbal extract were added, at equal volume, to ethanolic solution of DPPH (100µM). After 15 min at room temperature, the absorbance was recorded at 518 nm. The experiment was repeated for three times. Ascorbic acid were used as standard control. IC50 values denote the concentration of sample, which is required to scavenge 50% of DPPH radicals

Scavenging activity % = [A518 (control) – A518 (test)] / A518 (control)*100

### Invitro anti-inflammatory activity by HRBC membrane stabilisation method[27]

The principle involved here is stabilisation of human red blood cell membrane by hypotonicity induced membrane lysis.

**Preparation of Red blood cell (RBCs) suspension**

Fresh whole human blood (10 ml) was collected and transferred to heparin centrifuged tubes. The tubes were centrifuged at 3000rpm for 10 min and were washed three times with equal volume of normal saline. This washing process helps us to collect the pure blood cells from the whole blood. The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline.

**Hypotonicity-induced haemolysis[28]**

Different concentration of extract (100-500 µg/ml), reference sample, and control were separately mixed thoroughly with 1ml of phosphate buffer, 2 ml of hyposaline and 0.5 ml of HRBC suspension. Diclofenac sodium (100 µg/ml) was used as a standard drug. All the assay mixtures were incubated at 37°c for 30 min and centrifuged at 3000 rpm. The supernatant liquid was decanted and the haemoglobin content was estimated by a spectrophotometer at 560 nm. The percentage haemolysis was estimated by assuming the haemolysis produced in the control as 100%.

Percentage protection = 100 - (OD test/OD control) *100

**RESULTS AND DISCUSSION**

Literatures are full of scientific documentation today regarding medicinal plants and they have potential to cure various diseases.[29] Thus this future further encourage to manufacture a pharmaceutical products synthesized from medicinal plants as they are safe and dependable as compared to synthetic drugs, which are not only costly they have adverse effects too.[30]

**Antioxidant activity**

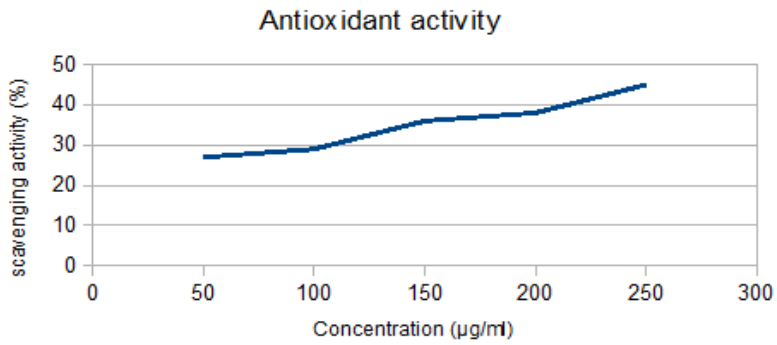
Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants through their scavenging power are useful for the management of those diseases. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of specific compound of plant extracts.[26]

DPPH is relatively stable nitrogen centered free radical that easily accepts an electrons or hydrogen whether it reacts with the suitable reducing agent as a result of which electrons become paired off and the solution loses colour depending on the number of electrons taken up.[31] Degree of decolourisation indicates scavenging potential of antioxidant extract in terms of hydrogen donating activity.[32] The results shows that the concentration of ectract (TPEE)

at 250µg/ml, showed maximum of 45% protection, whereas, Standard Ascorbic acid (100µg/ml) showed 69% of radical scavenging activity (Table 1).

**Table 1: Effect of TPEE on Inhibition of DPPH Assay.**

| Treatment (s) | Concentration (µg/ml) | % scavenging activity |
|---|---|---|
| control | - | - |
| T1 | 50 | 27 |
| T2 | 100 | 29 |
| T3 | 150 | 36 |
| T4 | 200 | 38 |
| T5 | 250 | 45 |
| Standard | 100 | 69 |




**Anti-inflammatory activity**

Protective effect on hypotonicity saline induced erythrocyte lysis is known to be very good index of anti-inflammatory activity of any agent. The investigation is based on the need for newer anti-inflammatory and anti-oxidant from natural sources with potent activity and lesser side effects as substitutes for chemical therapeutics.

**Membrane stabilisation**

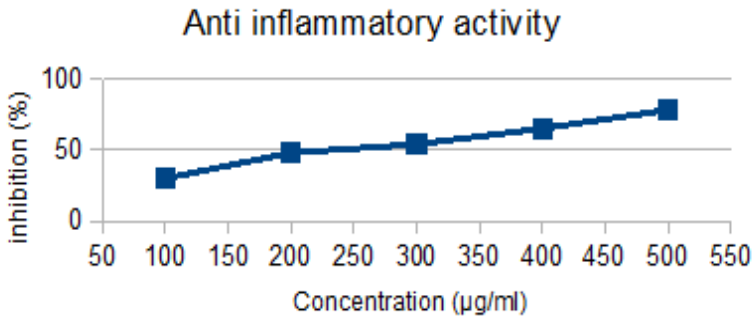
The HRBC membrane stabilisation has been used as a method to study the invitro anti-inflammatory activity because the erythrocyte membrane is analogous to the lysosomal membrane.[33,34] and its stabilisation implies that the extract may well stabilize lysosomal membranes. Stabilisation of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bacterial enzymes and proteases, which causes further tissue inflammation and damage upon extra cellular activity of these enzymes are said to be related to acute or chronic inflammation. The non-steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilising the lysosomal membrane.[35]

**Hypotonicity Induced Haemolysis**

The results showed that T. *procumbens* leaf extract at concentration range of 100-500µg/ml protect significantly the erythrocyte membrane against lysis induced by hypotonic solution (Table 2). Diclofenac sodium (100µg/ml) offered a significant protection against the damaging effect of hypotonic solution. At the concentration of 500µg/ml, extract(TPEE) showed maximum of 78% protection, whereas, Diclofenac sodium (100µg/ml) showed 58% inhibition of RBC haemolysis when compared with control.

**Table 2: Effect of TPEE on hypotonicity induced haemolysis of erythrocyte.**

| Treatment (s) | Concentration (µg/ml) | Absorbance at 660nm | % inhibition of haemolysis |
|---|---|---|---|
| Control | - | 0.31 | - |
| Standard | 100 | 0.13 | 58 |
| T1 | 100 | 0.22 | 30 |
| T2 | 200 | 0.16 | 48 |
| T3 | 300 | 0.14 | 54 |
| T4 | 400 | 0.11 | 65 |
| T5 | 500 | 0.07 | 78 |




**Phytochemical analyis**

In addition that, dried powdered samples were subjected to qualitative tests for the identification of phytochemicals constituents according to standard procedures. The preliminary phytochemical analysis showed the presence of phytoconstituents such as carbohydrates, tannin, flavonoid, saponin, terpenoid., etc were observed in *Tridax procumbens*. The leaves of *Tridax procumbens* was subjected to qualitative analytical tests for the various plant constituents.

**Table 3: Preliminary phytochemical analysis of *Tridax procumbens.***

| S.No | Tests | Observation |
|---|---|---|
| 1 | Test for Tannin | + |
| 2 | Test for Phlobactin | - |
| 3 | Test for Saponin | + |
| 4 | Test for Flavonoid | + |
| 5 | Test for Steroid | + |
| 6 | Test for Terpenoid | + |
| 7 | Test for Cardiac glycosides | + |
| 8 | Test for Leucoanthocyanin | - |
| 9 | Test for Anthocyanin | + |
| 10 | Test for Anthraquinone | - |
| 11 | Test for Protein | - |
| 12 | Test for Coumarin | - |
| 13 | Test for Glycosides | - |
| 14 | Test for Phenol | + |
| 15 | Test for Xanthoprotein | - |
| 16 | Test for Alkaloid | + |
| 17 | Test for Emodine | - |
| 18 | Test for Carbohydrates | + |

(+); Presence (-); Absence

## CONCLUSION

In the present study, results indicate that the ethanolic extract of the leaves of *Tridax procumbens* can be used in the treatment of inflammatory, oxidation and angiogenesis property due to the significant percentage of membrane stabilisation, DPPH assay and angiogenesis assay. These activities may be due to the presence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids, and phenols.

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**Conflict of Interest:** None.

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**REFERENCE**

1. Sawant, R. S. And Godghate, A. G. Preliminary Phytochemical analysis of leaves of *Tridax procumbens* L. International Journal of Science, Environment and Technology, ISSN 2278-3687, 2013; 2(3): 388-394.
2. Akujobi C, Anyanwu BN, Onyeze C and Ibekwe VI: Antibacterial activities and preliminary phytochemical screening of four medicinal plants. Journal of Applied Science, 2004; 7(3): 4328-4338.
3. Cowan, M.M. Plant products as antimicrobial agents. Clin. Microbial Rev., 1999; 12(4): 564-582.
4. General Guidelines for Methodologies on research and evaluation of Traditional Medicine. World Health Organization, Geneva, Switzerland, 2001; 1.
5. C.P. Malik, Medicinal uses, Chemical Constituents and Micro propagation of Three Potential Medicinal Plants, International Journal of Life science & Pharma Research, Jul-Sept 2012; 2(3): 58.
6. Trivedi, Pc. Herbal Medicine: traditional practices (Ed); Aavishkar Publishers, Jaipur, 2006; 322.
7. Lipipun V, Kurokawa M, Suttisri R, Taweechotipatr P, Pramyothin P, Hattori M, et al. Efficacy of Thai medicinal plant extracts against herpes simplex virus type 1 in vitro and in vivo. *Antiviral Res.*, 2003; 60(3): 175-80.
8. C.P. Malik, Medicinal uses, Chemical Constituents and Micro Propagation of Three Potential Medicinal Plants, International Journal of Life science & Pharma Research, Jul-Sept, 2012; 2(3): 57-76.
9. Hou WC, Lin RD, Cheng KT, Hung YT, Cho CH, Chen CI, et al. Free radical scavenging activity of Taiwanese native plants, Phytomedicine. 2003; 10: 170-175.
10. Kumpulainen JT, Salonen JT. Natural antioxidants and anticarcinogens in nutrition, health and disease. The Royal Society of Chemistry, UK., 1999; 178-187.
11. Halliwell B. Free radicals, antioxidants and human disease: curiosity, cause, or consequence Lancet, 1994; 344: 721-724.
12. Kuhnan J. The flavonoids. A class of semi essential food components; their role in human nutrition. World review of nutrition and dietics, 1976; 24: 117-191.
13. Younes M. Inhibitory action of some flavonoids on enhanced spontaneous lipid peroxidation following glutathione depletion. Planta Medica, 1981; 43: 240-245.
14. Schuler P. Natural antioxidants exploited commercially, in *Food Antioxidants*, Hudson BJF (Ed.). Elsevier, London, 1990; 99-170.

15. Cook NC, Samanan, S. Flavonoids-Chemistry, Metabolism, Cardio protective effects and Dietary sources. Nutritional Biochemistry, 1996; 7: 66-76.
16. Koleva II, Van Beek TA, Linseen JPH, de Groot A, Evstatieva LN Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochemical Analysis, 2002; 13: 8-17.
17. Gerard J Tortora, Sandra Reynolds, eds. Principles of Anatomy and Physiology. Harper Collins College Publishers, 1993, 7th edition, 695.
18. Rajendran V, Lakshmi KS, Bangladesh Journal of Pharmacol, 2008; 3: 121-4.
19. Russell GE, Welman WG, Reitief E, Immelman KL, Germishuizen G, Pienaar BJ, Wyk MV and Nicholas A. List of species of southern African plants, Mem. Bot. Surv. S. Africa, 1987; 2: 1-152.
20. Holm, L., Doll, J., Holm, E., Pancho, J., & Herberger, J. World weeds: Natural Histories and Distribution. John Willey & Sons, Inc. New York, 1997.
21. Powell M. A. Taxonomy of Tridax (Compositae). *The New York Botanical Garden,* 1965; 17: 47-96. https://doi.org/10.2307/2805391
22. C Pai, U Kulkarni, M Borde, S Murali, P Mrudula, Y Deshmukh. *British Journal of Pharmaceutical Research*, 2011; 1(4): 164-173.
23. Bessey CE, Principles and Practises of Plant Taxonomy, 1915; 127-130.
24. Graves WJ, Weed Initiated Pest Risk Assessment for *Tridax Procumbens* L., 2000.
25. Park JS, Rho HS, Kim DH, Chang IS. Enzymatic Preparation of Kaempferol from Green Tea Seed and its Antioxidant Activity, 2006; 54(8): 2951-2956.
26. Koleva II, Van Beek TA, Linseen JPH, de Groot A, Evstatieva LN Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochemical Analysis, 2002; 13: 8-17.
27. Sreekumari C, Yasmin. N, Raffiq Hussain and Babuselvam. M. In vitro anti-arthritic property of Rhizopora mucronata leaves. International Journal of pharma sciences and research, 2015; 6(3): 482-85.
28. Azeem AK, Dilip C, Prasanth SS, Junise V, Hanan Shahima. Anti-inflammatory activity of the glandular extracts of Thunnus alalunga. Asia Pac Journal for Med., 2010; 3(10): 412-20.
29. Lai, PK. And J. Rov. Antimicrobial and chemo preventive properties of herbs and spices. Curr. Med. Chem., 200; 47(2): 234-8.
30. Gordon MC, David JN. Natural product drug discovery in the next millennium. Pharm. Biol., 2001; 39: 8-17.

31. Ting Sun, Joseph powers R, Juming Tang. Evaluation of the antioxidant activity of asparagus, broccoli and their juices; Food Chemistry, 2007; 105: 101-106.
32. Mensor L, Fabio S, Menezes, Gilda G. Leitao, Alexandrae S. Reis, Tereza C, dos Santos, Cintia S. Coube *et al.* Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method, Phytotheraphy research, 2001; 15(2): 127-130.
33. Gandhidasan R, Thamaraichelvan A, Baburaj S. Anti-inflammatory action of Lannea coromandelica by HRBC membrane stabilisation. Fitoterapia, 1991; LXII(1): 81-83.
34. Shenoy S, Shwetha K, Prabhu K, Maradi R, Bairy KL, Shanbhag T. Evaluation of anti-inflammatory activity of Tephrosia purpurea in rats. Asian Pac J Trop Med., 2010; 3(3): 193-5.
35. Rajendran Vadivu, Lakshmi K S. Invitro and in vivo antiinflammatory activity of leaves of Symplocos cochinchinensis (Lour) Moore ssp Laurina. Bangladesh J Pharmacol, 2008; 3: 121-124.