www.jmscr.igmpublication.org
Impact Factor 5.84
Index Copernicus Value: 83.27
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v5i5.91

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

# In Vitro Antioxidant Activity of Methanolic Leaf Extract of *Tridax Procumbens* and *Tithonia Diversifolia*

Authors
**Okereke Stanly Chukwuma[1], Nwaogwugwu, Joel Caleb[2], Chukwudoruo Chieme Sunday[3]**
[1,2,3]Abia State University, Uturu, Abia State
Email: *okerekecstan@gmail.com*

**ABSTRACT**

**Objective:** *To analyze the antioxidant activities of the methanolic leaf extract of Tridax procumbens and Tithonia diversifolia.*

**Methods**: *This was done using various antioxidant models; 1,1-diphenyl-2-picryl hydroxyl (DPPH) quenching assay and superoxide anion($O_2^{\circ\circ}$) scavenging assay. Phenolic contents, flavonoid contents and ascorbic acid contents were also estimated*

**Results**: *The results of this study revealed that Thithonia diversifolia possessed a significantly higher ($P < 0.05$) phenolic content (115.00 ± 13.00mg/100g), flavonoid content (120.67 ± 11.01mg/100g) quercetin equivalent (QE) and ascorbic acid content (55.00 ± 4.58mg/100g) of vitamin C equivalent, when compared with the phenolic content (81.00 ± 2.65mg/100g), flavonoid content (85.00 ± 4.00mg/100g QE) and ascorbic acid content (67.33 ± 3.06mg/100g) of vitamin C equivalent, when compared with the phenolic content (81.00 ± 2.65 mg/100g), flavonoid content (85.00 ± 4.00 mg/100g QE) and ascorbic acid content(67.33 + 3.06 mg/100g of vitamin C equivalent) of Tridax procumbens respectively. The DPPH Quenching activity of the METP showed higher effective antioxidant activity at 80µg/ml and 100µg/ml to be 102.00 ± 1.00 and 109.00 ± 6.56 respectively which were significantly different when compared to the standard which had 90.00 ± 2.65 and 94.00 ± 2.00 at same concentrations. But the values of METD was observed to be 50.00 ± 0.87 and 50.10 ± 0.90 which decreased significantly compared to values of METP and that of BHT. The Superoxide Scavenging Activity of METP also showed significant increase at all levels of concentration of extracts when compared to the METD.*

**Conclusion**: *The result showed that the leaf methanolic leaf extract of Tridax procumbens shows more antioxidant activity than Tithonia diversifolia.*

**Keywords:** *Tridax procumbens L, Tithonia diversifolia, Antioxidant, total phenolic, Flavonoids, DPPH assay, Reducing power activity.*

## Introduction

The healing powers of plants have been helpful, with most pharmaceutical drugs derived from them. This brought about the term herbal medicine, which implies the use of herbs for their therapeutic or medicinal value. These plants are mostly used in traditional setting especially in the tropics where there are abundance of flora(Karou *et al*.,2007). Traditional herbal medicine has made outstanding achevements in such area as bone-setting,mental disorder, sickle-cell anemia, wound healing, anemia, liver and kidney problems,

diabetes, malaria and many others(Farnworth *et al*.,1985; Sofowara, 1993).

The interplay between free radicals, antioxidants, and co-factors is important in maintaining health, aging and age-related diseases. Free radicals induce oxidative stress, which is balanced by the body's endogenous antioxidant systems with an input from co-factors, and by the ingestion of exogenous antioxidants (Riley,1994).If the generation of free radicals exceeds the protective effects of antioxidants, and some co-factors, this can cause oxidative damage which accumulates during the life cycle, and has been implicated in aging, and age dependent diseases such as cardiovascular disease, cancer, neurodegenerative disorders, and other chronic conditions.

Two types of medicinal leaves *Tithonia diversifolia* (Hemsl) and *Tridax procumbens* L Gray is an impressive member of the sunflower family, Asteraceae. It is native to Central America and the West Indies, although it has become naturalized around the tropics. It serves various indigenous medicinal uses in many countries. In Nigeria, the decoctions of its various parts are used for the treatment of malaria, diabetes mellitus, sore throat, liver and menstrual pains (Elufioye and Agbedahunsi,2004; Owoyele *et al*., 2004) . Some of these indigenous medicinal uses have been scientifically authenticated. *T. diversifolia* has been reported to exhibit analgesic and anti-inflammatory properties (Owoyele *et al*., 2004). The antibacterial and antiplasmodial activities of the various parts of the plant have been demonstrated (Elufioye and Agbedahunsi, 2004; Obafemi *et al*., 2006).

*Tridax procumbens* Linn. Commonly known as coat button is found in the West African sub region and other Tropical Zones of the world. *T. procumbens* has been valued for its pharmaceutical properties (Sahoo and Chand, 1998). Investigations into the health maintaining properties of *Tridax procumbens* L. have resulted in the identification of a wide array of bioactive compounds like alkaloids, carotenoids, flavonoids, fumaric acid, β-sitosterol, saponins and tannins. A rich content of Carotenoids, Saponins, Oleanolic acid and ions like sodium, potassium and calcium has also been reported. The plant as a procumbent herb has known for its number of pharmacological activities like hepatoprotective activity (Ravikumar *et al*., 2005), anti-inflammatory (Prabhu *et al*., 2011), wound healing (Nia *et al*., 2003; Bhat *et al*., 2007), antidiabetic activity (Bhagwat *et al*., 2008), antioxidant activity (Chander *et al*., 2005) etc. Antioxidants are means for the substances or group of the substances that delay or inhibit oxidative damage to a molecule. The literature survey reveals that *Tridax procumbens* L. plant possesses good antioxidant activity (Harborne, 1984).

Due to presumptive treatment of diseases by indigenous people, using *T. diversifolia* and *T. procumbens* for medicinal purposes it is necessary to evaluate possible antioxidant properties of these two plants. The present study has been aimed at evaluating the invitro antioxidant activity of methanolic leaf extract of *Tithonia diversifolia and Tridax procumbens*. The health promoting benefits of antioxidants of plant origin are thought to be resulted from their potential effects against the reactive oxygen/nitrogen species.

## Materials and Methods

**Plant material:** The two plant sample was collected by uprooting the whole plants .The authentication of plant was done by a Taxonomist in Abia State University Uturu. Voucher specimen was deposited in College Herbarium (V.No. ABU/25/2016 and V.No. ABU/26/2016).The whole plants was washed with water and shade dried for one week.

**Extraction of plant material:** Dried materials was coarsely pulverized to powdered form 1.5 kg of powdered plants material was defatted with petroleum ether exhaustively extracted with 700 ml of methanol by maceration process. MeOH extract was concentrated using Vaccum Rotary Evaporator and residue was dried in Petri-dish till crystalline deep green mass (102.37 g) was available.

**Procedure**

**Determination of total phenolic content**

The concentration of phenolic in fractions was determined using spectrophotometric method (Singleton *et al*., 1999). Methanolic solution of the fractions in the concentration of 0.01 mg mL was used in the analysis. The reaction mixture was prepared by mixing 0.5 mL of methanolic solution of fraction, 2.5 mL of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 mL 7.5% $NaHCO_3$. Blank was concomitantly prepared, containing 0.5 mL methanol, 2.5 mL 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 mL of 7.5% of $NaHCO_3$. The samples were there after incubated at room temperature in dark for 45 min. The absorbance was determined using spectrophotometer at λmax = 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and a dilution series of gallic acid of concentration 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg $mL^{-1}$ was prepared and calibration line was construed. Based on the measured absorbance, the concentration of phenolic was read (µg $mL^{-1}$) from the calibration line; then the content of phenolic in different fractions was expressed in terms of Gallic acid equivalent (µg of GA $mg^{-1}$ of Fraction).

**Determination of total flavonoids content**

The content of flavonoids in the examined fractions was determined using spectrophotometric method (Quettier-Deleu *et al*., 2000). The sample contained 1 mL of methanol solution of the fractions in the concentration of 0.01 mg $mL^{-1}$ and 1 mL of 2% $AlCl_3$ solution dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at $\lambda_{max}$ = 415 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of rutin and a dilution series of rutin of concentration 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg $mL^{-1}$ was prepared and the calibration line was constructed. Based on the measured absorbance, the concentration of flavonoids was read (µg $mL^{-1}$) on the calibration line; then, the content of flavonoids in examined fractions was expressed in terms of rutin equivalent (µg of RU $mg^{-1}$ of Fraction).

**DPPH free radical scavenging activity**

**Principal:** The Capacity of biological reagents to scavenge the DPPH radical can be expressed as its magnitude of antioxidant ability. The DPPH alcohol solution is deep purple in colour with an absorbance peak of 517 nm. Which appears with the presence of the radical scavenger in the reactive system and when an odd electron of the Nitrogen in the DPPH is paired?

**Preparation of stock solutions:** An accurately quantity of fractions (10 mg) was dissolved in methanol and volume was brought up to 100 mL with methanol (100 µg $mL^{-1}$).

**Preparation of test solution:** The portion of stock solutions of different fractions were diluted appropriately with methanol to obtain a dilution series of concentration range of 25, 50, 75, 100, 125 µg $mL^{-1}$.

**Method:** The free radical scavenging activity of the *Tithonia diversifolia and Tridax procumbens* L. fractions and Ascorbic acid was measured in terms of hydrogen donating or radical scavenging ability using the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma-Aldrich) (Ravishankara *et al*., 2002; Taddei and Rosas, 2000). 0.1 mM solution of DPPH in methanol was prepared and 1.0 mL of this solution was added to 3 mL of test solution in water at different concentrations (25-125 µg $mL^{-1}$). After 30 min incubation in darkness at room temperature (23°C), the absorbance was recorded at 517 nm. Control sample contains all the reagents except the extract. Percentage inhibition was calculated using Eq. 1, while $IC_{50}$ values were estimated from the percentage inhibition versus concentration plot, using a non-linear regression algorithm. The data were presented as mean values ± standard deviation (n = 3).

$$\text{Percentage inhibition} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \quad (1)$$

where, "A" stands for Absorbance.

**Result/ Discussion**

**Table 1:** Quantitative Determination of Antioxidants for *Tithonia diversifolia*

| | METD | METP |
|---|---|---|
| TOTAL PHENOLIC CONTENT (TCP) µg galic Eg/mg | 15.00 ± 13.00[a] | 81.00 ± 2.659[a] |
| TOTAL FLAVONOID CONTENT (TFC) µg Quercetin Eg/mg | 85.00 ± 4.00[d] | 120.67 ± 11.00[d] |
| TOTAL ASCORBIC ACID CONTENT (AAC) µg Vit C Eg/100g | 67.33 ± 3.06[c] | 55.00 ± 4.58[c] |

Value is mean ± SD, mean along the column group with different ($P < 0.05$).

KEY:

METD: Methanolic extract of *Tithonia diversifolia*

METP: Methanolic Extracts of *Tridax procumbens*

The result of *Tithonia diversifolia* revealed that Phenolic content was observed to be 81.00 ± 2.66 followed by Flavonoid content with the value of 85.00 ± 4.00 and Ascorbic acid with 67.33 ± 3.06 while *Tridax procumbens* had higher value of Total phenolic content to be 115.00 ± 13.00 followed by flavanoid with the value of 120.67 ± 11.01 of quercetin equivalent (QE), Ascorbic acid content was found to have 55.00 ± 4.58 of Vitamin C equivalents.

**Table 2:** DPPH Quenching Activity of Methanolic extract of *Tithonia diversifolia* and *Tridax procumbens*

| CONC. Of Extract | METD | BHT | METP |
|---|---|---|---|
| 20µg/ml | 45.78 ± 2.64[a] | 68.33 ± 4.04[a] | 78.00±2.65[a] |
| 40µg/ml | 47.92 ± 0.80[b] | 73.67 ± 4.73[b] | 85.33±3.51[b] |
| 60µg/ml | 48.80 ± 0.85[c] | 84.00 ± 4.85[c] | 94.00 ± 4.00[c] |
| 80µg/ml | 50.00 ± 0.87[d] | 90.00 ±2.65[d] | 102.00±1.00[d] |
| 100µg/ml | 50.10±0.90[e] | 94.00±6.56[e] | 109.00±6.56[e] |

Values are mean ± SD, mean along the column group with different alphabetical superscript indicate a significant difference ($p<0.05$)

KEY:

METD: Methanolic extract of *Tithonia diversifolia*

BHT: Butylated hydroxyl toluene

METP: Methanolic Extracts of *Tridax procumbens*

The DPPH Quenching activity of the METP showed higher effective antioxidant activity at 80µg/ml and 100µg/ml to be 102.00 ± 1.00 and 109.00 ± 6.56 respectively which were significantly different when compared to the standard which had 90.00 ± 2.65 and 94.00 ± 2.00 at same concentrations. But the values of METD was observed to be 50.00 ± 0.87 and 50.10 ± 0.90 which decreased significantly compared to values of METP and that of BHT. - The Superoxide Scavenging Activity of METP also showed significant increase at all levels of concentration of extracts when compared to the METD.

**Table 3:** Superoxide Scavenging Activity of Methanolic Extract of *Tithonia diversifolia* and *Tridax procumbens*

| CONC. Of Extract | METD | BHT | METP |
|---|---|---|---|
| 20µg/ml | 6.00 ±2.00[a] | 78.00±2.65[a] | 40.33±2.52[a] |
| 40µg/ml | 22.67±1.53[b] | 85.33±3.51[b] | 47.33±4.04[b] |
| 60µg/ml | 30.67±2.52[c] | 94.00±4.00[c] | 61.00±3.00[c] |
| 80µg/ml | 37.00±5.57[d] | 102.00±1.00[d] | 71.00±1.00[d] |
| 100µg/ml | 49.67±3.51[e] | 109.00±6.56[e] | 80.00±4.00[e] |

Value are mean ± SD, mean along the column group with different alphabetical superscript indicates a significant difference ($p < 0.05$).

KEY:

METD: Methonolic extract of *Tithonia diversifolia*

BHT: Butylated Hydroxyl Toulene

METP: Methanolic Extracts of *Tridax procumben*

The Superoxide Scavenging Activity of Methanolic Extract of *Tithonia diversifolia* and *Tridax procumbens* also showed higher effective antioxidant activity at 80µg/ml and 100µg/ml to be 37.00±5.57[d] and 71.00±1.00[d] for 80µg/ml and for 100µg/ml (49.67±3.51[e] for METD and 80.00±4.00[e] for METP )respectively which were significantly different when compared to the standard which had 102.00 ± 1.00 at 80µg/ml and 71.00±1.00[d] at 100µg/ml concentrations.

**Discussion**

The methanolic leaf extract of Tithonia diversifolia on DPPH quenching activity at the concentration of 20,40,60,80 and 100 µg/ml showed no significant difference between METD and BHT while in *Tridax procumbens* the extract inhibited a concentration dependent antiradical activity by quenching DPPH radical and DPPH Scavenging activity when compared to the BHT. This suggests that *Tridax procumbens* has more DPPH Scavenging activities when compared to *Tithonia diversifolia*. This may be attributed to high phenolic content, Total Flavoind and Ascobic acid content of the methanolic leaf extract of *Tridax procumbens*.

The result of *Tithonia diversifolia* revealed that Phenolic content was observed to be 81.00 ± 2.66 followed by Flavonoid content with the value of 85.00 ± 4.00 and Ascorbic acid with 67.33 ± 3.06 while *Tridax procumbens* had higher value of Total phenolic content to be 115.00 ± 13.00 followed by flavanoid with the value of 120.67 ± 11.01 of quercetin equivalent (QE), Ascorbic acid content was found to have 55.00 ± 4.58 of Vitamin C equivalents. The higher antioxidant activities of Phenolic compounds in *Tridax procumbens* is mainly due to their redox properties which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers(Parr and Bowell,2000). The significant increase in the phenolic content of *Tridax procumbens* is attributed to its antioxidant activity as reported by Chander *et al*., (2005). The result of the analysis shows that there is a relationship between the phenol content of medicinal plants and antioxidant activity. This findings support earlier reports that plant metabolites like flavonoids, tannins, catechins and other phenolic. compounds possesses antioxidant activity (Rice-Evans et al., 1995) and have played a preventive role in the development of cancer, heart and age related diseases. They have also been reported to be chemo-preventive agents by lowering cholesterol and repairing damage cells (Kahkonen et al., 1999). An electron uptake on pairing with a suitable reducing agent resulted the solution to lose colour, as the electron or hydrogen is easily accepted by nitrogen centered free radical; DPPH (Blois, 2001). DPPH assay provides a good assessment for evaluation of *in vitro* antioxidant activity. The DPPH Quenching activity of the METP showed higher effective antioxidant activity at 80µg/ml and 100µg/ml to be 102.00 ± 1.00 and 109.00 ± 6.56 respectively which were

significantly different when compared to the standard which had 90.00 ± 2.65 and 94.00 ± 2.00 at same concentrations. But the values of METD was observed to be 50.00 ± 0.87 and 50.10 ± 0.90 which decreased significantly compared to values of METP and that of BHT. - The Superoxide Scavenging Activity of METP also showed significant increase at all levels of concentration of extracts when compared to the METD. The significant increase from the results of DPPH Quenching activity and the Superoxide Scavenging Activity of *Tridax procumbens* may be attributed to its reported pharmacological activities like hepatoprotective activity (Ravi-kumar *et al*., 2005), anti-inflammatory (Prabhu *et al*., 2011), wound healing (Nia *et al*., 2003; Bhat *et al*., 2007), antidiabetic activity (Bhagwat *et al*., 2008), antioxidant activity (Chander *et al*., 2005), hypotensive effect, immunomodulating property (Tiwari *et al*., 2004; Oladunmoye, 2006), anticancer activity (Vishnu *et al*., 2011).

**Conclusion**

The result of the study suggests that the methanolic leaf extract of *Tridax procumbens* shows more antioxidant activities than *Tithonia diversifolia.*

**References**


1. Tiwari,M. et al. (2006) Mechanism of 4-HPR-induced apoptosis in glioma
2. Bhagwat, D.A., Willeder, S.G. and Adnaik, R.S.(2008) Anti diabetic activity of leaf extract of Tridax procumbens. *International J Green Pharma*., 2:126-128
3. Bhat, R.S., Shankrappa, J. and Shivak-umar, H.(2007) Formation and evaluation of polyherbal wound treatments. *Asian J. of Pharm. Sci.*, 2 (1)11-17
4. cells: evidences suggesting role of mitochondrial-mediated pathway and
5. Blois, M.S (2001) Antioxidant activity of grape seed extract on peroxidation models *in vitro. J. Agric Food Chem*. 55: 1018-1018
6. Chandra, S., Gallardo, G., Fernandez-Chacon, R.,Schluter, O.M., Sudhof, T.C(2005) Alpha-synuclein cooperates with CSPalpha in preventing neruro degeneration. *Cell* 123:383-396
7. Elufioye, T.O. and Agbedahunsi, J.M. (2004). Antimalarial activities of *Tithonia diversifolia* (*Asteraceae*) and *Crossopteryx febrifuge* (*Rubiaceae*) on mice in vivo. *J. Ethnopharmacol*. 93 (2-3): 167-171.
8. Endoplasmic reticulum stress. Carcinogenesis, 27, 2047–2058
9. Harbone, J.B.(1984) Phytochemical methods, 2nd edition, Chapman and Hall publication , London New York, pp.288.
10. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha J, Pihlaja K, Kujala ST, Heinonen M (1999). Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem. 47: 3954-3962.
11. Nia, R.; Paper, D.H.; Essien, E.E.; Oladimeji, O.H.; Iyadi, K.C.; Franz, G.(2003)Investigations into in vitro radical scavenging and in-vivo anti-inflammatory potential of Tridax procumbens. *Nig. J. of Physiological sciences*, 18 (1-2) 39-43.
12. Obafemi, C.A., Sulaimon, T.O., Akinpelu, D.A. and Olugbade T.A. (2006). Antimicrobial activity of extracts and a germacranolide-type sesquiterpene lactone from *Tithonia dversifolia* leaf extract. *Afr. J. Biotechnol*. 5(12): 1254-1258.
13. Prabha, M.R., Pereira, P., Chowta, N. and Hegde, B.M. (1998). Clinical implications of hypocalcemia in malaria. *Indian J Med Res*. 108: 62-65.
14. Quettier, D.C., Gressier, B., Vasseur, J., Dine, T., Brunet, C., Luyckx, M.C., Cayin, J.C., Bailleul, F., Trotin, F. (2000): Phenolic compounds and antioxidant activities of buckwheat (Fagopyrum esculentum Moench) hulls and flour. *J. Ethnopharmacol*. 72, 35-42.
15. Ravikumar, V, Shivashangari, KS, Devaki, T. (2005). Effect of Tridax procumbens on liver antioxidant defense system during

lipopolysaccharide-induced hepatitis in D-galactosamine sensitized rats. *Mol. Cell Biochem*. 269(1-2): 131-136.

16. Ravishankara, M.N.; Shrivastava, N.; Padh, H.; Rajani, M. (2002) Evaluation of antioxidant properties of root bark of Hemidesmus Indicus (Anantmul). *Phytomedicine*, 9: 153- 160.
17. Riley, P.A (1994) Free radicals in biology ;oxidation stress and effects of ionizing radiation. *Int. J. Rad Biol*. 65: 27-33
18. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridh JB (1995). The relative antioxidant activities of plant-derived polyphenolics flavonoids. Free Radical Res. 22: 375-383.
19. Sahoo,V. and Chand, P.K (1998) Micropropagation of Vitex negundo L. a woody aromatic medicinal shrub through high frequency of axilary shoot proliferation. *Plant Cell Rep.* 18:301-307
20. Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M. (1999): Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol*. 299, 152-178.
21. Sofowara, A. (1993). Medicinal plants and traditional medicinal medicine in Africa. Spectrum Books Ltd. Ibadan, Nigeria. Pp 191-289.
22. Taddei, Rosas; Romero,A.J.(2000) Bioactivity studies of extracts from Tridax procumbens. *Phytomedicine*, 7(3):235-238.