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Inhibitory Activity of *Tithonia diversifolia* (Hemsl.) A. Gray leaves on Some Pro-Oxidant Induced Oxidative Stress in Rat Brain

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Abstract

This study examines the antioxidant potentials and also the ability of the aqueous extract of *Tithonia diversifolia* (Hemsl.) A. Gray leaves to inhibit some pro-oxidants (Fe^{2+} and sodium nitroprusside) induced lipid peroxidation in rat's brain homogenates *in vitro*. The ability of the extracts to inhibit 25 µM $FeSO_4$ and 7.0 µM sodium nitroprusside induced lipid peroxidation in isolated rat's brain determined. The antioxidative potentials of the extract as typified by the overall phenol content, reducing power, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging ability and iron II chelating ability were cojointly determined. The results of the study discovered that each pro-oxidant (Fe^{2+} and sodium nitroprusside) caused a considerably increase in ($p < 0.05$) malonaldehyde (MDA) content of the brain. Though, aqueous extracts of *Tithonia diversifolia* caused a substantial concentration-dependent decrease ($p < 0.05$) within the malonaldehyde (MDA) contents of the brain. Though, aqueous extracts from *Tithonia diversifolia* had an appreciably higher ($p < 0.05$) inhibitory impact on each Fe^{2+} and sodium nitroprusside induced lipid peroxidation within the rat's brain homogenates. This higher inhibitory effect of *Tithonia diversifolia* may be attributed to its considerably higher ($p < 0.05$) total phenol content, iron (II) chelating ability, reducing power and free radical scavenging ability. Therefore, Fe^{2+} and sodium nitroprusside induced oxidative stress within the brain may be probably managed by dietary intake of *Tithonia diversifolia* leaves. These antioxidant potentials of *T. diversifolia* extract could have contributed to the employment of the leaves in the management of disease.

Keywords: *Tithonia diversifolia*, Fe^{2+}, lipid peroxidation, Sodium nitroprusside, antioxidant

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1. Introduction

Reactive oxygen species (ROS) are continually produced for exact metabolic condition and are reduced by an effectual antioxidant complex in the body. Once the generation of these species surpasses the levels of antioxidant mechanism, it causes oxidative damage to tissues and biomolecules, eventually ensuing into disease conditions, specifically chronic diseases [1, 2]. Free iron within the cytoplasm and mitochondria might cause oxidative harm by acting catalytically within the production of ROS leading to impairment in cellular operation and integrity [3, 4]. Sodium nitroprusside (SNP) an anti-hypertensive drug, acts by relaxing smooth muscle, dilates peripheral arteries and veins. Malonaldehyde (MDA) is the outcome of lipid peroxidation. It is a reactive aldehyde and is one in all the numerous reactive electrophile species employed as a biomarker to quantify the level of oxidative stress in an organism [5]. Numerous plant extracts and secondary metabolites are shown to possess antioxidant or free radical scavenging properties [6] and it's been recognized as one of the mechanisms of their action [7]. In recent years, the extracts of many plants are screened for their antioxidant activities. The presence of active secondary metabolites in some plants and herbs utilized in managing several diseases as a result of oxidative stress

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has been documented. Samples of such plants are *Ocimum gratissimum* [8].

Tithonia diversifolia (Hemsl.) A. Gray is taken into account to be a medicative plant that's loosely utilized in medicine to treat numerous diseases, it's usually referred to as Mexican Sunflower, tree flower, bush helianthus or Japanese helianthus (English), "sepeleba" (Yoruba). Ethno botanical relevancy have shown that extracts from the plant exhibited anti-malaria, anti-diarrhoeic, anti-inflammatory, antibacterial, anti-proliferation properties and its effectiveness within the treatment of haematomas and wounds had been established in addition [9]. The leaf is reported to contain sequiterpene lactones taginin C as a vigorous part against *Plasmodium* [10], diversifolin, diversifolin methyl ether and tirotundin as effective constituent against inflammatory activity [11]. Though lots had been reported on the phytochemicals of *Tithonia diversifolia*, however, there's restricted data on the antioxidant and inhibitory potentials within the management of diseases related to oxidative stress. This study examines the antioxidant potentials and inhibitory effects of *Tithonia diversifolia* aqueous extract on some pro-oxidant (Fe^{2+} and sodium nitroprusside) induced lipid peroxidation in Rat's brain homogenates *in vitro*.

2. Materials and Methods

2.1 Collection of plant

Fresh leaves of *T. diversifolia* (Hemsl.) A. Gray were freshly purchased from Ijadu Farm, Olorunda, Ado-Ekiti, Nigeria. The plant was authenticated and documented by a senior taxonomist and an herbarium (UHAE/2014/086) number was documented at the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria.

2.2 Chemicals and reagents

Chemicals and reagents used was bought from Sigma–Aldrich, Inc., (St. Louis, MO). All chemicals used for the experiment were of analytical grade.

2.3 Preparation of extract

2.3.1 Aqueous extract preparation

The aqueous extract of the powered *Tithonia diversifolia* leaves was prepared using the method of [12]. The percentage yield of extraction calculated as 15.1 % percent yield.

$$\text{Percentage yield} = \frac{\text{Weight of the dry extract}}{\text{Weight of powdered leaves}} \times 100\%$$

2.4 Estimation of total phenol content

The total phenol content of *T. diversifolia* aqueous extract was assessed (as gallic acid equivalent) by the described protocol by McDonald et al. [13]. In short, 200 µL extract dissolved in 10 % DMSO (240 µg mL^{-1}) was incubated with one mL of Folin Ciocalteau chemical agent (diluted 10 times) and 800 µL of 0.7 mol L^{-1} Na_2CO_3 for 30 min at ambient temperature. Absorbance was read at 765 nm using spectrophotometer. All readings were repeated three times. Results expressed as mg GAE per hundred grams of dry aqueous extracts.

2.5 Determination of Ferric Reducing Antioxidant Property (FRAP) of *T. diversifolia* aqueous extract

The reducing property of the *T. diversifolia* extracts was studied by assessing the power of the extract to reduce $FeCl_3$ solution as described by Pulido et al. [14]. A 2.5 mL aliquot was mixed with 2.5 mL, 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL, 1% potassium ferricyanide. Solutions was incubated at 50 °C for 20min and then 2.5 mL, 10% TCA was added. Sample was then centrifuged at 650 g for 10 min. Thereafter, 5 mL of the supernatant was mixed with an equal volume of water and one mL, 0.1% ferric chloride. The above-stated process was also applied to a standard ascorbic acid solution, and finally the absorbance was read at 700 nm. The reducing ability was calculated as percentage inhibition.

2.6 Iron Chelation Ability Estimation

The Fe^{2+}chelating ability of *T. diversifolia* aqueous extracts was determined by employing a changed procedure by [15]. Newly made 500 µmol L^{-1} $FeSO_4$ (150 µL) was added to the solution containing 168 µL of 0.1 mol L^{-1} Tris-HCl (pH 7.4), together with 218 µL saline and the aqueous extracts (1-5 mL). Solution was incubated for 5 min, with the addition of 13 µL of 0.25% 1, 10-phenanthroline (w/v). Absorbance was read at 510 nm. Fe^{2+} chelating ability was determined and expressed as percentage inhibition.

2.7 Estimation of DPPH radical scavenging activity

The DPPH radical scavenging activity of *T. diversifolia*

aqueous extracts was assessed using somewhat modified modus defined by Tuba and Gulcin [16]. 0.3 mmol L^{-1}solution of DPPH was prepared in methanol, 500 µL of this solution was added to 1 mL of the extract (dissolved in 10 % DMSO) at different concentrations (1–5 mg mL^{-1}). Solution was mixed, incubated in the dark for 30 min at room temperature. Absorbance was measured at 517 nm against a blank samples lacking scavenger.

2.8 Estimation of Lipid Peroxidation

2.8.1 Preparation of Tissue Homogenates

Normal healthy rats were decapitated under mild diethyl ether anaesthesia and the brain was dissected, placed on ice and weighed. Brain tissue was afterwards homogenized in cold saline (1/10 w/v) with about 10 up-and-down strokes at about 1,200 rpm in a homogenizer. Homogenate was centrifuged for 10 min at 3,000 x g to yield a pellet that was superfluous, supernatant (S1) comprising primarily lipids (cholesterol, galactolipid, individual phospholipids, gangliosides), proteins, deoxyribonucleic acid, ribonucleic acid and water was kept for lipid peroxidation assay. The ethical committee of the Afe Babalola University approved this study.

2.9 Lipid Peroxidation Procedure

The lipid peroxidation assay was estimated using the adapted method of Ohkawa et al. [17]. Briefly 100 µL of the supernatant brain homogenate was mixed with a reaction mixture containing 30 µL of 0.1 M pH 7.4 Tris-HCl buffer, leaf extract (100 µL) and 30 µL of 250 µM freshly prepared $FeSO_4$ as the pro-oxidant. The volume was made up to 300 µL by water before incubation at 37 °C for 2 hr. The color reaction was developed by adding 300 µL 8.1% SDS (Sodium dodecyl sulphate) to the reaction mixture, this was followed by the addition of 500 µL of acetic acid/HCl (pH 3.4) mixture and 500 µL of 0.8% thiobarbituric acid (TBA). This mixture was incubated at 100 °C for 1 hr. Thiobarbituric acid reactive species (TBARS) produced were measured at 532 nm and expressed as malondialdehyde (MDA) produced (% control) using MDA standard curve.

Though, for sodium nitroprusside (SNP) induced lipid peroxidation, the procedure was carried out as above using 5 mM sodium nitroprusside (SNP) as the pro-oxidant.

3. Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 statistical package (GraphPad Software, USA). Data were analyzed using a statistical software package (SPSS for Windows, version 20, IBM Corporation, USA) using Duncan multiple range *post-hoc* test [25]. Values of mean ± standard deviations in triplicates were considered significantly different at $p < 0.05$.

4. Results

Aqueous extract of *T. diversifolia* (Hemsl.) A. Gray leaves has 15.1% as its yield. The overall phenolic content of aqueous extract of *T. diversifolia* leaves increases as the concentration (1-5 mg/ml) of extract increases. At 5 mg/ml, the extract of *T. diversifolia* has high phenol content of 28.62 ±1.93 mg GAE/100g dry extract (Table 1).

Table 1. Total Phenol contents of the Aqueous Extract of *Tithonia diversifolia* Leaves

Concentration (mg/ml)	(mg GAE/100g dry extract)
1	5.7 ± 0.14
2	11.10 ± 0.21
3	16.93 ± 0.25
4	23.06 ± 0.26
5	28.62 ±1.93

* Results are expressed as mean of three determinations ± standard error of mean (SEM)

Aqueous extract of *T. diversifolia* leaves show considerably ($P < 0.05$) higher reducing power (Figure 1). The trend of the reducing capacity of the extracts was concentration dependent (1-5 mg/ml).

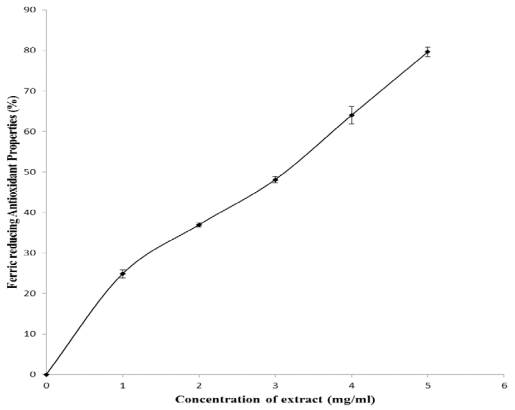


Figure 1. Reducing power of *Tithonia diversifolia* aqueous extract.

Aqueous extract of *T. diversifolia* leaves extract had higher chelating effect. IC_{50} values for chelating effect of aqueous extract of *T. diversifolia* leaves was 2.45 mg/ml. The trends of the chelating effect increase with increased concentration of *T. diversifolia* leaves of the extracts in a concentration-dependent manner (1-5 mg/ml) (Figure 2). This reveals that aqueous extract of *T. diversifolia* has a higher chelating ability.

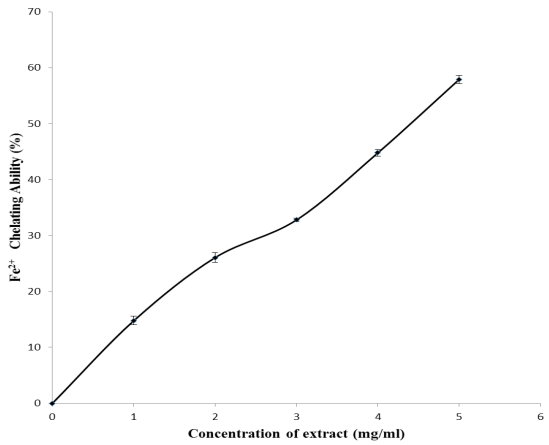


Figure 2. Fe (II) Chelating ability of aqueous extracts of *Tithonia diversifolia* leaves.

The scavenging effect of extracts in the range 1–5mg/ml on the 1,1-diphenyl-2-picrylhydrazyl radical increased with an increasing concentration of *T. diversifolia* extracts (Figure 3). IC_{50} value for 1,1-diphenyl-2-picrylhydrazyl scavenging by the aqueous extract of *T. diversifolia* was 2.67 mg/ml. In the present investigation *T. diversifolia* at different concentration validated significant 1,1-diphenyl-2-picrylhydrazyl scavenging activity indicating its ability to act as radical scavengers.

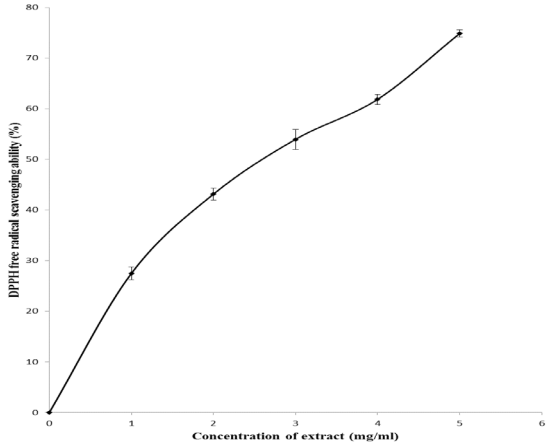


Figure 3. Free radical scavenging ability of aqueous extract of *Tithonia diversifolia*.

Table 3 revealed that incubation of the rat's brain in the presence of Fe (II) caused a significant increase ($p < 0.05$) in the MDA content of the brain (27.47 ± 2.51 µmoL/g tissue) when compared with the basal brain without Fe (II). However, the aqueous leaf extract of *T. diversifolia* inhibited MDA production in rat's brain in a concentration-dependent manner (1 – 5 mg/mL). Nonetheless, arbitrating by the EC_{50} (extract concentration causing 50 % enzyme inhibition) values in Table 2, the plant (EC_{50} = 0.64 ± 0.21mg/ml) had an expressively ($P \leq 0.05$) high inhibitory effect on Fe^{2+} induced lipid peroxidation in the liver homogenate.

Table 2. EC_{50} values of Inhibition of Fe^{2+} and Sodium nitroprusside (SNP) induced lipid peroxidation in rat brain by aqueous extract of *Tithonia diversifolia* leaves

Sample	EC_{50} (mg/ml) Fe^{2+} induced lipid peroxidation	SNP induced lipid peroxidation
Tithonia diversifolia	0.64 ± 0.21	0.68 ± 0.32

Values represent mean ± standard error of mean (SEM), number of samples n=3.
* EC_{50} (Extract concentration causing 50% inhibition)

Table 3. Inhibition of Fe (II) induced lipid peroxidation in rat's brain by aqueous extract of *Tithonia diversifolia* leaves in vitro

Treatments	*Tithonia diversifolia* concentration (mg/ml)	MDA (µmoL/g tissue)*
Normal	-	2.51 ± 0.14^{a}
Control	-	27.47 ± 2.11^{b}
Iron + leaf extract	1	6.95 ± 0.32^{c}
Iron + leaf extract	2	5.74 ± 0.66^{c}
Iron + leaf extract	3	4.56 ± 0.42^{d}
Iron + leaf extract	4	3.72 ± 0.56^{e}
Iron + leaf extract	5	2.88 ± 0.11^{f}

*Results are expressed as means of three experiments in duplicate ± SEM.
$^{a, b, c, d, e, f}$means without a common superscript differ significantly ($p \leq 0.05$) by Duncan's multiple range test.

Table 4 reveals that incubation of the rat's brain homogenates in the presence of 7 µM sodium nitroprusside caused a noteworthy increase ($p < 0.05$) in the MDA (22.25 ± 0.56 µmoL/g tissue) content of the brain compared to the basal brain. However, the plant (EC_{50} = 0.68 ± 0.32mg/ml) had a significantly ($P < 0.05$) high inhibitory effect on SNP induced lipid peroxidation in the brain homogenate.

Table 4. Inhibition of Sodium nitroprusside (SNP) induced lipid peroxidation in rat's brain by aqueous extract of *Tithonia diversifolia* leaves in vitro

Treatments	Tithonia diversifolia concentration (mg/ml)	MDA (µmoL/g tissue)*
Normal	-	1.65 ± 0.11^{a}
Control	-	22.25 ± 2.45^{b}
SNP + leaf extract	1	5.88 ± 0.16^{c}
SNP + leaf extract	2	4.74 ± 0.25^{c}
SNP + leaf extract	3	3.58 ± 0.21^{d}
SNP + leaf extract	4	2.46 ± 0.32^{d}
SNP + leaf extract	5	1.77 ± 0.24^{e}

*Results are expressed as means of three experiments in duplicate ± SEM. [a, b, c, d, e]means without a common superscript differ significantly ($p \leq 0.05$) by Duncan's multiple range test.

5. Discussion

In the present study, we have investigated the anti-oxidative and inhibitory effect of *Tithonia diversifolia* (Hemsl.) A. Gray leaves aqueous extracts *in vitro*. *Tithonia diversifolia* (Hemsl.) A. Gray leaves is distributed throughout the world. Consistent with this knowledge, it's evident that aqueous extract has higher yields and contain a higher total phenol content as the concentration increases (Table 1). This is according to earlier reports of [8]. Phenols are vital plant constituents due to their scavenging ability owing to their hydroxyl groups [18]. Several studies have shown that medicative plants with antioxidative properties would possible act as free radical scavenger, reducing agents, chelating agents for transition metals, slaking of singlet oxygen molecules and activators of antioxidative defense enzymes to suppress free radical damage in biological system [19]. There's a powerful correlation between thiobarbituric acid-reactive substance (TBARS) as a marker of lipid peroxidation and products that reveal oxidative damage to DNA. Adapted protocol to assess the antioxidant effect of *T. diversifolia* leaves embrace among others, ferric reducing antioxidant ability, the DPPH radical scavenging assay, and iron chelating ability a wide used methodology for assessing the antioxidant status of compounds or plant products. Likewise, intended IC_{50} values was used to validate the extent of scavenging power for the plant. Nonetheless, the lower the IC_{50} values the higher the scavenging activity. More importantly, the systematically lower IC_{50} value exhibited by the aqueous extract, for ferric reducing ability, iron chelating ability and DPPH radical scavenging ability implies that the extracts possess compounds with high radical-quenching ability that might terminate radical activities. Chelating iron may facilitates forestall the generation of hydroxyl group radicals. Iron is a metal catalyst in manufacturing hydroxyl group radicals from peroxide [8]. Aqueous extract of *T. diversifolia* considerably chelate Fe^{2+} in an exceedingly dependent manner (1-5 mg/mL). By chelating Fe^{2+}, the generation of hydroxyl group radicals within the Fenton reaction will be attenuated and thus forestall possible harm of hydroxyl radicals to biomolecules. Therefore, phenolic scavenge DPPH by donating electrons or chemical element to stabilize the radical. Our investigation disclosed that aqueous extracts of *T. diversifolia* leaves scavenged DPPH free radicals in a concentration dependent manner (1-5 mg/mL). However, the DPPH radical scavenging abilities of the aqueous extracts correlates with the total phenolic contents of the leaves.

Increase within the formation of TBARS in iron (II) sulphate-induced oxidative stress; as compared to normal recommend possible harm of tissue with overload of iron, which might cause considerable oxidative injury by increasing superoxide production. The finding that Fe^{2+} caused a major increase within the MDA content of the brain homogenate united with earlier report wherever Fe^{2+} was shown to be a potent initiator of lipid peroxidation [19]. The increased lipid peroxidation within the presence of Fe^{2+} may be attributed to the very fact that Fe^{2+} will catalyze one-electron transfer reactions that generate reactive oxygen species, like the reactive $OH^{\cdot}$, that is created from H_2O_2 through the Fenton reaction. Hence, doable depletion of iron might decrease oxidative stress throughout the total body [19]. Elevated Fe (II) content within the brain had been coupled to Parkinson's malady. Though, the etiology of the malady remains obscure, numerous studies point to the dominant role of Fe-induced oxidative stress mechanism.

Sodium nitroprusside (SNP) will cause brain injury through the discharge of cyanide or nitric oxide (NO) gas which might acts either alone or in conjunction with alternative reactive oxygen species (ROS) like superoxide radical to cause neuronic injury [20]. Though, aqueous extract from *Tithonia diversifolia* leaves ($p < 0.05$) inhibited 7 µM sodium nitroprusside induced lipid peroxidation in rat's brain in a very concentration dependent manner. This is often a sign that *T. diversifolia* extract was able to

scavenge the NO* made by sodium nitroprusside and chelate the Fe made as a result of the decomposition of the nitroprusside.

6. Conclusion

The aqueous extracts of *T. diversifolia* (Hemsl.) A. Gray was able to protect the brain against Fe^{2+} and sodium nitroprusside induced lipid peroxidation. These protective effect may be because of the antioxidant potentials like overall phenol content, Fe^{2+} chelating ability, free radical scavenging and reducing power. These antioxidant properties of *T. diversifolia* (Hemsl.) A. Gray may have contributed to its usage in traditional medicine.

7. Acknowledgment

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8. Conflict of Interest

The authors declare that they have no conflict of interests.

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