The Pharmaceutical and Chemical Journal, 2016, 3(1):120-124

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Inhibitory effect of ethyl acetate fraction of *Talinum triangulare* (jacq.) Willd on Fe^{2+} induced lipid peroxidation in albino rat tissue homogenates-*in vitro*

Afolabi OB, Ibitayo AO, Jaiyesimi KF, Olayide II, Adewumi DF

College of Science, Department of Chemical Science, Afe-Babalola University. P.M.B 5454, Ado- Ekiti, Ekiti State, Nigeria.

Abstract In the current study, total phenolic content (TPC) of ethyl acetate fraction of *Talinum triangulare* was determined using Folin-Ciocalteu method and the results indicated significant ($p<0.05$) difference within various concentrations examined with considerable increase as the concentration of the solvent extract increased, also, the ability of the solvent fraction of the plant to inhibit the formation of malondialdehyde (MDA) in Fe^{2+}-induced lipid peroxidation of some selected tissue homogenates was considered*in vitro*through TBARS and the results indicated significant ($p<0.05$) difference in the percentage inhibitions at moderate concentrations ranging from 0.33-3.33µg/ml. These results indicate phyto-nutritional endowment of *Talinum triangulare* which could be useful as aid in treating ailments and some common diseases.

Keywords Antioxidants, oxidative stress, total phenolic, TBARS.

Introduction

Free radicals cause damages, diseases and severe disorder to healthy cells by attacking them and these species are atoms or groups of atoms with an unpaired number of electrons which are produced when oxygen interacts with certain molecules [1]. They pose threat on vital cells and the danger comes from the damage they impose when they react with important cellular components such as DNA, or the cell membrane proteins and other macromolecules which may lead to a wide range of human diseases mostly heart disease, cancer and death or poor function of Cells [2]. The generation of these free radicals could be due to several factors one of which is environmental factors such as exposure to pollutants, alcohol, medications, infections, poor diet, toxins, radiation etc [3-4]. The antioxidants produced naturally by the body are not enough to neutralize all the free radicals in the body, therefore, a constant supply of external sources of antioxidants with outstanding ability to overhaul damaged molecules by donating hydrogen atoms to the molecules is always needed [5-6].

Plants are naturally endowed with numerous antioxidants properties that can in various ways mitigate the activities of reactive oxygen species (ROS) [7], these molecules interact with free radicals in an innocuous manner and alter the chain reaction that could possibly damage vital molecules and as well inhibit the oxidation of other molecules [3, 5]. Many phenolic compounds found in plants, particularly phenolic compounds, exhibit a wide range of biological effects which includes antioxidant, antibacterial, antiviral actions etc [8]. The health supporting effects of antioxidants from plants are thought to be as a result of their protective effects to neutralize reactive oxygen species (ROS), which are believed to play a significant role in the pathogenesis of various diseases and the oxidative deterioration of many products and prevention of lipid peroxidation and oxidative modification of low-density lipoproteins [9,10]. In the present study, solvent extraction of *Talinum triangulare*, a perennial deciduous plant with woody stems and succulent leaves popularly known as water leaf was examined. The present study was designed to verifying the total phenolic content and the ability of the plant to inhibit the formation of malondialdehyde (MDA) in tissue homogenates of Albino rats *in vitro.*

Materials and Methods

Collection of plant materials and preparation

Fresh water leaves, *Talinum triangulare* were bought in Ado-Ekiti, Ekiti State, Nigeria. Sample was taken to the Department of Plant Science in Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria for identification, which was carried out by a taxonomist in the department and Herbarium number UHAE 2013/76 was given after proper taxonomic investigations from the data base.

Chemicals

Chemicals and reagents used were all sourced from BDH Chemicals Ltd., (Poole, England) and all were of analytical grades and prepared using sterilized distilled water.

Preparation of Tissue homogenates

The rats were killed by cervical dislocation. Liver and brain tissues were quickly removed and placed on ice. Each of the tissues was homogenized in cold 0.1M Tris-HCl buffer pH7.4 (1:5 w/v) in a Teflon homogenizer. The homogenates were centrifuged for 10 min at 3000 g to yield a pellet that was discarded and the supernatant was used for the assay.

Preparation of Sample

The blended/powdered sample (120 g) of *Talinum triangulare* (*Tt*) soaked in solvent combination of 70 % ethanol for 72 h. The mixture was filtered using sterile Whatman paper G. No. 1 and re-concentrated. Portion of the ethanolic extract was weighed and reconstituted in distilled water, to this was added ml of petroleum ether (Pet ether). This was mixed thoroughly and then turned into separating funnels and left to stand. This procedure was repeated for the second time before re-concentration.

The percentage yield of extract was calculated as follows:

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

Determination of Lipid Peroxidation by Thiobarbituric Acid Reactive Species (TBARS)

A specific gram of each of the tissues was homogenized in cold 0.1 M Tris-HCl buffer pH 7.4 (1:10 w/v) in a Teflon homogenizer. The homogenates were centrifuged for 10 min at 3000 g to yield a pellet that was discarded and the supernatant was used for the assay. The supernatant with or without 50 µl of the freshly prepared pro-oxidant ($FeSO_4$), different concentrations of the plant extracts and an appropriate volume of distilled water which gives a total volume of 300 µl were incubated at 37 ^oC for 1 h. The color reaction was carried out by adding 200 µl 8.1 % sodium dodecyl sulphate (SDS), 500 µl 1.33 M acetic acid (pH 3.4) and 500 µl 0.6 % TBA. The reaction mixture was incubated at 97 ^oC for 1 h.The absorbance was read after cooling at a wavelength of 532 nm in a visible-ultraviolet spectrophotometer.

Estimation of Total Phenolic Content

Total Phenolic Content (TPC) was determined using Folin- Ciocalteu method [11]. Different concentration of the extract (20-100 µg/ml) were mixed with 500 µl of water and then with 100 µl of 10 % Folin-Ciocalteu reagent and allowed to stand for 6 min. Then 1 ml of 7 % sodium carbonate and 500 µl of distilled water were added to the reaction mixture. The absorbance was recorded after 90 min at 760 nm spectrophotometrically. Total Phenolic Content was calculated as garlic acid equivalents (µg/g GAE of the extract).

Statistical analysis

The results of replicate readings were pooled and expressed as mean±SD. One way analysis of variance was used to analyze the results and Duncan multiple tests was applied for the *post hoc* [12]. Statistical package for Social Science (SPSS) 10.0 for Windows was used for the analysis. The IC_{50} was calculated using non-linear regression analysis. The p value < 0.05 was considered statistically significant in the analytical data.

Results and Discussion

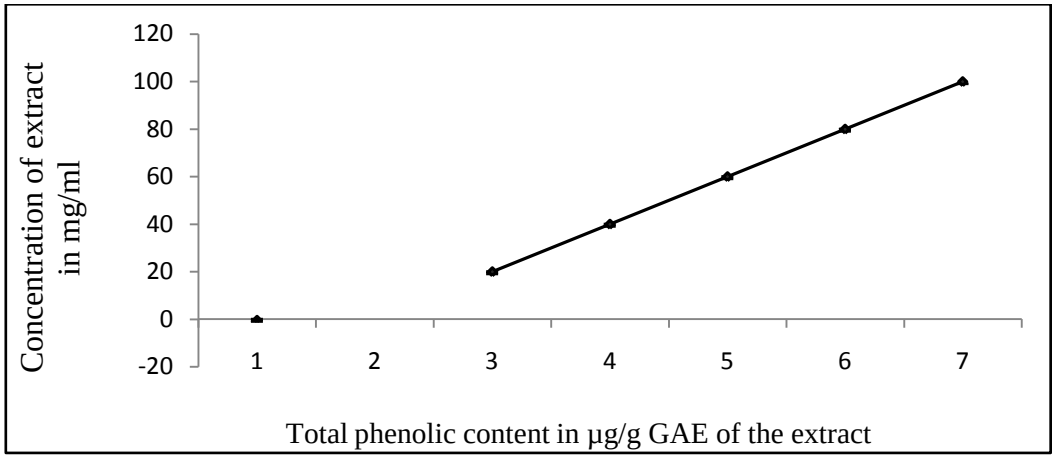

Figure 1: Total phenolic content in various concentrations observed in ethyl acetate extract of Tt

The results obtained indicated significant ($p<0.05$) difference in the Total phenolic content (TPC) observed in sample various concentrations of ethyl acetate extract of *Tt* as it increased with increase in the concentration of the extract (Figure 1). TPC was determined by interpolating the absorbance of the samples, based on a calibration curve constructed with standard garlic acid. The results were expressed in µg/g GAE of the dry sample per one gram garlic acid equivalents (GAE). The total phenolic content (TPC) of ethyl acetate fraction of *Talinum triangulare* was determined and expressed in µg/g GAE of the dry sample as represented in the results obtained (Figure 1), this result indicated significant ($p<0.05$) difference in total phenolic content (TPC) present in the sample with various concentrations of the solvent fraction examined as it increased with increase in the concentration of the extract (Figure 1). Phenolic compounds which are secondary metabolic products that occur all through the plant kingdoms had been reported to have demonstrated correlations with the antioxidant activities of most plant rich foods [13-14]. However, the anti-oxidative and protective roles of these compounds on cells has been established to be either by preventing the production of free radicals or by neutralizing/scavenging the free radicals produced in the body during metabolic processes, indicating that poly-phenolic compound- endowed plants would have high antioxidant properties [15].

Table 1: The Inhibitory effect of ethylacetate extract of *Talinum triangulare* (*Tt*) on iron II (Fe^{2+}) sulphate-induced lipid peroxidation in brain tissue homogenates.

Treatment	Conc. (µg/ml)	% Inhibition	Log. Equation	IC_{50} (µg/ml)	Brain homogenate (r^2)
Basal	-	53.17 ± 8.7			
Fe + *Tt*	3.33	31.40 ± 7.2			
Fe + *Tt*	2.67	27.80 ± 11.8	Y= 1.3693In (x)+29.359	0.70 ±0.48	r^2 = 0.0592
Fe + *Tt*	1.33	26.89 ± 17.5			
Fe + *Tt*	0.67	35.93 ± 8.7			
Fe + *Tt*	0.33	31.33 ± 12.6			

Results are expressed as means of three experiments ± standard deviation, Fe- iron II (Fe^{2+}) sulphate;*
Tt-Talinum triangulare,

The inhibitory potential of Ethyl acetate extract of *Talinum triangulare* against ROS - induced peroxidation in the brain of albino rats is shown in Table 1, the range of inhibitory power of the extract when incubated with Fe^{2+} varies considerably among the concentrations used in the brain homogenate with the highest inhibition 35.93±8.7 % observed at 0.67 µg/ml, this could be that, prevention of the production of free radicals or ability to neutralize/scavenge free radicals produced in the in the damaging tissue has been possibly orchestrated by the enriched antioxidant of which phenolic content (s) is/are among. Beneficial effects of antioxidant rich plant food on human health mainly reside in their potent anti-oxidative activity which have been reported to prevent or delay a number of chronic and degenerative ailments such as cancer, cardiovascular diseases, arthritis, aging, cataract, memory loss, stroke, Alzheimer's disease and inflammation [16]. The Membranous lipid peroxidation in the presence of Fe^{2+} is attributed basically to the fact that Fe^{2+} catalyzes one electron transfer reaction that generates reactive oxygen species (ROS) which is formed from H_2O_2 through Fenton reaction [17]. However, elevated Fe^{2+} content in

the brain has also been linked to these hosts of neurodegenerative diseases in humans as the presence of free Fe in the cell decomposes lipid peroxides, thereby generating peroxyl and alkoxyl radicals, which favors the propagation of lipid oxidation with the free Fe in the cytosol and mitochondria causing considerable oxidative damage by increasing superoxide generation which can react with Fe^{2+} to generate Fe^{3+} [18].

Table 2: The Inhibitory effect of ethylacetate extract of *T. triangulare* (*Tt*) on iron II (Fe^{2+}) sulphate-induced lipid peroxidation in liver tissue homogenates.

Treatment	Conc. (µg/ml)	% Inhibition	Log. Equation	IC_{50} (µg/ml)	Liver homogenate (r^2)
Basal	-	44.27 ± 6.2			
Fe + *Tt*	3.33	29.53 ± 18.2			
Fe + *Tt*	2.67	29.07 ± 12.4	Y= -16.53In (x)+50.931	1.90 ± 0.01	r^2 = 0.12
Fe + *Tt*	1.33	44.90 ± 12.7			
Fe + *Tt*	0.67	41.77 ± 12.9			
Fe + *Tt*	0.33	28.63 ± 14.2			

Results are expressed as means of three experiments ± standard deviation, Fe- iron II (Fe^{2+}) sulphate;* *Tt-Talinum triangulare*.

Considering Table 2, Significant ($p<0.05$) difference was noted in the inhibition of MDA produced within the concentrations ranging from 0.33-3.33 µg/ml when Fe^{2+} was incubated with the tissue homogenate showing formation of ROS and invariably indicating reduction in the activity of the induced oxidative stress in the tissue homogenate having been cushioned as the concentration of antioxidant in the extract increased [19]. The significant reduction in total ferric reducing ability of plasma has been implicated as the major cause of liver diseases both in pre-existing cirrhosis and ischemic-reperfusion injury due to increase in imbalance between antioxidant capacity and pro-oxidants, as they may interrelate with essential cellular targets among which are proteins, lipids and DNA, thereby, compromising cell viability and function [20]. However, liver cells, mainly hepatocytes have developed a comprehensive array of antioxidant defenses to prevent formation of ROS.

Conclusion

The current study on ethyl acetate extract fraction of *Talinum triangulare* has shown considerable antioxidant properties *in vitro*, this solvent used has been established to have high extracting power and with the phenolic antioxidant content having shown significant increase as the extract concentration increased. It could be that the phenolic compound contained in *Talinum triangulare* is responsible for the inhibition observed against the induced oxidative stress through MDA generated in the vital tissues examined. Beneficial effects of antioxidant rich plant food on human health mainly reside in their potent antioxidative activity which have been reported to prevent or delay a number of chronic and degenerative ailments such as cancer, cardiovascular diseases, arthritis, aging, cataract, memory loss, stroke, Alzheimer's disease, inflammation, infection.

References

1. Goldfarb, A. H. (1993). Antioxidants: role of supplementation to prevent exercise-induced oxidative stress. *Medicine and Science in Sports and Exercise*, *25*(2), 232-236.
2. Afolabi, O. B., Oloyede, O. I., Olayide, I. I., Obafemi, T. O., Awe, O. J., Afolabi, B. A., & Onikani, A. S. (2015). Antioxidant enhancing ability of different solvents extractable components of Talinum triangulare in some selected Tissue homogenates of Albino Rats-*In vitro*. *Journal of Applied Pharmaceutical Science*, *5*(09), 056-061.
3. Sies, H. (1997). Oxidative stress: oxidants and antioxidants. *Experimental Physiology*, *82*(2), 291-295.
4. Kohen, R., & Nyska, A. (2002). Invited review: Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicologic Pathology*, *30*(6), 620-650.
5. Afolabi, O. B., & Oloyede, O. I. (2014). Antioxidant properties of the extracts of Talinum Triangulare and its effect on antioxidant enzymes in tissue homogenate of swiss albino rat. *Toxicology International*, *21*(3), 307.
6. Birasuren, B., Kim, N. Y., Jeon, H. L., & Kim, M. R. (2013). Evaluation of the antioxidant capacity and phenolic content of *Agriophyllum pungens* seed extracts from Mongolia. *Preventive Nutrition and Food Science*, *18*(3), 188.

7. Afolabi, O.B., Ibitayo, A.O., & Fadaka, A.O. (2015). Overview of cellular damage in Abnormal absorption of Magnetic and Radio waves: Implications among Cell-Phone users. *Pharmacologyonline*, 3,1-7.
8. Cook, N. C., & Samman, S. (1996). Flavonoids—chemistry, metabolism, cardioprotective effects, and dietary sources. *The Journal of Nutritional Biochemistry*, *7*(2), 66-76.
9. Kaur, C., & Kapoor, H. C. (2001). Antioxidants in fruits and vegetables–the millennium's health. *International Journal of Food Science & Technology*, *36*(7), 703-725.
10. Servili, M., Selvaggini, R., Esposto, S., Taticchi, A., Montedoro, G., & Morozzi, G. (2004). Health and sensory properties of virgin olive oil hydrophilic phenols: agronomic and technological aspects of production that affect their occurrence in the oil. *Journal of Chromatography A*, *1054*(1), 113-127.
11. Odabasoglu, F., Aslan, A., Cakir, A., Suleyman, H., Karagoz, Y., Halici, M., & Bayir, Y. (2004). Comparison of antioxidant activity and phenolic content of three lichen species. *Phytotherapy Research*, *18*(11), 938-941.
12. Zar, J. H. (1984). Biostatistical Analysis. *Prentice-Hall, Englewood Cliffs, NJ*.
13. Sun, J., Chu, Y. F., Wu, X., & Liu, R. H. (2002). Antioxidant and antiproliferative activities of common fruits. *Journal of Agricultural and Food Chemistry*, *50*(25), 7449-7454.
14. Shahidi, F., Janitha, P. K., & Wanasundara, P. D. (1992). Phenolic antioxidants. *Critical Reviews in Food Science & Nutrition*, *32*(1), 67-103.
15. Amić, D., Davidović-Amić, D., Bešlo, D., & Trinajstić, N. (2003). Structure-radical scavenging activity relationships of flavonoids. *Croatica Chemica Acta*, *76*(1), 55-61.
16. Miller, A. L. (1996). Antioxidant flavonoids: structure, function and clinical usage. . *Alternative Medicine Review*, *1*(2), 103-11.
17. Zago, M. P., Verstraeten, S. V., & Oteiza, P. I. (2000). Zinc in the prevention of Fe^{+2} initiated lipid and protein oxidation. *Biological Research*, *33*(2), 143-150.
18. Oboh, G., Akinyemi, A. J., & Ademiluyi, A. O. (2012). Antioxidant and inhibitory effect of red ginger (Zingiber officinale var. Rubra) and white ginger (Zingiber officinale Roscoe) on Fe^{2+} induced lipid peroxidation in rat brain *in vitro*. *Experimental and Toxicologic Pathology*, *64*(1), 31-36.
19. Goode, H. F., Webster, N. R., Howdle, P. D., Leek, J. P., Lodge, J. P. A., Sadek, S. A., & Walker, B. E. (1994). Reperfusion injury, antioxidants and hemodynamics during orthotopic liver transplantation. *Hepatology*, *19*(2), 354-359.
20. Thorat, V. N., Suryakar, A. N., Naik, P., & Tiwale, B. M. (2009). Total antioxidant capacity and lipid peroxidation in liver transplantation. *Indian Journal of Clinical Biochemistry*, *24*(1), 102-104.