Original Research Article

In vitro Antioxidant Activity and Inhibition of Fe^{2+} and SNP Lipid peroxidation of African Mistletoes (*Tapinanthus globiferus)* from Three Selected Host Plants in Jos Plateau State Nigeria

ABSTRACT

Aim: The aim of this study was to investigate and compare the antioxidative properties of the mistletoe plant obtained from three different host species namely *Psidium guajava*, *Vernonia amygdalina* and *Moringa olifera lam*.

Study Design: Experimental Design

Place and Duration of Study: Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences and Department of Biochemistry, College of Health Sciences, University of Jos, Nigeria.

Methodology: Crude methanolic leaf extracts were studied for their antioxidative properties; Iron reducing and Iron-chelating activities, Nitric oxide (NO) radical and 2,2-diphenyl 1-picrylhydrazyl (DPPH) radical scavenging activities and the lipid peroxidation and thiobarbituric acid reaction (TBAR) methods. One way ANOVA was used for the result analysis with *P<.05* for significant difference.

Results: MSPG had significantly higher reducing property (0.16 – 0.20μg/mL); the chelating property of MSMO was significantly lower (45.7 – 58.9%); DPPH radical scavenging activity had no significant difference; and Nitric oxide scavenging activity was significantly higher in MSPG (72.1% in 75μg/mL) than the extracts from other hosts. MSPG had significantly higher TBAR inhibition using both $FeSO_4$ (77.8% at 125μg/mL) and Sodium nitroprusside (61.6±1.0% at 125μg/mL) with an IC_{50} of 30.27μg/mL . Extract of *Tapinanthus globiferus* leaves from *Psidium guajava* had more antioxidative activities in the TBARs followed by *Tapinanthus globiferus* leaf extract from *Vernonia amygdalina*.

Conclusion: From the study, mistletoes from *Psidium guajava* had higher antioxidant activity compared to other hosts, which probably justifies its use for treatment of cancer in traditional medicinal practice.

Keywords Antioxidant, traditional medicine, mistletoe, oxidative stress

1. INTRODUCTION

Oxidative stress has been linked to various diseases that are associated with lifestyle such as diabetes, cancer and cardiovascular diseases alongside others as a result of the activities of free radicals. These are unstable molecules that are highly reactive. They can be generated in the cells during enzymatic processes for example in the respiratory chain. These

free radicals are toxic in high concentrations leading to degenerative diseases but beneficial in lower concentrations; they are involved in immune response, cell signalling as well as maturity of cellular structures [1]. However their activities are quenched by antioxidants which are endogenously and exogenously (mostly via diet) made available to the body. Antioxidant activities have been linked to medicinal plants. Based on previous study a high population of the world population rely on medicinal plants for treatment and management of diseases [2]. The African mistletoe (*Tapinanthus globiferus)* has been identified as one of the numerous medicinal plants used in traditional medicine for treatment of oxidative stress induced diseases. It's an ever-green plant found growing as a shoot parasite on vascular plants, widely distributed along the West African Region and used to treat diabetes, hypertension, cancer and for treatment of metabolic disorders, epilepsy, irregular menstruation and menopause [3]. Its use dates back centuries before the practise of Christianity [4]. *Tapinanthus globiferus* belongs to Loranthaceae family. During draught farmers harvest and destroy them to avoid loss of produce [5]. *Tapinanthus globiferus* used for medicinal purpose is not based on species difference rather based on the host species and study carried out in Nasarawa State North Central Nigeria, revealed that it is widely and most commonly used plant for treatment of ailments [6]. The aim of this study was to compare the antioxidative properties of *Tapinanthus globiferus* from three different hosts *Psidium guajava* (guava), *Moringa olifera* (drumstick tree) and *Vernonia amygladina* (Bitter leaf).

2. MATERIALS AND METHODS

2.1 Equipment

The equipment used for the study include Water bath (Clifton® Water Bath, Sous vide Australia), Spectrophotometre (Jenway UV/Visible Spectrophotometer 7315, Bibi Scientific, United Kingdom), Micropipettes (10 - 100µl and 10 - 1000µl), Digital weighing balance, Bench

centrifuge and refrigerated centrifuge (MSE centrifuge, East Sussex United Kingdom) and Digital pH metre (Hauser® Digital pH meter).

2.2 Chemicals and Reagents

The chemicals used for the study include: Disodium hydrogen phosphate dodecahydrate, Aluminum Chloride, Sodium hydrogen carbonate, Sodium Dihydrogen phosphate dehydrate (all from Guangdong Guanghua Sci. Tech Co. Ltd); Tris Hydrochloride, Potassium acetate, 1,10 Phenanthroline, Follin-Ciocalteau Phenol Reagent, Trichloroacetic acid, Naphthylethelene diamine hydrochloride, sulfanilamide, Thiobarbituric acid (Lobal Chemie); Sodium Lauryl Sulphate, Gallic acid monohydrate (Tianjin Kermel); Glacial acetic acid, hydrogen peroxide, sodium hydroxide, methanol, sodium nitroprusside, sodium carbonate, L-ascorbic acid(BDH laboratories); Absolute methanol (Aldrich Sigma).

2.3 Preparation of Plant Samples

Tapinanthus globiferus leaves were obtained from three different hosts located at two different locations in Jos North L.G.A, Plateau State, namely: *Psidium guajava* and*Vernonia amygdalina* located at Alheri, Jos and *Moringa olifera lam* located at the Univesity of Jos Bauchi Road Campus Jos.

The *Tapinanthus globiferus* whole plants were identified at the Herbarium Department Federal College of Forestry Jos, Jos Plateau State, and the voucher specimen was deposited at the herbarium and given the number whereas the host plants were identified at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Jos. and voucher numbers were obtained for each of the host specimen *Vernonia amygdalina, Psidium guajava* and *Moringa olifera l*..The leaves of the *Tapinanthus globiferus* plants were dried in a well-ventilated room, grounded to powder and properly stored in an air tight container.

The mistletoe whole plants were identified at the Herbarium Department Federal College of Forestry Jos, Jos Plateau State, and the voucher specimen was deposited at the herbarium (as FHJ284) whereas the host plants were identified at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Jos. and voucher numbers were obtained for each of the host specimen *Vernonia amygdalina* (UJ/PCG/HSP/93/C02)*, Psidium guajava* 9UJ/PCG/HSP/95/M99) and *Moringa olifera l*.(UJ/PCG/HSP/10/M122). The leaves of the *Tapinanthus globiferus* plants were dried in a well-ventilated room, grounded to powder and properly stored in an air tight container.

3. METHODS

3.1 Methanolic Extraction

Extraction was done using methanolic extraction method [7].

Prior to further analysis, for each of the extracts, 1mg was re-dissolved in 1mL of distilled water.

3.2 Phytochemical Screening

Each plant extract was subjected to phytochemical screening based on the method described by [8], [9] and [10].

3.3 Total Antioxidant Assay

The comparative analyses of the antioxidant properties of the samples were determined using various methods.

3.3.1 Determination of Reducing Property

Iron reducing property of the extracts was determined using the method described by [11]. The extracts were mixed with 2.5ml 200nM sodium phosphate buffer (pH 6.6) and 2.5ml 1%

potassium ferricyanide. The mixture was incubated at 50ºC for 20minutes then 2.5ml 10% trichloroacetic acid was added then 1ml of distilled water and 1ml 0.1% ferric chloride were added to the mixture. The absorbance was measured at 700nm, and the ferric reducing antioxidant property was subsequently calculated and expressed as μg of Ascorbic Acid equivalent (AAE) using the formula:

$$\text{Reducing property} = \frac{\text{Absorbance of sample} \times \text{concentration of standard}}{\text{Absorbance of standard} \times \text{sample stock}}$$

3.3.2 Iron Chelating Property

Iron Chelating property of the extracts was determined using the method of [12]. Freshly prepared 500μM $FeSO_4$ (150μl) was added to a reaction mixture containing 168 μl 0.1M tris HCl (pH 7.4), 218 μl saline and the 40 μl of the extracts. The reaction mixture was incubated for 5 minutes then 13 μl 0.25% 1, 10 phenanthroline was added and absorbance measured at 510nm in a spectrophotometre and the chelating ability was calculated as % chelation using the formula:

$$\% \text{ inhibition} = \frac{Ab_{Ref} - Ab_{sample}}{Ab_{ref}} \times 100$$

3.3.3 Nitric oxide ($NO^{\bullet}$) Radical Scavenging Activity

Nitric oxide radical ($NO^{\bullet}$) scavenging property of the extracts was determined using the method as described by [13]. The Greiss reagent was prepared by dissolving 0.1ml sulfanilic acid 0.33% in 20% glacial acetic acid in 1ml of napthylethylenediamine dichloride at room temperature for 5 minutes 75μg of the extract was mixed with 350μl of distilled water then 1m of sodium nitroprusside was added and incubated at 37ºC for 2 hours. Then 500μl of Greiss reagent was added to the reaction mixture. The absorbance was measured at 570nm and percentage inhibition was calculated and expressed in percentage using the formula:

$$\% \text{ Inhibition} = \frac{Ab_{Ref} - Ab_{sample}}{Ab_{ref}} \times \frac{100}{1}$$

3.3.4 2,2-diphenyl 1-picrylhydrazyl (DPPH) Radical Assay

2,2-diphenyl 1-picrylhydrazyl (DPPH) radical scavenging property of the extracts was determined using the method described by [14]. DPPH was first prepared by dissolving in methanol and stored in the dark overnight.
To diluted solutions of 1mg/ml of the extracts, 1ml, 0.4mM methanolic solution containing DPPH radical was added, the mixture was left in the dark for 30 minutes and the absorbance measured at 516nm. The DPPH free radical scavenging activity was measured and expressed as percentage scavenging using the formula:

$$\% \text{ scavenging} = \frac{Ab_{Ref} - Ab_{sample}}{Ab_{ref}} \times \frac{100}{1}$$

3.3.5 Lipid peroxidation and Thiobarbituric acid reactions (*TBAR)* (Ohkawa*, 1979)*

Lipid peroxidation and Thiobarbituric acid reactions (*TBAR)* was carried out using the method described by [15]. Male Wistar albino rats weighing between 190 and 250 g were purchased from the Central Animal House, University of Jos, Jos Nigeria. They were housed in stainless steel cages under controlled conditions. The rats were allowed asses to food and water *ad libitum*. The animals were used in accordance with the procedure approved by the Animal Ethics Committee of the University of Jos, Jos Nigeria.

3.3.5.1 Preparation of tissue homogenates

The rats were decapitated under mild diethyl ether anaesthesia and the brain was rapidly isolated and placed on ice and weighed. This tissue was subsequently homogenized in cold saline (1/10w/v) in a pestle and the homogenate was centrifuged for 10minutes at 5000xg to yield a pellet that was discarded, and the supernatant was kept for lipid peroxidation.

TBAR species produced were measured at 532nm and the % induction was calculated using the formula:

$$\% \text{ induction} = \frac{\text{Absorbance of Induced Reference}}{\text{Absorbance of Basal Reference}} \times \frac{100}{1}$$

$$\% \text{ sample inhibition} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Basal Reference}} \times \frac{100}{1}$$

The same process was observed using sodium nitroprusside in the place of $FeSO_4$. Quercetin, a known naturally occurring antioxidant was used as a standard to compare the activities of the extracts using both $FeSO_4$ and sodium nitroprusside.

4. DATA ANALYSIS

The results of the replicates were collated and expressed as mean ± standard deviation (SD). One way analysis of Variance (ANOVA) (followed by Dunnett's and Tukey's multiple comparison tests) was used for result analysis and significance difference at *P=.05*, using Graphpad prism 7(Graphpad Software, 2365 Northside Dr. Suite 560, San Diego, CA 92108).

5. RESULTS

Most phytochemicals such as flavonoids, tannins etc. have been identified as natural antioxidants found in plants, thus, the qualitative analysis of the phytochemicals were carried out as and results shown in **table 1**.

5.1 Phytochemical screening

Table 1: Phytochemicals present in the extracts

Phytochemicals	*MSPG*	*MSMO*	*MSVA*
Alkaloids	++	++	++
Flavonoids	++	++	++
Phenolics	++	++	++
Saponins	++	++	+
Tannins	++	++	++

+Indicates the presence of the phytochemical

++ Abundant presence of the phytochemical

- Absence of the Phytochemical

Table 1 shows the phytochemicals present the extracts of the *Tapinanthus globiferus* from the different hosts.

5.2 Antioxidant Activities

5.2.1 Reducing Property of the *mistletoe* extracts

The results as indicated by table 2, showed that mistletoe from *Psidium guajava* (MSPG; 0.16μg of AAE/mL at 11.36μg/mL; 0.20μg of AAE/mL at 22.72μg/mL) had significantly ($P \leq 0.05$) higher reducing property than other extracts any of the extracts (MSVA; 0.13μg of AAE/mL (11.36μg/mL) and 0.16μg of AAE/mL (22.72μg/mL); MSMO; 0.063μg of AAE/mL (11.36μg/mL) and 0.16μg/mL (22.72μg/mL)) when compared with gallic acid (*P=.05*) compared with the gallic acid used as the standard.

Table 2: Reducing Property (μg of AAE/mL) **of the Mistletoe extracts from the host plants**

Sample	11.36 µg/mL	22.72 µg/mL
MS***PG***	0.16±0.009[a]	0.20±0.01[a]
MSMO	0.063±0.006[b]	0.16±0.002[b]
MSVA	0.13±0.003[c]	0.18±0.003[b]
GA	0.13± 0.06[c]	0.25± 0.03[a]

Values are presented as mean of replicates ±S.D
[abc] Values with the same subscript letter in the same column are not significantly different (*P=.05*)

MSPG- Mistletoe from *Psidium guajava*
MSMO – Mistletoe from *Moringa olifera l.*
MSVA- Mistletoes from *Vernonia amygladina*
GA – Gallic Acid

5.2.2 Iron Chelating Activity of the mistletoe extracts

Result showed in table 3 showed that the mistletoe extracts from *Moringa olifera* had a significantly lower chelating activity (MSMO; 45.7% at 40μg/mL and 58.9% at 60μg/mL) compared with other mistletoe extracts from *Psidium guajava* and *Vernonia amygladina* (MSPG; 62.1%, 69.4%; and MSVA: 63.9%, 68.9%) at *P=.05*.

Table 3: Iron Chelating Activity of the Mistletoe extracts from the three host plants

Sample	40 µg/mL (%)	60 µg/mL (%)
MS***PG***	62.1±1.5[a]	69.4±2.8[a]
MSMO	45.7±1.9[b]	58.9±6.4[b]
MSVA	63.9±5.7[a]	68.9 ± 1.9[a]

Values are presented as mean of replicates ±S.D
[abc] Values with the same subscript letter in the same column are not significantly different (*P=.05*)

MSPG- Mistletoe from *Psidium guajava*
MSMO – Mistletoe from *Moringa olifera l.*
MSVA- Mistletoes from *Vernonia amygladina*

5.2.3 Nitric Oxide Scavenging Activity of the Mistletoe extracts

Result showed in figure 1 showed that the mistletoe extract from *Psidium guajava* had significantly higher scavenging activity (MSPG; 72.1%) at the highest concentration (75μg/mL) at *P<.05* whereas extract from *Moringa olifera* (MSMO)had the lowest scavenging activity at all concentrations tested compared with the gallic acid used as the known standard.

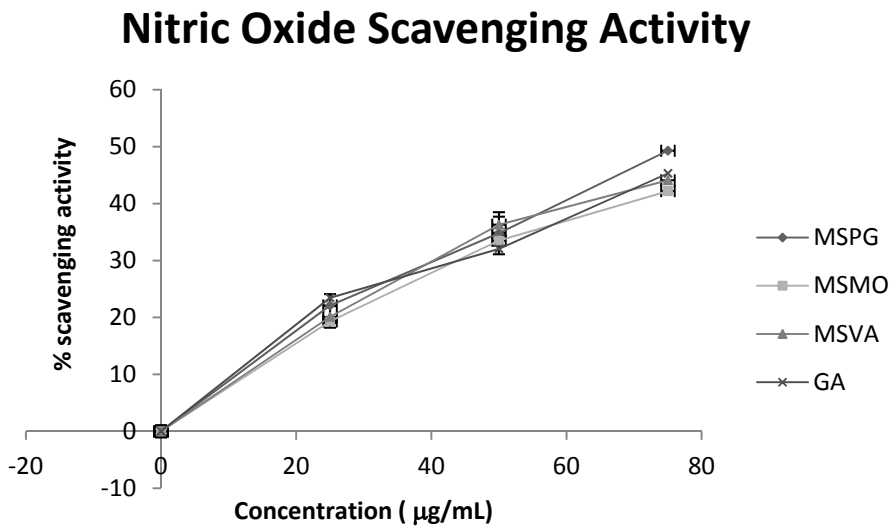

Fig 1: Nitric oxide Scavenging Activity of the mistletoes from the three host plants

5.2.4 DPPH Radical Scavenging Activity of the *Tapinanthus globiferus* extracts

The result of the DPPH scavenging activity using Gallic as the standard for comparing as shown in **figure 2** indicated that there was no significant difference at *P=.05*.

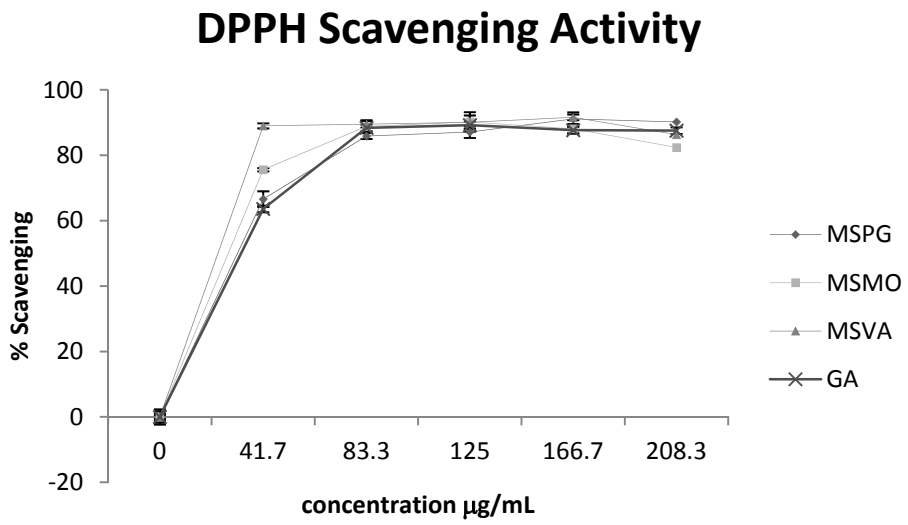

Fig 2: DPPH radical scavenging Activity of the mistletoes from the three plant hosts

5.2.5 Lipid peroxidation and Thiobarbituric acid reactions (*TBAR)* of the Mistletoe extracts

5.2.5.1 Lipid peroxidation and TBAR with $FeSO_4$

From the study carried out using $FeSO_4$, there was 94.4% induction. The result of the analysis as shown in **figure 3** indicated that at *P=.05* with mistletoes from the different hosts had no significant difference when compared to the standard, quercetin (86.6% at 125µg/mL), however, MSPG had higher inhibition (77.8% at 125µg/mL).

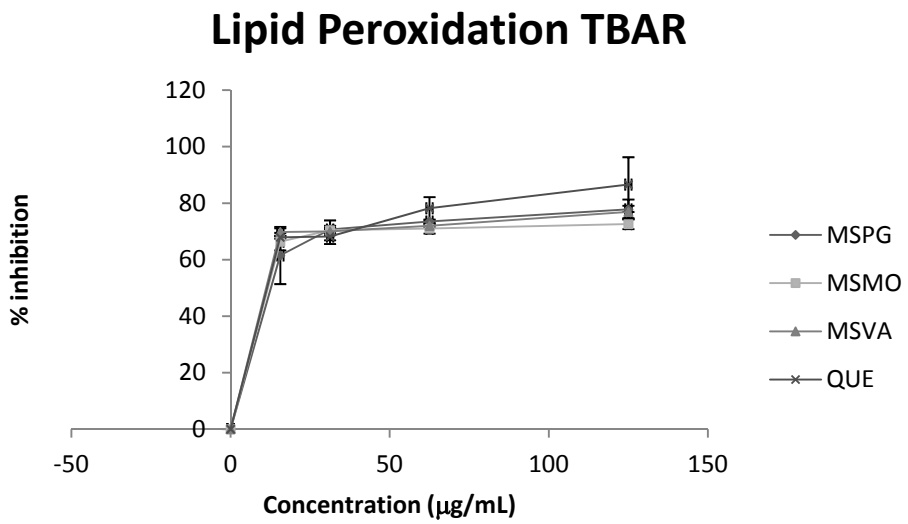

Fig 3: Lipid Peroxidation and TBAR ($FeSO_4$) of the mistletoes from the three host plant

5.2.5.2 Lipid peroxidation and TBAR with Sodium Nitroprusside

From the study carried out using Sodium nitroprusside, had 63.6% induction. There was significant difference at *P=.05*, as shown figure 4, with mistletoes from *Psidium guajava* having higher inhibition (61.6% at 125µg/mL) with IC50 at 30.27µg/mL whereas the extracts from *Moringa olifera* (54.64%) had the least inhibition. Inhibition increased with an increase in the concentration of each extract.

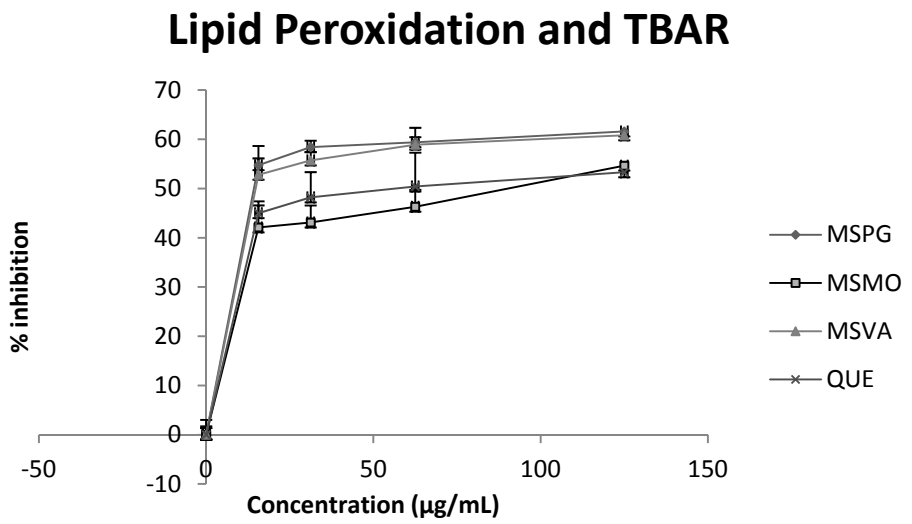

Fig 4: Lipid peroxidation and TBAR (Sodium Nitroprusside) of the mistletoes from the three host plants

6. **DISCUSSION**

Antioxidative properties have been associated with plants of medicinal values as such studies have been carried out on different medicinal plants as such antioxidants are sourced, mostly, from plant based foods. The antioxidant properties of plants have been attributed to the presence of phytochemicals [16]. However, their activities have been linked to their ability to scavenge free radicals; most notable being the polyphenols [17], as observed in the result in table 1. Polyphenols in foods contribute to oxidative stability [18].

The reduction of the ferric ion to ferrous ion increased with increase in the concentration of the extract (table 2) which agrees with the studies carried out [19]. Natural antioxidants possess the ability to reduce Ferric (Fe^{3+}) ion to ferrous (Fe^{2+}) ions. However, mistletoes from *Psidium guajava,* showed higher reducing property (0.20μg/mL) at the highest concentration.

Antioxidants are also regarded as good chelators. The extracts showed the ability to chelate iron by a decrease in the absorbance with an increase in the concentration of the extract (table 3) when compared to the reference without any extract containing only 1,10 phenanthroline and Iron (IV) sulphate. This agrees with the studies on the iron chelating properties of *Tapinanthus globiferus* by [20]. 1,10 phenanthroline is a chelator of Fe^{2+} that bind to form Fe-O-phenanthroline complex, which is a red chromogen. In the presence of a chelator, it cannot bind to the ferrous ion and this leads to decrease in the absorbance of the sample mixture [21]. From this study, mistletoes from *Moringa olifera l.* (58.9%) had the lowest chelating activity at the highest concentration (60μg/mL) compared to the mistletoes from *Psidium guajava* and *Vernonia amygladina*.

There was a decrease in the nitric oxide concentration with a corresponding increase in the concentration of the extracts (Fig. 1). The result indicates that the extracts have nitric oxide scavenging activity when compared with a known antioxidant, gallic acid. Nitric oxide is a natural radical generated in a biological system from the amino acid L-arginine by vascular endothelial cells, phagocytes and certain cells of the brain [22], which is capable of inhibiting effect of

oxidative stress but can also promote the process in the presence of a free radicals such as superoxide anions to form a peroxynitrite, which can cause DNA damage [23]. In the assay the extract from *Psidium guajava* (72.1% in 75μg/mL) had a higher scavenging property, compared to other mistletoes, in the highest concentration.

The DPPH method is referred to as an indirect method because DPPH is not a biological radical (cannot be generated *in-vivo)* [24]. The extracts were able to scavenge the radical which agrees with previous studies [25]. Though there was no significant difference in the activities, *Psidium guajava* had a higher scavenging activity as seen in Figure 2.

Lipid peroxidation refers to the oxidative deterioration of lipids. Studies have suggested that lipid peroxidation, which is a product of oxidative stress have been identified as a process involved in pathological conditions such as cancer [26]. Iron decomposes lipid peroxides, which produces peroxyl and alkoxyl radicals, which determines peroxidation chain termination [27]. When compared with the values of the quercetin standard, the mistletoe extracts all had the ability to inhibit lipid peroxidation, which agrees with previous studies by [18] by scavenging the free radicals generated by the $FeSO_4$ and the Sodium nitroprusside. This could be attributed to a decrease in absorbance of the MDA that which was formed during lipid peroxidation, which binds to the thiobarbituric acid to form a pink chromogen. There was higher inhibition of lipid peroxidation by mistletoe from *Psidium guajava* ($FeSO_4$; 77.8% ; SNP; 61.6%) at the highest concentration when compared to others as shown in figures 3 and 4 respectively. The outcome of this analysis may be due to the high phenolic and flavonoid contents which agrees with the finding that *Psidium guajava* leaf extracts have high phenolic acids responsible for their rich antioxidant properties [28], as polyphenolic plants tend to have high antioxidant activities.

7. CONCLUSION

In the course of this study, it was observed that the extract of the mistletoe leaves from the different hosts all had antioxidative activities, typical of plants. However the leaves from the *Psidium guajava* showed higher antioxidative ability compared to other mistletoes. Thus the use

of the mistletoe from *Psidium guajava* for treatment of cancer by the locals could be due to its high antioxidant properties attributed to its high polyphenolic content. However, *in vivo* studies could be carried out to ascertain the authenticity of these activities as environments differ within the two methods, which affect experimental conditions directly.

Conflict of Interests

None were declared

REFERENCES

1. Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., and Bitto, A. Oxidative Stress: Harms and Benefits for Human Health. Oxidative Medicine and Cellular Longevity. Review Article, 2017; Volume 2017, Article ID 8416763, 13 pages https://doi.org/10.1155/2017/8416763.
2. Singh, A., Singh, S., Prasad, A.M. Role of Medicinal Plants for health perspective: Special Reference to Antioxidant Potential. J Chem Biol Ther 2016, Vol 1(2):106 Doi:10.4172/2572-0406.1000106.
3. Adesina, S.K., Illoh, H.C., Imoh, J., Imoh, E. J. Ethnopharmacology, Chemistry and Medicinal Values: An Update. African Journal of Traditional, Complementary and Alternative Medicines (AJTCAM),2013; 10 (4): 161-170.
4. Ogunmefun, O.T., Olatunji, B.P., Adarabioyo, M.I. Ethnomedicinal Survey on the Uses of Mistletoe in South-western Nigeria. *European Journal of Medicinal Plants*. 2015; ISSN: 2231-0894. 8(4): 224 – 230
5. Mohammed, S.Z., Eldur, B.Z., Abbashar, A.A. Distribution and Host Range of *Loranthaceae* (*Tapinanthus globofenis* (A.Rich) Van Teigh) Along the Nile Banks in Central Sudan, International Journal of scientific and Technology Research 2014; Vol. 3, Issue 3, March 2014 ISSN 2277-8616.
6. Ibrahim, J.A., Egharevba, H. O., Jegede,A. I., Ugbabe, G. E., Muazzam, I., Kunle, O. F. and Gamaniel, K. S. Medicinal plants used and the perception of plant endangerment by the traditional medicine practitioners of Nasarawa State, Nigeria: A pilot study International Journal of Biodiversity and Conservation 2016; Vol. 8(1), pp. 8-20 DOI: 10.5897/IJBC2015.0842 Article Number: BD3333F56808 ISSN 2141-243X
7. Chu, Y., Sun, J., Wu, X. And Liu, R. Antioxidant and antiproliferative activities of common vegetables. Journal of Agricultural and Food Chemistry, 2002; 49(7), 3216 – 3222.
8. Sofowora, A. Medicinal plants and traditional medicines in Africa. Spectrum books Ltd Ibadan Nig. Pg 289. 1993.
9. Harborne, J.B. () Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. ISBN 13:978-0-412-23050-9 e-ISBN 13:978-94-009-5921-7 1973; Doi:10.1007/978-94-009-5921-7
10. Trease, G.E. and Evans, W.C. Textbook of Pharmacognosy ()12th Edition Tindall and Co., London: 343 – 383. 1983

11. Oyaizu, M. Studies on products of browning reaction: antioxidant activity of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition. 1986; 44: 307 – 315.
12. Minotti, G. and Aust, S.D. An investigation into the mechanism of citrate – Fe^{3+}-dependent lipid peroxidation. Free Radical Biology and Medicine Journal, 1987; 3: 379 – 387
13. Marcocci, L., Maguire, J.J., Droy-Lefaix, M.T and Packer, L. The nitric oxide – scavenging properties of *Ginkgbo biloba* extract EGb.761. Biochemical and Biophysical Research Communications. 1994; 201: 748 – 755.
14. Gyamfi, M.A., Yonamine, M., Aniya, Y.. Free radical scavenging action of medicinal herbs from Ghana: *Thonninga sanguine* in experimentally induced liver injuries. Gen Pharm 1999; 32: 661 – 667.
15. Ohkawa, H., Ohishi, N., Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry,1979; Vol 95 (2): 351 – 358
16. Dong-Pin, X., Ya L., Xiao, M., Tong Z., Yue Z., Jie, Z., Jiao-Jiao, Z. and Hua-Bin L. Natural Antioxidants in Foods and Medicinal Plants: Extraction, Assessment and Resources. Int J Mol Sci. 2017: 18(1):96. Doi: 10.3390/ijms18010096 PMCID: PMC5297730 PMID: 28067795
17. Yu-Jie, Z., Ren-You, G., Sha, L., Yue, Z., An-NA, L., Dong-Ping, X. And Hua-Bin, L.). Antioxidant Phytochemicals for the Prevention and Treatment of Chronic Diseases. Molecules 2015; 20: 21138-21156; doi: 10.3390/molecules201219753. www.mdpi.com/journal/molecules
18. Mattera, R., Benvenuto, M., Giganti, M.G., Tresoldi, I., Pluchinotta, F.R., Bergante, S., Tettamanti, G., Masuelli, L., Manzari, V., Modesti, A. and Bei, R., Effects of Polyphenols on Oxidative Stress – Mediated Injury in Cardiomyocytes. Review. Nutrient, 2017;20; 9(5). Pii:E523. Doi: 10.3390/nu9050523
19. Ogunbolude, Y., Ibrahim, M., Elekofehiniti, O., Adeniran, A., Abolaji, A.O., Rocha, J.B and Kandem J.P. Effects of *Tapinanthus globiferus* and *Zanthoxylum zanthoxyloid* extract on human leukocytes *in vitro*. Journal of International Ethnopharmacology 2014; 3(4), 167 - 72
20. Adekunle, A.S., Aline, A.B., Afolabi, O.K. and Rocha, J.B. Determination of free phenolic acids, flavonoid contents and antioxidant capacity of ethanolic extracts obtained from leaves of mistletoes (*Tapinanthus globiferus)* Asian J Pharm Clin Res, 2012; 5:36-41.
21. Alam, N., Bristi, N.J., Rafiquzzaman. Review of on in-vivo and in-vitro methods evaluation of antioxidant activity. Saudi International Journal. 2013; Vol 21. Doi: 10.1016/j.jsps.2012.05.02 143 - 152.
22. Boora, F., Chirisa, E. and Mukanganyama, S. Evaluation of Nitrite Radical Scavenging Properties of Selected Zimbabwean Plant Extracts and their Phytoconstituents. Journal of Food Processing, 2014; Volume 2014(2014), Article ID 918018, 7 pages. http://dx.doi.org/10.1155/2014/918018
23. Yuste, J.E., Tarragon, E., Campuzano C.M. and Ros-Bernai F. Implications of glial nitric oxide in neurodegenerative diseases. Font. Cell. Neurosci. 2015;9”32. Doi: 10.3389/fncel.2015.0032
24. Kasote, M.D., Katyare, S.S., Hegde, M.V., Bae, H. Significance of Antioxidative potentials of Plants and its relevance to Therapeutic alications. International Journal of Biological Sciences. 2015; Vol 11(8) 982 – 9911 Doi:10.7510/ijbs.12096 PMCID: PMC4495415
25. Emmanuel. H.M., Gusau, H. L., Gidado, L. M., Ali, B., Ugwah-Oguejiofor, C. J. , Yahaya, A. Comparative Studies on Phytochemical and Antioxidant activities of Tapinanthus

globiferus Piliostigma thonningii(A. Rich) and its host plant (Schum). Advance Pharmaceutical Journal 2017; 2(5): 179-184. www.apjonline.in
26. Guiseppina, B. Oxidative Stress and Lipid Peroxidation products in Cancer Progression and Therapy. Review ISRN Oncology. 2012; Vol.2012, Article ID:137289 21 pages http://dx.doi.org/10.5402/2012/137289\
27. Prisacaru, A. E.. Effect of antioxidants on polyunsaturated fatty acids – review. Acta Sci. Pol. Technol. Aliment. 2016; 15(2), 121–129. DOI: 10.17306/J.AFS.2016.2.12
28. Adedayo, O. A., Ganiyu O., Opeyemi B. O., Funmilayo, M. O. (2016). A comparative study *on* antihypertensive and antioxidant properties of phenolic extracts from fruit and leaf of some guava (*Psidium guajava* L.) varieties. Comp Clin Pathol (2016) 25: 363. https://doi.org/10.1007/s00580-015-2192-y