

IJSGS


 ISSN: 2488-9229
 FEDERAL UNIVERSITY
 GUSAU-NIGERIA

INTERNATIONAL JOURNAL OF SCIENCE FOR GLOBAL SUSTAINABILITY
Phytochemical Screening, *In vitro* Antioxidant Activity and Polyphenolic Content of the Leaves of Nigerian *Ziziphus spina* – Christi (L.) Willd
¹Ogbiko, C., ^{1,2}Okoh, E.V.C. and ³Abubakar, H.
¹Department of Pure and Applied Chemistry, Faculty of Science, Usmanu Danfodiyo University Sokoto, Sokoto State Nigeria

²Sokoto Energy Research Centre, Usmanu Danfodiyo University, Sokoto State Nigeria.

³Department of Biochemistry, Faculty of Natural Sciences, Ibrahim Badamasi Babangida University Lapai, Niger State Nigeria

 *Corresponding author Email & Phone No: cyrilogbiko@gmail.com; cyril.ogbiko@udusok.edu.ng; +2348080398933

Received on: August, 2020

Revised and Accepted on: November, 2020

Published on: December, 2020

ABSTRACT

Ziziphus spina is a neglected and underutilized species characterized as a multipurpose plant as different parts of the plant is being used for diverse trado-medicinal purposes. The study evaluated the phytochemical composition, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) antioxidant activity, total phenolic, and flavonoids content of the hydromethanol leaf extract and fractions with a view to validate its folkloric usage. Gallic acid, quercetin and ascorbic acid were used as the positive controls for the total phenol content, flavonoid content and the DPPH assay respectively. The result of the phytochemical screening confirmed the presence of flavonoid, tannins, saponins, phenolics, reducing sugars among others. While the standard antioxidant (ascorbic acid) had superior DPPH antioxidant capacity, n-butanol had better activity when compared to the crude extract and other fractions. Using the gallic acid and quercetin equivalent plots, the n-butanol fraction contained a higher concentration of total phenols and flavonoids in comparison with the crude extract and other fractions. There was a significant correlation between the DPPH antioxidant activity and the phenolic and flavonoid contents in the extracts. The results of this study showed that the leaf extracts of *Z. spina* possess promising antioxidant property capable of being exploited by pharmaceutical companies for the development of therapeutic agents aimed at treating free radical implicated diseases.

Keywords: Antioxidant, DPPH, Polyphenol, *Ziziphus spina*
1.0 INTRODUCTION

There is a renewed interest in the last decade to search for phytochemicals of native and naturalized plants for pharmaceutical and nutritional purposes which stem from the fact that plant derived products have great potential as sources of pharmaceuticals. The properties of medicinal plants have been investigated in recent scientific developments throughout the world, due to their potential antioxidant activities, with reduced side effects and economic viability (Auudy *et al.*, 2003). Recently, there has been an upsurge of interest in the therapeutic potentials of medicinal plants and some fruits as antioxidants in reducing such free radical induced tissue injury (Schuler, 1990). Free radicals are chemical compounds which contains an unpaired electron spinning on the peripheral layer around the nucleus. The family of free radicals

generated from oxygen is called reactive oxygen species (ROS) and those generated from nitrogen are called reactive nitrogen species (RNS). They are chemically aggressive molecules which react with different types of macromolecules in the body to cause damage to vital cell constituents such as DNA, proteins and lipids (Harman, 1981). They react with other molecules by extracting electrons from them in order to attain stability. ROS are ions, atoms or molecules that have the ability to oxidize reduced molecules. ROS include free radicals such as superoxide anion radicals (O_2^-) and hydroxyl radicals ($\text{OH}\cdot$), as well as non-free radicals hydrogen peroxide and singlet oxygen (Halliwell, 1995). In the body, free radicals are derived from two sources: endogenous sources, e.g. nutrient metabolism, ageing process, and exogenous sources like tobacco smoke, ionizing radiation, air

pollution, organic solvents, pesticides (Buyukokuroglu *et al.*, 2001). Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS (Kumpulainen and Salonen, 1999; Cook and Samman, 1996). Cells are equipped with natural mechanisms to fight against ROS and to maintain the redox homeostasis of cells. For example, antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) play important roles in scavenging free radicals and preventing cell injury (Bergendi *et al.*, 1999). In addition to antioxidant enzymes, non-enzymatic molecules, including thioredoxin, thiols, and disulfide-bonding play important roles in antioxidant defense systems. Some of the compounds are of an exogenous nature and are obtained from food, such as α -tocopherol, β -carotene, ascorbic acid, and some micronutrient elements like zinc and selenium (Farrukh *et al.*, 2006). These molecules inhibit lipid peroxidation in cell but when the mechanism of antioxidant protection becomes overwhelmed in the human body, antioxidant supplement may be required to help reduce oxidative damage.

Medicinal plants are an important source of antioxidants (Rice-Evans, 2004). It has been reported that the use of natural compounds containing antioxidant agents diminish the possibility of chronic diseases such as diabetes, cancer and cardiovascular diseases (Myojin *et al.*, 2008; Saha *et al.*, 2004; Horax *et al.*, 2005; Semiz and Sen, 2007). Plants contain many phytochemicals that are useful sources of natural antioxidants, such as phenolic diterpenes, flavonoids, tannins, phenolic acids (Lee *et al.*, 2004), and polyphenols (Bernardi *et al.*, 2008). The secondary metabolites like phenolics and flavonoids from plants have been reported to be

potent free radical scavengers (Mathew and Abraham, 2006). Several synthetic antioxidants are in use, but have however been reported in laboratory animals to have several side effects such as risk of liver damage, and carcinogenesis (Gao *et al.*, 1999; Osawa and Namiki, 1981). In order to avoid these effects, more effective, less toxic and cost effective antioxidants need to be developed. Medicinal plants appear to have these desired comparative advantages, hence the growing interest in natural antioxidants from plants.

Ziziphus spina - Christi is a spiny shrub or small tree (Figure 1) that strongly resists heat and drought. The genus *Ziziphus* is known for its medicinal properties as a hypoglycemic, hypotensive, anti-inflammatory, antimicrobial, antioxidant, anti-tumour, and liver protective agent and as an immune system stimulant (Said *et al.*, 2006). It is an evergreen tree but drops some of its leaves during the dry season (Von-Maydell, 1986). The plant leaves are simple, alternate, narrowly ovate and lanceolate, varying from 1 to 9 cm in length and 1 to 3.5 cm in width, glabrous above, minutely and densely pubescent beneath with three basal conspicuous veins running up to the apex and around 0.5 to 1 cm long petioles (Von-Maydell, 1986; Arbonnier, 2004). The bark is whitish brown or pale grey and deeply fissured. While the flowers (usually small, greenish-yellow, 2 mm long subsessile sepals, with five 1.5 mm long petals) have a sweet scent and are found in dense clusters in the axils of the leaves (El Amin, 1990), the fruit is a reddish-brown globose drupe of about 1–1.5 cm in diameter, with a hard stone surrounded by a sweet edible pulp. As part of our ongoing biological evaluation of medicinal plants for their therapeutic uses, the current study was carried out to determine the phytoconstituents, phenolic and flavonoid contents *viz a viz* the antioxidant activity of the leaves of the plant.



Figure 1: *Ziziphus spina* tree in its natural habitat

2.0 MATERIALS

2.1 Sample Collection, Authentication and Preparation

Fresh leaves of *Z. spina* were procured from Gusau central market in June, 2019. They were immediately transported fresh to the central laboratory of the Department of Pure and Applied Chemistry, Usmanu Danfodiyo University Sokoto, Sokoto State Nigeria. They were identified and authenticated by Mallam Abdulaziz Sani of the Botany Unit, Department of Biological Sciences, Usmanu Danfodiyo University Sokoto where herbarium specimen was deposited for future reference. The samples were washed with running tap water to remove dust and earthy impurities before being shade dried for 2 weeks to complete dryness and pulverized using a clean mortar and pestle. The pulverized sample was kept in an airtight glass container until ready for use.

2.2 METHODOLOGY

2.2.1 Extraction and Fractionation

500 g of the pulverized plant was macerated in 2.5 L of distilled water: methanol mixture for 72 hrs. The mixture was sieved using porcelain cloth and was further filtered using No. 1 Whatman filter paper. The filtrate was concentrated using rotary evaporator to obtain the crude hydromethanol extract (CME). Portion of CME was subjected to solvent-solvent extraction using solvents; pet ether, ethyl acetate and n-butanol to obtain the pet-ether fraction (PEF), ethyl acetate fraction (EAF) and the n-butanol fraction (NBF) respectively. All the fractions were concentrated in the rotary evaporator at 45°C and stored at 4°C till use.

2.2.2 Phytochemical Analysis

Simple chemical tests to detect the presence of carbohydrates, proteins and secondary metabolites were done in accordance with standard methods (Trease and Evans, 1978; Sofowora, 1982).

2.2.3 Screening Methods of Antioxidant Activity

Various methods are available to estimate the antioxidant activity of compounds in plant extracts since one assay method is insufficient to elucidate possible mechanisms of antioxidant effects (Dorman *et al.*, 2003; Erkan *et al.*, 2008).

2.2.4 Total Phenol Content (TPC)

The amount of total phenol content of *Z. spina* leaf extracts was determined by Folin-Ciocalteu reagent method as earlier described by Kim *et al.*, 2003 with slight modifications. The extract solution (0.5 ml) with a concentration of 1000 µg/ml was added to 4.5 ml of deionized distilled water and 0.5 ml of Folin-Ciocalteu's reagent which was earlier diluted with deionized water (1:10 v/v). After mixing the tubes, they were maintained at room temperature for 5 minutes followed by the addition of 5 ml of 7% sodium carbonate and 2 ml of deionized distilled water. The samples were incubated for 90 minutes at 23°C. The absorbance was measured by a spectrophotometer at 750 nm. The total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of extract. Gallic acid (McDonald *et al.*, 2001) was used as positive control. The standard curve was prepared using gallic acid in five different concentrations (50, 100, 150, 250 and 500 mg/L).

2.2.5 Total Flavonoid Content (TFC)

The total flavonoid content in the plant extracts was determined by the Aluminium Chloride method as described by Ebrahimzadeh *et al.*, 2008. Briefly, 0.5 ml of sample (5 g/L) was mixed with 1.5 ml of methanol and then, 0.1 ml of 10% aluminum chloride was added, followed by 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The mixture was incubated at room temperature for 30

min. The absorbance was measured by a spectrophotometer at 415 nm. The results were expressed as milligrams Quercetin equivalents (QE) per gram of extract (mg QE/g extract). Quercetin was used as positive control and the standard curve was prepared by quercetin in different concentrations (12.5, 25, 50, 80 and 100 mg/L).

2.2.6 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay

The ability of the methanol extract and petroleum ether, ethyl acetate and n-butanol fractions of *Z. spina* to scavenge 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radicals was estimated as previously described by Jain *et al.*, 2008. The extracts (3 ml) with six different concentrations (12.5, 25.0, 50.0, 75.0 and 100 µg/mL) were mixed with 1 ml of a 0.1 mM methanol solution of DPPH. The absorbance was measured by a spectrophotometer at 517 nm at 30 minutes intervals against a blank (pure methanol). The percentage of radical scavenging activity was calculated using the formula:

$$\text{DPPH radical inhibition (\%)} = [1 - (A_{\text{test}} / A_{\text{control}})] \times 100$$

Where A_{control} is the absorbance of the control and A_{test} is the absorbance of the sample extracts.

Lower absorbance values show higher free radical scavenging activity. Ascorbic acid was used as a reference standard in different concentrations (12.5, 25.0, 50.0, 75.0 and 100 µg/ml). The 50 % inhibitory concentration value (IC_{50}) is indicated as the effective concentration of the sample that is required to scavenge 50% of the DPPH free radicals.

2.2.7 Statistical Analysis

Results were expressed as means \pm standard deviations (SD). Statistical comparisons were made using the student t-test, one-way analysis of variance (ANOVA) using SPSS statistics 20.0 software package.

3.0 RESULTS

3.1 Percentage Yield of Crude Extract and Fractions

Table 1: Percentage yield of crude extract and fractions of *Z. spina*

Extract/Fractions	Percentage Yield
MCE*	5.21
NHF**	11.10
EAF**	46.90
NBF**	52.50

Key: MCE = Methanol Crude Extract; NHF = N-Hexane Fraction; EAF = Ethyl Acetate Fraction; NBF = N-Butanol Fraction; * = Crude Extract; ** = Fractions obtained from MCE.

The results of the extractive yield of the crude extract and its fractions are presented in table 1.

3.2 *In vitro* Phytochemical Screening

Crude extracts normally contain complex mixtures of biologically active compounds some of which might exhibit genotoxic or antigenotoxic effects (Yumnamcha *et al.*, 2014). It therefore imperative to screen natural products especially medicinal

plants for potential health hazards to serve as a guide for their intended medicinal purposes (Wafaa *et al.*, 2019). The presence of secondary metabolites in the analyzed sample is presented in table 2.

Table 2: Phytochemical screening of *Z. spina* leaves crude extract

S/N	Screened Phytoconstituents	Inference
1	Flavonoid	+
2	Tannin	+
3	Saponins	+
4	Alkaloid	+
5	Phenolics	+
6	Reducing Sugars	+
7	Anthraquinone	-
8	Terpenoid	-
9	Cardiac glycosides	-

Key: (+) = present; (-) = absent

The presence of secondary metabolites not only impart characteristic odor, taste, color, medicinal or poisonous properties on plants, they also aid in the biological actions for which they are identified in folk medicine (Evans *et al.*, 1982). The geographical distribution of plants, affect both the morphology and expression of phytochemicals hence could be attributed to the absence of anthraquinone and terpenoid reported in this study (Folkers *et al.*, 2008; Shen *et al.*, 2008). The results of the phytochemical composition showed that the plant has an appreciable number of phytochemicals.

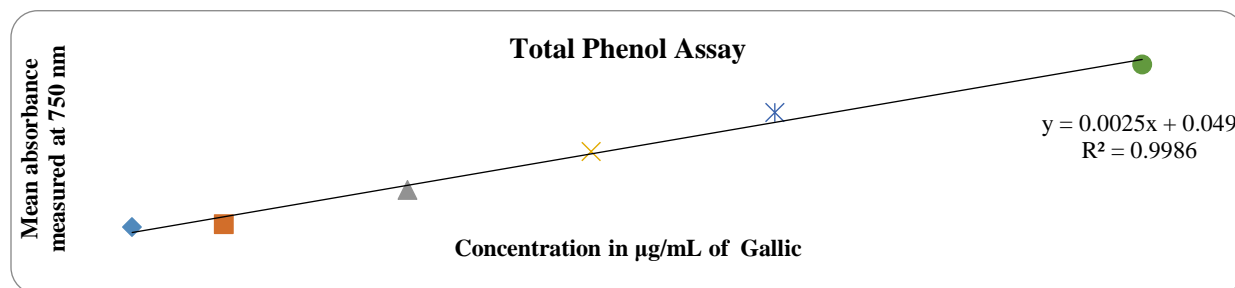
3.3 Total phenol content (TPC)

The total phenolic amount in the hydromethanol extract as well as the petroleum ether, ethyl acetate and n-butanol fractions are presented in table 3. All extracts contained significant amount of phenolic metabolites ranging from 15.18 ± 3.17 mg/g to 75.18 ± 4.17 mg/g (gallic acid equivalent). The n-butanol fraction showed the highest concentration of phenolics in the plant sample. The total phenol compounds were reported as gallic acid equivalent by reference to a standard curve ($y = 0.0025x + 0.049$; $R^2 = 0.9986$) as shown in Figure 2. Significant difference ($P < 0.05$) was observed in the phenolic content among the extract and various fractions.

Table 3: Total phenolic content in the extract and fractions of *Z. spina* gallic acid equivalent (GAE)

Extracts	Mean \pm SD
Hydromethanol ¹	53.10 ± 1.44^a
Petroleum ether ²	15.18 ± 3.17^c
Ethyl acetate ²	66.80 ± 2.55^a
N-butanol ²	75.18 ± 4.17^b

¹extract; ²fractions; mean with same letter are not significantly different at $p < 0.05$

**Figure 2: Mean absorbance against concentration of Gallic acid in µg/mL**

It has been reported that the Folin-Ciocalteu procedure is non-specific because it detects all phenolics (phenolic acids, flavonoids, and tannins) (Xie *et al.*, 2001), so it does not give details of the

quantity of the phenolic constituents of the extracts. It should however be noted that this method is rapid and provides useful overall evaluation of the phenolic content of the extracts.

3.4 Total flavonoid content (TFC)

Table 4 and figure 3 show the total flavonoid content of the hydromethanol extract and the petroleum ether, ethyl acetate and n-butanol fractions of the plant material, reported as mg quercetin equivalent/ g of extract with reference to a standard curve ($y = 0.0045x + 0.0433$, $R^2 =$

0.9913). Table 4 showed that the flavonoid content in the plant extract/fractions ranges from 158.38 ± 3.74 in the pet. ether fraction to 492.53 ± 4.62 in the n-butanol fraction mg QE/g of extract. Significant difference ($P < 0.05$) was observed in the phenolic content among the extract and various fractions.

Table 4: Total flavonoid content in the extract and fractions of *Z. spina* quercetin equivalent (QE)

Extracts	Mean \pm SD
Hydromethanol ¹	332.25 ± 7.10^a
Petroleum ether ²	158.38 ± 3.74^b
Ethyl acetate ²	391.00 ± 7.01^a
N-butanol ²	492.53 ± 4.62^c

¹extract; ²fractions; mean with same letter are not significantly different at $p < 0.05$

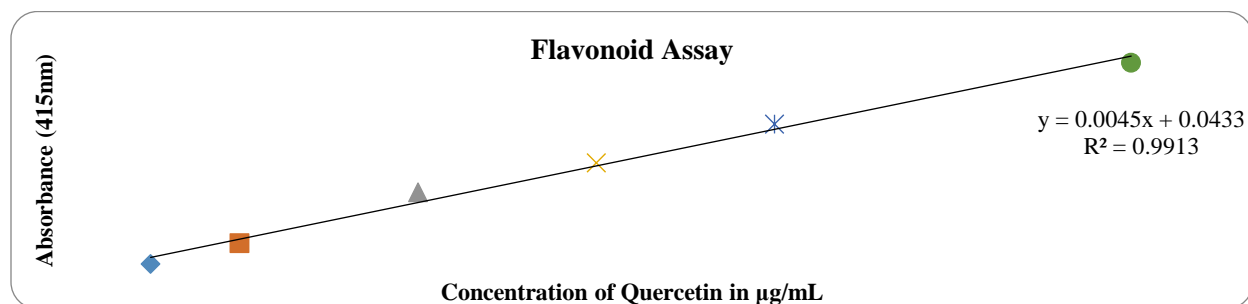


Figure 3: Mean absorbance against concentration of Quercetin in $\mu\text{g/mL}$

3.5 Free radical scavenging assay

The radical scavenging property of the extracts and fractions is shown in figure 4. Statistically significant differences were observed between the extracts and the control (ascorbic acid). Figures 5 and 6 show line fit plots for both total phenolic and

flavonoid contents in relation to the 50% inhibitory concentration as reported in the DPPH free radical scavenging ability (IC_{50}). There were significant correlation between the DPPH antioxidant activity and the phenolic and flavonoid contents in the plant extracts.

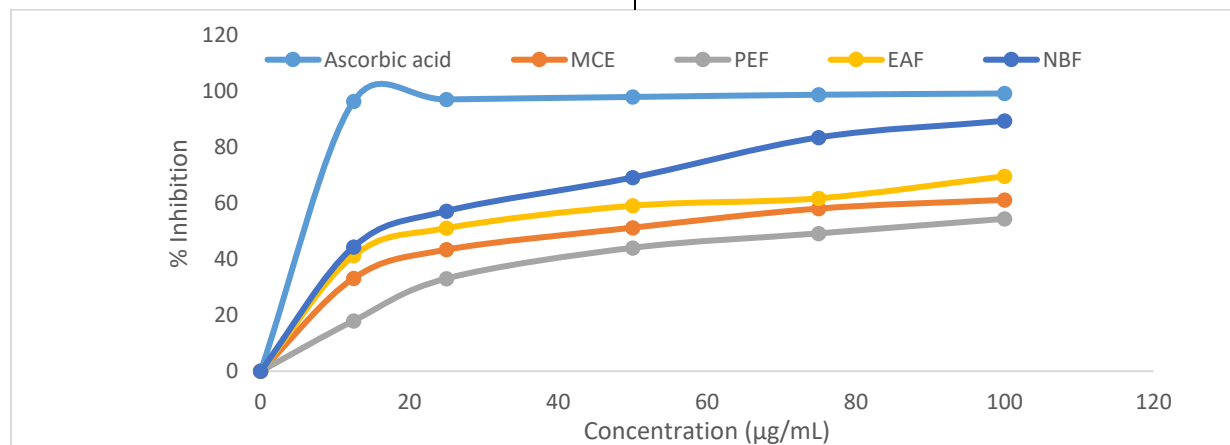


Figure 4: DPPH Radical Scavenging Assay of *Z. Spina* Leaf Extract and Fractions. Each Plot Represents Mean \pm SD, for $n = 3$

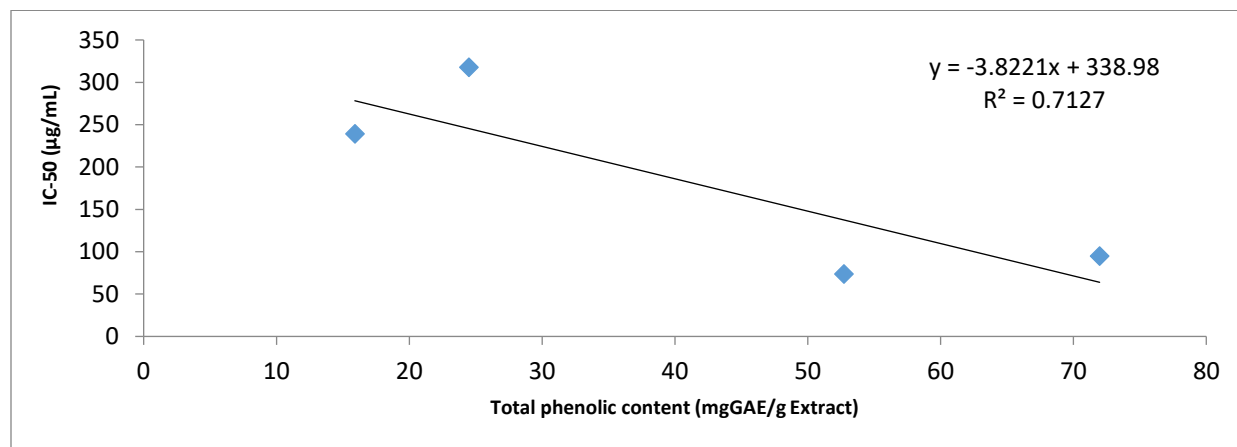


Figure 5: Correlation of Phenolic Content with IC₅₀ Values.

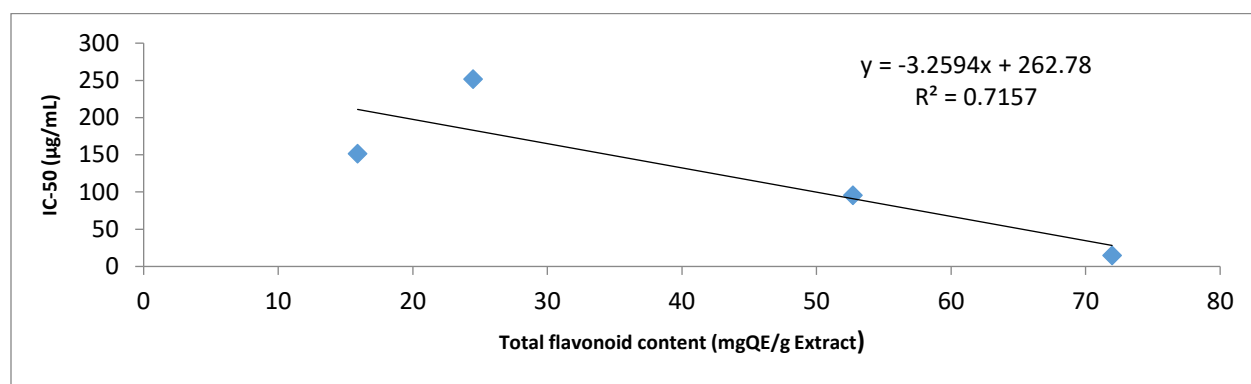


Figure 6: Correlation of Flavonoid Content with IC₅₀ values

4.0 DISCUSSION

4.1 Percentage yield of extract and fractions

The different extractive yields of the solvents are due to their difference in polarity which invariably will lead to the difference in the extracted bioactive compounds (Marinova and Yanishlieva 1997; Ogbiko *et al.*, 2020).

4.2 Antioxidant assay

The DPPH assay reports a dose-dependent inhibition in all extracts. As shown in figure 4, free radical inhibition increases as concentration of extract increase, however the percentage of free radical inhibition was higher in the n-butanol fraction than in others. There was a significant correlation of the polyphenolic contents of the extracts to their antioxidant activities (Figures 5 and 6). This suggests that the antioxidant activity of the plant extracts may be mainly due to the presence of phenols and flavonoid (Table 1) although other polyphenolics such as anthocyanidins, carotenoids, steroids among other

could as well partly offer some antiradical capabilities. This agreed to the report of several researchers who stated that plants with high contents of phenolics and flavonoids had high antioxidant activities (Maisuthisakul *et al.*, 2008). It is well recognized that free radicals are critically involved in various pathological conditions such as cancer, cardiovascular disorders, arthritis, inflammation and liver diseases (Martin *et al.*, 1993). Medicinal plant extracts possesses diverse phytochemicals with antioxidant activities due to their abilities to be donors of hydrogen atoms or electrons as well as to capture free radicals (Wu *et al.*, 2010). DPPH is a stable free radical with purple color, which changes into a stable yellow compound on reacting with an antioxidant. The extent of the reaction depends on the hydrogen-donating ability of the antioxidant. Ascorbic acid is a known and potent antioxidant agent used in medicines where it functions mainly as an antioxidant. The present results showed that standard antioxidants had stronger activity than the

tested extracts, probably because the former contain more purified compounds than the latter (Ogbiko *et al.*, 2020).

5.0 CONCLUSION

Crude extract and fractions of *Z. spina* depict varying antioxidants (free radical scavenging) properties in comparison with ascorbic acid. This is as a result of its phytoconstituents notably phenolics and flavonoids. The findings from this study lend support to the ethno-medicinal use of the plant in Nigerian folk medicine.

Conflict of Interest

The authors declare that they have no conflict of interest

REFERENCES

- Arbonnier, M. (2004). Trees, shrubs and lianas of West African dry zones. CIRAD, Margraf Publishers GmbH, Paris. 212 – 219.
- Audy, B., Ferreira, F., Blasina, L., Lafon, F., Arredondo, F., Dajas, R. & Tripathi, P.C. (2003). Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases. *Journal of Ethnopharmacology*. 84: 131-138.
- Bergendi, L., Benes, L., Durackova, Z. & Ferencik, M. (1999). Chemistry physiology and pathology of free radicals. *Life Science*. 65: 1865-1874.
- Bernardi, A.P.M., López-Alarcón, C., Aspée, A., Rech, S.B., Von-Poser, G.L., Bridi, R., Dutrafilho, C.S. & Lissi, E. (2008). Antioxidant activity in Southern Brazil *Hypericum* species. *Journal of Chilean Chemical Society*. 53: 1658-1662.
- Buyukokuroglu, M.E., Gulcin, I., Oktay, M. & Kufrevioglu, O.I. (2001). In vitro antioxidant properties of dantrolene sodium. *Pharmaceutical Research*. 44: 491- 494.
- Cook, N.C. & Samman, S. (1996). Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources. *Nutritional Biochemistry*. 7: 66-76.
- Dorman, H.J.D., Peltoketo, A., Hiltunen, R. & Tikkanen, M.J. (2003). Characterization of the antioxidant properties of de-odourised aqueous extracts from selected Lamiaceae herbs. *Food Chemistry*. 83: 255-262.
- Ebrahimzadeh, M.A. Pourmorad, F. & Bekhradnia, A.R. (2008). Iron chelating activity, phenol and flavonoid content of some medicinal plants from Iran. *African Journal of Biotechnology*. 7: 3188-3192.
- El Amin, H.M. (1990). Trees and shrubs of the Sudan. Ithaca Press Exeter, Ithaca. 211 – 217.
- Erkan, N., Ayranci, G. & Ayranci, E. (2008). Antioxidant activities of rosemary (*Rosmarinus Officinalis* L.) extract blackseed (*Nigella sativa* L.) essential oil, carnosic acid, rosmarinic acid and sesamol. *Food Chemistry*. 110: 76-82.
- Evans, R.E., Kileff, C. & Shelley, K. (1982). Herbal medicine: A living force in the Appalachians. Durham NC: Duke University Medical Center.
- Farrukh, A., Iqbal, A. & Zafar, M. (2006). Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. *Turkish Journal of Biology*. 30: 177-183.
- Folkers, A., Huve, K., Ammann, C., Dindorf, T., Kesselmeier, J., Kleist, E., Kuhn, U., Uerlings, R. and Wildt, J. (2008). Methanol emissions from deciduous tree species: dependence on temperature and light intensity. *Plant Biology*. 10(1): 65 - 75.
- Gao, J.J., Igalashi, K. & Nukina, M. (1999). Radical scavenging activity of phenylpropanoid glycosides in *Caryopteris incana*. *Bioscience, Biotechnology, and Biochemistry*. 63: 983-988.
- Halliwell, B. (1995). How to characterize an antioxidant: Biochemical Society Symposium. 61: 73-101.
- Harman, D. (1981). The aging process. *Procedure of the National Academic of Science*. USA. 78: 7124-7128.
- Horax, R., Hettiarachchy, N. & Islam, S. (2005). Total phenolic contents and phenolic acid constituents in 4 varieties of bitter melons (*Momordica charantia*) and antioxidant activities of their extracts. *Journal of Food Science*. 70(4): 275-280.
- Jain, A., Soni, M., Deb, L., Jain, A., Rout, S., Gupta, V. & Krishna, K. (2008). Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of *Momordica dioica* leaves. *Journal of Ethnopharmacology*. 115: 61-66.
- Kim, D.O., Jeong, S.W. & Lee, C.Y. (2003). Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry*. 81: 321-326.

- Kumpulainen, J.T. & Salonen, J.T. (1999). Natural antioxidants and anticarcinogens in nutrition, health and disease. The Royal Society of Chemistry. UK. 178-187.
- Lee, J., Hwang, W. & Lim, S. (2004). Antioxidant and anticancer activities of organic extracts from *Platycodon grandiflorum* A. De Candolle roots. *Journal of Ethnopharmacology*. 93: 409-415.
- Maisuthisakul, P., Pasuk, S. & Ritthiruangdej, P. (2008). Relationship between antioxidant properties and chemical composition of some Thai plants. *Journal of Food Composition and Analysis*. 21(3): 229-240.
- Marinova, E.M. & Yanishlieva, N.V. (1997). Antioxidative activity of extracts from selected species of the family Lamiaceae in sunflower oil. *Food Chemistry*. 58: 245-248.
- Martin, G.R., Danner, D.B. & Holbrook, N.J. (1993). Hepatoprotective activity of phenylthanoids from *Cistanche deserticola*. *Planta Medica*. 64: 120-125.
- Mathew, S. & Abraham, T.E. (2006). In vitro antioxidant activity and scavenging effects of *Cinnamomum verum* leaf extract assayed by different methodologies. *Food Chemistry & Toxicology*. 44: 198-206.
- McDonald, S., Prenzler, P.D., Antolovich, M. & Robards, K. (2001). Phenolic content and antioxidant activity of olive extracts. *Food Chemistry*. 73: 73-84.
- Myojin C., Enami, N., Nagata, A., Yamaguchi, T., Takamura, H. & Matoba, T. (2008). Changes in the radical-scavenging activity of bitter melon (*Momordica charantia* L.) during freezing and frozen storage with or without blanching. *Journal of Food Science*. 73: 546-550.
- Ogbiko, C., Okoye, F.B.C. & Eboka, C.J. (2020). Phytochemical, *In vitro* antimicrobial, antioxidant properties and polyphenolic content of methanol leaves extract of *Piliostigma thonningii* SCHUM MILNE-REDHEAD (Fabaceae). *African Journal of Pharmaceutical Research and Development*, 12(3): 299 – 312.
- Osawa, T. & Namiki, M. (1981). A novel type of antioxidant isolated from leaf wax of Eucalyptus leaves. *Agricultural and Biological Chemistry*. 45: 735-739.
- Rice-Evans, C. (2004). Flavonoids and isoflavones: absorption, metabolism and bioactivity. *Free Radical Biology & Medicine*. 36: 827-828.
- Saha, K., Lajis, N.H., Israf, D.A., Hamzah, A.S., Khozirah, S., Khamis, S. & Syahida, A. (2004). Evaluation of antioxidant and nitric oxide inhibitory activities of selected Malaysian medicinal plants. *Journal of Ethnopharmacology*. 92: 263-267.
- Said, A., Huefner, A., Tabl, E.S.A.A. & Fawzy, G. (2006). Two new cyclic amino acids from the seeds and antiviral activity of methanolic extract of the roots of *Ziziphus spina-christi*. Paper presented at the 54th Annual Congress on Medicinal Plant Research. *Planta Medica*. 72
- Semiz, A. & Sen, A. (2007). Antioxidant and chemoprotective properties of *Momordica charantia* L. (bitter melon) fruit extract. *Africa Journal of Biotechnology*. 6: 273-277.
- Schuler, P. (1990). Natural antioxidants exploited commercially, In *Food Antioxidants*, Hudson B.J.F. (ed.). Elsevier, London. pp. 99-170.
- Shen, H., Tang, Y., Muraoka, H. & Washitani, I. (2008). Characteristics of leaf photosynthesis and simulated individual carbon budget in *Primula mutans* under contrasting light and temperature conditions. *Journal of Plant Research*. 121: 191 - 200.
- Sofowora, A. (1982). Screening plants for bioactive agents. In *medicinal plants and traditional medicine in Africa*. Ibadan: Spectrum Books Ltd. pp. 289.
- Trease, E.A. & Evans, W.C. (1978). *Pharmacognosy*. (11th ed.). London: Churchill Livingstone Harcourt Health Service. 60 - 75.
- Xie, J., Zhu, L., Luo, H., Zhou, L., Li, C. & Xu, X. (2001). Direct extraction of specific pharmacophoric flavonoids from ginkgo leaves using a molecularly imprinted polymer for quercetin. *Journal of Chromatography*. 934: 1-11.
- Wafaa, G.S., Wael, M.A., Alaa, H.S., Mahmoud, H.S. & Ehab, A.E. (2019). Evaluation of the biological activity of *Moringa oleifera* leaves extract after incorporating silver nanoparticles, *in vitro* study. *Bulletin of the National Research Centre*. 43(2): 212-218.
- Wu, N, Zu, Y., Fu, Y., Kong, J., Zhao, X., Li, J., Li-Wink, M. & Efferth, T. (2010). Antioxidant activities and xanthine oxidase inhibitory effects of extracts and main polyphenolic compounds obtained from *Geranium sibiricum* L. *Journal of Agriculture Food Chemistry*. 76{2}: 231 - 239.

- Von-Maydell, H.J. (1986). Trees and shrubs of the Sahel: their characteristics and uses. Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH, Eschborn.
- Yumnamcha, T., Nongthomba, U. & Devi, M.D. (2014). Phytochemical screening and evaluation of genotoxicity and acute toxicity of aqueous extract of *Croton tiglium* L. International Journal Scientific Research Publications. 4(1): 1–5.