Global Scientific JOURNALS

GSJ: Volume 11, Issue 3, March 2023, Online: ISSN 2320-9186
www.globalscientificjournal.com

EVALUATION OF TOTAL FLAVONOID CONTENT AND *IN-VITRO* ANTIOXIDANT POTENTIAL OF METHANOL EXTRACTS FROM *DURIO ZIBETHINUS MURR.*

Adeniyi S.A. [*1], Olatunji G.A.[2] and Oguntoye O.S.[2]

[1] Department of Chemical Sciences, Igbinedion University, Okada, Benin City, Nigeria.
adeniyi.sunday@iuokada.edu.ng

[2] Department of Chemistry, University of Ilorin, Ilorin, Nigeria. m_ade49@yahoo.com, stevorol@unilorin.edu.ng

ABSTRACT

Oxidative stress (OS) accelerates the cellular ageing process since free radicals (FR's) or reactive oxygen species (ROS's) attack cell membranes and damage them. Hence, plant antioxidants can supplement the body's antioxidant defense system by increasing the efficiency of the cell membranes and tissues. In this study, the quantity of flavonoids and in-vitro antioxidant potential of methanol extracts of leaves, stem bark and root of Durio zibethinus M were estimated. Total Phenolic Contents (TPCs), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis-(3-ethylbenzothiozoline-6-sulfonic acid (ABTS) scavenging antioxidant activity of methanol extracts of Durio zibethinus M are determined spectrophotometrically at varying concentrations (10, 20, 50, 100, 150µg/mL) and a one-way ANOVA statistical analysis was done using GraphPad Prism. The statistical significance was found to be $P < 0.05$. Total flavonoid content in quercetin equivalent (QE) gave the highest levels of 2.74 ± 0.66, 4.71 ± 2.10 and 7.16 ± 0.76 mg QEg-1 sample weight for the leaves, stem bark and root extracts respectively. The plant extracts showed lower DPPH antioxidant activity with IC_{50} μgmL^{-1} (leaf- 1.467, stem-bark- 1.569 and root- 1.846) and ABTS (leaf- 1.929, stem-bark- 0.267 and root- ~ 2.002) compared to standard quercetin (DPPH- 1.490 and ABTS- 1.247 μgmL^{-1}). In this research, the descending order of total flavonoid content in the mist of methanol extracts of the plant was discovered to be root extract > stem bark extract > leaf extract. The antioxidant activity of the plant extract in decreasing order, follows the same trend with the flavonoid content, revealing that the extract's antioxidant activity is equivalent to the quantity of flavonoids present in the extracts.

Hence, the Durio zibethinus extracts have the potential to be a promising candidate for a plant derived antioxidant agent.

Key words: Total Flavonoid contents, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethylbenzothiozoline-6-sulfonic acid (ABTS), Antioxidants.

1.0 INTRODUCTION

Secondary metabolites such as polyphenols are abundantly available in medicinal plant parts (leaves, stems, roots, flowers and fruits) and play a defensive role against free radicals (Michalak, 2022). Flavonoids are phytonutrients that belong to the polyphenol class. There are various types of flavonoids, including anthocyanidins, flavonols, flavones, flavonones, and isoflavones (Syed et al., 2020). According to Mutha et al. (2021) and Mehmood et al. (2022), flavonoids derived from plants have antioxidant properties both in vitro and in vivo; they are also excellent scavengers of most oxidizable molecules, such as singlet oxygen and other free radicals associated to a variety of diseases. Flavonoids scavenge reactive species, bonded trace elements involved in free-radical formation, and hide reactive oxygen production, increase the cellular response and protect antioxidant defenses (Sharifi-Rad et al., 2020). Some antioxidants, such as phenolics and flavonoids, can be found in a variety of fruits, vegetables, and herbs, and have been shown to provide effective protection against oxidative stress caused by oxidizing agents and free radicals (Dhalaria et al., 2020; Rudrapal et al., 2022). The presence of phenolic compounds, particularly phenolic acids derivatives and flavonoids, in most herbal remedies have antioxidative and pharmacological properties. These phenolic derivatives and flavonoids are well-known for their ability to protect fatty acids from oxidation. They also help to increase the quality of plants that are employed as food ingredients (Kumar & Goel, 2019).

Polyphenols have antioxidant properties due to their redox properties, which allow them to act as hydrogen donors, reducing agents, single oxygen quenchers, and metal chelators. Polyphenolics have antibacterial, antiallergic, anti-inflammatory, antiviral, hepatoprotective, antithrombotic, anticarcinogenic, and vasodilatory properties; the bulk of these biological functions attributed to their free radical scavenging and antioxidant activity. (Sonter et al., 2021).

2.0 *DURIO ZIBETHINUS*

Durian (*Durio zibethinus* Murr.; Family Bombacaceae) is a fruit plant that is grown in Malaysia and Southeast Asia (Bhore et al., 2018). Durian is known as the "King of Tropical Fruit" because of its great nutritional value and appearance, which resembles the thorny thrones of Asian monarchs (Adeniyi et al., 2022). The fact that durian is considered by some botanists to be one of the ancient trees in the tropical rain forest is of scientific interest (Adeniyi et al., 2019). The durian fruit is considered to have medicinal and therapeutic properties, including the capacity to boost the immune system and heal wounds (Adeniyi & Olatunji, 2019). Durian fruit is reported to possess antioxidant (Ang et al., 2018), anti-cancer, anti-cardiovascular, anti-diabetic (Siburian et al., 2019; Sivakumari et al., 2020) and anti-obesity properties (Saminathan & Doraiswamy, 2020), as well as, the ability to promote digestion, cure insomnia, decrease blood pressure, and relieve the symptoms of

depression, anxiety, and stress (Aziz & Jalil, 2019). With this background, it was shown that the antioxidant activity of *Durio zibethinus* parts (leaves, stem-bark and root) has not been investigated. Hence, the present investigation was to estimate the amounts of flavonoids and to determine the *in vitro* antioxidant activity of methanol extracts of leaves, stem bark and root of *Durio zibethinus* Murr.

MATERIAL AND METHODS

3.0 Plant Preparation

Fresh plant materials (leaves, stem-bark and root) of *Durio zibethinus* were harvested from the premises of Crown Estate, Okada Edo State, Nigeria. The plant samples were submitted to the Herbarium of the Faculty of Life Sciences at the University of Ilorin, where voucher number UILH/001/1371 was assigned for standard identification and authentication. The samples were pulverized and extracted after being air-dried at ambient temperature.

3.1 Chemicals and Reagents

Reagents and chemicals which include 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethylbenzothiozoline-6-sulfonic acid), ABTS, quercetin and butylated hydroxytoluene (BHT) were products of Sigma-Aldrich, USA. Aluminum chloride, methanol, potassium acetate, potassium persulfate ($K_2S_2O_8$) and unless otherwise stated, all additional reagents used were analytical grade. However, solvents were redistilled before use as necessary.

3.2 METHODOLOGY

3.2.1 Determination of Total Flavonoid Contents

With minor modifications, the total flavonoid content (TFC) of *Durio zibethinus* extracts was evaluated using the aluminum chloride colorimetric method described by Martínez et al. (2022). To determine total flavonoid content, 0.5 mL of each extract stock solution (1 $mgmL^{-1}$) and each dilution of standard quercetin solution (10 to 100 μgmL^{-1}) were taken separately in test tubes. To each test tube 1.5 mL methanol, 0.1 mL aluminium chloride solution, 0.1 mL potassium acetate solution and 2.8 mL distilled water were added to the mixture and shaken vigorously. The sample blank for all of the dilutions of standard quercetin was made in the same way, by replacing the aluminum chloride solution with distilled water, filtering, and measuring absorbance at 510 nm against a suitable blank (Ayele et al., 2022). TFC was evaluated as milligram quercetin equivalent per gram of dried extract (mg QEg^{-1} DW). Triplicate analysis was carried out on the sample.

3.2.2 DPPH Scavenging Antioxidant Activity

The antioxidant activity of *Durio zibethinus* extracts was examined using a conventional approach of DPPH free radical antioxidant assay (Ehigie et al., 2021). 2.4 mL of each of the samples prepared in concentrations ranging from 10 to 150 gmL^{-1} was added to 0.8 mL of freshly prepared 0.1 mM DPPH solution in methanol and incubated in a dark chamber at room temperature. The reduction in

the optical density of DPPH was measured spectrophotometrically at 517 nm after 10 minutes of incubation, and the results were compared to a blank and standard control drug, quercetin (Pandey et al., 2022).

DPPH scavenging activity (%) =

$$\frac{\text{Abs. of DPPH alone - Abs. of sample alone}}{\text{(Abs. of DPPH alone)}} \times 100$$

3.2.3 ABTS Scavenging Antioxidant Assay

ABTS antioxidant potential of *Durio zibethinus* extracts was capacity was determined by mixing 1 mL of varied concentrations (10 to 150 gmL^{-1}) of each sample with 2 mL ABTS cations solution using a standard test method (Marín et al., 2019). The combination of ABTS standard solution (7 mM) with potassium persulfate (2.45 mM) generated ABTS•+ radical cations. The resulting solution was maintained in the dark for 12 hours at room temperature before being diluted with methanol to reduced optical density of 0.7 ± 0.01 as measured with a UV spectrophotometer at 734 nm. The optical density of the ABTS solution was measured at the same wavenumber after it had been allowed to react for 60 seconds with various concentrations of oil. This experiment was carried out in triplicate and quercetin standard control was used (Pellegrini et al., 2018).

ABTS scavenging activity (%) =

$$\frac{\text{Abs. of ABTS alone - Abs. of sample alone}}{\text{(Abs. of ABTS alone)}} \times 100$$

3.3 Statistical Analysis

GraphPad Prism 5.0 (San Diego, CA) was used to analyze the results obtained by using a one-way ANOVA statistical analysis with outcome triplicate values reported as mean ± standard deviation (± SD). Using GraphPad Prism 5.0, the concentrations of samples that showed a 50% inhibition (IC50) were calculated using a non-linear regression fit.

RESULTS AND DISCUSSION

4.0 Results of Total Flavonoid Content

The results of total flavonoid content of the three crude extracts (leaves, stem bark and roots) of *Durio zibethinus* are given in Table 1. Total flavonoid content in quercetin equivalent (QE) gave the highest levels of 2.74 ± 0.66, 4.71 ± 2.10 and 7.16 ± 0.76 mg QEg-1 sample weights for the leaves, stem bark and root extracts respectively.

Table 1: **Total Flavonoid content of methanol extracts from *Durio zibethinus* M**

Extract	Total Flavonoids (mg QE/g DW)
Leaf	2.74 ± 0.66
Stem bark	4.71 ± 2.10
Root	7.16 ± 0.76

The values are means ± SD of three replicates. QE = Quercetin equivalent, DW = Dried weight.

Results obtained for total flavonoid content of the three crude extracts (leaves, stem bark and roots) of *Durio zibethinus* showed that all the extracts have some number of total flavonoids. In this research, it was observed that the root extract has a significant quantity of flavonoids compare to other extracts. But the minimum quantity of flavonoids was obtained in leaf extract. The results were similar to those reported by other authors (Fábio et al., 2019; Ayele et al., 2022).

4.1 Results of *In-Vitro* Antioxidant Activity

The results of *in-vitro* antioxidant activity with DPPH bioassay are presented in Figure 1. The activities recorded for the leaves, stem bark and root extracts range from 1.80 to 14.74 %, 0.09 to 16.20 % and 0.31 to 19.67 % respectively, while the standard quercetin ranges from 16.55 to 62.22 % with corresponding IC50 values of 1.467, 1.569 and 1.846 µgmL^{-1} for leaves, stem bark and root respectively compared to standard quercetin 1.490 µgmL^{-1} at concentrations (10 to 150 µgmL^{-1}).

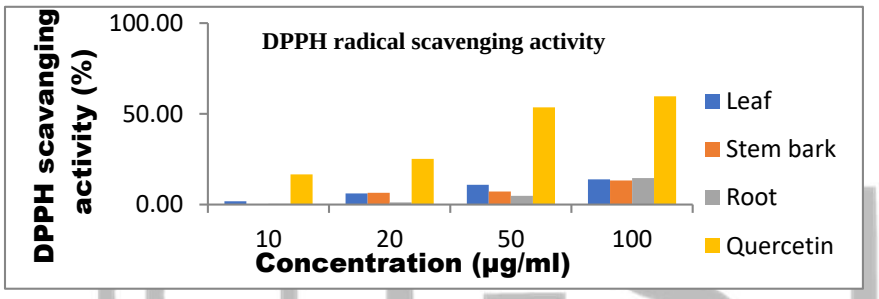

Figure 1: DPPH radical scavenging activity of the methanol extracts of *Durio zibethinus*

Results of in vitro antioxidant activity with ABTS bioassay are presented in Figure 2. The activities recorded for the leaves, stem bark and root extracts range from 7.08 to 18.79 %, 5.13 to 19.69 % and 10.83 to 40.30 % respectively, while the standard quercetin ranges from 32.59 to 62.87 % with corresponding IC_{50} values of 1.929, 0.267 and ~ 2.002 µgmL^{-1} for leaves, stem bark and root respectively compared to standard quercetin 1.247 µgmL^{-1} at concentrations (10 to 150 µgmL^{-1}).

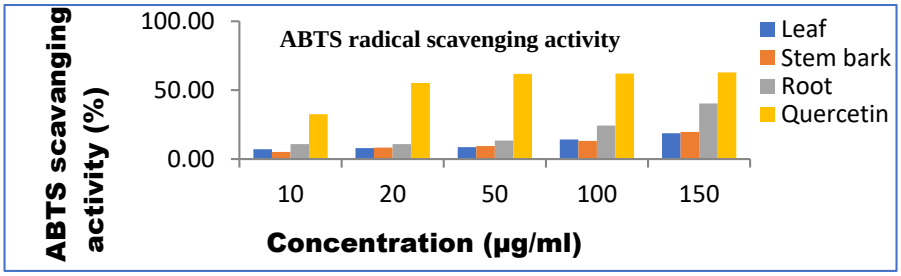

Figure 2: ABTS radical scavenging activity of the methanol extracts of *Durio zibethinus*.

Two antioxidant bioassays (DPPH and ABTS assays) were used to determine the antioxidant activity of the *Durio zibethinus* extracts. The plant extracts exhibited low dose-dependent activities in the DPPH assay as compared to the standard antioxidant, quercetin (Figure 1). However, the root extract of *Durio zibethinus* was found to be the most potent scavenger among the three extracts. Hence, the

decreasing order of antioxidant activity among the studied plant part extracts was established to be root extract > stem bark extract > leaf extract. This order is similar to the report given by Udem et al. (2018).

The plant extracts exhibited low dose-dependent activities in the ABTS assay, compared to the standard antioxidant, quercetin (Figure 2). When investigating the effect of pH on the antioxidant activity of various compounds, ABTS can be used. ABTS' solubility in organic and aqueous media also made it an important assay for determining the antioxidant activity of samples on a regular basis (Platzer et al., 2021). However, the study has shown that the root extract of *Durio zibethinus* was discovered to be the most potent scavenger among the three extracts. Hence, the decreasing order of antioxidant activity among the studied plant part extracts was ascertained to be root extract > stem bark extract > leaf extract.

Plant extracts displayed low antioxidant activity in the two complementary DPPH and ABTS antioxidant assays (Figures 1 and 2). The variations in antioxidant activities reported by the DPPH and ABTS assays appear to be attributable to differences in each antioxidant assay's pathway (Lin et al., 2022).

CONCLUSION

Our findings suggest that *Durio zibethinus* extracts are a viable antioxidant source that might be employed as a natural preservative in food, medicine, pharmaceuticals, and non-food materials. To isolate the phytoconstituents of the plant that show a broad spectrum of pharmacological activity, further phytochemical analysis is required.

5.0 References

Adeniyi S.A. & Olatunji G.A. (2019). Evaluation of macro and micro elements in *Durio zibethinus* Murr. and their biological roles on human physiology. Global Scientific Journals, 7(10): 1114-1122.

Adeniyi, S.A., Olatunji, G.A. & Oguntoye, O.S. (2022). Total Phenolic Contents and Anti-lipooxygenase Activity of the Methanolic Extracts from *Durio zibethinus* Murr. Journal of Scientific Research, 14 (2), 617-623.

Adeniyi S.A., Oke V.J. & Olatunji G.A. (2019). GC-MS analysis of phytochemical constituents in methanol extract of wood bark from *Durio Zibethinus* Murr. International Journal of Medicinal Plants and Natural Products, 5(3): pp 1-11.

Ang, A.M.G., Nalda, C.M.D.R. & Sabejon, S.E. (2018). Brine shrimp lethality and antioxidant activity of the leaf, rind and seed ethanolic extracts of *Durio zibethinus* L. Asian Journal of Biological and Life Sciences, 7(3):105-111

Ayele, D.T., Akele, M.L. & Melese, A.T. (2022). Analysis of total phenolic contents, favonoids, antioxidant and antibacterial activities of *Croton macrostachyus* root extracts. BMC Chemistry 16:30.

Aziz, N.A.A. & Jalil, A.M.M. (2019). Bioactive Compounds, Nutritional Value, and Potential Health Benefits of Indigenous Durian (*Durio Zibethinus* Murr.): A Review. Foods, 8(3): 96.

Bhore, S.J., Husin, N.A., Rahman, S. & Karunakaran, R. (2018). A review on the nutritional, medicinal, and genome attributes of durian (*Durio zibethinus* L.), the king of fruits in Malaysia. Bioinformatics, 14(6):265-270.

Dhalaria, R., Verma, R., Kumar, D., Puri, S., Tapwal, A., Kumar, V., Nepovimova, E. & Kuca, K. (2020). Bioactive compounds of edible fruits with their anti-aging properties: A comprehensive review to prolong human life. Antioxidants, 9(11): 1123. [doi:10.3390/antiox9111123]

Ehigie, F.A., Akinyemi, A.O., Ayoade, E.T., Ogundola, F.A., Oyelere, F.S. & Olabinri, P.F. (2021). Evaluation of the antioxidant potential of aqueous extracts of *Moringa oleifera* leaf and *Cocos nucifera* Husk: A comparative analysis. Pharmaceutical and Biomedical Research, 7(4): 295-302.

Fábio, J., Ana, C.G., Gilberto, A. & Luís, R.S. (2019) Exploring the phenolic profile, antioxidant, antidiabetic and anti-hemolytic potential of *Prunus avium* vegetal parts. Food Research International, 116:600–610.

Kumar, N. & Goel, N. (2019). Phenolic acids: Natural versatile molecules with promising therapeutic applications. Biotechnology Reports, 24(e00370): pp 1-10.

Lin, C., Ying-Mei, W. & Gui-Ju, S. (2022). Variations of Antioxidant Properties and Enzymes Activities during the Whole Fruit Fermentation of Two Kiwifruit (*Actinidia chinenesis* Planch.). International Journal of Fruit Science, 22(1): 860-871.

Marín, F.R., Hernández-Ruiz, J. & Arnao, M.B. (2019). A colorimetric method for the determination of different functional flavonoids using 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) and peroxidase. Preparative Biochemistry and Biotechnology, 49(10): 1033–1039.

Martínez, S., Fuentes, C. & Carballo, J. (2022). Antioxidant Activity, Total Phenolic Content and Total Flavonoid Content in Sweet Chestnut (*Castanea sativa* Mill.) Cultivars Grown in Northwest Spain under Different Environmental Conditions. Foods, 11: 3519.

Mehmood, A., Javid, S., Khan, M.F., Ahmad, K.S. & Mustafa, A. (2022). In vitro total phenolics, total flavonoids, antioxidant and antibacterial activities of selected medicinal plants using different solvent systems. BMC Chemistry, 16(64): 1-10.

Michalak, M. (2022). Plant-derived antioxidants: Significance in skin health and the ageing process. International Journal of Molecular Sciences, 23(2): 585.

Mutha, R.E., Tatiya, A.U. & Surana, S.J. (2021). Flavonoids as natural phenolic compounds and their role in therapeutics: an overview. Future Journal of Pharmaceutical Sciences, 7(25): 1-13.

Pandey, R.A., Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A. & Chang C-M. (2022). Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiose*. Molecules, 27(4): 1326.

Pellegrini, N., Vitaglione, P., Granato, D. & Fogliano, V. (2018). Twenty-five years of total antioxidant capacity measurement of foods and biological fluids: merits and limitations. Journal of the Science of Food and Agriculture, 100(14): 5064-5078.

Platzer, M., Kiese, S., Herfellner, T., Ute Schweiggert-Weisz, U., Miesbauer, O. & Peter Eisner, P. (2021). Common trends and differences in antioxidant activity analysis of phenolic substances using single electron transfer based assays. Molecules, 26(5): 1244.

Rudrapal, M., Khairnar, S.J., Khan, J., Bin Dukhyil, A., Ansari, M.A., Alomary, M.N., Alshabrmi, F.M., Palai, S., Deb, P.K. & Devi, R. (2022). Dietary polyphenols and their role in oxidative stress-induced human diseases: Insights into protective effects, antioxidant potentials and mechanism(s) of action. Frontier in Pharmacology, 13(806470): 1-15.

Saminathan, V. & Doraiswamy, R. (2020). Phytochemical analysis, antioxidant and anticancer activities of durian (*Durio zibethinus* Murr.) fruit extract. Journal of Research in Pharmacy, 24(6): 882-892.

Sharifi-Rad, M., Anil Kumar, N.V., Zucca, P., Varoni, E.M., Dini, L., Panzarini, E., Rajkovic, J., Fokou, P.V.T., Azzini, E., Peluso, I., Mishra, A.P., Nigam, M., El Rayess, Y., El Beyrouthy, M., Polito, L., Iriti, M., Martins, N., Martorell, M., Docea, A.O., Setzer, W.N., Calina, D., Cho, W.C. & Sharifi-Rad, J. (2020). Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. Frontiers in Physiology, 11(694): 1-21.

Siburian, R., Aruan, D.G.R., Barus, T., Haro, G. & Simanjuntak, P. (2019). Phytochemical screening and antidiabetic activity of n-hexane, ethyl acetate and water extract from durian leaves (*Durio zibethinus* L.). Oriental Journal of Chemistry**,** 35(1):487-490.

Sivakumari, K., Mohamad Sitheek, A., Rajesh, S. & Ashok, K. (2020). Anti-proliferative effect of durian fruit (*Durio zibethinus*) against hl-60 cells and its phytochemical analysis. Journal of Advanced Scientific Research, 11(5): 174-181.

Sonter, S., Mishra, S., Dwivedi, M.K. & Singh, P.K. (2021). Chemical profiling, in vitro antioxidant, membrane stabilizing and antimicrobial properties of wild growing *Murraya paniculata* from Amarkantak (M.P.). Scientific Reports, 11(9691): pp 1-15.

Syed, L.B., Asad, U., Sidra, M., Noreen, K., Lubna, G., Benjamin, G.P., Abdul-Hamid, E. & Mariusz, J. (2020). Important flavonoids and their role as a therapeutic agent. Molecules, 25(22): 5243.

Udem, G.C., Dahiru, D. & Etteh, C.C. (2018). In vitro Antioxidant Activities of Aqueous and Ethanol Extracts of *Mangifera indica* Leaf, Stem-Bark and Root-Bark. Pharmacognosy Communications, 8(3):119-124.