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Evaluation of the total phenolic, total flavonoid, and radical scavenging properties of the stem bark and leaves of Pterocarpus osun

Adewale Elijah Fadeyi[1&2*], Oluremi Isola Adeniran[1]
[1]Chemistry Department, University of Abuja, Nigeria, benadole@yahoo.com
[2*]Chemistry Advanced Research Centre, Sheda Science and Technology Complex, Abuja, Nigeria, wale.fade@gmail.com

*Correspondence: wale.fade@gmail.com

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Abstract

Introduction: Medicinal plants have the capacity to scavenge free radicals due to their potential to stabilize harmful molecules, which contributes to oxidative stress that results in non-communicable diseases like cancer, diabetes. Methodology: Folin-Ciocalteu colorimetric method and the aluminium chloride method respectively, were used to evaluate the total contents of phenolic and flavonoid of the Pterocarpus osun leaf and stem bark extracts. Purpose of study: The goal of the current investigation was to evaluate the total content of flavonoid, and total content of phenol present in methanol extracts of the leaf and stem bark of P. osun. Using the DPPH assay to measure the extracts' capacity to scavenge free radicals, inhibition concentrations of 0.0625, 0.125, 0.250, 0.500, and 1.00 mg/mL were noted. Results: The IC50s for the stem bark and the leaf extracts are 3.44, and 48.14 mg/mL, respectively. The amount of flavonoid in the stem bark was 2.32, and 1.50 mg quercetin/g extract for the leaf. The total phenolic content was 8.61 and, 6.61 mg gallic acid equivalent (GAE)/g extract for the stem bark and for the leaf, respectively. Conclusion: The results show that there is a correlation between radical scavenging properties and poly phenolics.

Keywords: Flavonoids, Free radicals, In vitro, Phenolics, Pterocarpus osun

1. Introduction

Secondary metabolites like phenolic acids, tannins, polyphenols, flavonoids, and others, which are frequently referred to as antioxidants, are found in herbs and medicinal plants (Sinh et al, 2021). According to Unuofin et al. (2020), the many health benefits that medicinal plants and spices provide are due to the large concentration of these antioxidants. Several health conditions that affect people, such as cancer, Alzheimer's, heart, kidney, and liver conditions, fibrosis, coronary artery disease arthritic disease brain disorders, and old age, are caused by the production of free radicals in the body (Setchell et al., 2014). By providing the necessary number of electrons to stabilize the free radicals, the antioxidants found in herbs and spices can neutralize them.

2. Literature review

The uptake of electrons stabilizes the free radicals, making them non-reactive to cellular DNA (Martemucci et al., 2022). According to studies, the common phenolic and flavonoid chemicals compounds in plants have strong antioxidant action (Farasat et al., 2014). Scavenging reactive oxygen species (ROS), as well as boosting natural enzyme activity in antioxidants, metal chelation, and blocking ROS-producing pro-oxidant enzymes, phenolic and flavonoids have been shown to have numerous antioxidant mechanisms (Kocyigit et al., 2018). The most prevalent polyphenols in our diet are flavonoids and phenolic acids, which are found in large quantities in a variety of foods, including fruits, vegetables, cereals, and drinks. Because of their potent antioxidative qualities and their observable contributions to the prevention of numerous oxidations associated with stress diseases, nutritionists have recently placed a lot of emphasis on dietary polyphenols. One of the key mechanisms for creating radicals that are unstable found in food and in biological systems is the oxidation process. Epidemiological data, as well as in vitro and in vivo investigations, are used to infer the preventative properties of plant polyphenols and their application in therapy of illnesses (Halliwell et al., 1990; Gulcin et al., 2020). Some investigations have demonstrated that a wide variety of phytochemicals with antioxidant properties in herbs for medicinal purposes, vegetables, and fruits, which form the basis of their beneficial health effects (Llaurado et al., 2020).

In conjunction with vitamin C, vitamin E, and carotenoids, polyphenols have significant antioxidant capacity, according to Ellong et al. (2015). All plants naturally contain phenolic chemicals, which are organic compounds produced as a byproduct of secondary plant metabolism (Yasin et al., 2015). They can be organized into many classes, such as flavonoids, phenolic acids stilbenes, and lignans, based on the presence of multiple phenolic molecules attached to varying degrees of complex structures (Tan et al., 2018). Vegetables, grains, olives, legumes, as well chocolate, and beverages including coffee, tea, and wine all contain significant amounts of phenolic chemicals, according to Genwali et al. (2013). Despite the fact that phenolics are most commonly recognized for their antioxidant effects, there is mounting evidence that they also have other beneficial health effects, such as antidiabetic, cancer-preventing, anti-inflammatory properties, cardioprotective, osteoprotective, neuroprotective, antiasthmatic, antihypertensive, antiaging, disinfectant, cerebrovascular defense, cholesterol-lowering, hepatoprotective, antimicrobial, and antiviral capabilities. Previous research has shown that the B-ring hydroxyl groups of flavonoids are what give them their known antioxidant effects by donating hydrogen atoms during free radical reactions (Hernandez-Rodringuez et al., 2019).

In addition, a number of techniques, that also comprise metal-ion chelation, neutralizing singlet oxygen, hydrogen donation, and free radical scavenging, and functioning as a potential base for oxidants like superoxide along with hydroxyl, phenolics are a valuable source of antioxidant compounds (Almokhtar, Ata, Azab, 2019). The antioxidants obtained from plants have more advantages over synthetic antioxidants like butylatedhydroxyanisole (BHA) due to their natural origin. Nigeria is home to a wide variety of medicinal plants, and a lot of the populace uses herbal medicines as their primary means of healthcare. These sorts of plants can serve as a source of organic antioxidants that are naturally occurring. However, there is a lack of quantitative data on the polyphenol composition *Pterocarpus osun*. In our previous investigation (Fadeyi *et al.*, 2022) the

nutritional value and antibacterial qualities of the stem bark and leaves of Pterocarpus osun were evaluated. The ability to neutralize free radicals is correlated with the overall phenolic content.

Tables and Figures

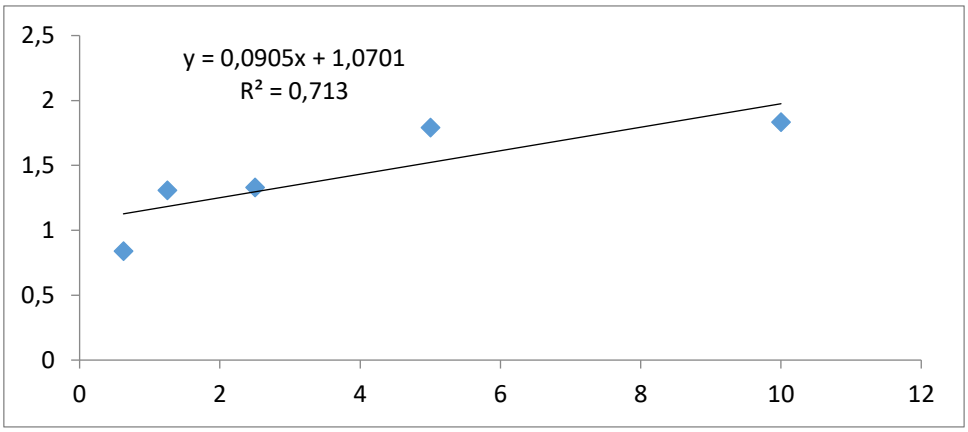

Figure 1: Garlic acid standard calibration curve

Table 1: TPC and TFC of stem bark and leaf of *P. osun,* and their ratio

	TPC	TFC	TFC/TPC
Stem bark	8.61±1.21	2.32±0.13	0.269
Leaf	6.82±1.03	1.50±0.53	0.220

Note: TPC is measured mg of gallic acid equivalent, GAE/100 g sample powder weight, and TFC is mg quercetin equivalent, QE/100 g sample powder weight.
TPC = total phenolic content, TFC total flavonoid content

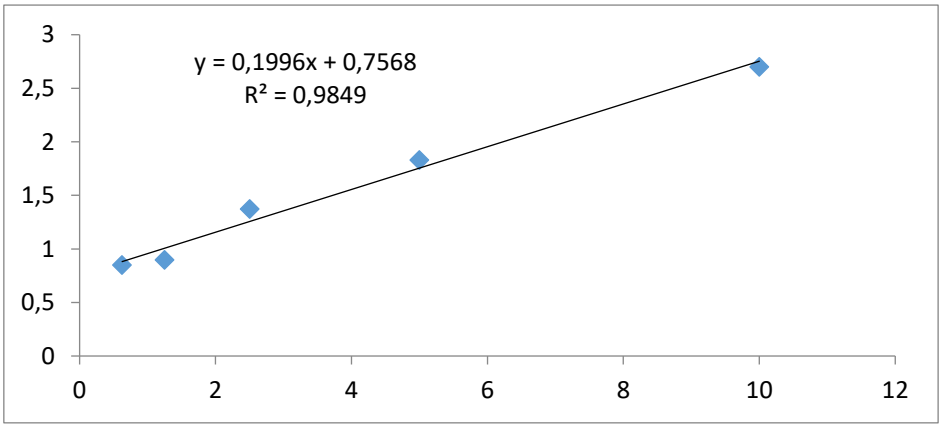

Figure 2: Quercetin standard calibration curve

Table 2: Free Radical removing capabilities of stem bark and leave of *P. osun,* and ascorbic acid (Fadeyi et al., 2022).

Concentration (mg/mL)	%RSA Stem bark	%RSA Leaf	%RSA Ascorbic acid
0.0625	64.80±0.17	57.21±0.20	81.27±0.31
0.125	81.10±0.08	59.30±0.24	79.77±0.22
0.250	83.12±0.15	79.36±0.15	79.43±0.16
0.500	81.30±0.11	74.16±0.21	77.69±0.16
1.0	78.21±0.13	62.60±0.32	79.05±0.26
IC50	**7.25 mg/mL**	**3.44 mg/mL**	**48.14 mg/mL**

Key: AA – Antioxidant Activity

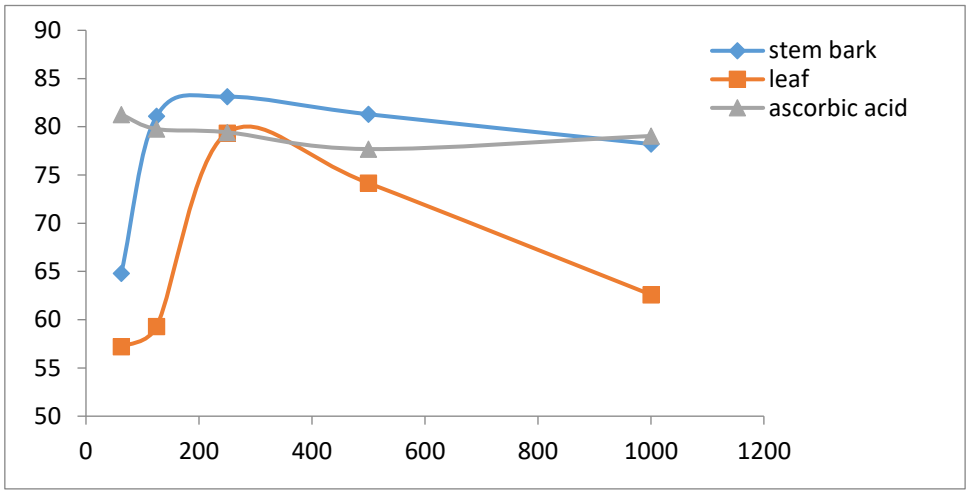

Figure 3: %Inhibition vs. concentration of DPPH radical scavenging capabilities

3. Research methodology

Chemicals: Gallic acid, Folin-Ciocalteu (F-C) reagent, (DPPH) 2,2-diphenyl-2-picrylhydrazyl, quercetin, ascorbic acid (vitamin C), aluminum chloride, sodium carbonate, and methanol. The solvents are redistilled before use.

3.1. Plant materials

Stem bark and leaves of *P. osun* were harvested from the medicinal garden of Sheda Science and Technology Complex (SHESTCO), FCT, Abuja, Nigeria. The plant's leaf was submitted to the herbarium of the National Institute of Pharmaceutical Research (NIPRD), Abuja. The stem bark and leaf were air dried and blended to powder using a hammer mill. The powdered plant parts were stored in plastic bags until further use.

3.2. Extraction of samples

Individually, 50 g of each of the dried and pulverized plant parts were soaked for two days in 200 mL of methanol (cold extraction technique). Filtration of the extracts was carried out and the solvents were recovered using a rotary evaporator.

3.3. Preparation of standard

Folin-Ciocalteu colourimetric technique involving reactions caused by redox was used to quantify the quantity of phenolic compounds in plant extracts (Amezquita *et al.,* 2019). Gallic acid solutions in methanol were serial diluted to concentrations (0.625, 1.25, 2.50, 5.00, and 10.00 mg/mL). One milliliter of gallic acid solution was put into a 20-milliliter test tube. Then, 5 milliliters of Folin-Ciocalteu reagent (10%), and with addition of 4 milliliters of Na_2CO_3 (7%) resulting to a total amount of 10 milliliters. The resulting mixture of blue solution was thoroughly agitated and allowed to stay in water bath for 30 minutes at 40 ^oC. Absorbance was measured at 760 nm and compared to the absorbance of the blank solution. Three replications of each experiment were run throughout. The calibration curve was plotted using the mean values of absorbance obtained at various gallic acid concentrations.

3.4. Assessment of radical scavenging tendencies

Employing the DPPH (2,2-diphenyl-1-picrylhidrazyl) technique as described by (Sungthong *et al.,* 2018), the radical scavenging capability was measured. From the sample initial solution in methanol, different sample concentrations were made. 1 ml each of 0.1 mM DPPH solution was added to them, and they were then covered, uniform mixed, and made to stand for 30 min. in a dark cupboard. At 517 nm, the absorbance was read on a UV-visble spectrophotometer. The experiment was repeated thrice, and the absorbance of the solution with no sample (the blank) made up of DPPH solution in methanol. The following formula was used to get the percent inhibition:

$$\% \text{ Inhibition} = \frac{Ablank - Asample}{Ablank} \times 100\%$$

Ablank = absorbance of blank

Asample = absorbance of sample

The percent inhibition values obtained are plotted on the y-axis against logarithmic values of concentrations on the x-axis, and the linear regression curve generated. The equation for linear regression, y = ax + b, was used to determine the IC50 value.
In the equation, y is replaced with 50 and x is the value of the IC50.

3.5. Measurement of the total phenolic compounds

Folin-Ciocalteu reagent (FCR) was employed in the measurement of total quantity of phenolic compounds in the samples. 50 mL of the extract solution was combined with 2.0 mL of 7.5% Na_2CO_3 and 2.5 mL of 10% Folin-Ciocalteu reagent prior being incubated at 45°C for a period of 15 min. Sample absorbance was measured at 765 nm, using a UV-visible spectrophotometer. Gallic acid absorbance was plotted versus its concentration to create garlic acid standard curve. The total quantity of phenol of the samples was determined and reported as GAE, (gallic acid equivalents), measured in milligrams per gram of extract (mg GAE/g extract). The measurements were made in triplicate (Munteanu *et al.,* 2021).

3.6. Estimation of total flavonoid compounds

The quantity of flavonoids in the sample was evaluated using the aluminum chloride technique. Quercetin was employed as the standard. For this, a standard curve for quercetin was generated. The serial concentrations of quercetin made in methanol from the standard quercetin solution are 0.1, 0.5, 1.0, 2.5, and 5mg/mL. 0.1 mL of each quercetin was mixed with 0.5 mL of distilled water and 0.1 mL of 5% sodium nitrate, the mixture was incubated for 6 minutes afterwards, 0.15 mL of a 10% aluminum chloride solution was added, and 0.2 mL of a 1M sodium hydroxide solution was consecutively added 5 min thereafter. The absorbance of this reaction combination was noted at 510 nm on a UV-visible spectrophotometer The experiment was repeated with methanol extracts of the samples, and then, the total concentration of flavonoid was determined as mgQE/g (mgQE = milligrams per gram). The experiment was repeated three times.

4. Data analysis

The data are presented as the average standard deviation after each experiment was run in triplicate. Using Microsoft Office Excel 2007, a linear correlation coefficient study was performed. The equation for linear regression is:

$Y = mx + c.$

Where, x = concentration of the extract,

m = slope of the calibration curve, and

c = intercept.

Concentrations of extracts were estimated using this regression equation. The total flavonoid and phenolic composition of each extract was evaluated using the concentration data.

5. Results and discussions

By using 2,2-diphenyl-1-picrilhidrazyl (DPPH), the ability of the extracts to scavenge free radicals was evaluated. The 2,2-diphenyl-1-picrilhidrazyl radical changed from purple to yellow as an indication of increased stability due to electron donation from antioxidants. The absorbance was taken at 517 nm using a ultraviolent- visible (UV-visible) spectrophotometer, and the percentage inhibition was computed. The potentials of DPPH to scavenge free radical of methanol extracts of *P. osun* leaf and stem bark are shown in Table 2. This suggests that the plant is effective in scavenging free radicals. When compared to ascorbic acid, which is employed as the standard control, the stem bark and leaf extracts show great activity. However, when the ascorbic acid, the leaf and the stem bark extracts are compared, the IC50 values of 7.25, 3.44, and 48.14 mg/mL, respectively are recorded in Table 2. The graph of percentage DPPH radical scavenging activities that are inhibited at various concentrations is shown in Figure 3. In the stem bark and the leaf extracts of *P. osun*, respectively, the phenolic contents are 8.61 and 6.82 mg of GAE/100 gm (Table 1). The standard garlic acid and quercetin calibration curves are shown in Figures 1 and 2 respectively. Both the stem bark and the leaf have total flavonoid concentrations of 2.32 and 1.5 mg QE/100 g accordingly (Table 1). The secondary metabolites component in the methanol extract of *P. osun*, responsible for the strong radical scavenging activity demonstrated by the plant.

The total amount of phenolic components in the two *P. osun* extracts was determined and stated in GAE (gallic acid equivalents) using the equation on the calibration graph. Utilizing the formula of

the standard quercetin calibration graph, the total flavonoids were determined, and their values were expressed in mg of the equivalent of quercetin (QE) per 100 g. Flavonoid molecules function as free radical scavengers because they contain hydroxyl radicals, which may prompt the release of protons from the hydrogen ions. Hydrogen ions, which only have one proton and no electrons, are released when radical electrons from the nitrogen atom in the DPPH molecule bind to the hydrogen ions (Zheng et al., 2022). For instance, iron, copper, manganese, and cobalt are all chelated by flavonoids. According Cherrak *et al., (*2016), flavonoids' hydroxyl (OH) and carbonyl groups can form durable complexes with metal. Phenols function as antioxidants by donating hydrogen atoms to radicals of DPPH to reduce them to a less reactive state. The ability of phenolic substances to scavenge free radicals depends on the number and position of hydroxyl molecules inside the molecular structure. The production of antioxidant activity increases with the quantity of hydroxyl groups. Antioxidant capabilities may be impacted by how it interacts with DPPH free radicals (Shahidi *et al,* 2015). Due to the -OH groups that are connected to the aromatic ring's carbon, phenols and flavonoids have the capability to operate as free radical scavengers.

Antioxidant activity in polyphenolic compounds is highly correlated with the side-chain composition and structure of the aromatic ring (Vuolo *et al.,* 2018). The DPPH technique depends on an antioxidant's ability to supply an electron, a hydrogen atom, or the stable free radical DPPH, which has a deep violet hue. When an odd electron pairs up in the presence of an antioxidant or free radical scavenger, the dark violet steady free radicals, or DPPH radicals, become reduced to the corresponding hydrazine, or DPPH-H structure, and the mixture's initial deep violet color turns to a pale yellow. The decrease in absorbance corresponds to the quantity of the antioxidant total phenolic content in both extracts, according to an evaluation of the Folin-Ciocalteu approach, using gallic acid as the standard. The absorbance values obtained at various gallic acid concentrations were used to generate the calibration curve (Figure. 1). By transferring electrons from phenolic compounds to phosphomolybdic phosphotungstic acid complexes in an alkaline solution, the Folin-Ciocalteu method produces blue-colored complexes that may be read at 765 nm in a UV-vis spectrophotometer. From the outcome of this study, it can be concluded that *P.osun* stembark and leaves can a lead to discovering novel antimicrobial agents for wide spectrum of pathogens causing diseases in our society and oxidative stress related illnesses because of the phenolic and flavonoid contents.

6. Conclusion

The methanol extract of *P. Osun* stem bark and leaf are rich in flavonoids and phenols, hence high antioxidant capacity as shown by the result of the free radical depleting capacity of both stem bark and the leaf extracts. Therefore, the use of the *Pterocarpus osun* in traditional medicine for various therapeutic purposes such as antioxidant, antibacterial, antifungal, and many more can further be exploited.

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ORCID

Adewale Elijah Fadeyi https://orcid.org/0000-0001-7152-7131
Oluremi Isola Adeniran https://orcid.org/0000-0001-7235-6549

References

1. Almokhtar, A. A., Ata, S. I. E., & Azab, E. A. (2019). Oxidative stress and antioxidant mechanisms in human body. *J Appl Biotechnol Bioeng*, 6: 43–47.
2. Amezquita, P. M., Beltrán-Morales, F. A., Manríquez-Rivera, G. A., Cota-Almanza, M. E., Quian-Torres, A., & Peralta-Olachea, R. G. (2019). Nutritional Value of Conventional, Wild and Organically Produced Fruits and Vegetables Available in Baja Califo rnia Sur Markets. *Terra Latinoamericana*, *37*(4), 401–406.
3. Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R., & Koirala, N. (2019). Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. *Plants*, *8*(4). 1-12.
4. Ellong, E. N., Billard, C., Adenet, S., & Rochefort, K. (2015). Polyphenols, carotenoids, vitamin C content in tropical fruits and vegetables and impact of processing methods. *Food and Nutrition Sciences*, *6*(03), 299.
5. Fadeyi, A. E., Adeniran O. I., Akiode O. S. (2022). Nutrients, Phytochemical, Antioxidant and Antimicrobial Analysis of *Pterocarpus osun* stem bark and leaf for their nutritional, medicinal capacity. Indo. J. Chem. Res., 10(1), 58-67. Retrieved from: http://ojs3.unpatti.ac.id/index.php/ijcr
6. Farasat, M., Khavari-Nejad, R. A., Nabavi, S. M. B., & Namjooyan, F. (2014). Antioxidant activity, total phenolics and flavonoid contents of some edible green seaweeds from northern coasts of the Persian Gulf. *Iranian journal of pharmaceutical research: IJPR*, *13*(1), 163.
7. Ganesan K., & Xu, B. (2017). Polyphenol-Rich dry common beans (Phaseolus vulgaris L.) and their health benefits. *Int J Mol Sci*, 18: 2331.
8. Genwali, G. R., Acharya, P. P., & Rajbhandari, M. (2013). Isolation of gallic acid and estimation of total phenolic content in some medicinal plants and their antioxidant activity. *Nepal journal of science and technology*, *14*(1), 95-102.
9. Gulcin, İ. (2020). Antioxidants and antioxidant methods: An updated overview. *Archives of toxicology*, *94*(3), 651-715.
10. Halliwell, B., & Gutteridge, J. M. (1990). [1] Role of free radicals and catalytic metal ions in human disease: an overview. *Methods in enzymology*, *186*, 1-85.
11. Hernández-Rodríguez, P., Baquero, L. P., & Larrota, H. R. (2019). Flavonoids: Potential therapeutic agents by their antioxidant capacity. In *Bioactive compounds* (pp. 265-288). Woodhead Publishing.
12. Kocyigit, A., Guler, E. M., & Dikilitas, M. (2018). Role of antioxidant phytochemicals in prevention, formation, and treatment of cancer. *Reactive Oxygen Species (ROS) in Living Cells; InterchOpen: London, UK*, 21-45.

13. Llauradó Maury, G., Méndez Rodríguez, D., Hendrix, S., Escalona Arranz, J. C., Fung Boix, Y., Pacheco, A. O., & Cuypers, A. (2020). Antioxidants in plants: A valorization potential emphasizing the need for the conservation of plant biodiversity in Cuba. *Antioxidants*, *9*(11), 1048.

14. Martemucci, G., Costagliola, C., Mariano, M., D'andrea, L., Napolitano, P., & D'Alessandro, A. G. (2022). Free radical properties, source and targets, antioxidant consumption and health. *Oxygen*, *2*(2), 48-78.

15. Munteanu, I. G., & Apetrei, C. (2021). Analytical Methods Used in Determining Antioxidant Activity: A review. *International Journal of Molecular Sciences*, *22*(7), 1–30.

16. Nimse, S. B., & Pal, D. (2015). Free radicals, natural antioxidants, and their reaction mechanisms. *RSC Adv*, 5: 27986–28006.

17. Paixao, N. R., Perestrelo, J. C., Marques., & Camara, J. S. (2007). Relationship between antioxidant capacity and total phenolic content of red, rose, and white wines. *Food Chemistry* **105**: 204-214.

18. Purwaningsih, I., Fathiah, F., Amaliyah, N., & Kuswiyanto, K. (2023). The Phenolic, Flavonoid, and Anthocyanin Content From Methanol Extract of Senggani Fruit and Its Antioxidant Activity. *Indonesian Journal of Chemical Research*, *10*(3), 195-202.

19. Setchell, K. D., Brown, N. M., Zimmer-Nechemias, L., Wolfe, B., Jha, P., & Heubi, J. E. (2014). Metabolism of secoisolariciresinol-diglycoside the dietary precursor to the intestinally derived lignan enterolactone in humans. *Food & function*, *5*(3), 491-501.

20. Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects–A review. *Journal of functional foods*, *18*, 820-897.

21. Sinh, J. N., N'guessan, A. A., Kouadio, O. K., & Gonnety, J. T. (2021). Acceptability, nutritional and antioxidant properties of spice formulations based on Coelocaryon oxycarpum (Cox), ginger and pepper. *Journal of Applied Biosciences*, *166*(1), 17231-17241.

22. Sungthong, B., & Srichaikul, B. (2018). Antioxidant activities, acute toxicity and chemical profiling of torch ginger (Etlingera elatior Jack.) inflorescent extract. *Pharmacognosy Journal*, *10*(5).

23. Tan, B. L., Norhaizan, M. E., Liew, W. P. P., & Sulaiman Rahman, H. (2018). Antioxidant and oxidative stress: a mutual interplay in age-related diseases. *Frontiers in pharmacology*, *9*, 1162.

24. Unuofin, J. O., & Lebelo, S. L. (2020). Antioxidant effects and mechanisms of medicinal plants and their bioactive compounds for the prevention and treatment of type 2 diabetes: an updated review. *Oxidative medicine and cellular longevity*, *2020*.Enrique C, Davies KJA. Mitochondrial free radical generation, oxidative stress, and aging. *Free Radical Biology and Medicine* 2000; 29(3):222-230.

25. Vuolo, M. M., Lima, V. S., & Junior, M. R. M. (2019). Phenolic compounds: Structure, classification, and antioxidant power. In *Bioactive compounds* (pp. 33-50). Woodhead Publishing.

26. Yasin, B. R., El-Fawal, H. A., & Mousa, S. A. (2015). Date (Phoenix dactylifera) polyphenolics and other bioactive compounds: A traditional islamic remedy's potential in prevention of cell damage, cancer therapeutics and beyond. *International journal of molecular sciences*, *16*(12), 30075-30090.

27. Zheng, Y. Z., Deng, G., & Zhang, Y. C. (2022). Multiple free radical scavenging reactions of flavonoids. *Dyes and Pigments*, *198*, 109877.

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