Relative Solvent-Based Antioxidant Potentials of Baphia Nitida Leaf Extracts

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Abstract

Safety concerns due to artificial antioxidants added to especially, food products has intensified the search for natural sources of antioxidants capable of preventing, quenching and/or repairing effects of free radical damages. The current research explores the *in vitro* antioxidant activity of *Baphia nitida* aqueous, methanol and ethanol leaf extracts using ferric ions (Fe^{3+}) reducing antioxidant power assay (FRAP), hydrogen peroxide scavenging activity (HPSA), evaluation of total antioxidant capacity (TAC) with phosphomolybdenum and determination of total phenols content (TPC) using Folin–Ciocalteu reagent. The results showed that all the extracts have good antioxidant activity as indicated by the parameters analysed. But the aqueous extract had the highest peroxide scavenging activity and total phenolic content (significantly at $p<0.05$) indicating that the plant possesses water soluble phenolic phytochemicals capable of quenching aqueous hydrogen peroxides radicals. The result of FRAP showed that the alcoholic leaf extracts have the highest reducing capacity than the aqueous extract (significantly at $p<0.05$), pointing out that the leaves of the plant have more alcohol-soluble phytochemicals that can donate electron to neutralise one electron-deficient and potentially damaging free radicals. While the results for TAC shows a significant pattern (at $P<0.05$) of methanolic extract > aqueous extract > ethanolic extract indicating that the methanolic extract is having higher total phytochemicals with antioxidant potentials. Hence, all the three leaf extracts of *B. nitida* have antioxidant potentials to deal with free radicals which could be due to different mechanisms based on the phytochemicals extracted by each solvent. Therefore, the leaf extracts of the plant are potential sources of natural antioxidants, and may be good candidates for pharmaceutical plant-based products.

Keywords: *Baphia nitida,* Antioxidant Capacity, Phytochemicals, Methanolic Extract, Ethanolic Extract, Aqueous Extract.

Introduction

Antioxidants are any substance that can eliminate reactive oxygen species (ROS) directly or indirectly, acting as a regulator of the antioxidant defence, or inhibiting the production of those species.[1] The ROS are produced due to the partial reduction of molecular oxygen resulting in the formation of superoxide anion (O_2^-), hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$)[2] that may cause oxidative stress without a proper antioxidant defences system in place.[3] Oxidative stress can be considered as any condition in which oxidant metabolites can exert their damaging effects [4] on biological molecules such as lipids, proteins and DNA which lead to lipid peroxidation and oxidative DNA damage because of increased production of ROS and/or altered cellular mechanisms of (antioxidant) protection.[5] This could result in pathogenesis of lifestyle-related diseases, such as atherosclerosis, hypertension, diabetes mellitus, ischemic diseases and malignancies.[4]

Antioxidants can be classified into enzymatic or non-enzymatic, preventative or repair-systems, endogenous or exogenous, primary or secondary,

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hydrosoluble or liposoluble and natural or synthetic.[6] Antioxidants may be present in foods as endogenous factors or may be added to preserve their components from quality deterioration.[7] The synthetic antioxidants such as butylated hydroxyanisole (BHA) are commonly used in food formulations. However, due to safety concerns, interest in natural antioxidants has intensified.[7] Antioxidants of nutritional origin thus play important roles in complementing *in vivo* antioxidants in "fighting" free radicals encountered by the body's cells.[8]

According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs.[9] Cam wood (*B. nitida*) is a widely distributed shrub or short tree with immense medicinal benefits. Geographically, it is found in the wetter parts of coastal regions, rain and secondary forests and on abandoned farmland from sea-level up to 600m altitude and can grow to a height of about 9m.[10] It is widely endowed with a wide range of ethno-pharmacological benefits hence, has been used by the inhabitants of many West African countries for medicinal purposes.[11] It is used to treat constipation, ringworm, sprains, swollen joints, parasitic skin diseases, wounds, ulcers, boils, venereal diseases and gastrointestinal problems. Furthermore, it also has haemostatic, anti-inflammatory, analgesic and hepatoprotective activities.[10] The dye of the plant has also been reported to have antimicrobial activity.[12]

The ethanolic leaf extracts of *B. nitida* have been reported to possess a dose dependent antioxidant activity.[13] Therefore, this study aimed to compare the *in vitro* antioxidant capacity of the different extracts (aqueous, methanolic and ethanolic) of *B. nitida* (Camwood) in order to determine the extract with the highest antioxidant potential. Thus, this will give an insight on the extracts that dissolve most of the active phytochemicals responsible for the antioxidant capacity of this plant. This could be useful in exploring the applications of *B. nitida* leaves as a natural source of antioxidants.

Materials and Method

Collection of Plant Material

The leaves of *B. nitida* were collected from different locations within Bayero University, Kano, Nigeria (Old Campus) on 17th September 2015. It was identified at Herbarium Unit, Department of Plant Biology, Faculty of Science, Bayero University, Kano with a voucher number "BUKHAN 0051."

Sample Preparation

The fresh leaves were shade dried in the Department of Biochemistry, Bayero University Kano and then ground into powdered form using mortar and pestle. The powdered form of the leaf was stored in a clean and dry container. The leaf powder (300g) was divided into three equal parts of 100g each. Each portion of the portion of the powder (100g) was soaked in 500 ml of distilled water, methanol and ethanol respectively. The mixtures were left to stand for 48 hours and then filtered using Whatman No. 1 filter paper. The aqueous filtrate was evaporated at 45°C using a water bath while the alcoholic filtrates (methanolic and ethanolic) were evaporated using the rotary extractor at 45°C. The extracts were all weighed and 100µg/ml was prepared for each extract using deionised water as solvent.

Estimation of Parameters

The antioxidant capacity of the different leave extracts of *B. Nitida* was determined using four different methods, which are ferric ions (Fe^{3+}) reducing antioxidant power assay (FRAP) developed by Oyaizu[14] with slight modification by Gulcin[15], hydrogen peroxide scavenging activity by Ruch *et al.*,[16], evaluation of antioxidant capacity by phosphomolybdenum method of Prieto *et al*.,[17] and determination of total phenols content using Folin–Ciocalteu reagent developed by Singelton *et al.,*.[18] All analyses were carried out in triplicate and the data was statistically analysed by one-way ANOVA with Tukey multiple comparison post-test using GraphPad InStat® software (2000), version 3.05 (32 bit for Win 95/NT) by GraphPad Inc.

Results and Discussion

The results for *in vitro* FRAP and HSPA analyses of the different leaf extracts (aqueous, methanol and ethanol) of the *B. nitida* (Camwood) leaves are presented in Figure 1. The result for TAC analysis of the same three leaf extracts of the same plant is presented in Figure 2 while the result of TPC determination is presented in Figure3.

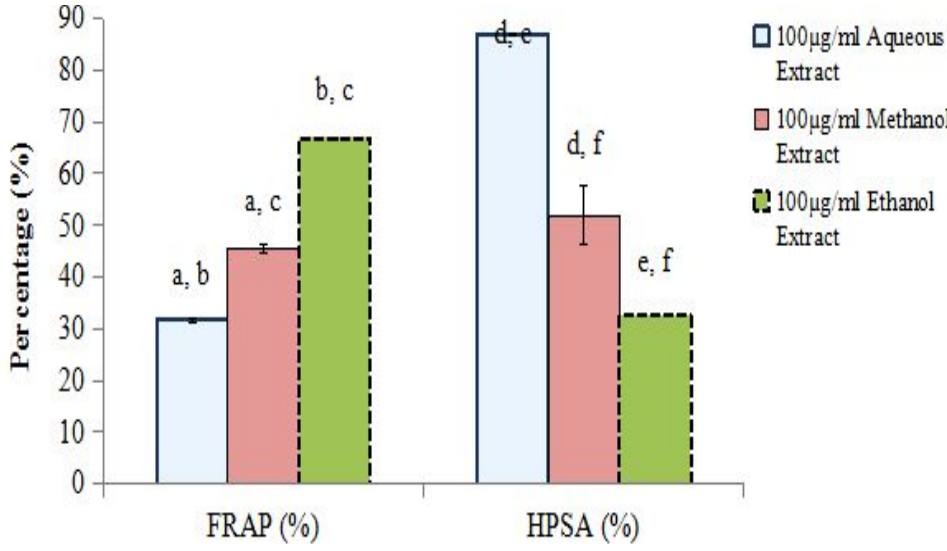

Figure 1: Ferric ions (Fe^{3+}) reducing antioxidant power assay (FRAP) and hydrogen peroxide scavenging activity (HPSA) of the different leaf extracts (aqueous, Methanolic and ethanolic) of the *B. nitida* (Camwood)

Values are presented as mean ± standard deviation, *n* = 3. Bars bearing same alphabets are significantly different at p<0.05 when compared to each other.

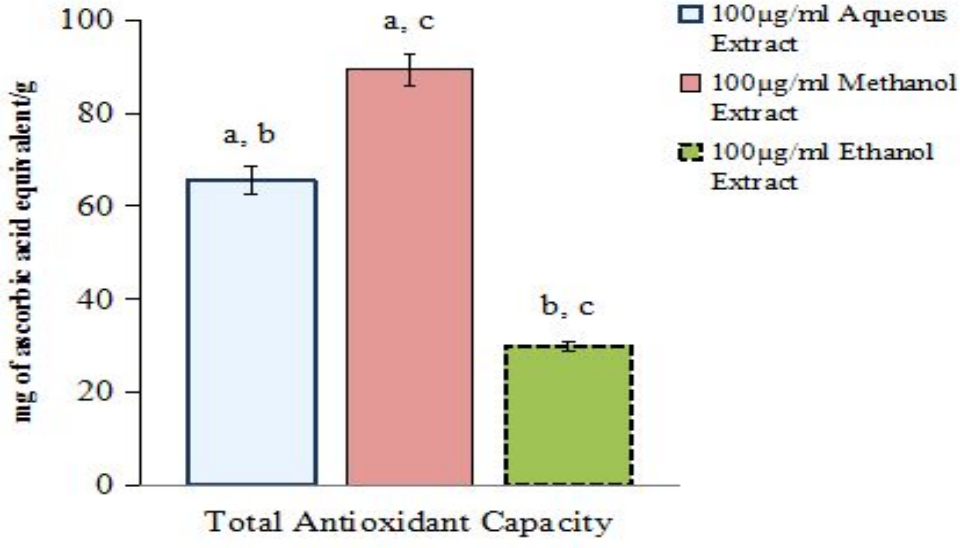

Figure 2: Total Antioxidant Capacity (TAC) of the different leaf extracts (aqueous, methanolic and ethanolic) of the *B. nitida* (Camwood)
Values are presented as mean ± standard deviation, *n* = 3. Bars bearing same alphabets are significantly different at p<0.05 when compared to each other.

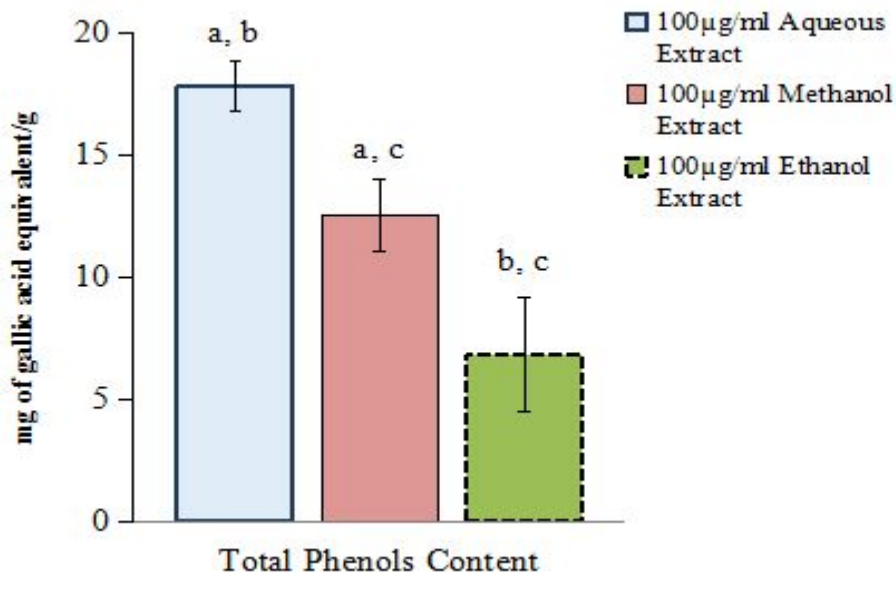

Figure 3: Total Phenolic Content (TPC) of the different leaf extracts (aqueous, methanolic and ethanolic) of the *B. nitida* (Camwood)
Values are presented as mean ± standard deviation, *n* = 3. Bars bearing same alphabets are significantly different at p<0.05 when compared to each other.

The results of hydrogen peroxide scavenging activity (HPSA) (Figure 1) and total phenolic content (TPC) (Figure 3) showed a similar pattern (at P<0.05) of aqueous extract> methanolic extract>ethanolic extract. The result of FRAP (Figure 1) showed that the alcoholic leaf extract shave the highest reducing capacity than the aqueous extract (significantly at p<0.05). While the result of total anti-oxidant capacity (TAC) (Figure 2) showed a pattern of anti-oxidation (significantly at P<0.05) of methanolic extract > aqueous extract > ethanolic extract. Therefore, the results (Figure 1-3) indicate that, all the different extracts have some anti-oxidant capacity depending on the type of phytochemical extracted by each solvent. This is because there are many different phytochemicals that are responsible for the antioxidant properties of any plants and their solubility depend on the specific properties of the solvent such as polarity. This finding is similar to the findings of Akande *et al*.,[13] who reported the dose dependent anti-oxidant activities of the leaf extracts of *B. Nitida*.

The pattern showed by HPSA and TPC results (Figure 1 and Figure 2, respectively) of the different leaf extracts of *B. nitida* agrees with the findings of Oyedemi *et al*,[19] which reported that the aqueous extract of *S. henningsii* contain high level of phenol content that might account for the strong activity observed against hydrogen peroxide (H_2O_2) radicals. This scavenging activity may be due to the presence of hydroxyl groups attached to the aromatic ring structures of phenolic compounds and thus help to quench the radicals.[20] On the other hand, the result of FRAP (Figure 1) showed that the alcoholic leaf extracts (ethanolic > methanolic) having higher reducing power than aqueous leaf extracts is probably due to high flavonoids content of the plant[21] which are readily soluble in alcoholic extracts[22] and are associated with pre-eminence reducing power capability.[23] This corresponds to the findings of Asha, *et al*[24] who reported that ethanolic leaf extract of *Euphorbia hirta* Linn had the highest reducing power than other extracts. The higher TAC observed in the methanolic leaf extract of the plant over that of the aqueous and methanolic leaf extracts in Figure 2, is similar to the findings of Nandhakumar and Indumathi[25] which demonstrated that (generally) methanol extracts are having higher antioxidant activity than the aqueous extracts. This is probably due to the higher presence of phenolic and flavonoids constituents soluble in the methanol extracts.[22] Hence, this study revealed that, it is the phytochemicals extracted or dissolved by a

particular type of solvent that determine the nature of how the antioxidants present in the plant can deal with free radicals.

Conclusion

In conclusion, the results of this study have shown that all the various leaf extracts of *B. nitida* have antioxidant property to scavenge free radicals which could be due to different mechanisms based on the phytochemicals extracted by each solvent. Therefore, this study provides evidence that the leaf extracts of *B. nitida* are potential sources of natural antioxidants and they may be good candidates for pharmaceutical plant-based products.

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