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Total Phenolic Contents and Antioxidant Activity of Nine Medicinal Plants used in Nigerian Traditional Medicine

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ABSTRACT

In Nigerian ethnobotany, several medicinal plants have been used for decades for the management of various ailments. Although several ethnobotanical studies have been conducted to document the most frequently used medicinal plants in the treatment of oxidative stress associated diseases, there is need to validate the therapeutic potentials of these plants. Therefore, this study was conducted to determine the radical scavenging ability as well as the total phenolic contents of nine medicinal plants used in Nigerian ethnobotany for the treatment of inflammation, diabetes and related medical conditions.

The methanol extracts of nine medicinal plants selected from Southwestern Nigeria ethnomedicinal plants were evaluated for their antioxidant activity using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay and their total phenolic content was determined using Folin-Ciocalteu reagent. All the extracts tested showed significant DPPH scavenging activity. Amongst the nine plants, *Bridelia ferruginea*, *Piper guineense* and *Nauclea diderrichii* had the highest antioxidant activity with IC_{50} of 11.46, 15.02 and 18.12 µg/mL, respectively, compared with the standard drugs; ascorbic acid (IC_{50} = 1.40 µg/mL) and gallic acid (IC_{50} = 0.79 µg/mL). The results of total phenolic content showed *N. diderrichii* with the highest phenolic content of 347.77 mg gallic acid equivalent per gramme of extract (GAE/g extract), while *Holarrhena floribunda G.Don.* (Apocynaceae) had the least phenolic content of 12.58 mg GAE/g extract. The result obtained from this study revealed that some of the plant extract exhibited free radical scavenging ability and could serve as candidates in the search for natural antioxidants.

Keywords: Antioxidant activity, *Bridelia ferruginea*, DPPH, Ethnomedicine, *Nauclea diderrichii*

Introduction

Humans are constantly faced with changing environmental conditions which often lead to the production of various free radicals which must be dealt with in order to ensure their survival.[1] Reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals and peroxyl radicals, are natural byproducts of the normal metabolism of oxygen in living cells and they play important roles in cell signaling. These ROS also play an integral function in the pathogenesis of many acute and chronic human diseases including atherosclerosis, ageing, cancer, diabetes, immunosuppression and stroke.[2] The human body is equipped with an inherent antioxidative mechanism with which the deleterious effects of the ROS are waded off. These antioxidants such as glutathione and superoxide dismutase help deactivate the damaging effects of free radicals, thereby rendering them incapable of attacking their targets in biological cells.[3, 4] The lack of balance between ROS and the inherent antioxidant potential of the body has necessitated the use of dietary and/or herbal supplements especially during the occurrence of diseases. Although several synthetic antioxidants including butylated hydroxyanisole and butylated hydroxytoluene are commercially available, they are quite expensive and there are major concerns about their safety.[5] Researches have revealed that many medicinal plants possess antioxidant activity which may offer protection against certain diseases.[6] Therefore, in recent years, considerable attention has been directed towards the identification of medicinal plants with antioxidant ability that may be beneficial for humans, as this will serve as a replacement to synthetic antioxidants, which are being restricted due to their side effects such as carcinogenicity.[7]

Natural antioxidants from plants, the most prominent representatives of which are phenols, tannins, and flavonoids, can protect the human body from free radicals and retard the progress of many chronic diseases.[8] For example, studies have shown that the utilisation of medicinal plants with high level of antioxidants may be an effective treatment option for hepatic damages.[9, 10] In addition, the morbidity and mortality associated with many degenerative disorders have been shown to have been alleviated by the consumption of natural antioxidants.[11] These natural antioxidants exhibit their activity by various mechanisms including the scavenging of free radicals, formation of complexes with pro-oxidant metal ions, quenching the formation of singlet-oxygen and inhibition of enzymes responsible for the generation of free radicals.[5] The high potency associated with natural antioxidants have stimulated the interest of scientists in the discovery of novel antioxidants from plant sources as more and more medicinal plants are currently being investigated for their antioxidant properties.

In Nigerian traditional medicine, various medicinal plants have been utilised for the treatment of several diseases, including inflammation, wounds, cancer and diabetes.[12] Although several ethnobotanical studies have been conducted to document the most frequently used medicinal

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plants in the treatment of oxidative stress associated diseases,[13-15] there is need to validate the therapeutic potentials of these plants, as this approach may lead to the discovery of effective and potent drug candidate. In view of this, this study was designed to investigate the DPPH radical scavenging ability as well as the total phenolic contents of nine extracts of selected medicinal plants used in Nigerian ethnomedicine in the management of inflammation, diabetes and related medical conditions.

Materials and Methods

Chemicals and Reagents
1,1-Diphenyl-2-picrylhydrazyl (DPPH) (Sigma Aldrich, USA), Folin-Ciocalteu reagent (Lobs chemie Laboratory, India), anhydrous sodium carbonate (Sigma Aldrich, USA), ascorbic acid (Sigma Aldrich, USA) and gallic acid (Sigma Aldrich, USA). All other chemicals and reagents were of analytical grade.

Plant collection and authentication
Nine medicinal plants identified from a previous ethnobotanical study in Oyo and Ondo State of South-West Nigeria in November 2013 were used in this study.[16] The plants were collected, identified and authenticated at the Forest Herbarium Ibadan (FHI) in Forestry Research Institute of Nigeria (FRIN), where voucher specimen numbers were assigned and deposited. The plant materials were air-dried for three weeks and milled into coarse powder.

Plant extraction
Plant materials (150-400 g) were extracted into methanol at room temperature (25 – 29°C) for 72 h. After the removal of methanol *in vacuo* using a rotary evaporator, percentage yields were calculated and plant extracts were stored in the refrigerator (4°C) until used.

1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay
The ability of the plant extract to scavenge DPPH free radicals was assessed following a method described earlier in the literature.[17] The stock solutions of the various plant extracts were prepared in methanol to achieve the concentration of 1 mg/mL. Serial dilutions were made with methanol to obtain concentrations of 500, 250, 125, 62.5, 31.25, 15.63 and 7.81 µg/mL. Gallic acid and ascorbic acid were used as positive controls at concentrations of 50, 25, 12.5, 6.25, 3.125, 1.563 and 0.781 µg/mL, while negative control contained all the reagents except the extract or standard drugs. The assay mixture contained 100 µL each of the extract or standard, 100 µL of methanol and 150 µL of freshly prepared DPPH solution (0.04 mg/mL; 2 mg in 50 mL of methanol). The mixture was incubated at room temperature in the dark for 30 min. The change in colour from deep violet to light yellow was measured spectrophotometrically at 517 nm using UV Spectrophotometer (Paul Baucher, SPECTRA max PLUX, Analytik and Biotechnologie, 4051 Basel, Germany). The ability of the extract to scavenge DPPH radical was calculated using the equation:

$$\% \text{ Inhibition } = \frac{\text{Mean OD control} - \text{Mean OD sample}}{\text{Mean OD Control}} \times 100$$

The experiment was conducted in triplicate and the IC_{50} values were determined as the concentration of the extracts that caused 50% DPPH radical inhibition. The lower the IC_{50} value, the higher the antioxidant activity of the extract.

Determination of total phenolic content
The concentration of phenolics in the plant extracts was determined using the Folin–Ciocalteu's method as described by Kaur and Kapoor (2002).[18] Briefly, the reaction mixture was prepared by vigorously mixing 0.5 mL solution of methanol extract (1 mg/mL) of each plant with 2.5 mL of 10% Folin-Ciocalteu's reagent for 5 min followed by the addition of 2.5 mL of 7.5% $NaHCO_3$. The blank solution which contained 0.5 mL methanol, 2.5 mL of 10% Folin-Ciocalteu's reagent and 2.5 mL of 7.5% of $NaHCO_3$ was concomitantly prepared. Thereafter, the reaction mixture was incubated at room temperature (25 – 29 °C) for 2 h. The absorbance of the mixture was then measured spectrophotometrically at 765 nm. The same procedure was repeated for the standard (gallic acid) at concentrations of 50, 40, 30, 20, 10, and 5 µg/mL, and the calibration curve was constructed. Based on the measured absorbance, the concentration of phenolics (mg/mL) was determined from the calibration curve and the phenolic content expressed in terms of gallic acid equivalent (mg of GA/g of extract) in 1 mg/mL. The experiment was carried out in triplicates.

Statistical analysis
All experiments were carried out in triplicate and were expressed as mean ± standard error of means (SEM). IC_{50} values were determined by nonlinear regression using the GraphPad prism software version 5.0® (GraphPad Inc, San Diego, CA, USA). The dose-response curve was obtained by plotting the percentage of inhibition versus the concentration.

Results and Discussion

In this study, we have determined the phenolic composition and the DPPH radical scavenging activities of nine medicinal plants used in the Nigerian ethnobotany for the treatment of several diseases. Several medicinal plants used in Nigerian traditional medicine have been shown to possess the ability to protect the human body against cellular oxidation.[19-21] Medicinal plants contain numerous antioxidant components and several methods have been designed to estimate the antioxidant capacity of each compound separately and these include measuring the ability of the plant to scavenge free radicals, inhibit lipid peroxidation or chelate metals.[22]
Plants with antioxidant principles usually have the potential of scavenging the DPPH radical due to their hydrogen-donating ability. The DPPH radical is a free radical that accept an electron or hydrogen radical to form a stable diamagnetic molecule. The amount of a plant extract that is needed to reduce the concentration of DPPH by half (IC_{50}) in the DPPH assay is a measure of its antioxidant activity; a lower IC_{50} value corresponding to a higher antioxidant power.[17] The nine medicinal plants investigated in this study are presented in Table 1 and the result obtained from the DPPH radical scavenging assay of the plant extracts is shown in Table 2. The methanol crude extract of *Bridelia ferruginea* Benth. (Euphorbiaceae) had the highest DPPH scavenging, with an IC_{50} value of 11.46 µg/mL, followed by the methanol crude extract of *Piper guineense* Schumach. & Thonn (Piperaceae), with an IC_{50} value of 15.02 µg/mL, compared to ascorbic acid and gallic acid with IC_{50} values of 1.41 and 0.71 µg/mL, respectively (Table 2). Amongst the plant extracts, *Calyptrochilum christyanum* (Rchb.f.) Summerh (Orchidaceae) displayed the least DPPH scavenging activity. The methanol crude extracts of *Nauclea diderrichii* (De Wild.) Merr. (Rubiaceae), *Bridelia ferruginea* and *Calyptrochilum christyanum* had high phenolic contents with values of 347.77, 304.76 and 153.38 mg/g gallic acid equivalent in 1 g of the extract, respectively, as compared to *Holarrhena floribunda* G. Don. (Apocynaceae) with the least phenolic content of 12.58 mg/g gallic acid equivalent in 1 g of the extract (Table 2). Interestingly, the extracts of *Bridelia ferruginea* and *Nauclea diderrichii* that displayed significant DPPH scavenging activity also had a higher quantity of phenolics contents which showed linear correlation in their antioxidant abilities. Phytochemicals especially polyphenols and flavonoids constitute a major group of compounds that acts as primary antioxidants by scavenging free radicals.[23]
Bridelia ferruginea has been used in Nigerian ethnomedicine for the management of several ailments including diarrhea, female sterility, rheumatic pain, intestinal disorders, piles, rheumatic pain and various skin diseases.[24, 25] Previous phytochemical investigation revealed that the methanol extract of the plant contains alkaloids, tannins, saponins, flavonoids and anthraquinones.[26] An earlier study reported the antioxidant potential of *B. ferruginea* and suggested that its high phenolic content might be responsible for this effect.[27] In fact, several active flavonoids with antioxidant property including rutin, myricetin, isomyricetin and isoquercetin have been isolated from the dried leaves of *B. ferruginea*.[28] These secondary metabolites present in *B. ferruginea* could have been responsible for the significant DPPH radical scavenging activity observed in this study.
Piper guineense, a climber that resides commonly in the tropical rain forest, is commonly called *'Iyere'* by the Yoruba speaking people of southwestern Nigeria. It is used in Nigerian ethnomedicine for several purposes including culinary, medicinal, cosmetic and insecticidal applications.[29] It is widely used in local practice for the management of

Table 1: Plant species analysed for antioxidant properties in this study.

Plant	Family	Local name	Part used	FHI No	% Yield
Acanthospermum hispidum D. C.	Asteraceae	Dagunro gogoro	Aerial part	110050	6.35
Alchornea laxiflora (Benth.) Pax & K. Hoffm.	Euphorbiaceae	Pepe	Leaf	110155	13.44
Bridelia ferruginea Benth.	Euphorbiaceae	Irasa	Stem bark	109985	4.8
Calyptrochilum christyanum (Rchb.f.) Summerh	Orchidaceae	Ela	Whole plant	110054	2.17
Heliotropium indicum L.	Boraginaceae	Apari igun	Aerial part	110156	5.66
Holarrhena floribunda G. Don.	Apocynaceae	Dagba	Leaf	110053	3.46
Ipomoea asarifolia (Desr.) Roem. & Schult.	Convolvulaceae	Gboro ayaba	Leaf	110052	6.72
Nauclea diderrichii (De Wild.) Merr.	Rubiaceae	Opepe	Stem bark	110049	2.75
Piper guineense Schumach. & Thonn.	Piperaceae	Iyere	Leaf	110051	6.43

rheumatism, bronchitis, gonorrhea, infertility and mental illness.[30] A recent study revealed that *P. guineense* was among the frequently used herbal recipe for the management of cancer and inflammatory diseases by traditional healers.[31] A recent report demonstrated the antioxidant potential of the seeds of *P. guineense* and also showed that the methanol extract of the seed significantly altered the levels of several liver enzymes including alanine transaminase, aspartate transaminase and alkaline phosphatase, indicating that the extract may possess antioxidant protective roles.[32]

Nauclea diderrichii is a savannah tree that is widely used in furniture, arts and building industries in several parts of Nigeria.[33] It is used in traditional folklore medicine for the treatment of anaemia, diabetes, stomach-ache, inflammation, jaundice, measles and wounds [34]. A recent study reported that the butanol fraction of *N. diderrichii* displayed a better α-amylase inhibitory activity (IC_{50} = 137.8 µg/mL) than the standard α-amylase inhibitor, acarbose (IC_{50} = 177.5 µg/mL).[16] A previous study revealed that *N. diderrichii* displayed strong antioxidant property which was attributed to its high phenolic and flavonoid contents; a finding supported further in this work. Thin layer chromatography (TLC) autobiography of *N. diderrichii* methanol extract revealed that it contained catechin, quercetin and kaempferol [35]. Since these flavonoids have been shown to possess strong antioxidant properties, they may be responsible for the antioxidant activity observed in this study.

Table 2: DPPH free radical scavenging (RSA) activity extracts, expressed as IC_{50} values (µg/mL), and the total phenolic contents, (TPC) of the investigated plant extracts.

Plant Extract	DPPH RSA (IC_{50} in µg/mL)	TPC (mg GAE/g extract)
Acanthospermum hispidum	28.93 ± 0.39	29.46 ± 0.18
*Alchornea laxiflor*a	33.98 ± 0.34	39.96 ± 2.42
Bridelia ferruginea	11.46 ± 0.11	304.77 ± 0.66
Calyptrochilum christyanum	50.49 ± 0.62	153.39 ± 3.54
*Heliotropium indicu*m	48.41 ± 0.41	27.82 ± 0.94
Holarrhena floribunda	40.66 ± 0.53	12.58 ± 0.29
Ipomoea asarifolia	24.27 ± 0.37	24.74 ± 3.33
Nauclea diderrichii	18.12 ± 0.59	347.77 ± 2.34
Piper guineense	15.02 ± 0.28	16.18 ± 0.73
Gallic acid	0.79 ± 0.21	-
Ascorbic acid	1.41 ± 0.34	-

Values expressed as mean ± SEM.

Conclusion

This study has revealed that all the investigated plant extracts had noticeable antioxidant effects. In particular, the extract of *Bridelia ferruginea* and *Nauclea diderrichii* displayed remarkable DPPH radical scavenging activity and have high phenolic contents. Our results suggest these extracts are potential sources of plant-derived antioxidant compounds.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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