Antioxidant content and \textit{in vitro} 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity of selected medicinal plants

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\textbf{ABSTRACT}

\textbf{Background/Aim:} The medicinal plants and their derivatives have long been recognized as important sources of antioxidants in the prevention and treatment of various diseases. This study investigated phytochemicals, antioxidant content, and \textit{in vitro} 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activities of methanolic and aqueous leaves extracts of \textit{Anogeissus leiocarpus}, \textit{Ipomoea asarifolia}, \textit{Bauhinia rufescens}, \textit{Guiera senegalensis}, and \textit{Moringa oleifera}.

\textbf{Materials and Methods:} The extracts were subjected to qualitative phytochemical analysis to identify the bioactive constituents in each plant. Total phenolics and proanthocyanidin contents were determined using Folin–Ciocalteu and vanillin-methanol assays while antioxidant free scavenging activity was estimated using DPPH assay.

\textbf{Results:} The phytochemical screening revealed the presence of alkaloids, flavonoids, and tannins in all the five plants investigated. The total phenolic and proanthocyanidin contents of methanolic extracts were significantly higher ($p < 0.05$) as compared with aqueous extracts. DPPH-free radical scavenging activity of methanolic extracts of \textit{A. leiocarpus} and \textit{M. oleifera} was similar to vitamin C. \textit{In vitro} DPPH radical scavenging activity of methanolic extracts of \textit{A. leiocarpus} and \textit{M. oleifera} were better as compared with \textit{B. refescens}, \textit{I. asarifolia}, \textit{G. senegalensis}, and aqueous extracts. The antioxidant activities of both extracts were in a dose-dependent manner. Furthermore, the lower IC\textsubscript{50} of methanolic extracts of \textit{A. leiocarpus}, \textit{B. refescens}, and \textit{M. oleifera} correlated with strong scavenging activities of these plants.

\textbf{Conclusion:} This study demonstrated the antioxidant content and DPPH-free radical scavenging activities of five medicinal plants. Thus, the study underscored these plants as potential sources of natural antioxidants that can be explored for the treatment of oxidant-related diseases.

\textbf{ARTICLE HISTORY}
Received September 26, 2017
Accepted December 11, 2017
Published January 06, 2018

\textbf{KEYWORDS}
Antioxidants; DPPH; free radicals; medicinal plants; phytochemicals

\textbf{Introduction}

Medicinal plants contain bioactive ingredients that have potential therapeutic effects against various diseases. Many bioactive compounds with antioxidant activities in plants have been suggested to play a protective role against oxidative stress-related diseases [1,2]. The consumption of these plants has been reported to lower the risk of atherosclerosis, cancer, hypertension, stroke, and hepatic diseases [3–6]. The antioxidant activities of medicinal plants have been attributed to the presence of polyphenols such as flavonoids, phenolic acids, tannins, anthocyanin [5–7], and β-carotene, vitamins C and E [7] which has the ability to scavenge free radicals that are generated in the living system. Oxidative stress occurs when there is an imbalance between oxidant and antioxidant molecules in favor of the oxidant which results in excess production of reactive oxygen species (ROS). The ROS generated are capable of destroying the internal redox balance that may cause tissue damage or premature aging [8–11]. The medicinal plants also serve as a

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source of supplement or functional foods that can safeguard the health of the individual. Several natural antioxidants have been isolated from various plant sources such as cereals, algae, leafy vegetables, fruits, and seeds [12]. The free radical scavenging activities of antioxidant polyphenols in plants and plant products have been attributed to their ability to donate protons to ROS. Phytochemicals such as flavonoids, essential oils, and anthocyanin have received attention as sources of natural antioxidant in health promotion as well as cosmetics because they are safer than synthetic antioxidant [13]. The use of herbs have less adverse effects as they are commonly direct toward aiding the body's own healing process rather than addressing symptoms caused by specific diseases as in the case of synthetic drugs [14].

*Anogeissus leiocarpus* belongs to the family Combretaceae and is widely used in African traditional medicine for the treatment of various diseases [15]. In Nigeria, rural populations used it as a chewing stick for oral hygiene. The plant has been shown to possess antibacterial activity [15]. The leaves and stem bark of the plant have also been reported for the treatment of jaundice, cough, and fever [16]. *Ipomoea asarifolia* is a hairless, succulent perennial weed of the family, Convolvulaceae. The plant has been reported to have anti-inflammatory activity by decreasing the levels of interleukin 1β, interleukin 6, and tumor necrosis factor α in a murine model of peritonitis [17]. *Bauhinia rufescens* is a shrub that belongs to the family Fabaceae. The plant has been shown to possess therapeutic effects against fibrosis, dysentery, and jaundice [18]. *Guiera Senegalensis* is a semi-evergreen shrub that can grow up to 3 m high and belongs to the family Combretaceae. The leaves have a high reputation as a “cure-all” in Africa, where those are taken in decoctions or mixed with foods for the treatment of a wide range of disease conditions [19]. It is known as being active against diseases such as cough, fever, diarrhea, dysentery, rheumatism, leprosy, and is given to women to promote the flow of milk after childbirth [19]. The plant has also been reported to possess hypoglycaemic effect in type 2 diabetic patients [20]. *Moringa oleifera* is a member of Moringaceae. The leaves contain nutrients, especially essential amino acids, vitamins, and β-carotene [21]. Apart from nutritional benefits, the hypoglycemic and hypolipidemic effects of leaf extract of *M. oleifera* have been reported [22]. The leaf extract has also been shown to enhance hepatic glutathione restoration [23]. Ethnobotanical surveys indicated that stem bark, leaves, and root extracts of these medicinal plants have been used for the treatments of various diseases in Nigeria [16,24–26].

Therefore, this study was designed to compare the antioxidant contents and DPPH free scavenging activities of aqueous and methanolic extracts of *A. leiocarpus*, *I. asarifolia*, *B. rufescens*, *G. senegalensis*, and *M. oleifera* used in the treatment of various diseases.

**Materials and Methods**

**Plant materials**

All the plants except *M. oleifera* were collected from Arkila area of Sokoto, Nigeria. Fresh *M. oleifera* was bought from Sokoto central market, Nigeria. The leaves of all the five plants were collected in October 2015 and air dried. The plants were identified and authenticated at the Botany Unit, Department of Biological Science, Usmanu Danfodiyo University, Sokoto, Nigeria. The voucher specimens were deposited at the herbarium of the same institution with the following voucher numbers: *M. oleifera* (UDUH/ANS/0225), *A. leiocarpus* (UDUH/ANS/0180), *I. asarifolia* (UDUS/ANS/0140), *B. rufescens* (UDUH/ANS/0210), and *G. senegalensis* (UDUH/ANS/0144).

**Preparation of plant extracts**

Total of 10 g of each of the plant leaves was washed with water, air-dried, and ground to a powder with mortar and pestle. The powdered leaves were exhaustively extracted with water and methanol separately at room temperature for 48 h with stirring at intervals and later filtered with a muslin cloth. The aqueous and methanolic extracts obtained were concentrated to dryness at 40°C, using a rotary evaporator under reduced pressure. The dried extracts were weighed and percentage yield (data not provided) of all the extracts calculated and recorded, then stored for subsequent analysis.

**Phytochemical screening**

Methanolic and aqueous extracts of each of the plants were subjected to qualitative phytochemical analysis to identify the bioactive constituents of each plant.

**Test for alkaloids**

Total of 2 ml of test solution was mixed with 2N HCl. The aqueous layer formed was decanted and few drops of Mayer’s reagent were added. The formation of cream colored precipitate indicates the presence of alkaloids.
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Test for saponins

The leaf extract (0.5 g) was stirred with 10 ml of distilled water in a test tube. The formation of frothing which persists on warming in a water bath for 5 minutes indicates the presence of saponins.

Test for tannins

The leaf extract (0.5 g) was stirred with 10 ml distilled water and then filtered. Then ferric chloride solution (5%) was added drop by drop to the extract and the colored produced was noted. The presence of tannins was observed by the formation of dark-green color.

Test for cardiac glycosides

A quantity (2.5 ml) of 50% $H_2SO_4$ was added to 5 ml of the extract in a test tube. The mixture was heated in a water bath for 15 minutes, cooled, and neutralized with 10% NaOH. Then 5 ml of Fehling solution was added and the mixture was boiled. A brick-red precipitate indicates the presence of cardiac glycosides.

Test for flavonoids

A few (three) drops of 1% ammonia solution were added to 10 ml of the plant extracts in a test tube. A yellow coloration observed indicates the presence of flavonoids.

Determination of total phenolic contents

The Folin–Ciocalteau reagent method was employed for the estimation of total phenolic contents of each of the extracts according to Lister and Wilson [27]. A solution of crude extracts of the plants was prepared and then 100 µl of the supernatant was taken from each prepared extract and mixed with 0.5 ml (1/10 dilution) of Folin–Ciocalteau reagent. Then the mixture was incubated at room temperature for 1 minute. Thereafter, 1.5 ml of 2% $Na_2CO_3$ (w/v) solution was added. The final mixture was shaken and incubated in the dark at room temperature for 1.5 hours. The absorbance of the sample was measured at 765 nm using spectrophotometer. Standard calibration curve for gallic acid was prepared in the range of 20–100 µg/ml in the same manner. The results were expressed as milligram gallic acid equivalent per gram of extract.

Determination of proanthocyanidin contents

The total proanthocyanidin of the extracts was determined using the procedure reported by Asowata-Ayodele et al. [28]. A volume of 0.5 ml of 0.1 mg/ml of extract solution was mixed with 3.0 ml of 4% vanillin-methanol solution and 1.5 ml of hydrochloric acid and then vortexed. The mixture was allowed to stand for 15 minutes at room temperature, followed by the measurement of the absorbance of the extract at 500 nm. Total proanthocyanidin contents were expressed as catechin (mg/g) from the standard curve.

Determination of DPPH radical scavenging activity

The DPPH-free radical scavenging activity of both aqueous and methanolic extracts was determined as described by Chew et al. [29] with slight modification. Total of 1 ml of diluted extracts (20, 40, 60, 80, and 100 µg/ml in ethanol) was added to 1 ml of DPPH (0.15 mm in methanol) and control consisting of 1 ml each of DPPH and ethanol. The reaction mixture was mixed thoroughly and then incubated in the dark at room temperature for 30 minutes and the absorbance was measured at 517 nm by a spectrophotometer. The ascorbic acid was used as a positive control while ethanol was used as a blank. The DPPH scavenging ability of the plant extracts was calculated using the following equation:

\[
\text{% scavenging activity} = \frac{AC - AS}{AC} \cdot 100
\]

where AC is absorbance of control (DPPH + ethanol) and AS is absorbance of sample/extract.

Data Analysis

The data are expressed as mean ± standard deviation ($n = 3$). Curve Expert (version 1.4) was used for the determination of IC$_{50}$. Microsoft Excel 2010 was used for bar chart and line graphs. Statistical package for the social sciences (version 15) was used for boxplot. Kruskal Wallis test followed by Dunn’s Post hoc test was used for the comparison between multiple groups while unpaired t-test was applied for comparison between two independent groups using GraphPad InStat (version 3). $p < 0.05$ was considered to be statistically significant.

Results

The phytochemical screening of plant extracts (Table 1) indicates the presence of alkaloids, flavonoids, and tannins in both aqueous and methanolic extracts of all the five plants. However, saponins were not detected in A. leiocarpus, B. refescens, and I. asarifolia in both aqueous and methanolic extracts. Cardiac glycoside was below detection limit in I. asarifolia and M. oleifera of both extracts.
Figures 1 and 2 show the result of the total phenolic and proanthocyanidin contents of aqueous and methanolic extracts. The result indicated that total phenolic contents of methanolic extracts were significantly ($p < 0.05$) higher than the corresponding aqueous extracts. The total phenolic content of aqueous extract of *B. refescens* was significantly ($p < 0.05$) higher as compared with *G. senegalensis*. Furthermore, the total phenolic content of methanolic extract of *A. leiocarpus* was significantly ($p < 0.05$) higher as compared with *G. senegalensis*. The proanthocyanidin content of aqueous extract of all the five plants were significantly ($p < 0.05$) lower as compared with their corresponding methanolic extract. Proanthocyanidin content of methanolic extract of *B. refescens* was significantly ($p < 0.05$) higher as compared with *G. senegalensis*. There was no significant difference in the proanthocyanidin content of aqueous extracts of the five medicinal plants.

The mean percentage inhibitions of aqueous and methanolic extracts against DPPH are presented in Figures 3 and 4, respectively. Figures 5 and 6 showed the results of half maximal inhibitory concentration ($IC_{50}$) of aqueous and methanolic extracts of the five medicinal plants.
Antioxidants in medicinal plants. The mean percentage inhibition of methanolic extract of *A. leiocarpus* and *M. oleifera* was comparable to vitamin C and had shown better inhibition of DPPH as compared with *B. refescens*, *G. senegalensis*, and *I. asarifolia*. Also, the methanolic extracts had shown better free radical scavenging activity against DPPH than the corresponding aqueous extracts. The mean percentage inhibitions of these medicinal plants were in dose-dependent manner. The IC$_{50}$ of aqueous and methanolic extracts of the medicinal plants were higher as compared to that of vitamin C, but the methanolic extract of *A. leiocarpus*, *B. refescens*, and *M. oleifera* has shown comparable results.

**Discussion**

Phytochemicals are natural bioactive compounds in plants that have been recognized for their biological role as antioxidants that are capable of scavenging free radicals associated with oxidative assault [30,31]. These phytochemicals are compounds such as flavonoids, tannins, polyphenols, proanthocyanidins, and alkaloids that are considered important in the prevention and treatment of chronic diseases caused by oxidative stress [32]. In this study, phytochemical screening, antioxidant content, and *in vitro* DPPH-free radical scavenging activity of aqueous and methanolic extracts of *A. leiocarpus*, *B. refescens*, *G. senegalensis*, *I. asarifolia*, and *M. oleifera* were investigated. The preliminary phytochemical screening revealed the presence of flavonoids, alkaloids, and tannins in all the five medicinal plants while cardiac glycosides and saponins were below detection limit in some of the plants. The results indicated that these plants are rich sources of various natural antioxidants that can be isolated for the treatment of oxidative stress-related diseases. Cardiac glycosides were below detection limit in both aqueous and methanolic extracts of *I. asarifolia* in this study which corroborated the findings of Jegede et al. [33]. On the contrary, flavonoids were detected by this study in both methanolic and aqueous extracts of *I. asarifolia* which was not detected in their study. These variations may be attributed to the differences in the season or the location where the plant was obtained. Phenolic compounds are capable of acting as reducing agents, donors of hydrogen, metal ion chelators, or quenchers of singlet oxygen which can be attributed to their redox potentials [34].

The result showed a considerable amount of total phenolics and proanthocyanidins in the methanolic extract as compared with the aqueous extract.
This indicates that solvent system has significant influence on the extraction of bioactive components from medicinal plants. Studies have revealed that total phenolic and flavonoid contents of methanolic extract of *P. californicum* [35], *M. oleifera* [36], and antioxidant activity of buckwheat methanolic extract [37] was higher than other solvent systems. The highest amount of total phenolics was observed in methanolic extract of *A. leiocarpus* and *B. refescens* while methanolic extract of *B. refescens* and *M. oleifera* demonstrated the highest amount of proanthocyanidins as compared with the rest of...
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Therefore, it is evident from this study that solvent system plays a critical role in the extraction of antioxidant bioactive components from plants and as such for effective extraction and isolation of antioxidants, it is important to carefully select the solvent system as well as extraction method to achieve better results.

DPPH is a stable nitrogen-containing free radical that produces deep purple color in methanol solution. The assay is based on the reduction of purple colored DPPH in the solution of methanol

Figure 5. Mean IC$_{50}$ of aqueous extract of medicinal plants. Values are mean ± SD, n = 3, AqE = aqueous extract.

Figure 6. Mean IC$_{50}$ of methanolic extract of medicinal plants. Values are mean ± SD, n = 3, MeOHE = methanolic extract.
to form yellow colored, diphenylpricyl hydrazine in the presence of hydrogen donating antioxidants. The decrease in absorbance is proportional to the antioxidant activity of the plant extract. This study shows that the antioxidant free radical scavenging activities of the extracts were in dose-dependent manner. The highest inhibition of DPPH corresponds with lower IC₅₀. This shows that methanolic extract of A. leiocarpus, B. refescens, and M. oleifera with the highest reduction of DPPH and lower IC₅₀ performed better than the methanolic of G. senegalensis and I. asarifolia and the corresponding aqueous extract of all the medicinal plants. The high antioxidant activity of methanolic extract of these three plants further buttresses the results of total phenolic and proanthocyanidins. This study provided the evidence of DPPH-free radical scavenging activity of these plants and could be a reflection of the total activities of various components rather than individual component.

**Conclusion**

This present study demonstrated various antioxidant contents and DPPH free radical scavenging activity of five medicinal plants used traditionally in the treatment of different ailments in Nigeria. The extracting solvents significantly influence the antioxidant contents and DPPH-free radical scavenging activity of these plants. Methanolic extracts have shown better DPPH-free radical scavenging activity than aqueous extracts. The results of this study indicated the potential of these plants as natural sources of antioxidants. Further studies are needed to possible isolate and characterize these bioactive components with a suitable solvent system for the treatment of free radical-induced diseases.

**Conflict of Interest**

The authors declare no potential conflict of interest.

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