Original Article:

Effects of Methanol Extract on Bioactive Property and *in Vitro* Antioxidant Activity of Palma Christi (*Anthocleista nobilis* G. Don.) Root

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ABSTRACT

Background: Plants have diverse phytochemicals with different solubility levels and medicinal efficacy. This study aimed to determine the presence of phytochemical constituents and *in vitro* antioxidant activity of methanol root extract of *Anthocleista nobilis*.

Objectives: Determining the bioactive principles and free radical scavenging properties of *A. nobilis* root.

Methods: The preliminary phytochemical investigations were performed using standard analytical procedures. The *in vitro* antioxidant properties were assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, nitric oxide (NO) scavenging activity, ferric reducing antioxidant power (FRAP) assay, total antioxidant capacity (TAC), and hydrogen peroxide (H_2O_2) scavenging activity.

Results: The phytochemical analyses revealed the presence of several compounds: saponins, reducing sugar, alkaloids, tannins, flavonoids, cardiac glycosides, and mucilages. The concentrations of these compounds were different. A considerable quantity of phytochemicals was found in the methanol extract. The impact of the extract on DPPH, NO, FRAP, TAC, H_2O_2 radicals were dependently and ascendingly concentrated. Solvent extract demonstrated better antioxidant activity, with DPPH and NO showing maximum antioxidant capability in conjunction with IC50 values of 5.45 µg/mL.

Conclusion: The study results prove that *A. nobilis* is a possible source of natural antioxidants, justifying its use in indigenous medicine.

1. Introduction

Humans have used plants to treat various infectious and non-infectious diseases since the dawn of time. The plants also were the primary source for many orthodox medicines [1]. They have long been used as a source of food and drugs. Not only do they function as high-nutrient vegetables, but their different parts (root, leaf, and fruit) are also used for remedial health purposes. The

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beneficial effects of plant products can be due to their phytochemical and antioxidant elements, such as phenolic compounds, vitamins, flavonoids, and saponins [2].

Recently, there has been a growing interest in natural antioxidants. These chemicals reduce the harmful effects of free radicals on the human body. The free radical scavenging properties of natural antioxidants are used in various medical applications because of their efficacy and protection [3]. Consumption of fresh fruits and vegetables rich in plant polyphenols (antioxidants) as food has been documented to protect against several diseases, including cancer, cardiovascular diseases, diabetes, asthma, etc. [4], suggesting that the mechanism of action of the secondary metabolites can be traced back to their antioxidant properties.

Wild plants are a potential source of these phytochemicals and antioxidants that play a vital role in promoting health, especially in rural communities with better access to these plants. These biomolecules have beneficial effects, such as antioxidant, anti-inflammatory, antimicrobial, and anticancer [5], and as such, hold therapeutic applications in the formulation of valuable drugs. Medicinal plants tend to be rich in secondary metabolites, commonly used in conventional medicines to fight and cure various diseases, thus increasing their demands. Traditional medicine plays a crucial role among rural communities in developing countries. This therapy provides health care in the absence of an efficient primary health care system. Traditional medicine depends on the diversity of plant species and related knowledge of their use as herbal medicines [5].

The use of plants in preventive, management, and curative measures of different ailments lies in the presence of bioactive phytochemical constituents found in plant's parts (root, stem, twig, leaf, flower, and seed). These chemicals include quinones, tannins, saponins, alkaloids, flavonoids, triterpenes, and phenolic derivatives. Some of these secondary metabolites are synthesized by plants for specific purposes. Others may be by-products of plant metabolism that do not have a known biological function. The study of these compounds has demonstrated their role in many biological activities. For example, tannins are high molecular weight molecules; they are found in many plants and have various properties. They are donors of protons or lipid-free radicals formed during peroxidation and have been documented to inhibit the chain reaction of lipid auto-oxidation [6]. One of the key roles of these phenolic compounds is to protect the plants against herbivores [7]. They act as antihemorrhagic agents and combat infections.

Flavonoids represent a very large variety of natural compounds belonging to the polyphenol family. Currently, flavonoids have shown remarkable pharmacological effects, such as antiviral, antimicrobial, and anticancer activities [8]. Many plants used in traditional medicines contain saponins that can also account for their medicinal function, such as insecticides, antibiotics, and fungicides [9]. Alkaloids are known for their role as anti-inflammatory, anti-nociceptive, and antipyretic properties [10]. Interestingly, phenolic and polyphenolic compounds have been implicated in the literature to exhibit antioxidant properties.

Antioxidants are molecules that stop oxidation processes. The antioxidant activity of phenolic compounds is derived from the ability to act as a reducing agent, i.e., donating hydrogen, electrons and stabilizing reactive oxygen species [11]. Reactive oxygen species (ROS), like oxygen singlet, hydroxyl ions, superoxide ions, hydrogen peroxide, etc., are potent reactive and toxic molecules generated routinely in all cells during metabolism [12]. The ROS cause severe oxidative injury to proteins, enzymes, lipids, and DNA strands through peroxidation and covalent bonding, leading to tissue injury [13]. A free radical is characterized as an atom or molecule with unpaired electrons; it is associated with different harmful conditions, such as inflammation, cancer, and neurodegeneration, hence justifying numerous studies on the impacts of antioxidants in preventing and treating various disease processes [14]. The formation of free radicals and damage to healthy cells can be prevented by inhibiting the oxidation process (i.e., through the use of antioxidants), thereby treating and controlling chronic illnesses, such as cardiovascular disease, diabetes, obesity, and certain types of cancer [15]. An antioxidant molecule works by slowing or discontinuing the effect of oxidation of other molecules, unlike one that involves electron transfers from one oxidizing agent material [13]. Oxidation reactions create free radicals that cause chain reactions leading to cellular damages [12]. An antioxidant acts by ending these chain reactions, eliminating free radical intermediates, and stopping oxidants from oxidizing beneficial molecules [14].

2.2-Diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and 2.2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) methods could be used to assess antioxidant function in many plant extracts [16]. Natural and artificial antioxidants, such as butylated hydroxyanisole, butylated hydroxyl toluene, and tetra-butylhydro-quine, also scavenge free radicals [17]. However, synthetic/artificial antioxidants are not favored because of concerns over their safety,

structural instability, and carcinogenicity [17]. In general, synthetic antioxidants are harmful to human health [18]. In contrast, natural antioxidants, such as phytochemicals, are safer alternatives than synthetic ones since they offer various therapeutic properties and are devoid of known side effects [18].

A. nobilis (G. Don.) is a member of the Loganiaceae family. It is a small to medium-sized tree that grows up to 30 m. It is native to tropical African habitats, such as the Mascarene Islands and Madagascar, and is seen in the southern, western, and eastern parts of Nigeria. It is also a ubiquitous flora found densely grown in the forest, farmland, swampy land, and roadsides in tropical rainforests. The bark is smooth and pale gray. The inside bark is cream-yellow and granular, while the twig has two spines above the leaf axis. Leaves are simple, broad and opposite, crowded at the end of the branches, and the petiole is 1.0 to 6.0 cm long. It is a photoautotroph plant. It displays the root tap system, and the root can be erect, bent, or curved. It is commonly referred to as candelabrum, cabbage tree, cabbage palm, or palma christi in English. It is also known locally as Uko nkirisi in the Igbo language, Apa-Ora in the Yoruba language, Kwari in the Hausa language, Ogugu in the Ilaje language, and Duwa kuchi in the Nupe language [19]. Traditionally, *A. nobilis* is used to treat fever, stomach ache, diarrhea, and gonorrhea. It is also used as a potent purgative, diuretic, and poultice to treat sores in parts of West Africa [20]. It is used as a steam bath to treat leprosy, venereal diseases, and dysmenorrheal. Its root decoction is usually used to regulate menstruation and as an abortifacient. In the Mbano group in Imo State, Nigeria, root bark decoctions are primarily used to treat diabetes mellitus, gastrointestinal worms, malaria, and jaundice [20]. In contrast, in the Okitipupa division area of Ondo State, Nigeria, a root tincture is used as an antioxidant to treat arthritis.

A. nobilis is a potent natural antioxidant and anti-inflammatory cure in folkloric medicine, emphasizing the root being more potent than its leaves, stem, and bark. However, there is little information (or not enough information) in the open scientific literature, pointing out the bioactive principles responsible for its benefits, as reported in the folkloric medicine of Southern Nigeria [20]. Hence, we decided to determine the biochemical composition and *in vitro* biological (antioxidant) effect of methanol extract of the roots of *A. nobilis*.

2. Materials and Methods

Reagents and the chemicals

We obtained DPPH, butylated hydroxy toluene (BHT), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4'-sulphonic acid, hydrogen peroxide, ferrous chloride, potassium ferricyanide, catechin, ascorbic acid, tannic acid, quercetin, nicotinamide adenine dinucleotide (NADH), trichloroacetic acid (TCA), phosphate buffer, sulfanilic acid, glacial acetic acid, naphthyl ethylenediamine dichloride, Folin-Ciocalteu reagent, sodium carbonate, vanillin, aluminum chloride, and potassium acetate from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). All other chemicals and reagents used, including solvents, were of analytical grade.

Collection of plant material sample

The sample of the plant material (roots of *A. nobilis*) was obtained in November 2019 within the premises of Ondo State University of Science and Technology (OSUSTECH), Okitipupa, Nigeria. The samples were identified by the herbarium Curator, Botany Program of the Department of Biological Sciences, Faculty of Science, OSUSTECH, Okitipupa (Herbarium no./Accession no.: OAUSTEHHx102), Nigeria.

Plant material preparation

The fresh roots of *A. nobilis* were detached from the whole plant, rinsed in water, and spread over the laboratory tables, where they were dried under room temperature. The plant material was then kept in the oven at 40°C for 10 min and then reduced to a fine powder with the assistance of a mechanical grinder.

Extracts preparation

For 48 hours, 200 g of powdered plant material was macerated in 1 L of methanol. The mixture was tapped using porcelain cloth and further filtered using No.1 Whatman filter paper. The filtrate was concentrated using a rotary evaporator, and the raw concentrate was then deposited at 4°C before further tests.

Determination of phytochemical assays

The phytochemical properties of the plant extract were determined using preliminary qualitative and preliminary quantitative assays. Chemical studies were carried out on the methanol extract of the plant sample as well as the powdered form of the plant sample using standard methods (Standard analytical methods described by Edeoga et al.

(2005), Trease and Evans (1996) and Obadoni and Ochuko (2002). On the calculation of the IC50 value, the stated authorities and standard analytical methods refers).

Preliminary qualitative phytochemical assay of the methanol root extract

The following standard laboratory protocols described by Edeoga et al. [21], Trease and Evans [22], and Obadoni and Ochukwo [23] were used for qualitative analysis of the sample to check for the presence of saponins, alkaloids, phenols, flavonoids, tannins, mucilage, cardiac glycosides, anthraquinones as well as triterpenoids:

Detection of Saponins (foam test)

Two milliliters of the sample were mixed with 6 mL distilled water and shook vigorously for 15 minutes. The creation of bubbles or persistence foam confirms a positive result.

Detection of Alkaloids

A small volume (0.2 g) of the sample was dissolved in the dilute H2SO4 and filtered thoroughly. To 1 mL of the filtered solution, a few drops of Meyer's, Wagner's, and Hager's reagents were added. The appearance of white or creamy precipitates, reddish-brown precipitates, and yellow precipitates for Meyer's, Wagner's, and Hager's reagents, respectively, confirmed positive results.

Test for Phenol

To 2 mL of the extract, 2 mL of 50% aqueous ferric chloride was added; the blue color formation suggests the presence of phenols in the sample extract.

Check of Flavonoids

To 2 mL of the extract, a few drops of 20% sodium hydroxide were added, and strong yellow color formation was observed. Then, a few drops of 70% diluted hydrochloric acid were added. The disappearance of the yellow color suggests the presence of flavonoids in the sample extract.

Testing for Tannins

To check the presence of tannins, 10% alcoholic ferric chloride was added to 2 mL of the extract. The formation of brownish blue or black color suggests the presence of tannins.

Testing for Mucilage

Two grams of the extract were added separately to 25 mL of absolute alcohol in continuous stirring and filtered. The precipitate was dried in the air and tested for its swelling properties. The development of swelling is suggested by the presence of mucilage.

Cardiac glycoside screening

About 0.5 mL of glacial acetic acid and 3 drops of 1% aqueous ferric chloride solution were added to 1 mL of the extract. The development of a brown ring on the interface suggests the presence of cardiac glycosides in the sample extract.

Anthraquinone test

One gram of the powdered plant sample was deposited in a dry test tube, and 20 mL of chloroform was added to it, and the mixture was heated in a steam bath for 5 minutes. When it was hot, the extract was filtered and allowed to cool down. An equivalent amount of 10% ammonia solution was applied to the filtrate. The mixture was shaken, and the upper aqueous layer was noted for the presence or absence of bright pink coloration to indicate the presence or absence of anthraquinones.

Triterpenoid test

To 2 mL of the extract, 5 drops of concentrated sulfuric acid were applied, shaken, and allowed to stand. The existence of a greenish-blue color suggests the presence of triterpenoids.

Preliminary quantitative phytochemical assay of the *Anthocleista nobilis* root methanol extract

The amount of each phytochemical, comprising saponin, alkaloids, phenols, flavonoids, tannins, mucilage, and cardiac glycosides present in the raw powdered sample was analyzed using standard laboratory procedures defined by Herborne [24].

Estimation of saponins

About 20 g of grounded sample was put into a conical flask followed by the addition of 100 mL of 20% methanol. It was then heated over a hot water bath for 4 h with constant stirring at around 55°C. The mixture was filtered, and the residue was re-extracted with 200 mL of 20% methanol. The extract was reduced to 40 mL over a water bath at approximately 90°C. The extract concentrate was washed twice with 10 mL of 5% aqueous so-

dium chloride. The residual solution has been heated in a water tank. After evaporation, the sample was dried to a constant mass in the oven. The content of saponin was measured as a percentage.

Estimation of Alkaloids

Five grams of the sample was weighted in a 250 mL beaker, and 200 mL of 10% acetic acid in methanol was added and covered and allowed to stand for 4 h. The solution was then diluted, and the extract was concentrated in a water bath to one-quarter of the original amount. Concentrated ammonium hydroxide was applied dropwise to the extract before the precipitation was completed. The entire solution was allowed to be settled, and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue, if present, is an alkaloid that is dried and measured.

Estimation of Phenols Using Spectrophotometric Process

The fat-free sample was boiled with 50 mL phenolic methanol for 15 minutes. Then, 5 mL of the extract was pipetted into a 50-mL container, followed by the addition of 10 mL of distilled water. Next, 2 mL of ammonium hydroxide solution and 5 mL of concentrated amyl alcohol were added. The sample was made to label, and the color produced was measured at 505 nm below room temperature after 30 minutes.

Estimation of Flavonoids

About 10 mg of plant sample was collected repeatedly at room temperature with 100 mL of 80% aqueous methanol. The total solution was filtered through the Whatman filter paper No. 42 (125 mm). The filtrate was then moved to the crucible and evaporated over the water bath to dryness and weighted to a constant weight.

Estimation of Tannins

About 200 mg of the sample was measured in a 50-mL bottle of plastic. The 50-mL volumetric flask was made up of the label. Then, 5 mL of the filtrate was pipetted into the test tube and combined with 2 mL of 0.1 M FeCl3 in 0.1 N HCl and 0.008 M potassium ferrocyanide. Absorbance was estimated at 120 nm within 10 minutes.

Estimation of the Mucilage

About 2 g of dry samples were blended with 10 mL of distilled acid water (pH=3.7). Then, 200 mL of purified water (the same pH as above) was added and blended for 20 minutes. After isolating the waste products using the Buchner funnel, the remaining solution was centrifuged, and 96% ethanol was applied (4 times the solution volume). The final solution maintained the mucilage precipitation at 4°C for 24 hours. The precipitate was separated by vacuum filtration using the Bucher funnel and then weighed after drying.

Estimation of Cardiac Glycosides

The extract (1 g) was branded with 50 mL of distilled water and filtered. To the filtered (1 mL), 4 mL of the alkaline pirate solution was added. The mixture was boiled for 5 minutes, allowing it to cool down. The absorbency was read at 490 nm.

In vitro determination of antioxidant assays

Plant extract antioxidant ability was evaluated using DPPH radical scavenging, nitric oxide scavenging, FRAP, total antioxidant power, and hydrogen peroxide scavenging assays.

DPPH radical scavenging assay

The scavenging capacity of the plant extract on DPPH was calculated using the method stated by Wintola and Afolayan [25], with changes in concentrations. One milliliter of DPPH was dissolved in methanol (0.135 mM) and mixed with 1 mL of varying concentrations ranging from 0.2 to 1 mg/mL of plant extract and standard. The mixture was thoroughly vortexed and left in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm using a spectrophotometer. The scavenging capability of the plant extract on DPPH was determined as follows: DPPH scavenging operation (%)=[(Abs control – Abs sample)/(Abs control)] ×100, where Abs control is the absorbance of DPPH + methanol, and Abs sample is absorbance DPPH radical + sample (extract/standard [ascorbic acid solution]). The IC50 value of DPPH radical scavenging assay had been determined thus: DPPH Inhibition (%)=[(AO – A1)/AO] ×100, where AO is the absorbance of standard, and A1 is the absorbance of the test sample.

Nitric oxide (NO) scavenging activity

The degree of inhibition of nitric oxide radical generation *in vitro* was assessed by the method of Awah and Verla [26]. Sodium nitroprusside in aqueous solution, at physiological pH, naturally produces nitric oxide, which interacts with oxygen to create nitric ions measured spectrophotometrically at 546 nm. Sodium nitroprusside (100

mM), phosphate-buffered saline (pH 7.4), and gray reagent (1% sulfanilamide, 2% H3PO4, and 0.1% naphthyl ethylene dihydrochloride) had been used as reagents. The reaction was triggered by adding 2.0 mL of sodium nitroprusside, 0.5 mL of PBS, to 0.5 mL of root extract (50 mg), and incubation at 25°C for 30 minutes. Griess reagent (0.5 mL) was applied and incubated for another 30 minutes. Control tubes were prepared without the extract. The absorbance was read in a spectrophotometer at 546 nm against the blank reagent (GENESYS 10-S, USA).

Ferric Reduction of Antioxidant Power (FRAP) Assay

The ferric reduction of the antioxidant potential of the extract was evaluated as defined by Zhao et al. [27]. The increase in the absorbance of the reaction mixture increased the reduced power.

Total Antioxidant Capacity (TAC) Assay

The total antioxidant ability was calculated by the phosphomolybdenum method as defined by Ohikhena et al. [28]. In short, 0.3 mL of methanol extract and standard (0.025-0.4 mg/mL) were placed in test tubes and dissolved in 3 mL of reagent solution (0.6 M sulfuric acid, 4 Mm ammonium molybdate, and 28 mM sodium phosphate) [The solvent used is methanol, and the appropriate expression should be "methanol extract", not solvent extract. What was referred to as normal, is the referenced standard substance used in any experiment]. The test tubes were sealed and incubated at 95°C in a water bath for 95 minutes. The mixture was allowed to cool down to room temperature, and the absorbency was measured at 695 nm. Ascorbic acid and BHT were used as standards. The percentage inhibition was determined as follows: [(absorbance of sample – absorbance of control)/(absorbance of the sample)]×100.

Hydrogen Peroxide (H_2O_2) Scavenging Activity

The following equation calculated the percentage of H_2O_2 scavenging: percentage of the scavenging behavior of H_2O_2=[(A0-A1)/A0]×100, where A0 and A1 were the absorbance rates of the control and the test extract, respectively [29].

Statistical analysis

The data obtained in chemical contents were analyzed by 1-way ANOVA. Analytical determinations were made in triplicate, and values were considered significant at $P<0.05$. Results are expressed as the mean and standard error of the mean.

3. Results

Phytochemical Findings

Table 1 presents different phytochemical components in the methanol root extract of *A. nobilis*. Phytochemical screening on plant material has demonstrated the presence of saponins, phenols, alkaloids, tannins, flavonoids, cardiac glycosides, and mucilage, but the absence of anthraquinones and triterpenoids. The roots of the plant contain carbohydrate stores, such as mucilage. The result showed that *A. nobilis* methanol root extract has no toxic secondary metabolites or lipid stores, such as sterols (steroid compounds) and terpenes.

Table 2 presents the quantitative concentration of the tested phytochemicals. The amount of all tested phytochemicals as per the solvent used was in the following order:

flavonoids (17.17[a]±0.27) >saponins (14.25b±0.02) >phenols (7.9c±0.44) >alkaloids (6.85c±0.48) >mucilage (5.53c±0.1) >tannins (4.65c±0.04) >cardiac glycosides (3.41d±0.13)

The highest concentration was found in flavonoid while cardiac glycosides had the least, i.e., the results were arranged in ascending order of concentration. According to the results, there are some variations in the concentrations of different phytochemicals tested.

Antioxidant findings

Table 3 presents *in vitro* antioxidant activity results for the methanol extract of the roots of *A. nobilis*. The antioxidant activity of the roots of *A. nobilis* was evaluated and confirmed using five functional, analytical chemical index methods: radical scavenging activity (DPPH), nitric oxide scavenging (NO), ferric reduction antioxidant power (FRAP), total antioxidant capacity (TAC), and hydrogen peroxide scavenging activity (HPSA). The experimental extract obtained utilizing solvent, methanol, and proportion could inhibit the DPPH radical, NO scavenging, FRAP, TAC, and HPSA. The potential for antioxidants varied considerably among the functional indexes and was as follows: 29.91a±7.96% with the DPPH method, 28.99a±12.72% with the NO method, 7.60b±1.65% with the FRAP method, 5.20b±1.39% with the TAC method, and 0.15c±0.02% with the HPSA method. A good correlation (R2=0.96) has been observed between the polyphenol of the roots of *A. nobilis* and the antioxidant activity, suggesting that polyphenol compounds are the major contributors to the antioxidant ability of the root of *A. nobilis*.

Table 1. Preliminary qualitative phytochemical analysis of *A. nobilis* methanol root extract

Phytochemicals	Inference
Saponins	+++
Phenols	++
Alkaloids	++
Tannins	++
Flavonoids	+++
Cardiac glycosides	+
Anthraquinones	_
Triterpenoids	_
Mucilage	++

Concentration of phytochemicals: +++ high; ++ moderate; + low; - absent.

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4. Discussion

In recent years, the focus has been on botanicals with antioxidant properties of the plants. It is believed that these botanicals may prevent or protect tissues from the harmful effects of free radicals. The results of the phytochemicals analyses on the plant extract correlate with the availability of flavonoids and other polyphenols to the antioxidant activity, as seen in the study. Preliminary phytochemical assessments of *A. nobilis* roots show the presence of flavonoids, saponins, reducing sugar, alkaloids, mucilage, tannins, and cardiac glycosides with varying concentrations. At the same time, anthraquinones and triterpenoids were absent or not detected. This finding corresponds with the report by Yakubu and Quadri [30].

Yakubu and Afolayan [31] reported the absence of steroids and flavonoids, while Bamishaiye et al. [32] reported the absence of cardiac glycosides in the extract of Moringa oleifera leaf. These secondary metabolites have numerous therapeutic and biological properties [33]. Phytochemical screening may help detect the chemical components of the plant extract and the predominant one. It may also be used to discover bioactive agents for raw products used in the partial synthesis of certain helpful medicines [24].

The search for medicinal products and dietary supplements from plants has accelerated recently. Natural science researchers combine Earth's phytochemicals and guiding principles that could be developed to treat various diseases. As part of their normal metabolic activities, all plants produce chemical and biochemical compounds. These phytochemicals are divided into primary metabolites, such as sugars and fats, which occur in all plants, and secondary metabolites found in a smaller range of plants

Table 2. preliminary quantitative phytochemical analysis of *A. nobilis* methanol root extract

Phytochemicals	Concentration (%), Mean±SD
Saponins	14.25b±0.02
Phenols	7.9c±0.44
Alkaloids	6.85c±0.48
Tannins	4.65c±0.04
Flavonoid	17.17a±0.27
Cardiac glycosides	3.41d±0.13
Mucilage	5.53c±0.1

* P<0.05. Different letters in superscript down the column showed significant differences in the chemical response.

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Tables 3. *In vitro* antioxidant indices of *A. nobilis* methanol root extract

Chemicals	Level (% Concentration)
2,2-Diphenyl-1-picrylhydrazyl scavenging activities (% inhibition)	29.91a±7.96
Nitric oxide (% radical scavenging activity)	28.99a±12.72
Ferric reducing antioxidant power	7.60b±1.65
Total antioxidant capacity	5.20b±1.39
Hydrogen peroxide scavenging activities	0.15c±0.02

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*P<0.05. Different letters in superscript down the column showed significant differences in the chemical response when compared with 4.61% being value of standard ascorbic acid.

and serve more specific functions [34]. These secondary metabolites and pigments may have therapeutic effects in humans and maybe refined to produce drugs [34].

Plants used for the treatment of illnesses contain bioactive compounds with biological effects. Some of these chemicals are responsible for the characteristic smell, pungency, and color of the plant, whereas others give a plant its culinary, medicinal, or toxic value [35]. Results of the qualitative phytochemical constituents of root extract of *A. nobilis* showed that the tested phytochemicals were present in this plant (Table 1). The flavonoids in the extract of medicinal plants have been implicated in possessing anti-allergic, anti-inflammatory, antiviral, antiproliferative, and anti-carcinogenic activities, as well as some aspects of mammalian metabolism [36].

Oxidative stress, caused by an inability of the biological system to neutralize excessive free radical products, has been associated with all kinds of human diseases and aging [26]. Antioxidants (free radical scavengers) interact with and neutralize free radicals, thus preventing them from damaging cells in the biological system [37]. The body makes some antioxidants that use them to neutralize free radicals. These antioxidants are referred to as endogenous antioxidants. However, the body also relies mainly on external (exogenous) food sources to obtain the rest of the antioxidants it needs [38]. These exogenous antioxidants are commonly known as dietary antioxidants. In recent years, the focus has been on dietary plants with antioxidant properties. It is believed that these plants can prevent or protect tissues from the harmful effects of free radicals [39].

Free radicals and Reactive Oxygen Species (ROS) have been involved in various human diseases [40]. The beneficial medicinal effects of plant materials are typically the result of combinations of secondary metabolites present in the plant; through additive or synergistic action of several chemical compounds at single or multiple target sites associated with a physiological process [41]. It can therefore act as a moderate radical scavenger that can decrease autooxidation in the biological system or even a dietary product that contains unsaturated fat [42].

People believe that the antioxidant capacity of a food-based product such as an herb can help protect oxidative stress both *in vitro* and in vivo [26]. In this study, the *in vitro* antioxidant potential of *A. nobilis* methanol root extract was evaluated by assessing the percentage concentration of DPPH, nitric oxide scavenging (NO), FRAP, TAC, and HPSA. Interestingly, the high content of DPPH and NO (Table 3) may suggest considerable antioxidant potentials, and they are a class of antioxidant compounds acting as free radical terminals [41]. The high DPPH and NO radical scavenging capability of the root extract of this plant may result from the increased concentration of these organic compounds. Gupta et al. [43] recorded similar findings with extracts from Cassia fistula; hence, the observed antioxidant capacity of extract of this plant could result from its organic constituents. The DPPH stable scavenging model, and NO radicals and other antioxidant compounds as revealed by this study, is a broadly used assay for assessing the free radical scavenging capability of different samples [26].

Plants contain natural antioxidants that have beneficial effects on the maintenance of health, disease management, and the harmful effects of toxic agents [44]. Phenolic compounds are a class of antioxidants that may inhibit and scavenge free radicals due to their redox properties [45]. The present study revealed that the extract exhibited variance concentrations and substantial scavenging activities. A similar trend of DPPH free radical scavenging activity has also been reported earlier by Vishmu [46]. In the solvent extract tested, DPPH and NO

exhibited maximum radical scavenging activity. This outcome could have been due to a higher concentration of flavonoids, essential biocompounds required for the extract scavenging activity. Meanwhile, findings from the present study indicate that *A. nobilis* root possesses antioxidant properties. Further research on the active ingredients of the plant needs to be carried out to confirm the safety of its consumption and therapeutic application. The high content of flavonoids seen in the methanol extract of the plant can support its ethnomedicinal use in the management of arthritis and other related diseases.

Biochemical compounds have vital roles in medicinal products and nutrition. They possess a strong antioxidant capability and have been implicated in protecting the body against tissue damage, cancer and improving blood circulation by enhancing capillaries, arteries, and veins [47]. The high content of compounds observed in this study could therefore point to the plant's potential to prevent or control diseases. It can synergistically contribute to the significant antioxidant potency of the plant and promote its ethnomedicinal use in the treatment of oxidative stress-related diseases and disorders. Alkaloids and saponins are relevant for their pain and inflammatory relief, and antispasmodic effects. Saponins have many pharmacological activities, including expectorant, anti-inflammatory, vasoprotective, gastroprotective, and antimicrobial properties. They are also helpful in reducing the risk of coronary heart disease in human diets [47]. The high content of saponin observed in the plant may, therefore, encourage the consumption and traditional use of the plant to treat various ailments.

In this study, appreciable values for scavenging activities were obtained. It indicates that the plant is a potent source of antioxidants. DPPH assay is a common spectrophotometric technique used to determine antioxidants' activity. The advantage of this process is that the antioxidant activity is usually measured at an ambient temperature, so the danger of heat-induced breakdown of the tested molecule is eliminated. Free radical scavenging is the known mechanism inhibiting lipid oxidation by antioxidants [48].

The scavenging effect of *A. nobilis* extract on H_2O_2 was low compared to other biocompounds. The disparity in concentration may be due to solvent polarity. Naczk and Shahidi reported [49] that solvent polarity has a crucial role in the rising solubility of phenolic biocompounds. Meanwhile, it is evident from the literature that hydrogen peroxide is naturally produced in human bodies, air, water, plants, food, and microorganisms at low concentrations [50]. It is promptly decomposed into water and oxygen, which may provide hydroxyl radicals (OH), capable of initiating lipid peroxidation and DNA damage. Therefore, the efficiently scavenging hydrogen peroxide effect observed in the methanol extract of the plant material could be attributed to the presence of phenolic groups that neutralizes it into the water through donating electrons to hydrogen peroxide [18]. Solvents like methanol, ethanol, and their mixtures with different quantities of water have been used to extract phenolics, mainly from plant materials. The phenolic contents of plants are also dependent on the solvent types, the polarity, and several intrinsic (i.e., genetic) and extrinsic factors, such as environmental, developmental stage, and handling methods [51].

In the present study, the potential antioxidant activities of *A. nobilis* roots extract were assessed by the FRAP method. This method was based on reducing ferric ions into their ferrous form. The results showed that methanol extract had higher FRAP values than HPSA and TAC. Thus, the antioxidant activities of HPSA and TAC were the lowest. The highest antioxidant activities were exhibited by DPPH, followed by NO. These observed activities could ascribe the occurrence of secondary or subsidiary metabolites, such as phenols, flavonoids, etc., in *A. nobilis*. There was a strong association between phenols, tannins, and flavonoids detected during the phytochemical screening and the observed radical scavenging activity. It demonstrates that the phytochemical constituents of *A. nobilis* root extract are the principal enhancers of its antioxidant potential.

In conclusion, the results obtained in this research prove that the extracting solvent considerably affected the phytochemistry and antioxidant activity of the plant material. The results showed that the plant material had a reasonable concentration of phytochemicals, which correlated well with its antioxidant effects. The methanol extract showed the highest antioxidant capabilities based on the DPPH and NO radicals scavenging assays. The high phytochemical content and antioxidant activity of the root of *A. nobilis* is indicative of its ability to maintain health and treat various diseases and, therefore, promotes its use in ethnomedicine. *A. nobilis* may possess vital ability in combating oxidative stress. It is therefore highly recommended to expand its application in health maintenance.

Ethical Considerations

Compliance with ethical guidelines

The protocol for experimentation was approved on January 12, 2020, by the Ethics Committee (Ref. no: OSUSTECH/NOEC/BIOSC_FSC/365/5) on Experimental Use and Maintenance of Laboratory Equipment(s) of

the School of Science, Ondo State University of Science and Technology, Okitipupa, Nigeria. All ethical considerations were observed throughout the research.

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Conflict of interest

The author declared no conflict of interest.

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