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Phytochemical Screening and Antioxidant Activities of *Rytigynia nigerica* (S. Moore) Robyns Roots Extracts

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Abstract

Rytigynia nigerica (Rubiaceae), a medicinal herb native to West Africa, is known for its antimalarial and anticancer properties and is traditionally used to treat various ailments. However, the biological activities of *R. nigerica* have not yet been fully studied. However, this study was designed to extract, analyse, and evaluate the antioxidant potential and phytochemical screening in the root extracts of *R. nigerica*. The roots were obtained from the Forestry Research Institute of Nigeria (FRIN), Ibadan and authenticated. The air-dried and pulverised root samples were extracted with methanol using the Maceration method and then partitioned into *n*-hexane and ethyl acetate using the liquid-liquid extraction method. The phytochemical screening was evaluated using the standard method, while antioxidant activity was investigated viz 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) assays. The phytochemical screening analysis revealed the presence of saponins, cardiac glycosides, tannins, phenols, reducing sugars, alkaloids and resins in the root extract. The antioxidant activity of the extracts was significant when compared to reference standards. The percentage inhibition of the antioxidant extracts and reference standard are as follows: n-hexane extract (58.50 -45.08%), ethyl acetate extract (90.62-67.82%), Methanol extract (70.81-52.97%), Vitamin C (95.66-91.63%) and butyl hydroxyanisole (94.76-90.96%). The antioxidant inhibition of the free radical was concentration-dependent. The results obtained in this study indicate that *R. nigerica* root extracts exhibit antioxidant properties, suggesting potential pharmaceutical applications.

Keywords: Rytigynia nigerica root; 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH); phytochemical constituents.

INTRODUCTION

Historically, Natural products have attracted a lot of interest because they are primarily found in nature and made of organic materials from living things (Pezzuto *et al.,* 2005). Natural products are chemical compounds isolated from living organisms (Bhat *et al.,* 2005). It can be synthesised artificially or organically through partial or entire synthesis, and it is essential in advancing organic or medicinal chemistry by offering challenging synthetic targets (Chintoju *et al*., 2015).

Natural products have played a crucial role in developing medications, dietary supplements, cosmetics, and agrochemicals (Beghyn *et al*., 2008). Natural medicines not only meet most of the primary healthcare needs of the populations in developing nations, but they are also gaining popularity in developed nations due to spiralling healthcare costs and widespread financial austerity. About 49% of people in the USA have experimented with natural remedies for illness prevention and treatment (WHO, 2009). Natural remedies are said to contain "active components" or "active principles," which are substances recognized to have therapeutic advantages. Nearly half of the chemical medications for treating human diseases approved by the United States Food and Drugs Agency (FDA) between the 1940s and the end of 2014 were derived from or inspired by natural sources (Newman *et al*., 2012; Newman *et al*., 2016). Functional groups, chirality and structural complexity of molecules from natural products are more similar to those of drugs than those of molecules from combinatorial chemistry (Cragg & Newman, 2003; Atanasov *et al*., 2015). In natural remedies, the concentrations of the active components

are rarely very high. The bottleneck in using natural products in medication research has been the labour-intensive and time-consuming extraction and isolation process.

Furthermore, a number of chronic and degenerative illnesses, including cancer, pulmonary, neurological, and digestive disorders, have been linked to the overproduction of reactive oxygen species (ROS) (Liu et al., 2018). Antioxidants, which can be produced internally or obtained through external supplementation, subtly control the levels of ROS under physiological settings. Malnutrition and antioxidant deficiencies together may make people more susceptible to oxidative stress, which raises the chance of developing cancer (Liu et al., 2018). In addition, the antioxidant defence can be overwhelmed during continuous inflammation such as in chronic obstructive lung illnesses, inflammatory bowel disease, neurodegenerative disorders, cardiovascular diseases, and aging (Chelombitko, 2019). Certain antioxidant vitamins, such as vitamin D, are vital in regulating metabolic processes that contribute to the normal functioning of organs. In several clinical studies, antioxidant supplements have been demonstrated to reduce endogenous antioxidant depletion, hence mitigating related oxidative damage (Forman & Zhang, 2021).

The Nigerian species of *Rytigynia nigerica* (Figure 1) is the focus of this study. According to Burkill (1985), the species have been reported as having antimalarial and anticancer effects and are used to treat other common diseases in West Africa. The stomatal type was paracytic and epidermal cell shape was irregular in both species. A higher epidermal cell number was recorded in *R. nigerica.* The parenchyma cell of the petiole was generally polyhedral to roughly oval in both species. In *R. nigerica,* the adaxial surface of the petiole was almost flattened and crystalloid deposits of calcium oxalate were found in the sclerenchyma tissue. Furthermore, in *R. nigerica*, the midrib surface was pubescent, no cell inclusion was recorded and the vascular bundle was V-shaped (Ajayi et al., 2011). Leaves of *R. nigerica* were generally for the treatment of hypostomatic, malaria and cancer (Ajayi *et al*., 2011). The phytochemical screening of *R. nigerica* revealed the presence of steroids, tannins, saponins, reducing compounds, terpenes, and flavonoids in the ethanolic extracts. Phlobatannins, anthraquinones, cardiac glycosides, and cyanogenetic compounds were absent (Ajayi *et al*., 2011).

Figure 1. Picture of *R. nigerica*.

These medicinal plants have been used for centuries, but very little research has been done to examine their phytochemical constituents or antioxidant properties. This study aims to investigate the phytochemical constituents of extracts of these plants and evaluate their antioxidant properties.

MATERIALS AND METHODS

Equipment/Apparatus and Chemicals/Reagents Used

The equipment and apparatus used include a steam bath, round-bottom flask, weighing balance, beakers, distillation flask, sample vials, ice chest, syringes, UV-visible spectrophotometer, foil papers, and separating funnel. All solvents were general-purpose chemicals obtained from the Local Vendors: Methanol, Ethyl acetate, *n*-Hexane and were distilled before use. Concentrated H_2SO_4, HCl, ferric chloride, Fehlings' solution A and B, Ammonia solution, benzene, Iodine crystals, glacial acetic acid, Molisch reagent, magnesium powder, ascorbic acid, butylated hydroxylanisole, α-tocopherol and 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH).

Collection, Identification and Preparation of Plant Sample

The fresh leaves of *Rytigynia nigerica* Robyns were collected from the Forestry Research Institute of Nigeria (FRIN), Oyo State, Nigeria. They were identified and authenticated at FRIN (Forestry Research Institute of Nigeria). The leaves and stem of *Rytigynia nigerica* Robyns were air-dried under shade, separated and pulverized before extraction.

Extraction of plant sample

The air-dried roots of *Rytigynia nigerica* were ground and about 1.0 kg of sample of root samples were obtained. 1.0 kg of the root sample was carefully charged into an aspirator bottle and extracted with methanol for 24 hrs using a maceration method. The mixtures were decanted and concentrated into a paste via distillation and dried using a vacuum desiccator. 150 g of the root extract was obtained. 89.77 g of the methanol extract of *Rytigynia nigerica* leaves was partitioned first into hexane and later into ethyl acetate using the liquid-liquid extraction method described by (Ferrone et al., 2017) was employed The resulting hexane and ethyl acetate extracts were concentrated to a paste using a rotary evaporator under reduced pressure at 40 ^{0}C and dried using a vacuum desiccator.

Phytochemical screening of *Rytigynia nigerica* roots (Sofowora, 1993; Okwu, 2001)**.**

Screening for Alkaloids

To 1% HCl was added 0.2 g of extract, the mixture was filtered and then drops of Dragendorff's reagent were added. The creation of a precipitate showed the presence of alkaloids.

Screening for Saponin

To each extract (0.2 g) 5 mL of distilled water was added. The formation of froth upon warming and letting stand for about 10 minutes indicates the presence of saponin.

Screening for Tannins

A total of 0.2 g of extract was measured and dissolved in distilled water. The resulting solution was filtrate and ferric chloride was added to it. The presence of a green-blue-dark or blue-green precipitate detected tannins.

Screening for Steroids (Salkowski's Test)

A total of 0.2 g of extract was weighed and dissolved in 2 mL of chloroform. A lower layer of concentrated H_2SO_4 was then applied. The reddish-brown colour of cardenolides during the interphase corroborated their deoxy-sugar properties.

Screening for Cardiac-active Glycoside (Keller-Killani Test)

From the extracts, 0.2 g was weighed and dissolved in 2 mL of glacial acetic acid containing one drop of ferric chloride solution. 1 mL of concentrated sulphuric acid was then added. Brown ring inter-phase indicated the presence of cardiac glycosides.

Screening for Carbohydrates

About 5 mL of extract was measured and placed in a test tube in a slanting position and a few drops of Molisch reagent were poured through the side of the test tube holding 1 mL of sulphuric acid solution. Without mixing, the acid created a layer beneath the aqueous solution. A reddish-brown solution suggested the presence of carbohydrates.

Screening for Reducing Sugars

About 0.2 g of the extract was mixed with distilled water before being filtered. The filtrate was then heated for two minutes with equal volumes of Fehlings' solution A and B. The presence of orange precipitate generated proved the presence of reducing sugar.

Screening for Flavonoids (Shinoda Test)

About 3 mL of extract solution was measured and a small amount of magnesium powder was added. A few drops of strong hydrochloric acid were also added to the mix. The production of a crimson precipitate confirmed the presence of flavanones.

Screening for Resins

About 5 mL copper acetate was added to 5 mL of extracts, agitated vigorously and allowed to separate. The appearance of a green colour verified the existence of tar.

Test for Anthraquinones (Born – Trager's Test)

About 4 mL of benzene was added to 0.2 g of extracts and the mixture was filtered. The filtrate was shaken with 2 mL of the Ammonia solution. The production of pink, red or violet colour in the ammonical portion (Lower layer) was indicative of the presence of anthraquinones.

Screening for Phenols

About 0.2 g of the extracts were dissolved in ferric chloride solution. The appearance of a dirty green precipitate after dissolving suggested the presence of a phenolic chemical.

Screening for Phlobatannins

For the extracts, dissolution of 0.5 g each was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl formation. The formation of red precipitate indicated the presence of phlorotannins.

Antioxidant Assay

The radical scavenging activity was evaluated using the approach reported by Ibok et al., 2023. In methanol, a 0.5 mM solution of 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) was produced, and 3 mL of this solution was combined with 1 mL of the oil sample in methanol (Ibok et al., 2023). The reduction in DPPH absorption at 517

nm was evaluated after a 10-minute incubation period. The percent inhibition was calculated, and the decrease in absorption was compared to the control group's drop in absorption. Using recognized standards such as ascorbic acid and butylated hydroxylanisole, a similar process was used. The tests and analyses were repeated three times, the results were averaged, and the activities were calculated as a function of the percentage inhibition (%I) was then calculated using the following. Equation:

$$I(\%) = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

The percentage yield of *Rytigynia nigerica* roots extracts (*n*-hexane, ethyl acetate and methanol)

The roots crude extracts of *Rytigynia nigerica* yield (w/w) obtained in this study are presented in Table 1. The semi-polar ethyl acetate extract had the lowest yield (23.5%), followed by the non-polar *n*-hexane extract (33.5 %) and the polar aqueous-methanol extract (43.0%; Table 1). The yield of the extracts obtained in the study showed the level of the components present in the plant extracts based on their polarity. It is reported that the yield of plant extracts can be significantly influenced by the solvent type used, as each solvent has a different polarity and affinity for various compounds. However, the *n*-hexane extract in this study had a better yield than the ethyl acetate extract, in contrast to the literature report based on lipophilic compounds. This indicated that the root extract contained more lipophilic compounds.

Table 1. Yields and properties of the root wood extracts of Rytigynia nigerica roots.

Extract	Weight (g)	Yield (%)	Description
Aqueous Methanol	38.60	43.0	Dark brown viscous
Ethyl acetate	21.10	23.5	Dark brown
n-Hexane	30.07	33.5	Dark brown

%Yield = [Weight of the extract / Weight of bulk extract] × 100

Phytochemical screening of the *Rytigynia nigerica* leaves

The phytochemical screening of *Rytigynia nigerica* root extracts involved using three different solvent extractions—polar, semi-polar, and nonpolar—to obtain a comprehensive chemical profile of the plant. Each solvent targets specific compounds based on their polarity, allowing for a more thorough investigation of the plant's constituents. As presented in Table 2, the results indicated the presence of saponins, cardiac glycosides, tannins, phenols, reducing sugars, alkaloids, and resins, all compounds commonly associated with medicinal activity.

The phytochemical screening of *Rytigynia nigerica* root extracts, utilising polar, semi-polar, and nonpolar solvents, provided a comprehensive chemical profile of its constituents. Each solvent selectively extracted different classes of compounds based on polarity, enabling an in-depth investigation of bioactive compounds present in the plant (Sofowora, 1993; Okwu, 2001). As summarised in Table 2, the analysis revealed saponins, cardiac glycosides, tannins, phenols, reducing sugars, alkaloids, and resins—all known for their medicinal properties. Saponins and tannins possess anti-inflammatory and antimicrobial effects, while phenols contribute antioxidant benefits. Alkaloids and cardiac glycosides are linked to anti-diabetic and cardioprotective activities, supporting the potential of *R. nigerica* in traditional medicine for diverse therapeutic applications (Ogunlana et al., 2008). These findings lay the groundwork for further exploration into the medicinal properties of the plant. In many traditional medicinal systems, plants containing these compounds are utilised for treating inflammation, infections, heart ailments, and wounds. This supports the role of *Rytigynia nigerica* in traditional medicine and suggests that it could serve as a source of therapeutic agents for modern medicine, potentially providing alternatives for managing various health issues.

Table 2. Constituents of the various extracts of *Rytigynia nigerica* roots.

Constituents	Hexane Extract	Ethylacetate Extract	Methanol Extract
Saponins	+	+	+
Cardiac glycoside	+	+	+
Tannins	+	+	+
Phenols	+	+	+
Alkaloids	+	+	+
Reducing sugar	+	+	+
Flavonoids	-	+	-
Carbohydrate	-	-	+
Anthraquinones	-	-	-
Resin	+	+	+
Phlobatannins	-	+	+

Key:
+ = Present.
- = Not present.

Antioxidant activities of *R. nigerica* root extracts

The DPPH radical has a distinct purple colour with a maximum absorbance at 517 nm in the visible spectrum. When an antioxidant donates an electron or a hydrogen atom to DPPH, the radical stabilizes, and the colour changes from purple to bright yellow. This decolorization is proportional to the antioxidant activity of the tested substance. As the antioxidant compounds in the extracts neutralize the DPPH radicals, the absorbance value at 517 nm decreases, indicating a reduction in free radical concentration. The extent of this reduction reflects the strength of the antioxidant activity in the extracts (Brand-Williams et al., 1995).

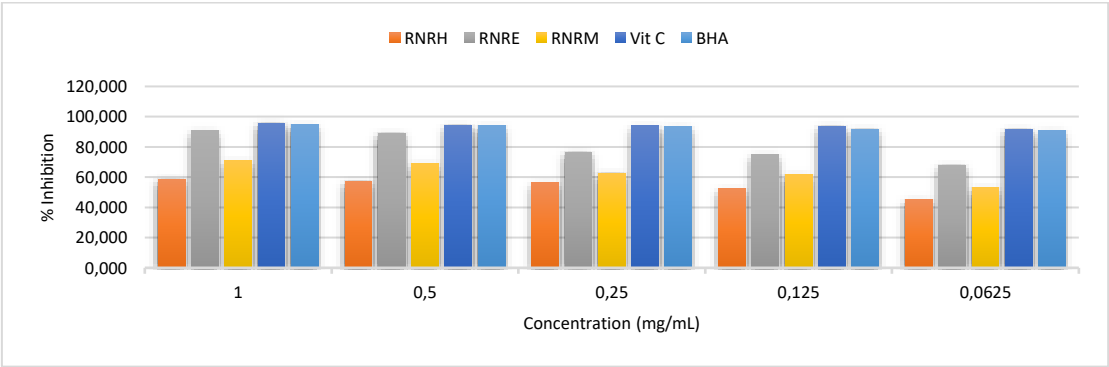
Phenolic compounds, well-known for their antioxidant action, are present in significant amounts in the ethyl acetate and methanol extracts. Phenolic

compounds contribute to antioxidant activity through their ability to donate electrons or hydrogen atoms, neutralizing free radicals and thus preventing oxidative chain reactions. Phenolics are effective antioxidants because their hydroxyl groups readily participate in free radical scavenging, forming more stable molecules that halt oxidative damage to cells and biomolecules (Ogunlana et al., 2008). These compounds can protect biological structures, such as proteins, lipids, and nucleic acids, from damage caused by oxidative stress, which is implicated in many chronic diseases.

The results of the DPPH assay are presented in Figure 2, with the antioxidant activities of the extracts compared to known antioxidant standards such as vitamin C and butylated hydroxyanisole (BHA). The antioxidant potential of the extracts and standards were ranked as follows: Vitamin C, serving as a potent reference standard, displayed the highest radical scavenging activity (95.66-91.63%). Known for its electron-donating solid ability, vitamin C neutralises free radicals quickly, resulting in a rapid decline in DPPH absorbance (Table 6). Its effectiveness underscores the benchmark against which the plant extracts were measured. This was followed by Butylated Hydroxyanisole, a synthetic antioxidant commonly used in food preservation, demonstrating high antioxidant activity. Although slightly less active than vitamin C, the capacity of BHA (94.76-90.96%) to stabilise radicals highlights its efficacy in halting oxidative reactions, which provides a further point of comparison for evaluating the plant extracts (Table 7). RNRE, among the plant extracts, exhibited the highest antioxidant activity (90.62-67.82%), close to that of BHA and vitamin C. Ethyl acetate (Table 4) is an effective semi-polar solvent for extracting phenolic compounds, which are abundant in *Rytigynia nigerica* and is known to possess strong antioxidant properties. This high antioxidant activity is attributed to phenolic compounds with hydroxyl groups that donate electrons to free radicals, converting them into stable products and stopping radical chain reactions. RNRM extract showed moderate antioxidant activity (Table 5), lower than RNRE but still significant. Methanol, as a polar solvent, extracts phenolic compounds, though often different in profile compared to those extracted by ethanol. Despite the slightly lower activity, RNRM demonstrated effective radical scavenging (70.81-52.97%), supporting its role as a potential antioxidant source. The activity may be due to a combination of phenolic and other antioxidant compounds present in the methanol extract, though possibly in lesser quantities or types than those in the ethanol extract. The hexane extract displayed the lowest antioxidant activity among the tested samples. Hexane, a nonpolar solvent, extracts mainly lipophilic compounds like terpenoids and other nonpolar constituents that generally possess lower antioxidant activity compared to polar phenolics. The lower antioxidant activity of RNRH (Table 3) suggests a lesser content of electron-donating compounds, indicating that its radical-scavenging potential (58.50 -45.08%) is weaker than the more polar extracts.

The results confirm that the solvent used for the extraction and its polarity significantly influences the antioxidant activity of the extracts. The ethanol and methanol extracts, richer in phenolic compounds, demonstrated more muscular antioxidant activities due to the higher concentration of polar antioxidant compounds capable of donating electrons. In contrast, the nonpolar hexane extract had a lower antioxidant capacity, likely due to its different profile of extracted compounds with less electron-donating potential.

These findings suggest that *Rytigynia nigerica* root has a substantial antioxidant capacity, particularly in its ethanol and methanol extracts. This antioxidant property supports the traditional use of *Rytigynia nigerica* in herbal medicine, as oxidative stress reduction is a crucial mechanism in the prevention of chronic diseases such as cardiovascular disorders, diabetes, and cancer. The strong antioxidant properties of the ethanol and methanol extracts of *Rytigynia nigerica* root underscore its potential as a natural source of antioxidants for health applications.



RNRH = Hexane extract of *R. nigericia* roots
RNRE = Ethyl acetate extract of *R. nigericia* roots
RNRM = Methanolic extract of *R. nigericia* roots
BHA = Butyl hydroxyanisole
Vit C = Vitamin C

Figure 2. Antioxidant activities of *R. nigerica* root extracts.

Table 3. Absorbance reading of *n*-Hexane extract of *R. nigericia* roots.

Concentration (mg/mL)	Absorbance			Average	Blank	% Inhibition
	1st	2nd	3rd			
1.00	0.49	0.491	0.492	0.491	1.183	58.495
0.5	0.504	0.505	0.509	0.506	1.183	57.227
0.25	0.519	0.515	0.518	0.517	1.183	56.269
0.125	0.564	0.561	0.567	0.564	1.183	52.325
0.0625	0.654	0.646	0.649	0.650	1.183	45.083

Table 4. Absorbance reading of Ethyl acetate extract of *R. nigericia* roots.

Concentration (mg/mL)	Absorbance			Average	Blank	% Inhibition
	1st	2nd	3rd			
1.00	0.11	0.112	0.111	0.111	1.183	90.617
0.5	0.134	0.129	0.131	0.131	1.183	88.898
0.25	0.28	0.275	0.276	0.277	1.183	76.585
0.125	0.301	0.3	0.298	0.300	1.183	74.669
0.0625	0.381	0.379	0.382	0.381	1.183	67.822

Table 5. Absorbance reading of Methanol extract of *R. nigericia* roots.

Concentration (mg/mL)	Absorbance			Average	Blank	% Inhibition
	1st	2nd	3rd			
1.00	0.35	0.34	0.346	0.345	1.183	70.809
0.5	0.371	0.368	0.367	0.369	1.183	68.836
0.25	0.446	0.442	0.441	0.443	1.183	62.553
0.125	0.451	0.45	0.468	0.456	1.183	61.426
0.0625	0.555	0.556	0.558	0.556	1.183	52.973

Table 6. Absorbance reading of Vitamin C extract of *R. nigericia* roots.

Concentration (mg/mL)	Absorbance			Average	Blank	% Inhibition
	1st	2nd	3rd			
1.00	0.051	0.051	0.052	0.051	1.183	95.661
0.5	0.064	0.065	0.067	0.065	1.183	94.477
0.25	0.07	0.071	0.068	0.070	1.183	94.111
0.125	0.075	0.076	0.078	0.076	1.183	93.547
0.0625	0.1	0.099	0.098	0.099	1.183	91.631

Table 7. Absorbance reading of Butyl hydroxyanisole (BHA) extract of *R. nigericia* roots.

Concentration (mg/mL)	Absorbance			Average	Blank	% Inhibition
	1st	2nd	3rd			
1.00	0.061	0.062	0.063	0.062	1.183	94.759
0.5	0.07	0.071	0.073	0.071	1.183	93.970
0.25	0.075	0.078	0.079	0.077	1.183	93.463
0.125	0.099	0.098	0.099	0.099	1.183	91.660
0.0625	0.11	0.111	0.1	0.107	1.183	90.955

CONCLUSION

The findings of this study reveal that the root extracts of *Rytigynia nigerica* contain a variety of phytochemicals, notably alkaloids and steroids, which distinguish this plant as a unique source of bioactive compounds. The presence of these phytochemicals contributes to the root's significant antioxidant and anti-diabetic properties, with activity comparable to standard medications used in this investigation. The antioxidant capacity, likely driven by phenolic content, underscores the potential of *R. nigerica* in combating oxidative stress, while its anti-diabetic effects suggest possible benefits for managing blood glucose levels. These results support the traditional use

of *R. nigerica* in herbal medicine and highlight its potential as a natural therapeutic source. Further studies should explore the mechanisms of action and isolate specific bioactive compounds, paving the way for the development of *R. nigerica*-based therapies in oxidative stress-related diseases and diabetes management.

Competing Interests: The authors have not declared any conflict of interest.

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