Kashmir Journal of Science
https://kjs.org.pk ISSN: 2958-7832

Kashmir Journal of Science (2024), 3(4):10-22

Research Paper

Phytochemicals, Nutritional and Elemental Composition of different plant parts of *Rinorea oblongifolia* (C.H Wright) C. Marquand ex Chipp

Adeniyi Success Ohunayo[1*], Olusola O. Elekofehinti[2], Muftau Kolawole Oladunmoye[1], Anthony Kayode Onifade[1]

[1]Department of Microbiology, Federal University of Technology Akure

[2]Department of Biochemistry, Federal University of Technology Akure

*Corresponding Author Email: ***adeniyi.ohunayo@eksu.edu.ng***

ARTICLE INFO

Article history:

Received: 09 November 2024
Revised: 26 December 2024
Accepted: 27 December 2024
Available online: 29 December 2024

Keywords:
Rinorea oblongifolia
Phytochemicals,
Proximate,
Mineral element composition

Abstract

Antioxidants are essential in managing chronic diseases by neutralizing free radicals and reducing oxidative stress. *Rinorea oblongifolia*, a medicinal plant, contains a rich profile of phytochemicals and minerals, adding to its therapeutic potential. This study provides a detailed analysis of the proximate, phytochemical, mineral, and antioxidant properties of its leaves, stems, and roots. The plant parts were collected, air-dried, and ground into powder for evaluation. Phytochemical screening detected alkaloids, saponins, tannins, phenolics, flavonoids, steroids, and terpenoids, with the highest concentrations in the leaves. Proximate analysis showed that the leaves had the highest moisture (9.17%), crude protein (9.91%), and carbohydrate (75.26%) contents, while roots had the highest ash (2.45%) and crude fat (4.61%). Mineral analysis showed that the roots were rich in iron, calcium, and copper, while leaves contained more potassium, sodium, phosphorus, and magnesium. Antioxidant assays (DPPH, FRAP, ABTS) revealed significant free radical scavenging, especially in the leaves. These findings support *oblongifolia*'s potential for nutritional supplements and therapeutic uses, with further research needed to investigate mechanisms and standardize extraction protocols.

Introduction

Medicinal plants are a significant source of compounds having medicinal properties. (Dare *et al*., 2011).It is well known that a large number of herbal products have promising futures (Monjane, 2017); however, in developing countries, people often resort to herbal medicinal plants without proper awareness or information on the associated risks, particularly in cases of excessive or chronic use. In most developing countries, many unregistered and poorly regulated herbal products are sold freely with little or no restraint. This lack of control leads to insufficient knowledge about their mechanisms, possible adverse reactions, contraindications, and interactions with conventional pharmaceuticals and functional foods (Ekor 2014).The *Violaceae* family which includes several species known for their diverse morphological and ecological traits (Smith *et al.*, 2009), includes the plant species *Rinorea oblongifolia* (C.H. Wright), which is found in the tropical rainforests of Nigeria, Liberia, Cameroon, and Uganda. In Nigeria, it is particularly common in Ogun State, especially within the Omo Forest Reserve. *Oblongifolia* is considered one of the less common tree species within the genus *Rinorea*. In Cameroun, it is a significant medicinal plant with a variety of traditional medical applications, such as the treatment of diabetes, headaches, and neurological disorders. (Attah *et al.*, 2016; Erhenhi and Obadoni, 2015; Jiofack, 2008). *Rinorea oblongifolia* plays a crucial role in its native habitat. The plant's ability to grow in challenging environmental conditions makes it a candidate for further ecological studies in forest dynamics (Mawuli *et al.*, 2020). The specific epithet "oblongifolia" refers to the shape of the leaves, which are typically elongated and lanceolate. It is a species of significant ecological and medicinal value, contributing to the biodiversity of tropical ecosystems and offering potential benefits in traditional and modern medicine (Ngo *et al.*, 2017). Despite all of this indigenous usage, there are no pharmacological results on these ethnomedical claims in the literature. This study provides a detailed analysis of the proximate, phytochemical, mineral and antioxidant properties of *Rinorea oblongifolia* leaves, stems and roots.

Materials and Methods

Plant sample collection and preparation:

Rinorea oblongifolia (C.H. Wright) leaves was collected from Agbogi village, Ikire district, Osun state and authenticated by Mr Odewo S.A of federal institute of technology, Ibadan with specimen No.FHI.113606. The plant parts were sorted and washed thoroughly with distilled water to remove any form of dirt, then air dried for four weeks at room temperature (28-30℃).

The samples were pulverized using an electric blender to increase the surface are and to hasten the process of extraction and then stored at room temperature in air tight polythene bag prior to use.

Plants extraction: Fifteen grams (15g) of powdered plant materials were dissolved in 100ml of distilled water using maceration technique. The container was tightly closed and left for three days at room temperature (28-33℃) with periodic stirring to ensure maximum extraction of the bioactive compounds. The dissolved plant extracts were filtered and decanted, and the filtrates were concentrated using a rotary evaporator at 40c.

Phytochemical screening: To evaluate the chemical components of crude plant samples, phytochemical screens were performed using standard procedures for qualitative screening of phytochemicals of interest (Evans, 2009). The phytochemicals examined included alkaloids, flavonoids, saponins, tannins, phenols, and steroids.

Test for flavonoids: 5ml of plant extract was mixed with 4–3 drops of sodium hydroxide solution. The emergence of a strong yellow color upon the addition of dilute acid indicates flavonoid presence.

Test for saponin (foam test): 5ml of extract was diluted with water to make up to 15ml in a cylinder container. The suspension was shaken in a graduated cylinder for 15 minutes, and saponins were identified by the production of two cm of foam.

Test for tannins (ferric chloride test): The extract was diluted in 5ml of distilled water, and a few drops of neutral 5% ferric chloride solution were added; a dark green hue indicated the presence of tannin compounds.

Test for phenols (lead acetate test): After dissolving 3 milliliters of plant extract in distilled water and adding 3 milliliters of a 10% lead acetate solution, a large white precipitate formed, signifying the presence of phenolic compounds.

Test for steroids: Chloroform was used to extract the material, and a few drops of acetic anhydride were added to the extract. After treatment, the extract was heated and allowed to cool. After that, strong sulfuric acid was applied. The presence of steroids was verified by the development of a blue green solution.

Test for alkaloids: Along the test tube's sidewalls, two drops of Meyer's reagent (potassium mercuric iodide solution) were added to two milliliters of plant sample extract. Alkaloids are present when a white, creamy precipitate appears.

Proximate Analysis

Determination of moisture content: The air oven method was used to determine the moisture content. After being cleaned, the crucibles were dried in an oven. After letting them cool in the desiccator, their weight was recorded. After that, 1.0g of the sample was put into the crucibles and allowed to dry at a temperature of 103 to 105 degrees Celsius. After cooling in a desiccator, the dry samples were weighed. After that, they were put back in the oven, and the procedure was repeated until the weights remained constant. The following formula was used to determine the moisture content:

$$\% \text{ Moisture content} = \frac{\text{weight loss} \times 100}{\text{Weight (g) of sample}}$$

Determination of Ash Content: A finely powdered sample was weighed into a clean, dried, previously weighted crucible with a lid (W1) to quantify the amount of ash present. After removing the cover, the sample was lit over a low flame to char the organic matter. After that, the crucible was heated to 600 degrees Celsius for six hours in a muffle furnace until it had entirely ashed. After that, it was moved straight to desiccators, chilled, and weighed right away (W_2).

$$\% \text{ Ash content} = \frac{\text{weight loss} \times 100}{\text{Weigh (g) of sample}}$$

Determination of crude protein Procedure: The crude protein was determined using microKjeldah method as described by the Association of Official Analytical Chemists, 2000.

$$\% \text{ N (wet)} = \frac{(A-B) \times 1.4007 \times 100}{\text{Weigh (g) of sample}}$$

A= volume (ml) std HCl × normality of std HCl

B= volume (ml) std NaOH × normality of std NaOH

Determination of crude Fat: Using the Soxhlet device, crude fat was calculated. A weighted filter paper was filled with 10g of the sample, which was then carefully folded. The pre-weighed thimble (W1) was filled with this. After inserting the thimble containing the sample (W2) into the Soxhlets apparatus, n-hexane (which has a boiling range of 60 to 80 degrees Celsius) was used for 6 hours of extraction under reflux. After extraction, the thimble was cooled in a desiccator and weighed after being dried in an oven set at 100°C for roughly 30 minutes to remove the solvent (W3). Next, the amount of fat removed from a certain amount of sample was computed: The method used for this was gravimetric. A thimble was filled with 5g of the sample. Petroleum ether at 60–80°C was used for the three-hour extraction process. The extractant was later distilled

$$\% \text{ Fat} = \frac{\text{weight of lipid X 100}}{\text{Weight of sample}}$$

Determination of Crude Fibre: Crude fiber was calculated by weighing the fat-free extract that was left over after estimating the ether extract. The digestible fraction is then hydrolyzed away by heating it repeatedly with diluted acid and diluted alkali. After drying, the residue is weighed. The fiber content is this weight less the weight of ash.

Determination of Total Carbohydrate: By adding the percentages of moisture, ash, crude protein, and fat (ether extract) and deducting from 100% CHO = 100- (Sum of the percentages of moisture, ash, fat, protein, and crude fiber), the percentage carbohydrate content of the sample was calculated. The proportion of the sample's total carbohydrate content was calculated from the difference in values. 100 - (% fat + % ash + % wet + % protein) is the total amount of carbohydrates.

Mineral composition: The Gallenkamp Flame Analyzer was used to assess sodium and potassium, while the Atomic Absorption Spectrophotometer (N1100A model) was used to determine calcium, magnesium, iron, zinc, and copper. Using the Spectrumlab 752S Spectrophotometer and phosphovanado molybdate colorimetric methods, the phosphorus level was ascertained.

Results

Comparism qualitative phytochemical composition of *Rinorea oblongifolia* plant parts *(*leaves, stem and root) are presented in Table 1 below. Saponin, steroid and alkaloid were present in all

the parts evaluated. Flavonoid and tannin were detected in the stem and root while alkaloid was abundant in the leaves.

Table 1: Phytochemical Screening of *Rinorea oblongifolia*

Phytochemical Test	Leaves	Stem	Root
Saponin	+	++	+
Flavonoid	++	+	+
Alkaloid	+++	ND	ND
Phenolic	++	++	+
Tannin	ND	ND	ND
Steroid test	+	+	+

KEY ND = Not Detected; + = Trace;++ = Moderately Present; +++ = Abundant

The proximate analysis of *oblongifolia* stem, root, and leaf are presented in Table 2. below. Moisture, crude fibre, crude protein and carbohydrate were recorded highest in the leaves while ash and crude fat were recorded highest in the root.

Table 2: Proximate Analysis of *Rinorea oblongifolia*

Proximate Analysis	Leaves	Stem	Root
Moisture	9.17	7.67	8.45
Ash	1.34	1.53	2.45
Crude fat	4.31	4.51	4.61
Crude protein	9.91	9.45	9.62
Crude fibre	3.81	4.23	4.44
Carbohydrate	75.26	70.69	70.80

Table 3 shows the elemental composition of *oblongifolia* leaf, stem and root. Iron, calcium and copper were recorded highest in the root while Sodium, potassium, phosphorous and magnesium were observed in large quantities in the leaf.

Table 3: Elemental Composition of *Rinorea oblongifolia*

Mineral Composition	Leaves	Stem	Root
Fe (Mg/100g)	12	11.7	12.7
Na (Mg/100g)	10.2	8.6	8.9
K (Mg/100g)	14	12.2	11.7
Ca (Mg/100g)	6.3	6.8	6.9
Cu (Mg/100g)	6.1	6.2	6.9
P (Mg/100g)	13.9	13.1	12.2
Zn (Mg/100g)	2.73	2.92	2.92
Mg (Mg/100g)	2.22	2.19	2.12

KEY: Fe: Iron**;** Na: Sodium**;** K: Potassium**;** Ca: Calcium**;** Zn: Zinc**;** Cu: Copper**;** P: Phosphorous; Mg: Magnesium

Table 4-6. shows the antioxidant screening of *oblongifolia* leaves plant parts across different concentrations. Table 5 shows the antioxidant screening of *oblongifolia* stem across different concentrations of different reagents.

Table 4: Antioxidant Screening of *Rinorea oblongifolia* leaves

Con. (ug/ml)	DDPH	ABTS	FRAP	NO	TPH (mg GAE/g)	TFC (mg QE/g)
100	31.23	37.6	42.00	13.56	28.75	2.21
200	48.26	43.1	56.74	18.23	28.66	2.31
300	63.15	54.0	64.21	27.65	29.21	2.34
400	76.00	65.2	74.23	36.42	28.65	2.31
500	86.43	73.6	83.00	47.32	29.00	2.30

DDPH: Diphenyl-1-Pierylhyhydrazyl; ABTS: Azino – Bis-3-Ethyl Benzothiazolin-6-Sulfonic Acid; FRAP: Ferric Reducing Ability of Plasma; NO: Nitric Oxide; TPH: Total Phenol; TFC: Total Flavonoid Content

Table 5: Antioxidant Screening of *Rinorea oblongifolia* Stem

Con. (ug/ml)	DDPH	ABTS	FRAP	NO	TPH (mg GAE/g)	TFC (mg QE/g)
100	31.89	37.10	43.11	14.01	24.01	2.22
200	48.91	42.84	59.30	18.23	25.94	2.29
300	63.81	56.92	66.40	26.18	30.01	2.31
400	77.52	67.37	73.56	37.91	28.68	2.30
500	86.32	74.12	83.36	42.52	26.86	2.39

KEY: DDPH: Diphenyl-1-Pierylhyhydrazyl; ABTS: Azino – Bis-3-Ethyl Benzothiazolin-6-Sulfonic Acid; FRAP: Ferric Reducing Ability of Plasma; NO: Nitric Oxide; TPH: Total Phenol; TFC: Total Flavonoid Content

Table 6: Antioxidant Screening of *Rinorea oblongifolia* root

Con. (ug/ml)	DDPH	ABTS	FRAP	NO	TPH (mg GAE/g)	TFC (mg QE/g)
100	34.32	37.10	50.11	13.99	26.01	2.34
200	43.36	47.94	57.33	17.93	26.94	2.20
300	63.10	55.02	62.40	26.77	30.51	2.22
400	70.21	62.77	73.56	36.54	25.68	2.22
500	84.43	77.02	83.66	41.63	28.86	2.21

KEYDDPH: Diphenyl-1-Pierylhyhydrazyl; ABTS: Azino – Bis-3-Ethyl Benzothiazolin-6-Sulfonic Acid; FRAP: Ferric Reducing Ability of Plasma; NO: Nitric Oxide; TPH: Total Phenol; TFC: Total Flavonoid Content

Discussion

Rinorea oblongifolia is a plant containing a wide range of bioactive compounds and has been found to possess significant pharmacological properties. In Table 1,saponins were found in

moderate amounts in the leaf and stem and root. Alkaloids are present in abundance in the leaves, but are not detected in other parts of the plant. Research has shown that both saponins and alkaloids possess analgesic, anti-malarial, and anticancer properties (Thawabteh *et al.*, 2019). The high abundance of alkaloids in *oblongifolia* indicates potential for medicinal use, particularly in pain management and cancer treatment. The leaf's richness in flavonoids supports the local use of this plant for pain relief (Liga *et al.*, 2022). Phenolic compounds moderately present in both leaves, stem, and root, are known for their antioxidant properties and potential for preventing chronic diseases. Steroids, present in trace amounts across the plant, are known for their anti-inflammatory and immunomodulatory activities. The consistent presences of steroids in oblongifolia indicate its potential for managing inflammation and related disorders. The discovery of these phytochemicals corroborates the findings of Karioti *et al* (2011) that reported the presence of saponin, alkaloid, flavonoid, phenols from the plant parts of *Viola odorata* a flowering plant sharing the same family with *oblongifolia.*

In Table 2, the proximate analysis of *oblongifolia* plant parts reveal significant variations in the nutritional composition. The leaf of this plant had the highest moisture content (9.17%), followed closely by the root (8.45%), and the stem (7.67%). High moisture content in leaves is typical, as leaves generally contain more water to facilitate photosynthesis and transpiration processes.*Rinorea oblongifolia*'s moisture content is similar to that of other medicinal plants, such *Moringa oleifera* leaves, which have been reported to have a moisture content of 13.67% (Salihu *et al.*, 2024). High moisture content indicates the potential for higher perishability and necessitates appropriate drying methods for storage and preservation. Ash content representing the total mineral content, is highest in the root (2.45%), followed by the stem (1.53%), and the leave (1.34%). The high ash content in the root indicates a higher mineral concentration, which is consistent with observations made in other medicinal plants with roots, such as ginseng, known for its elevated mineral content (Jang *et al.*, 2022). This mineral richness in the root highlights its potential application in formulations designed for mineral supplementation.

Crude fat content is highest in the root (4.61%), with the stem (4.51%) and leaves (4.34%) showing slightly lower values. The presence of fats in all parts of the plant is notable, as these fats could contribute to the plant's caloric value and serve as a source of essential fatty acids. Compared to many other green vegetables, such spinach, which has a crude fat level of about 0.39%, *oblongifolia* has a greater crude fat content (USDA, 2020). This suggests that *oblongifolia* could provide a significant energy source. The leaves contain the highest crude protein content (9.91%), slightly higher than the root (9.62%) and stem (9.45%). Protein

content in leaves is crucial for dietary considerations, especially in regions where plant-based diets are predominant. The protein content of *oblongifolia* is similar to that of *Amaranthus sp*, which also have a high protein level of 9.8%. This highlights the potential of *oblongifolia* leaves as a valuable protein source in dietary applications. The highest crude fibre content is found in the root (4.44%), followed by the stem (4.23%) and leaves (3.81%). High fibre content is beneficial for digestive health, promoting bowel regularity and preventing constipation. The fiber content in *oblongifolia* is comparable to that of kale, which contains approximately 4.1% fiber (USDA, 2020). This suggests that *oblongifolia* could be effectively used as a dietary fibre supplement.

Carbohydrate content is relatively consistent across all parts of the plant, with the leaves (75.26%), stem (70.69%), and root (70.80%) showing similar values. Carbohydrates are a vital energy source, and the high carbohydrate content indicates that *oblongifolia* can contribute significantly to energy intake. The carbohydrate levels are higher than those found in many other leafy greens, such as lettuce, which contains about 2.97% carbohydrates (USDA, 2020). The findings in this study are similar to the study by Fernández-Bobey *et al*. (2023) where several chemical constituents were found in the family of *Violaceae.* Table 3 rich mineral composition of the different plant parts, which is important for understanding its nutritional value. The plant's iron content is highest in the root, which is essential for oxygen transport and enzyme function. According to Fernández-Bobey *et al*. (2023), the root has ahigher iron content than spinach, which suggests it could be a useful source for iron supplements. The leaves have the highest potassium levels, which are essential for heart and muscle health. Potassium prevents gastroenteritis and is a natural treatment for constipation (Hilda *et al.*, 2023).

Calcium and copper are found more in the root than other plant parts evaluated. The leaves have the highest phosphorus content, which is crucial for bone health and energy production (Serna and Bergwitz, 2020). The zinc levels are consistent across all parts of the plant, suggesting that all parts of *oblongifolia* could contribute to immune support and wound healing. The leaves have the highest magnesium level, which is similar to spinach's 79 mg/100g content (Serna and Bergwitz, 2020). Instant uptake of potassium and magnesium is crucial for regulating bowel movements and managing diarrheal conditions. Table 4. – 6 shows that both plant parts exhibit high DDPH, ABTS, and FRAP activities, with significant NO scavenging abilities. The rich phenolic and flavonoid content further supports their use as effective antioxidants. The stem shows significant DDPH scavenging activity, which is crucial for protecting cells from oxidative damage. This similar DDPH values have been observed in other

medicinal plants like green tea. However, antioxidant activity has been noted in another species from the *Violaceae* family, specifically *Rinorea dentata*, indicating that this plant family may have significant potential for antioxidant properties (Ibukun *et al.*, 2020). Comparative analysis shows that both the stem and root of *oblongifolia* exhibit strong DDPH and ABTS activities, indicating substantial free radical scavenging abilities. The root shows slightly higher DDPH activity at lower concentrations, while the stem exhibits comparable or slightly higher ABTS activity at higher concentrations.

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