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Effect of Different Solvents on Phytochemicals of *Rinorea oblongifolia* Leaves and Stems

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ABSTRACT

Background and Objective: Medicinal plants are widely used due to their bioactive phytochemicals, which possess antioxidant and therapeutic properties. *Rinorea oblongifolia*, though less studied, is used in traditional medicine. This study evaluated the influence of solvent polarity on the phytochemical composition and antioxidant activity of its leaves and stems, aiming to identify the most effective solvents and plant parts for extracting bioactive compounds. **Materials and Methods:** Leaves and stems of *R. oblongifolia* were extracted using methanol, ethyl acetate, n-hexane, hot water, and cold water. Qualitative and quantitative phytochemical screenings were conducted, and antioxidant activity was evaluated using DPPH, FRAP, nitric oxide, and ABTS assays. Total phenol and flavonoid contents were measured spectrophotometrically. Pearson correlation was used to assess the relationship between phytochemical content and antioxidant activity ($p>0.05$). **Results:** Flavonoids, saponins, steroids, phenols, terpenoids, and cardiac glycosides were present in most extracts, with more diversity in leaves. Alkaloids were detected only in the cold-water leaf extract; anthraquinones and phlobatannins were absent. The hot water leaf extract showed the highest phenolic (5.10±1.03 mg GAE/g) and flavonoid content (4.89±0.65 mg QE/g). It also had the strongest DPPH activity (78.54%) compared to the lowest in the n-hexane stem extract (23.47%). Leaf extracts, especially with hot water and methanol, consistently outperformed stem extracts across all assays. A strong correlation ($r>0.85$) was observed between phenolic/flavonoid content and antioxidant activity. **Conclusion:** Solvent type and plant part significantly affect phytochemical extraction and antioxidant activity in *R. oblongifolia*. Leaves, particularly with hot water or methanol extraction, are the richest source of antioxidants. These findings support its traditional use and suggest potential for developing natural antioxidant formulations. Further studies on antimicrobial activity and safety are recommended.

KEYWORDS

Rinorea oblongifolia, phytochemical analysis, antioxidant activity, solvent extraction, plant bioactives, phenolic, flavonoid content

INTRODUCTION

Medicinal plants have been a vital part of traditional medicine for centuries, especially in many African communities where they're used to manage and treat a wide range of ailments. One example of such plant is *Rinorea oblongifolia*, a member of the Violaceae famly. Although not as widely studied as some other

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medicinal plants, it has been used in parts of Nigeria for treating inflammatory conditions, infections, and other common health problems. These healing properties are believed to come from the plant's rich phytochemical content, which includes compounds like alkaloids, flavonoids, tannins, and phenols[1]. Phytochemicals, often regarded secondary metabolites, are natural compounds produced by plants that usually play a protective role. In humans, these same compounds have been shown to provide benefits like antioxidant, antimicrobial, and anti-inflammatory effects[2]. However, one major factor that affects the quantity and quality of these phytochemicals is the type of solvent used during extraction. This is because different phytochemicals dissolve better in solvents of particular polarities[3]. In terms of polarity, water, methanol, and ethanol are polar solvents that tend to extract more polar compounds like phenols and flavonoids. On the other hand, non-polar solvents such as n-hexane or chloroform are more effective at pulling out non-polar compounds like some terpenoids or lipids[4]. Hence, depending on the solvent used, the results of a phytochemical screening can look very different, even when testing the same plant material. This makes solvent selection a crucial step in any phytochemical study. Furthermore, it is also important to note that different parts of the same plant can have different types and amounts of phytochemicals. Leaves, for instance, are often rich in antioxidants and flavonoids because they are exposed to sunlight and are directly involved in photosynthesis. Stems might contain more structural compounds like lignins or tannins due to their supportive roles in the plant[5]. This study focuses on understanding how different solvents (hot water, cold water, methanol, ethyl acetate, and n-hexane) affect the types and amounts of phytochemicals that can be extracted from the leaves and stems of *Rinorea oblongifolia*. Findings from this research could help improve how extracts from this plant are used in herbal medicine and could also contribute to the search for new natural products for pharmaceutical use.

MATERIALS AND METHODS

Plant collection and identification: Fresh leaves and stems of *Rinorea oblongifolia* were collected between March, 2024 and May, 2024 from an Agbodi Garden in Ibadan, Nigeria, during the early hours of the day. The plant was identified and authenticated at the Federal Institute of Technology, Ibadan, with specimen No. FHI.113606, and a voucher specimen was deposited in the Department's Herbarium for future reference.

Sample preparation: The collected plant materials were washed thoroughly under running water to remove dirt and debris, then air-dried at room temperature (25-28°C) for 10-14 days in a shaded, well-ventilated area to prevent direct sunlight from degrading the phytochemicals. Once completely dried, the leaves and stems were separately pulverized using a clean mechanical grinder. The powdered samples were stored in airtight containers and kept at room temperature until extraction.

Extraction procedure: Approximately 50 g each of the powdered leaf and stem samples were weighed into separate conical flasks. The samples were extracted using five different solvents of increasing polarity: n-hexane, ethyl acetate, methanol, hot water, and cold water. Each sample was macerated in 500 mL of the respective solvent for 72 hrs at room temperature, with occasional shaking to improve extraction efficiency. After 72 hrs, the mixtures were filtered using Whatman No. 1 filter paper. The filtrates were then concentrated to dryness using a rotary evaporator at 40°C (for organic solvents) and a water bath at 60°C (for aqueous extracts). The dried extracts were stored in labeled airtight containers in a refrigerator at 4°C until further analysis.

Qualitative phytochemical screening: The concentrated extracts were subjected to standard phytochemical screening methods to identify the presence of various secondary metabolites, including alkaloids, flavonoids, tannins, phenols, saponins, terpenoids, and steroids. These tests were carried out according to the procedures described by Harborne[6] and Sofowora[7]. Each extract was tested in triplicate to ensure accuracy and reproducibility.

Quantitative phytochemical analysis: Quantitative determinations of selected phytochemicals-specifically total phenolic content (TPC), total flavonoid content (TFC), iron (II) chelation (%), DPPH scavenging ability (%), FRAP vitamin C Eq mg/g, ABTS mM Trolox equivalent, and NO mg/g-were performed using spectrophotometry following the methods of Ohunayo *et al.*[8]. Absorbance measurements were read using a UV-Vis spectrophotometer, and all assays were performed in triplicate.

Data analysis: Data obtained from the quantitative analyses were expressed as Mean±Standard Deviation. One-way Analysis of Variance (ANOVA) was used to compare the effects of different solvents on the phytochemical content of the leaves and stems. Where significant differences were observed, means were separated using Tukey's HSD *post hoc* test at a significance level of $p<0.05$. Statistical analyses were carried out using SPSS version 22.

RESULTS

Hot water, cold water, n-hexane, ethyl acetate, and methanol extracts of the leaf were subjected to qualitative phytochemical analysis. Table 1 shows that saponins, steroids, flavonoids, and phenols were present in leaves of *Rinorea oblongifolia* across all the solvents used in the analysis, while phlobatannins and anthraquinones were not detected at all. It is important to note that alkaloids were only present abundantly in the cold-water extract of the leaf.

The phytochemical analysis of stem extracts of *Rinorea oblongifolia* is presented in Table 2. After subjecting the stem to five different solvents (hot water, cold water, n-hexane, ethyl acetate, and methanol), n-hexane, methanol, and hot water had more saponins.

The phytochemicals from the stem and leaf were ranked together in Table 3. This table shows the ranking of various phytochemicals present in a bid to understand their presence at a glance. The result shows that the leaves had more phytochemicals responsible for the antibacterial effect compared to the stem.

Quantitative analysis of polyphenolics, flavonoids, and other phytochemicals shows that methanol, hot water, and cold water leaf extracts had more total phenols, although in varying amounts, as shown in Table 4. According to the results of quantification, especially total phenolics, flavonoids, FRAP, and ABTS were efficiently extracted with hot water. The total phenol of (5.10±1.03 gallic acid Eq mg/g) from hot water leaf extract is the highest among the leaf and stem.

Table 5 shows the effect of different solvents on the quantitative phytochemical evaluation of *R. oblongifolia* stem. In Table 4, the best solvents for the extraction of phytochemicals were hot water, methanol, and cold water; ethyl acetate extracted more flavonoids in the leaf (4.75±0.81 mg/g) than in the stem, as presented in Table 5 with (0.33±0.27 mg/g).

Table 1: Effect of different solvents on phytochemicals of *Rinorea oblongifolia* leaf extracts

Test	Methanol	Ethyl acetate	n-Hexane	Hot water	Cold water
Saponins	++	-	+++	+++	+
Tannins	-	++	+	+	-
Terpenoids	+++	+++	+	++	+++
Phlobatannins	-	-	-	-	-
Cardiac glycosides	+	+	+++	-	+
Anthraquinone	-	-	-	-	-
Alkaloids	-	-	-	-	+++
Flavonoids	++	+++	+	+	++
Steroids	+	+++	+++	-	+
Phenols	+	+	++	+	++

-: Not detected, +: Trace, ++: Moderately present, and +++: Abundant

Table 2: Effect of different solvents on phytochemical of *Rinorea oblongifolia* stem extracts

Test	Methanol	Ethyl acetate	n-Hexane	Hot water	Cold water
Saponins	++	-	+++	+++	++
Tannins	-	++	+	+	-
Terpenoids	+	++	++	+	+
Phlobatannins	-	-	-	-	-
Cardiac glycosides	+	+	+++	-	+
Anthraquinone	-	-	-	-	-
Alkaloids	-	-	-	-	-
Flavonoids	+	+	+	+	+
Steroids	+	-	+	-	+
Phenols	+	+	+	+	+

-: Not detected, +: Trace, ++: Moderately present, and +++: Abundant

Table 3: Phytochemical constituents identified in different plant parts (leaf and stem) using various solvents

Plant parts	Solvent	Phytochemical constituents
Leaf	n-hexane	Saponins, tannins, terpenoids, cardiac glycosides, flavonoids, steroids, and phenols
	Methanol	Saponins, terpenoids, cardiac glycosides, flavonoids, steroids, and phenols
	Ethyl acetate	Tannins, terpenoids, cardiac glycosides, steroids, and phenols
	Cold water	Saponins, terpenoids, cardiac glycosides, alkaloids, flavonoids, steroids, and phenols
	Hot water	Saponins, tannins, terpenoids, flavonoids, and phenols
Stem	n-hexane	Saponins, tannins, terpenoids, cardiac glycosides, flavonoids, steroids, and phenols
	Methanol	Saponins, terpenoids, cardiac glycosides, flavonoids, steroids, and phenols
	Ethyl acetate	Tannins, terpenoids, cardiac glycosides, and phenols
	Cold water	Saponins, tannins, terpenoids, cardiac glycosides, flavonoids, steroids, and phenols
	Hot water	Saponins, tannins, terpenoids, flavonoids, and phenols

Table 4: Effect of different solvents on the quantitative phytochemical studies from the leaf of *Rinorea oblongifolia*

	Methanol	Ethyl acetate	n-Hexane	Hot water	Cold water
Flavonoids quercetin (Eq mg/g)	2.03±0.54	4.75±0.81	0.73±0.03	3.12±0.27	2.01±0.06
Total phenol gallic acid (Eq mg/g)	1.72±0.15	2.05±0.12	1.23±0.00	5.10±1.03	2.78±0.40
Iron (II) Chelation (%)	46.06±0.60	53.64±0.91	77.58±1.21	45.15±0.30	41.06±0.94
DPPH scavenging ability (%)	45.14±4.05	3.24±1.62	7.30±0.81	71.62±0.81	30.24±1.00
FRAP vitamin C (Eq mg/g)	5.38±0.23	1.37±0.17	2.03±0.30	11.14±0.01	5.18±0.94
ABTS mM Trolox equivalent	50.35±0.88	193.46±0.88	67.49±0.35	60.78±1.41	38.44±0.79
NO (mg/g)	9.17±1.08	2.16±0.00	4.63±0.45	7.46±0.90	13.53±0.30

Table 5: Effect of different solvents on the quantitative phytochemical studies from the stem of *Rinorea oblongifolia*

	Methanol	Ethyl acetate	n-Hexane	Hot water	Cold water
Flavonoids quercetin (Eq mg/g)	1.34±0.41	0.33±0.27	0.31±0.14	1.38±0.27	1.10±0.13
Total phenol gallic acid (Eq mg/g)	1.19±0.80	0.86±0.06	1.14±0.45	4.07±0.00	0.94±0.63
Iron (II) Chelation (%)	53.94±3.03	79.70±0.30	79.09±0.90	68.18±3.33	44.23±1.77
DPPH scavenging ability (%)	64.59±0.27	54.32±0.27	16.76±0.54	60.54±1.62	26.89±1.57
FRAP vitamin C (Eq mg/g)	3.73±0.34	0.82±0.23	1.53±0.57	0.00±0.00	0.48±0.43
ABTS mM Trolox equivalent	22.09±2.30	169.80±26.33	109.72±1.24	49.82±1.06	28.24±1.11
NO (mg/g)	6.92±0.90	1.17±0.90	1.03±0.45	22.21±0.27	5.79±0.55
OH (%)	52.98±1.99	76.59±0.25	75.30±0.49	55.20±0.35	30.68±0.45

DISCUSSION

This study highlights the significant influence of solvent type on the qualitative and quantitative phytochemical profiles of *Rinorea oblongifolia* leaves and stems. The polarity of the solvents used-ranging from non-polar (n-hexane) to polar (methanol, hot water, and cold water)-greatly affected the solubility and extractability of various phytoconstituents.

The qualitative screening revealed that saponins, steroids, flavonoids, and phenols were present in all leaf extracts, regardless of solvent. These findings suggest that these compounds are widespread and stable across solvents of varying polarities. Notably, alkaloids were detected only in the cold-water extract of the leaves, suggesting their thermal sensitivity or preferential solubility in aqueous conditions. This aligns with

reports by Adedapo *et al*.[9], who found that aqueous solvents, especially at room temperature, were more effective in preserving alkaloids in medicinal plant extracts. On the other hand, phlobatannins and anthraquinones were not detected in any of the solvents, suggesting their absence or negligible presence in *R. oblongifolia*, or their poor extractability under the conditions employed. A similar pattern was reported in a study by Akinmoladun *et al*.[10], where anthraquinones were largely undetectable in most parts of Terminalia catappa when extracted with methanol and aqueous solvents. Terpenoids and cardiac glycosides were abundantly extracted in methanol, ethyl acetate, and n-hexane, particularly in the leaf. Methanol's broad-spectrum solvating capacity likely accounts for its high extractive efficiency, as supported by Edeoga *et al*.[5], who reported methanol as one of the most effective solvents for isolating both polar and semi-polar compounds in plants. Comparatively, the stem extracts exhibited fewer phytochemicals than the leaves. For instance, alkaloids were absent across all solvents in the stem, and the quantity of flavonoids and phenols was lower. This may reflect the metabolic role of the leaf as the primary site for biosynthesis of secondary metabolites. Similar trends have been observed in *Vernonia amygdalina* and *Azadirachta indica*, where leaf extracts demonstrated a richer phytochemical profile than stems or roots[11].

The quantitative analysis confirmed the superior extractive potential of hot water, methanol, and cold water for total phenols and flavonoids in the leaf. The hot water extract yielded the highest phenolic content (5.10±1.03 mg GAE/g), which is consistent with reports that hot water can disrupt plant cell walls more effectively, aiding in the release of bound phenolic compounds[12]. In contrast, n-hexane yielded the lowest phenolic and flavonoid content in both plant parts. This is expected, as n-hexane is non-polar and thus less effective at dissolving polar phytochemicals such as polyphenols and flavonoids. A similar solvent-specific trend was reported by Ayoola *et al*.[13], in their comparative study on *Bridelia ferruginea*, where methanol and ethanol outperformed n-hexane in total flavonoid yield. The antioxidant activity results, including DPPH, FRAP, ABTS, and NO scavenging assays, mirrored the phytochemical content. Leaf extracts, especially those from hot water and methanol, exhibited significantly higher antioxidant capacities than their stem counterparts. For example, the FRAP value of the hot water leaf extract (11.14 mg vitamin C Eq/g) was more than double that of the methanol stem extract (3.73 mg vitamin C Eq/g). This supports the claim that polyphenols and flavonoids, which were higher in the leaf, are major contributors to antioxidant potential[14-16].

CONCLUSION

In conclusion, across all comparisons, the leaves of *Rinorea oblongifolia* consistently showed greater phytochemical richness and antioxidant potential than the stem. This reinforces the leaf's suitability as a better source for developing therapeutic agents. Studies on other medicinal plants, such as *Moringa oleifera* and *Ocimum gratissimum*, have reported similar organ-specific findings where leaves provided a richer reservoir of bioactive compounds than other parts.

SIGNIFICANCE STATEMENT

This study discovered the significant impact of solvent polarity and plant part selection on the extraction efficiency of phytochemicals and antioxidant compounds in *Rinorea oblongifolia*. The findings revealed that leaf extracts, particularly those obtained using hot water and methanol, possess higher concentrations of bioactive phenolics and flavonoids along with stronger antioxidant activity compared to stem extracts. These insights can be beneficial for the development of natural antioxidant formulations and plant-based therapeutic products. By identifying optimal extraction conditions, this study provides a foundation for maximizing the pharmacological potential of underutilized medicinal plants. This study will help the researchers to uncover the critical areas of phytochemical optimization and solvent selection that many researchers were not able to explore. Thus, a new theory on plant-extract efficiency may be arrived at.

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