SHORT COMMUNICATION

Qualitative and quantitative phytochemical profiling of white water lily (*Nymphaea lotus* L.) leaves in South-west Nigeria

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Abstract

Nymphaea lotus *L., an important aquatic medicinal plant, is a valuable source of bioactive compounds. However, its phytochemical profile remains underexplored in regions such as South-West Nigeria. This study was designed to investigate the phytochemical composition of Nymphaea lotus leaves (NLL) collected from various aquatic bodies in southwest Nigeria. Qualitative and quantitative assays for saponins, tannins, flavonoids, cardiac glycosides, anthraquinones, terpenoids, steroids, alkaloids, and phenols were conducted using standard procedures. The qualitative and quantitative analyses revealed a rich phytochemical profile in Nymphaea lotus leaves. The qualitative results indicated that terpenoids, alkaloids, flavonoids, saponins, tannins, steroids and phenols were abundantly present in the leaves (++ve), while cardiac glycosides and anthraquinones were detected in lower concentrations (+ve). Quantitative analyses showed that terpenoids were the most abundant (3.10% w/w), followed by saponins (1.70% w/w), alkaloids (1.05% w/w), tannins (0.91 ± 0.003% w/w), phenolic compounds (0.62 ± 0.002% w/w), and flavonoids (0.61 ± 0.018% w/w). These findings confirm that Nymphaea lotus leaves are rich in bioactive compounds, particularly terpenoids. These compounds highlight the potential of Nymphaea lotus as a natural feed additive in animal nutrition.*

Keywords: Phytochemical screening; Secondary metabolites; Aquatic plant; *Nymphaeaceae*; Bioactive compounds.

Profilage Phytochimique Qualitatif et Quantitatif des Feuilles de Nénuphar Blanc (*Nymphaea lotus* L.) dans le Sud-Ouest du Nigéria

Résumé

Nymphaea lotus L., une plante médicinale aquatique importante, constitue une source précieuse de composés bioactifs. Cependant, son profil phytochimique reste peu exploré dans des régions comme le Sud-Ouest du Nigeria. Cette étude a été conçue pour analyser la composition phytochimique des feuilles de Nymphaea lotus (FNL) collectées dans différents plans d'eau du Sud-Ouest du Nigéria. Des tests qualitatifs et quantitatifs ont été réalisés pour détecter les saponines, tanins, flavonoïdes, glycosides cardiotoniques, anthraquinones, terpénoïdes, stéroïdes, alcaloïdes et phénols, en suivant des procédures standardisées. Les analyses qualitatives et quantitatives ont révélé un profil phytochimique riche dans les feuilles de Nymphaea lotus. Les résultats qualitatifs ont indiqué que les terpénoïdes, alcaloïdes, flavonoïdes, saponines, tanins, stéroïdes et phénols étaient abondamment présents (++ve), tandis que les glycosides cardiotoniques et les anthraquinones ont été détectés à des concentrations plus faibles (+ve). Les analyses quantitatives ont montré que les terpénoïdes étaient les composés les plus abondants (3,10 % p/p), suivis des saponines (1,70 % p/p), des alcaloïdes (1,05 % p/p), des tanins (0,91 ± 0,003 % p/p), des composés phénoliques (0,62 ± 0,002 % p/p) et des flavonoïdes (0,61 ± 0,018 % p/p). Ces résultats confirment que les feuilles de Nymphaea lotus sont riches en composés bioactifs, notamment en terpénoïdes. Ces composés mettent en évidence le potentiel de Nymphaea lotus comme additif alimentaire naturel en nutrition animale.

Mots-clés : Dépistage phytochimique ; Métabolites secondaires ; Plante aquatique ; *Nymphaeaceae* ; Composés bioactifs.

Introduction

Phytogenic feed additives have gained increasing interest as potential replacements for antibiotics in animal production (Adebiyi *et al*., 2025). The rise of antibiotic resistance in both humans and animals is one of the most critical challenges in global health, creating an urgent need for alternative therapeutic strategies (Catry *et al.,* 2003; European Commission, 2003). Among these alternatives, medicinal plants have gained significant attention due to their long-standing use in traditional or folk medicine and their potential to yield unresearched and novel bioactive compounds. Medicinal plants are not only cost-effective but also easily accessible sources of nutrients and therapeutic agents that can significantly enhance human and animal health.

Nymphaea lotus, commonly known as the water lily or Egyptian lotus, is an abundant aquatic plant that has been reported to possess both antimicrobial and antioxidant properties, making it a valuable resource in traditional medicine. Belonging to the Nymphaeaceae family, *N. lotus* is a rooted, tuberous herb that thrives in temperate and tropical regions worldwide (Wiersema, 1982). The plant is rich in tannins, alkaloids, flavonoids, saponins, terpenoids, phenols, and anthraquinones as well as primary metabolites (Abelti *et al*., 2023). These phytochemicals contribute to its use in treating various ailments, including indigestion, heart diseases, stomach aches, and cancer, as well as serving as an aphrodisiac, astringent, cardiotonic, sedative, and anti-inflammatory agent (Duke, 2007).

The medicinal properties of the leaves of *Nymphaea lotus* have largely been attributed to the presence of gallic and ellagic acids. Also, the presence of these compounds in *Nymphaea lotus* contributes to its antioxidant activity, which has been associated with potential health benefits such as protection against oxidative stress, inflammation, and chronic diseases (Sowemimo *et al*., 2007; Thippeswamy *et al*., 2011; Gupta & Pandey, 2014).

Despite its medicinal potential, *Nymphaea lotus* remains under-researched, particularly in the context of its phytochemical composition and bioactivity, especially in regions like Nigeria where it is prevalent. This gap in comprehensive information underscores the importance of the present study. Therefore, this study aimed to investigate the phytochemical constituents and potential bioactivity in *Nymphaea lotus* leaves.

Materials and Methods

Sample Collection and Identification

Fresh leaves of *Nymphaea lotus* (water lily) were collected from lakes and ponds in Ibadan, Oyo State, Nigeria. The plant was identified and authenticated as *Nymphaea lotus* leaves by a taxonomist in the Department of Botany, Faculty of Science, University of Ibadan, Oyo State, Nigeria.

Sample preparation

The leaves were carefully selected from freshly harvested *Nymphaea lotus* plants, free from visible damage or contaminants. The selected leaves were rinsed under tap water to remove impurities. After rinsing, the leaves were cut into small, uniform pieces and air-dried for seven days at an ambient temperature of 30-34^{0}C. Once fully dried, the leaves were ground into a fine powder using a laboratory blender. The powdered sample was then stored in a Ziploc bag under cool, dry conditions until further analyses.

Qualitative phytochemical screening

The preliminary phytochemical screening of the plant leaves was conducted to detect the presence or absence of alkaloids, saponins, flavonoids, tannins, anthraquinones, terpenoids, cardiac glycosides, phenols, and steroids. The analyses were performed using established standard methods as documented by Ayoola *et al*. (2008) and Shaikh and Patil (2020).

Quantitative analysis of phytochemicals in Nymphaea lotus leaves

Total alkaloids, flavonoids, saponins, tannins, terpenoids, and phenol contents of leaves of Nymphaea lotus were quantitatively determined in triplicate using the method described by Krishnaiah *et al. (*2009) and Tambe and Bhambar (2014).

Results and Discussion

Qualitative phytochemical screening

The qualitative phytochemical screening of *Nymphaea lotus* leaves (NLL) revealed the presence of several bioactive compounds, including tannins, terpenoids, phenolics, saponins, alkaloids, flavonoids, steroids, and anthraquinones (Table 1). These findings align with the reports by Chatepa *et al*. (2024) and Lata and Dubey (2010), which suggest that the medicinal properties of plants are often attributed to the presence and quality of such bioactive constituents.

The presence of alkaloids was confirmed using Dragendorff's, Mayer's, and Wagner's tests, all indicating an abundant presence (++ve) of alkaloids in the leaves. Alkaloids are often linked to therapeutic effects, including analgesic and antimicrobial activities (Cushnie *et al*., 2014). Similarly, flavonoids were detected in abundance, as indicated by the Ammonia/H_2SO_4, Aluminium solution, and Ethyl acetate/Ammonia tests. Flavonoids are recognised for their potent antioxidant, anti-inflammatory, and anti-cancer properties. (Khan *et al*., 2022; Liu *et al.,* 2023).

The frothing test indicated a strong presence (++ve) of saponins. This finding is particularly interesting given that saponins have been reported to exhibit immunomodulatory, anti-cancer, and cholesterol-lowering effects (Khan *et al.*, 2022,). Likewise, the Ferric chloride test revealed an abundance of tannins (++ve). Tannins are known for their astringent properties and have been associated with various biological activities, including antioxidant, antimicrobial, and wound-healing properties (Sieniawska and Baj, 2017). The high tannin content may explain some of the traditional uses of *N. lotus* in treating gastrointestinal disorders and skin conditions. The result of this study conformed with the report by Mahmud *et al.* (2020).

Borntrager's test confirmed the presence of anthraquinones (+ve), which, although not as abundant as some other compounds, are significant due to their well-documented laxative and anti-inflammatory properties (Malik & Müller, 2016). The Salkowski test revealed a substantial presence of terpenoids (++ve), a diverse class of compounds known for their wide range of biological activities, including antimicrobial, anti-inflammatory, and anticancer properties (Ludwiczuk *et al*., 2017). The Keller-Killiani test confirmed the presence (+ve) of cardiac glycosides, compounds that are widely recognised for their effectiveness in treating heart conditions, particularly by enhancing the force of cardiac contractions (Prassas & Diamandis, 2008). *N. lotus* leaves also demonstrated a substantial presence (++ve) of phenols in the ferric chloride test. Phenolic compounds are renowned for their potent antioxidant properties, which play a crucial role in reducing inflammation and oxidative stress (Liu *et al.*, 2023). The high phenolic content suggests that *N. lotus* leaves could be highly effective in managing oxidative stress-related disorders and age-related degenerative diseases. Additionally, the Liebermann-Burchard test revealed a significant presence (++ve) of steroids. Phytosterols, the plant-based steroids, are known for their anti-inflammatory effects and their involvement in regulating various metabolic functions (Martel *et al*., 2016).

Table 1: Qualitative analysis of *Nymphaea lotus* leaves

Bioactive compound	Test used	Occurrence (Indicating Positive Test)
Alkaloids	Dragendorff's test	++
	Mayer's reagent test	++
	Wagner's test	++
Flavonoids	Ammonia/H_2SO_4	++
	Aluminium solution	++
	Ethyl acetate/Ammonia	++
Saponins	Froth test	++
Tannins	Ferric chloride	++
Anthraquinones	Borntrager's test	+
Terpenoids	Salkowski test	++
Cardiac glycosides	Keller-Killiani test	+
Phenols	Ferric Chloride Test	++
Steroids	Liebermann-Burchard test	++

Keys: +ve = present, ++ve = abundant

Quantitative phytochemical constituents

The quantitative analysis of *Nymphaea lotus* leaves (NLL) revealed the presence of several bioactive phytochemicals, with the yields expressed as a percentage of the leaf's dry weight. The notable phytochemicals identified include alkaloids (1.05% w/w), saponins (1.70% w/w), terpenoids (3.10% w/w), flavonoids (0.61 ± 0.018% w/w), phenols (0.62 ± 0.002% w/w), and tannins (0.91 ± 0.003% w/w)(Table 2). The presence of these key secondary metabolites suggests significant potential for improving animal nutrition and health

The alkaloid content of NLL obtained in this study suggests potential antimicrobial and immunomodulatory benefits when included in livestock diets. Alkaloids have been reported to exhibit antibacterial and antiparasitic properties, which may help in reducing disease incidence in farm animals (Cushnie *et al*., 2014). Furthermore, certain alkaloids can stimulate appetite and improve feed intake, contributing to better growth performance (Klotz, 2015). Additionally, the presence of alkaloids contributes significantly to the medicinal value of plants and may enhance the potential application of *Nymphaea lotus* in natural therapeutics and animal nutrition. However, excessive alkaloid intake can have toxic effects; it can affect the nervous system, liver function, and overall metabolism of animals. Therefore, appropriate inclusion levels should be evaluated to ensure safety in livestock diets.

The moderate level of saponins in *Nymphaea lotus* suggests its potential role as a functional feed ingredient in animal diets. Saponins have also been reported to enhance immune function and provide antimicrobial protection as well as improve gut health in animals by reducing pathogenic microbial loads and enhancing nutrient absorption (El Aziz *et al*., 2019; Muhammad *et al.*, 2022; Adebiyi *et al*., 2024).

This study revealed that terpenoids were the most abundant phytochemicals in *Nymphaea lotus* leaves (NLL). The relatively high yield (0.031 g per 1 g of NLL sample) is particularly noteworthy, as terpenoids play crucial roles in plant defence mechanisms and exhibit diverse biological activities. These compounds are widely recognised for their antioxidant, anticancer, and anti-inflammatory properties, contributing to the aromatic and medicinal attributes of plants. The high concentration of terpenoids in *Nymphaea lotus* suggests that its

leaves may possess significant antioxidant potential, which could help mitigate oxidative stress in both humans and animals (Ludwiczuk *et al.*, 2017; Tetali, 2019; Tohfa *et al*., 2024) Flavonoids are well-recognised for their antioxidant, anti-inflammatory, and anticancer activities and even at lower concentrations, they may still contribute significantly to the plant's medicinal properties (Shraim *et al*., 2021; Hasin *et al*., 2024). Despite being present in lower concentration, flavonoids can still make substantial contributions to the strong antioxidant properties of NLL just as observed in previous studies.

Table 2. Quantitative phytochemical screening *Nymphaea lotus* Leaves

sample	Alkaloids (%w/w)	Saponins (%w/w)	Terpenoids (%w/w)	Flavonoids (%w/w)	Phenols (%w/w)	Tannins (%w/w)
Leaf sample	1.05±0.00	1.70±0.00	3.10±0.00	0.61±0.018	0.62±0.002	0.91±0.003

The moderate total phenolic content suggests that most phenolic compounds present in the leaf matrix of *Nymphaea lotus* are readily extractable. Phenolic compounds are well-known for their potent antioxidant properties (Naresh *et al*., 2019). The presence of phenolics in *Nymphaea lotus* leaves suggests potential applications in feed formulations designed to combat oxidative stress, improve meat quality and promote the overall health of animals.

Tanins are often considered antinutritional factors at high concentrations; however, the concentration of tannins in NLL, as observed in this study, is within a tolerable range. At moderate levels, tannins can reduce methane production in ruminants and improve protein utilisation by forming complexes with dietary proteins, thus enhancing ruminal bypass protein (Min et al., 2003).

Conclusion

In conclusion, the qualitative phytochemical analysis of *Nymphaea lotus* leaves revealed a significant presence of alkaloids, saponins, tannins, terpenoids, flavonoids, steroids, and phenols, with anthraquinones and cardiac glycosides present in lower quantities. The quantitative analysis further demonstrated a rich phytochemical composition with terpenoids being the most prominent. The presence of these bioactive compounds supports the traditional use of *Nymphaea lotus* in herbal medicine and indicates its potential application in animal nutrition. Further research on the biological activities of these phytochemicals, as well as their effects in feed formulations, is recommended to explore their full potential.

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Date received: 9th April, 2025

Date accepted: 10th July,2025