Phytochemical Screening and Acute Toxicity Studies of *Nymphaea Lotus* Extract and Fractions

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ABSTRACT

Nymphaea lotus (Linn), an herbaceous aquatic plant from the *Nymphaeaceae* family, is widely utilized for its therapeutic properties. This study evaluates the phytochemical composition and acute toxicity of the methanol leaf extract and its aqueous residual and *n*-Butanol fractions in rats and mice. Phytochemical screening revealed the presence of various bioactive compounds; the quantitative determination of the bioactive constituents was carried out using Spectrophotometric analysis which revealed the aqueous residual fraction rich in tannins and the *n*-Butanol fraction rich in flavonoids. Acute toxicity tests, following OECD 425 guidelines, indicated that the LD50 is above 5000 mg/kg, confirming the non-toxic nature of the extract and fractions. Hematological analysis and histopathological examinations of major organs (stomach, intestine, kidney, and liver) showed no significant differences between treated and control groups. The findings support the plant's safe use in traditional medicine and highlight its potential for further pharmacological research.

Keywords: *Nymphaea lotus*, Phytochemical Screening, Acute Toxicity, OECD 425, Herbal Medicine

INTRODUCTION

The use of plants as medicine is an ancient practice that is common to all societies, especially the African Society. These practices continue to exist in the developing nations. According to an estimate by World Health Organization (WHO, 2005), around 80 % of the world's population in developing countries relies on traditional plant medicine for their primary health care needs; of which major portions involve the use of plant extracts or their active principles (WHO 2012; Welz *et al*., 2018). It is on this basis that researchers keep working on medicinal plants to produce and/or develop the basic medicines for physiological or therapeutic use (Malviya *et al.*, 2011).

Nymphaea lotus (Linn) is an aquatic plant of ethno-pharmacological importance, widely used in traditional medicine for its sedative, analgesic, anti-inflammatory and antipyretic properties (Duke, 2008). Phytochemical studies have revealed the presence of alkaloids, saponins, tannins, and flavonoids, which contribute to these therapeutic effects.

Statement of the Problem

Despite the widespread use of *Nymphaea lotus* in traditional medicine, there is limited scientific evidence supporting its safety profile. The lack of toxicity studies poses a challenge to its acceptance for broader medicinal and pharmacological applications. Assessing its acute toxicity is critical to confirm its safety for human use and to provide baseline data for further investigations.

Significance of the Study

The findings of this study are significant in several ways:

1. It provides scientific validation for the safe use of *Nymphaea lotus* in traditional medicine.

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2. The study establishes baseline data on the acute toxicity profile, which is essential for further pharmacological and clinical research.
3. By identifying key phytochemical constituents, this study highlights the potential of *Nymphaea lotus* as a source of bioactive compounds for drug development.

Aims and Objectives

The aim of this study is to evaluate the phytochemical composition and acute toxicity of *Nymphaea lotus* methanol extract and its fractions. The specific objectives are:

1. To identify the phytochemical constituents present in the methanol extract and its aqueous residual and *n*-Butanol fractions.
2. To determine the acute toxicity (LD50) of *Nymphaea lotus* extract and fractions in rats and mice following OECD 425 guidelines.
3. To assess the effects of the extract and fractions on hematological parameters in treated animals.
4. To evaluate the histopathological changes, if any, in the major organs of treated animals.

MATERIALS AND METHODS

Plant Material Collection and Extraction

Fresh leaves of *Nymphaea lotus* were collected and authenticated at the Department of Botany, Ahmadu Bello University, Zaria. The plant material was shade-dried, pulverized, and subjected to cold maceration using methanol. The methanol extract was concentrated under reduced pressure, and successive solvent partitioning was performed to obtain aqueous residual and *n*-Butanol fractions.

Phytochemical Screening

Phytochemical screening of the crude methanol extract and its fractions was conducted using standard qualitative methods to detect alkaloids, flavonoids, saponins, tannins, glycosides, and phenolic compounds. Spectrophotometric analysis was used for the quantitative determination of the bioactive constituents.

Acute Toxicity testing

The acute toxicity study was carried out following OECD 425 guidelines (2001).

- **Animals**: Healthy rats and mice were used for the experiment.
- **Dose Administration**: The methanol extract and fractions were administered at a single dose of 5000 mg/kg body weight orally via cannula.
- **Observation Period**: Animals were observed for clinical signs of toxicity (e.g., fur changes, movement, diarrhea, sleep, convulsions) at 30 minutes, 4 hours, 24 hours, 48 hours, and daily for 14 days.

Hematological Analysis

On the 14th day, blood samples were collected from all animals in EDTA bottles for hematological analysis. Parameters such as WBC, RBC, lymphocytes, neutrophils, platelet count, and PCV were analyzed using a Biobase Automatic Hematology Analyzer.

Histopathological Examination

Major organs (stomach, intestine, kidney, and liver) were harvested, processed, and stained using hematoxylin and eosin (H&E) for histopathological examination under a light microscope.

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RESULTS

Phytochemical Screening

The phytochemical screening revealed the presence of tannins, flavonoids, alkaloids, glycosides, and phenolic compounds. The aqueous residual fraction was particularly rich in tannins, while the *n*-Butanol fraction was abundant in flavonoids; determined by spectrophotometric quantitative analysis (Table 1)

Table 1: Quantitative Phytoconstituents of Flavonoid and Tannin Rich Fractions from n-butanol and residual aqueous fractions of Methanol Leaf Extract of *Nymphaea lotus* Linn

Phystoconstituents	**n-Butanol Fraction**		**Aqueous Residual Fraction**	
	Flavonoids	**Tannins**	**Flavonoids**	**Tannins**
Weight (mg/g)	75.80	18.66	23.32	266.33
Percentage (%)	7.58	1.87	2.33	26.63

Acute Toxicity Findings

The LD_{50} of *N. lotus* extract and its fractions was determined to be >5000 mg/kg, indicating practical non-toxicity. Behavioral observations revealed no significant abnormalities in treated groups compared to controls (Table 2).

Table 2: Behavioral Observations in Rats and Mice

Parameter	**30 min**	**4 hrs**	**24 hrs**	**48 hrs**	**7 days**	**14 days**
Fur/Skin	Normal	Normal	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal	Normal	Normal
Sleep	Alert	Alert	Alert	Alert	Alert	Alert
Movement	Normal	Normal	Normal	Normal	Normal	Normal
Mortality	None	None	None	None	None	None

Hematological Indices

No significant differences were observed between control and treated groups in hematological parameters (Table 3).

Table 3: Hematological Parameters of Control and Treated Groups

Parameter	Control (Rats)	Treated (Rats)	Control (Mice)	Treated (Mice)
WBC (×103/µL)	4.90 ± 0.00	5.13 ± 0.39	5.03 ± 0.00	4.67 ± 0.12
RBC (×106/µL)	5.10 ± 0.00	6.00 ± 0.10	6.10 ± 0.00	6.27 ± 0.20
Platelet (×103/µL)	167.03 ± 0.00	213.00 ± 0.00	227.03 ± 0.00	191.00 ± 18.00
PCV (%)	37.03 ± 0.03	38.33 ± 1.76	39.00 ± 0.00	40.33 ± 0.00

Histopathological Examination

Histopathological analysis revealed no significant pathological changes in the stomach, intestine, kidney, and liver of treated animals (Figures 1–4).

DISCUSSION

The application of medicinal plants as medicines has been exponentially expanding because of their pharmacological properties. This is associated with the presence of health-relevant secondary metabolites such as flavonoids and tannins (Silva *et al;* 2022) Rege and Co-workers (2020) reported the presence of cardiac glycosides, saponins, steroid/terpenoids, flavonoids, tannins and alkaloids in the methanol leaf extract of *N.*

lotus. The presence of .phenolic compounds (tannins, and flavonoids), saponins, terpenes and steroids were also reported by Abdulrahman *et al.*, (2020).

This study highlights the non-toxic nature of *Nymphaea lotus* methanol extract and its fractions. The absence of mortality, normal behavioral patterns, and consistent hematological indices suggest the plant's safety. Histopathological findings further support these vie; as no significant lesions were observed. These results align with previous reports emphasizing the safe use of *N. lotus* in traditional medicine (Adeneye *et al;* 2006; Ogbonnia *et al;* 2010). There is a general notion among the consumers of herbal medicines; the belief that herbal medicines are always safe and effective, because they are ''natural". However, shreds of evidences suggests otherwise. There has been several adverse effects (sometimes life threating), due to consumption of traditional medicines or herbal products (Awodele *et al;* 2012). Therefore, contrary to the popular notion and believe, herbal medicines can be harmful to health (Alastar and Wood; 2012). Therefore, the need to evaluate the toxicity of herbal products cannot be overemphasized.

Previously we reported the acute toxicity profile of the methanol leaf extract of *Nymphaea lotus* in rats (Rege *et al;* 2020) using Lorkes Method. Also Sharaibi *et al* (2015) reported the sub-chronic toxicity profile of the leaf extract in rats using varying doses of 50,100, and 200 mg/kg. Higher doses of 250, 500 and 1000 mg/kg in repeated doses orally for 28 days in rats were also used for its sub-chronic toxicity profiling (Rege *et al;* 2023) with same result of safety profile of the plant *N. lotus* . The current study is in agreement with the previous findings of the safety profile of the *N. lotus* plant. .

The presence of flavonoids and tannins, known for their antioxidant, analgesic, antipyretic and anti-inflammatory properties, adds to the plant's therapeutic potential. Further studies are recommended to explore its pharmacological applications.

CONCLUSION

The present study demonstrates that *Nymphaea lotus* methanol extract and its fractions are rich in bioactive compounds and exhibit no acute toxicity at doses up to 5000 mg/kg. These findings validate the plant's safety in traditional medicine and warrant further pharmacological investigations.

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