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*Article*

# Pharmacognostic and acute toxicity study of the rhizome of *Nymphae lotus* L. (Nymphaeaceae)

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**Abstract:** *Nymphaea lotus* belongs to the family Nymphaeaceae and traditionally used in the treatment of aphrodisiac, anodyne, astringent, cardiotonic, sedative, demulcent, analgesic and as anti-inflammatory agent. The objective of the study was to evaluate the phytochemical, physicochemical analysis and safety margin of *N. lotus* rhizome with the hope of assisting in its standardization for quality, purity and safety. The powdered sample of the rhizome was extracted with aqueous and methanol and evaluated for physicochemical parameters of the plant. The extracts were subjected to qualitative and quantitative phytochemical analysis and acute toxicity study. The physicochemical parameters evaluated include: moisture content (7.4%), total ash (10.3%), water soluble (7.1%), acid insoluble (2.8%), ethanol extractive value (16.7%), and water extractive value (22.0%). The quantitative phytochemical analysis revealed that alkaloids (166.0 mg/g) was the highest phytochemical detected in the rhizome while the lowest was saponins (22.0 mg/g). $LD_{50}$ of both extracts was above 5000 mg/kg and did not cause mortality in all the tested rats. The results of this finding may be useful in laying down standards and for the compilation of a suitable pharmacopoeia parameters on *N. lotus*.

**Keywords:** *Nymphaea lotus;* standardization; phytochemicals; safety margin

## 1. Introduction

Medicinal plants have played a key role in world health including developing countries. In spite of the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care (Calixto and Barz, 2000). The use of herbal remedies is on the increase with concern and uncertainty about the quality, safety and efficacy of these remedies (Mohammad *et al.*, 2017). There is also the problem of incorrect diagnosis, imprecise dosage, and low hygiene standards, lack of regulation of herbal medicines in many countries, ineffective monitoring and control of the sales of unregistered products (De-Smet, 1995). It is therefore essential to lay down the standardization specifications of medicinal plants which are used as drugs.

*Nymphae lotus*, commonly known as water lily, belongs to the family Nymphaeaceae. The flowers are white, sometimes with a tinge of pink (Afolayan *et al*., 2013). It is a perennial plant that grows up to 45 cm in height; it is herbaceous aquatic plant, whose leaves float or submerge in water. The plant is native to the Nile and is grown in various parts of East Africa and Southeast Asia (Abu-Zaida *et al.,* 2008). The plant has different names depending on the tribes, for instance the Igbo call it 'Ijikara', Yoruba call it 'Iyeye 'while the Hausa call

it 'Bado' (Jethro, 1994). It is commonly seen in freshwater ecosystem where it is scattered and seen flating on top of water bodies (Carlini, 2003). It is among the earliest aquatic macrophytes that have been identifid in Nigerian freshwaters (Akinjogunla *et al.,* 2009). It is used in traditional medicine system as an aphrodisiac, anodyne, astringent, cardiotonic, sedative, demulcent, analgesic and as anti-inflammatory agent (Aduema *et al.,* 2018). The plant is known to contain a lot of chemical compound. It has a calming and sedative effect on the nervous system, therefore it can be used in the treatment and management of insomnia and anxiety disorders (Afolayan *et al*., 2013).

Despite the medicinal applications of *N. lotus*, there is dearth of information on the standardization parameter. Therefore, this study was carried out to determine the phytochemical, physicochemical parameters and safety margin of *N. lotus* rhizome.

## 2. Materials and Methods

### 2.1. Collection, identification and preparation of the plant material

The plant material was collected at Kabo Local Government Area of Kano State. The plant was taxonomically authenticated at the Herbarium unit, Department of Plant biology, Bayero University Kano, Nigeria with Voucher specimen number BUKHAN356. The rhizome was washed, cleaned and all foreign matter removed, it was then air-died and comminuted to powder form, stored in an air-tight container for subsequent use.

### 2.2. Chemo-microscopic studies on the rhizome of *Nymphae lotus*

Powdered sample (rhizome) of *Nymphae lotus* was used for this study to detect the presence of cell wall materials and cell inclusions. Finely ground sample of plant was cleared in a test tube containing 70% chloral hydrate solution. It was then be boiled on a water bath for about thirty minutes to remove obscuring materials. The cleared sample was mounted with dilute glycerol onto a microscope slide. Using various detecting reagents the presence of cell wall materials and cell inclusions was detected in accordance to WHO (2011) guidelines.

### 2.3. Cell wall materials

#### 2.3.1. Test for cellulose

A drop or two of iodinated zinc chloride was added to the powdered sample and allowed to stand for a few minutes and observed under a microscope. It stained cellulose cell wall blue to blue- violet.

#### 2.3.2. Test for lignin

The powdered plant material was moistened on a slide with a small volume of phloroglucinol and allowed to stand for about two minutes or until almost dry. A drop of hydrochloric acid was added and view under a microscope. Pink stained or cherry red was observed, for the presence of lignin.

#### 2.3.3. Test for suberized or cuticular cell walls

A drop or two of Sudan red was added to the cleared powdered sample and allowed to stand for few minutes and observed under a microscope. Orange red or red colour was observed presence of suberin or cutin on the cell.

#### 2.3.4. Test for gum and mucilage

To a small portion of the cleared powdered sample of the plant, a drop of ruthenium red was added. Appearance of pink coloration was considered positive for gums and mucilage.

### 2.4. Cell inclusions/cell contents

#### 2.4.1. Test for starch grains

To a small portion of the cleared powder sample of the plant, N/50 iodine was added. Appearance of blue-black or reddish-blue coloration on some grains would be considered positive for starch.

#### 2.4.2. Test for calcium oxalates and calcium carbonates

To a small portion of the cleared powdered sample of the plant, HCl was added, dissolution of crystals in the powdered drug without effervescence was considered positive for calcium oxalate while slow dissolution with effervescence was considered positive for calcium carbonate.

### 2.4.3. Inulin

A drop of 1-naphthol and that of sulphuric acid was added to the powdered sample and viewed under the microscope. Spherical aggregations of crystals of inulin turned brownish red and dissolve.

### 2.4.4. Test for tannins

To a small portion of the cleared powdered sample of the plant, 5% ferric chloride solution was added. Appearance of greenish black colour was considered as positive for tannins.

## 2.5. Physicochemical parameters

Powdered sample was subjected to physicochemical analysis such as water and alcohol soluble extractives, total ash, acid insoluble ash, water soluble ash and moisture content were determined (WHO, 2011).

## 2.6. Elemental analysis of powdered rhizome of *Nymphae lotus*

### 2.6.1. Acid digestion of the samples

0.5 grams of the powdered plant material was weighed into 10 different beakers each of 50 ml, to which 2.5ml of hydrochloric acid (HCl) and 7.5ml Nitric Acid ($HNO_3$) were added to each beaker. The 10 beakers used were placed in an open space for 2 hours and mixture of hydrochloric acid (HCl) and nitric acid (HNO3) in 1:1 ratio was added to each beaker. It was kept on a hot plate at 100$^{o}$C-170$^{o}$C for 1- 4 hours. After the contents in beakers is about to dried; 5 ml of Hydrochloric acid (HCl) was added to each beaker and be kept on the hot plate until the entire liquid content in the beakers got evaporated. Then, 5 ml of de-ionized water was added to each beaker and the solutions were poured in sterile bottles and tested for the quantification of the metals. The concentration of Fe, Mg, Zn, Cu was read using the flame atomic absorption spectrophotometer (FAAS), AA 500 model, Atomic Emission Spectrophotometer, HACH Spectrophotometry (DR/4200) and Atomic Absorption Spectrophotometer were used for other elements detected The elemental analyses of the plant materials were carried out in Ahmadu Bello University Zaria, Multi-user Research Laboratory,. The mineral elements estimations indicated the amount of macro, trace elements and heavy metals present in the Plant samples. The mineral elements detected include; Zinc (Zn), Magnesium (Mg), Lead (Pb), Manganese (Mn), Selenium (Se), Copper (Cu), Iron (Fe), Cadmium (Cd), Arsenic, Nickel and these were done by Spectrophotometric methods. Before determining the concentration of any element in the sample, calibration curve of the element in the sample was prepared using prepared standard stock solutions for the elements as reported by AOAC, 2000; 2005; Akpabio and Ikpe (2013).

### 2.6.2. Extraction method

50 g of the powder sample of *Nymphae lotus* rhizome was macerated with 500 ml of aqueous and methanol successively. The extracts was evaporated to dryness on water bath.

### 2.6.3. Qualitative phytochemical screening of the aqueous and methanol extracts of *Nymphae lotus* rhizome

The aqueous extracts was subjected to phytochemical screening in order to identify the phytochemical constituents of the plant using the standard phytochemical reagents and procedures (Sofowora, 2006; Evans, 2009).

### 2.6.4. Quantitative phytochemical screening of the aqueous extract of *Nymphae lotus* rhizome

#### 2.6.4.1. Preparation of fat free sample

About 2 g of the sample was weighed and defatted with 100 ml of diethyl ether using a soxhlet apparatus for 2 hours.

#### 2.6.4.2. Alkaloid determination

About 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol were added and covered and allowed to stand for 4 hours. This was filtered and the extract is concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation is completed. The whole solution was allowed to settle and the precipitates were collected and wash with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed (Harborne, 1973).

#### 2.6.4.3. Flavonoid determination

10 g of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter upper No. 42 (125 mm). The filtrate was transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight (Bohm and Kocipal – Abyazan, 1994).

#### 2.6.4.4. Saponin determination

The method of Obadoni and Ochuko (2001) was used. Out of the grinded samples 10 g was weighed for each and put into a conical flask and 100 ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55$^{o}$C. The mixture was filtered and the residue re-extracted with another 200 ml, 200% ethanol. The combined extracts were reduced to 40ml over water bath at about 90$^{o}$C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 ml of n – butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

#### 2.6.4.5. Tannin determination

About 500 mg of each sample was weighed into a 50 ml plastic bottle and 50 ml of distilled water was added and shaken for 1hour on a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up of the mark. Then 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1M $FeCl_3$ in 0.1M HCl and 0.008M potassium ferrocyanide. The absorbance was measure at 120 mm within 10 min (Van-Burden and Robinson, 1981).

### 2.6.5. Acute toxicity studies of aqueous and methanol extracts of *Nymphae lotus* rhizome

The lethal dose was determined by Lorke's method. Phase I: Nine wistar rats were used. They were divided into three groups of three animals each. Each groups of animals were administered different doses (10, 100, and 1000 mg/kg) of the extracts and then observed for 24 hours to monitor their behavior as well as mortality. Phase II: Three animals were used. They were divided into three groups of one animal each. The animals were administered higher doses (1600, 2900 and 5000 mg/kg of the extracts and observed for behaviour as well as mortality (Lorke, 1983). The oral median lethal dose ($LD_{50}$) was calculated as the geometric mean of the minimum toxic dose and maximum tolerated dose.

## 3. Results and Discussion

Chemo-microscopical examination of powdered stem of *Nymphae lotus* revealed the presence of cellulose, tannins, starch, lignin, calcium oxalate, suberin, aleurone grain and mucilage, calcium carbonate (Table 1). The chemo-microscopic features are most valuable in the identification of powdered drug as their identification is largely based on the form, the presence or absence of certain cell types and cell inclusions. These are very important diagnostic pharmacognostic parameters for the identification and authentication of crude drugs especially in powdered plants (Chanda, 2011).

**Table 1. Chemomicroscopical studies of *Nymphae lotus* rhizome.**

| Constituents | Inference |
|---|---|
| Starch | + |
| Gum and Mucilage | + |
| Cellulose cell walls | + |
| Lignin | + |
| Aleurone grain | + |
| Calcium oxalate crystals | + |
| Calcium carbonate | - |
| Suberized/Cuticular cell wall | + |
| Inulin | + |

The physicochemical parameters assessed the moisture content, total ash, acid insoluble, water soluble, alcohol and water extractives values (Table 2) revealed the moisture content (7.37%) which is relatively low when compared with BHP, (1990) that reported that percentage of moisture content in any crude drug should be within 12-14 %. However, it indicated less chances of microbial degradation of the drug during storage. The lower the value, the less likelihood of degradation of drug and suggests better stability of the product. Moisture is considered an adulterant because of its added weight as well as the fact that excess of moisture promotes mold and bacterial growth (Prasad *et al*., 2012; Nuhu *et al*., 2016). Total ash value (10.33%) represents both the physiological and non-physiological ash from the plant. The non-physiological ash is an indication of inorganic residues after the plant drug is incinerated. The acid insoluble ash values (2.80%) obtained in this study indicated that the plant was in good physiological condition and contained little extraneous matter such as sand, silica and soil. The total ash value is used as criteria to judge the identity and purity of drugs (WHO, 2011; Prasad *et al.*, 2012).

The ethanol extractive value (16.7%) and water extractive value (22.0%) showed that both water and alcohol potentials in the extraction of the active constituents. Tiwari and Mishra (2010) stated that solvent choice in research involving plant depend on phytochemicals to be extracted as well as the cost and easy access of the solvents.

**Table 2. Physicochemical Constants of *Nymphae lotus* powdered rhizome.**

| Parameters | Values (%w/w) ± SEM* |
|---|---|
| Moisture content | 7.37 ± 0.33 |
| Ash content | 10.33 ± 0.33 |
| Acid insoluble ash | 2.80 ± 0.58 |
| Water soluble ash | 7.10 ± 0.06 |
| Water extractive value | 22.00 ± 0.00 |
| Ethanol extractive vale | 16.67 ± 0.33 |

*****Average values of three determinations

Trace metals which include Fe and Mn detected in *Nymphae lotus* were above the FAO/WHO (1984) permissible limit for edible plants as shown in Table 3. While others, Pb, Zn, Cd and Cu were found to be within the safety limit.

**Table 3. Elemental analysis of *Nymphae lotus* powdered rhizome.**

| Elements | Concentration (ppm) | FAO/WHO (1984) limit* (ppm) |
|---|---|---|
| Iron(Fe) | 76.301 | 20.00 |
| Copper (Cu) | 0.044 | 3.00 |
| Lead (Pb) | 0.446 | 0.43 |
| Zinc (Zn) | 0.348 | 27.40 |
| Nickel (Ni) | -0.015 | 1.63 |
| Manganese (Mn) | 23.718 | 2.00 |
| Aluminum (Al) | 20.485 | - |
| Cadmium (Cd) | -0.013 | 0.21 |
| Selenium (Se) | 0.441 | - |
| Chromium (Cr) | 0.033 | - |
| Arsenic (As) | 0.729 | - |

Phytochemical analysis of plant revealed the presence of constituents which are known to exhibit medicinal as well as action on the human body (Vaghasiya *et al.*, 2008; Yadav and Agarwala, 2011). Phytochemical analysis shows the presence of many medicinally important secondary metabolite types of phytoconstituents like alkaloids, cardiac glycosides, saponins, triterpenes, which indicates that the plant possesses high profile values and can be used to treat various kinds of diseases. The presence of these secondary metabolites suggests that the plant might be medicinal importance. The results of qualitative phytochemical screening of aqueous and methanolic rhizome extracts of *N. lotus* are shown in Table 4.

Quantitative estimate of phytochemical present in methanol rhizome extract of *N. lotus* shows alkaloids to be highest (166.0 mg/g), followed by tannins (90.0 mg/g), then flavonoids (80.0 mg/g) and the lowest was saponins ( 22.0 mg/g) as in Table 5.

Alkaloids are beneficial chemicals to plants serving as repellant to predators and parasites. This probably endows these group of agents its antimicrobial activity. However, when ingested by animals, they affect glucagon, thyroid stimulating hormones and inhibit certain enzymatic activities (Okaka *et al.,* 1992). Flavonoids general serve as flavoring ingredients in plants. Besides their role as flavoring agents they are also expressed in plants in response to microbial infection suggesting their antimicrobial activity (Kujumgiere *et al.,* 1999). Flavoniods have also been implicated as antioxidants both in physiological and diseased states. For instance tea flavonoids have been reported to reduce the oxidation of low-density lipoprotein, lower the blood level of cholesterol and triglycerides (Erdman, 2007). Tannins in this study were indicated to be present but in low concentration in both plant parts. This bioactive compound is known to have potentials anti-viral activity (Cheng *et al.,* 2002) as well as potential prophylactic and therapeutic effect against cancer cells, but via different mechanisms (Narayanan *et al.,* 1999). Saponins are known bioactive substances that can reduce the uptake of cholesterol and glucose at the gut through intra-lumenal physiochemical interaction (Eyong *et al.,* 2011). Saponins as a class of natural products are also involved in complexation with cholesterol to form pores in cell membrane bilayers (Francis *et al.,* 2002) as such may be used as anticholesterol agents or cholesterol lowering agent.

**Table 4. Qualitative Phytochemical screening of aqueous and methanolic rhizome extracts of *Nymphae lotus*.**

| **Metabolite** | **Inference** | |
|---|---|---|
| | **Aqueous Extract** | **Methanol Extract** |
| Alkaloid | + | + |
| Flavonoid | + | + |
| Saponins | + | + |
| Cardiac glycoside | + | + |
| Tannins | + | + |
| Steroid/ Triterpenes | + | + |
| Anthraquinones | - | + |
| Carbohydrate | + | + |

**Table 5. Quantitative phytochemical screening of methanol extract of *Nymphae lotus* rhizome.**

| **Metabolite** | **Quantity (mg/g)** |
|---|---|
| Alkaloids | 166.00 ± 0.57 |
| Flavonoids | 80.00 ± 0.29 |
| Saponins | 22.00 ± 0.12 |
| Tannins | 90.00 ± 0.50 |

In order to determine the safety margin of drugs and plant products for human use, toxicological evaluation was carried out in experimental animals using Lorke's method to predict toxicity and to provide guidelines for selecting a "safe'' dose in animals and also used to estimate the therapeutic index ($LD_{50}/ED_{50}$) of drugs (Olson *et al.,* 2000; Rang *et al*., 2012). In this study, median lethal dose ($LD_{50}$) of the extracts (aqueous and methanol) of the *N. lotus* rhizome was carried out orally in rats. The $LD_{50}$ was found to be greater than 5000 mg/kg when administered orally in rats (Table 4) and all the animals remain alive and did not manifest any significant visible signs of toxicity at these doses. These studies showed the extracts of *N. lotus* rhizome are practically non-toxic when administered using the oral route (Table 6). This is based on the toxicity classification which states that substances with $LD_{50}$ values of 5000 to 15,000 mg/kg body weight are practically non-toxic (Loomis & Hayes, 1996).

**Table 6. Acute toxicity studies of aqueous and methanol extracts of *Nymphae lotus* rhizome when administered orally to Wistar Rats.**

| Experiment | Dose (mg/kg ) | Number of dead rat after 24 hours | |
|---|---|---|---|
| | | Aqueous Extract | Methanol Extract |
| Phase 1 | 10 | 0/3 | 0/3 |
| | 100 | 0/3 | 0/3 |
| | 1000 | 0/3 | 0/3 |
| Phase 2 | 1600 | 0/1 | 0/1 |
| | 2900 | 0/1 | 0/1 |
| | 5000 | 0/1 | 0/1 |

## 4. Conclusions

The standardization for the rhizome of *N. lotus* will be useful for the compilation of suitable pharmacopoeia parameters and also serve as a basis for proper identification and safety usage of *N. lotus* rhizome. *N. lotus* rhizome possess secondary metabolites which include alkaloids, tannins, flavonoids, cardiac glycosides and saponins. The values of Fe and Mn in the plant were above the FAO/WHO (1984) permissible limit for edible plants. However, Pb, Zn, Cd and Cu were found to be within the safety limit. The Acute toxicity (LD50) of the aqueous and methanolic extract of *N. lotus* rhizome was found to be greater than 5000 mg /kg and is considered safe for use. Nonetheless, further studies are encouraged to evaluate toxicity at much higher doses.

## Conflict of interest

None to declare.

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