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Phytochemical and Elemental Constituents of the extract of waterlily *(Nymphaea lotus*) Collected from Dadin-Kowa, Yamaltu Deba Local Government Area, Gombe State.

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Abstract

Nymphaea lotus commonly known as white water lily (English) or Bado (Hausa) belongs to the family Nymphaeaceae. It has been used in traditional medicines for the treatment of different diseases. The studies determined the phytochemical and elemental content of the plant using standard method. The extract of the leaves and root were obtained using sonicator extractor, with ethyl acetate and aqueous solvents. The qualitative phytochemical analysis of the leave and root extract showed the presence of alkaloid, flavonoid, tannin, saponin, terpenoid and glycosides. However, the result showed the absence of sterol and steroid in both the leave and root extract. The elemental composition of the extract were determined using atomic absorption spectrophotometer and flame photometer. The result shows the element were in the range of 3.36±0.09mg/kg to 6.73±0.25mg/kg for Na, 3.22±0.02mg/kg to 6.61±0.11mg/kg for K, 0.87±0.31mg/kg to 6.60±0.38mg/kg for Ca, 2.77±0.07mg/kg to 3.93±0.06mg/kg for Fe, 0.28±0.01mg/kg to 0.62±0.02mg/kg for Mg, and 0.70+0.01mg/kg for Cu. The result indicate that *Nymphaea lotus* contained some bioactive constituents as well some mineral element which are essential to maintain the human body metabolism.

Keyword: Phytochemical, mineral, sonicator, AAS, flame photometry
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Introduction

Medicinal plants constitute the fundamental substrate used in the traditional healthcare system. Medicinal plants grow naturally around us. Over centuries, cultures around the world have learned how to use plants to fight illness and maintain health. These readily available and culturally important traditional medicines form the basis of an accessible and affordable healthcare regime and are an important source of livelihood for indigenous and rural populations (Sourabie *et al*., 2013).

Several thousands of plants have been claimed to possess medicinal properties and are employed in the treatment of many ailments. Many of these indigenous medicinal plants are used as spices for food plants and for medicinal purposes (Khan *et al.*, 2011). Medicinal remain the dominant form of medicine in most countries. Over three fourth of the earth's population depend primarily on raw plant products to meet their daily health care needs (Kirtikar and Basu, 2002). *Nymphaea lotus* is an aquatic flowering plant that belongs to family Nymphaeaceae, usually consist of white blossom and perpetual rhizomes affixed down with mud, floating or submerged solitary leaves and flashy flowers.The Hausas called it Bado, Yoruba Iyeye and the Igbo Ijikara in Nigeria (Abdulrahman *et al.*, 2020).

The seeds are consumed raw by infants and other category of people. The roots are cooked and eaten as green or dried and ground into powder for use as thickening agent or flour (Bimakr, 2017).Water lily is not only an ornamental plant but also an important water purification, because the roots of water lily can absorb the poisonous substances like mercury, lead, etc. and filterer the microorganism in water, it plays an important role in decontaminating water, afforesting and landscaping (Longbap *et al.*, 2018). The plant is widely distributed in most tropical areas of Africa. *Nymphaea* is employed as an effective cure in treating several diseases, ranging from diabetes, rapid aging, infection cancer, kidney stone elimination e.t.c. Phytochemical are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients, they protect plants from disease and damage and contribute to the plant's color, aroma and flavor (Mamta *et al.*, 2013).

Minerals are key elements of the body, they are needed in the build-up and function of important biomolecules in the human body. Although, minerals are not a source of energy in the body but they are necessary for the maintenance of normal biochemical processes in the body. Based on the body needs, these essential minerals can be classified as either a macro or micro (trace) minerals. Macro-minerals are nutritionally important minerals such as sodium, calcium, phosphorus, magnesium and potassium (Abdulrahman *et al.*, 2020). Traditional herbal remedies have a long and proper usage in treatment of pain related ailments. Traditional medicine practice is most popular in developing countries, is unique in its own way because it takes a historical view of the patience situation.

Materials and Method

Sample Collection

The waterlily leaf and root were obtained from Dadin-kowa Yamaltu Deba local Government, Gombe State of Nigeria for phytochemical and elemental constituents analysis. The samples were collected at four locations before Dadin-kowa dam in figure 1 the sampling area are divided into Difa, with longitude 11^0250'E and latitude 10^0250'N Dadin kowa with longitude11^0250'E and latitude 10^020'N Hinna longitude 11^030'and Unguwar canteen with longitude 11^0350'E and latitude 10^020'N.

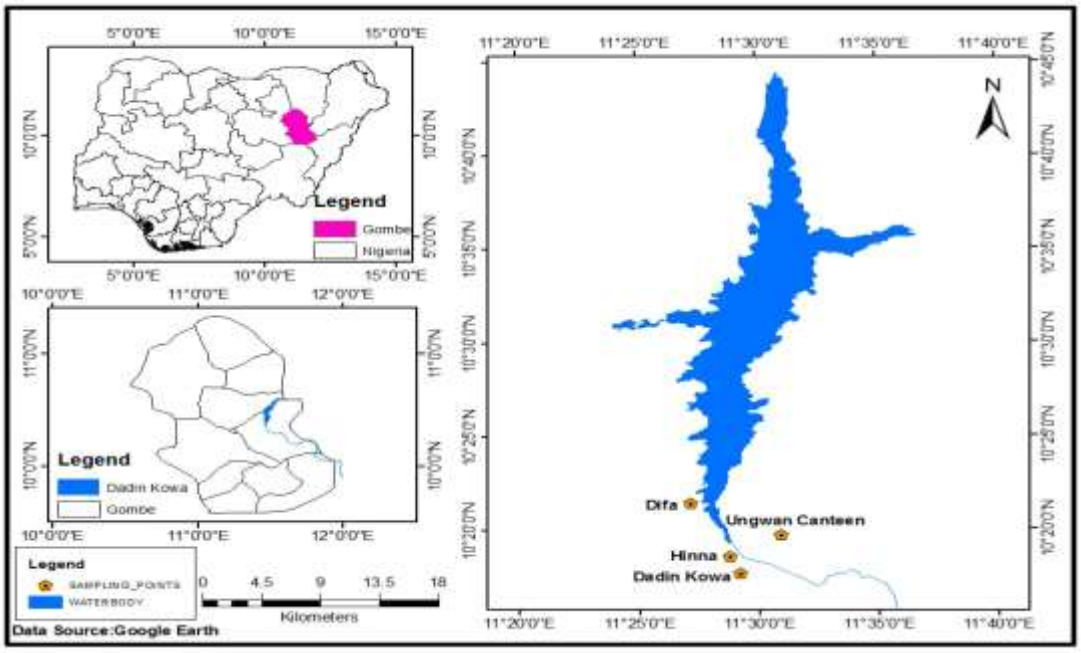

Figure 1. Map of the study area

Reagents used

All chemicals and reagent are analytical grade. Blank solution were also prepared alongside and bulked together for use as dilutants.

Apparatus

Centrifuge tube (50ml and 15ml) VWR ultrasonic cleaner USC-300T, Atomic absorption spectrophotometer (Shimadzu Japhan AA-6800 model), hot plate (model VWR VELP scientific Germany), glasswares (pyrex USA), Weighing balance (model PC 440 Metter, Deltarange Ltd).

Sample Preparations

The leave and root of *Nymphaea lotus* were thoroughly washed to remove sand and the part were dry under shade. The samples were ground using wooden mortar and pestle to ensure homogeneity. The powdered sample was passed through a fine (2 mm mash) sieve to remove any remaining residue. The fine powdered sample was then stored into labeled plastic containers prior to use (Njagi, 2008).

Method of Extraction

Ultrasonic-Assisted Extraction (UAE)

Approximately 0.5 g of the homogenized plants was weighted into 50 ml centrifuge tube and add 10 ml of 1:1 v/v of ethyl acetate and aqueous solvent and shake for 1 minute immediately, and sonicated for 2 minutes, then centrifuged at 400 rpm for 10minites take 4minutes of the liquid of the upper layer and transfer into 15 ml centrifuge tube containing 0.4ml of $MgSO_4$ and shake for 1minites then centrifuge for 5 minutes at 5000 rpm (Abdul Mudalip, 2021).

Preliminary Qualitative Phytochemicals Screening

The preliminary phytochemical analysis of the plants extracts was performed using standard procedures to detect the presences of bioactive components in the leaves and root of *Nymphaea lotus*.

Test for Alkaloids

To 2.00 ml of the extracts few drops of concentrated Hydrochloric acid and Dragendorf's reagent were added. Formation of white precipitate indicated the presence of alkaloids (Gowri *et al.*, 2022).

Test for Flavonoids

Wagner's Test: A fraction of extract was treated with Wagner's test reagent [1.27 g of iodine and 2 g of potassium iodide in 100 ml of water] and observed for the formation of a reddish brown colour precipitate (Gowri *et al.*, 2022).

Test for Tannin

To 2.00 ml of each extract 1 $cm^3$of distilled water and 3 drops of 10 % ferric chloride solution were added. Formation of blue or green black color was an indication of the presences of tannins. (Gowri *et al.*, 2022).

Test for saponins

A 2.00 ml of extracts were shaken in the test tube for 30 seconds. Formation of foam which persisted for 10 minutes was an indication of the presence of saponins (Gowri *et al.*, 2022).

Test for Terpenoids

To 1.00 ml of each of the extract, a 3.00 ml of chloroform was added. The resultant solution was carefully mixed with 2.00 ml concentrated sulphuric acid. Formation of a reddish brown colour at the interface was an indication of the presence of terpenoids (Gowri *et al.*, 2022).

Test for glycosides (Keller-Kiliani test)

Each 2.0 ml of filtered sample was added with 0.5 ml glacial acetic acid, three drops of 1% aqueous $FeCl_3$ solution, and 0.5 ml H_2SO_4 concentrated. A brown ring formed between the layers, which showed the entity of cardiac steroidal glycosides, (Gowri *et al.*, 2022).

Test for steroids

It was carried out by Salkowski's test. About 2 ml of sample was mixed with 2 ml of chloroform. Then, 2 ml of concentrated H_2SO_4 was added to it. If steroids are present, the chloroform layer will appear red, and the acid layer will show greenish-yellow fluorescence. (Gowri *et al.*, 2022).

Test for Sterols

Liebermann-Burchard Test: Take extract and add with 2 ml chloroform. 1-2 ml acetic anhydride and 2 drops of concentrated H_2SO_4 were dropped into the test tube. First red, then blue and finally green colour indicates the presence of sterols (Gowri *et al.*, 2022).

Elemental Analysis

Preparation of Samples (Wet Digestion)

Each plant material (2 g) were taken in 50 ml flask and add 6.5 ml of mixed acid solution that is, Nitric acid (HNO_3), Sulfuric acid (H_2SO_4) and Perchloric acid ($HClO_4$) (5:1:0.5) The sample boiled in acid solution in fume hood on hot plate (model VWR VELP scientific Germany) till the digestion has been completed.

Thereafter, few drops of distilled water were added and allowed to cool. Digested samples were transferred in 50 ml volumetric flasks and the volume was made up to 50 ml by adding distilled water in them. The extract was collected in labeled plastic bottles. The solutions were analyzed for the elements of interest utilizing Atomic Absorption Spectrometer Shimadzu AA-670 and flame photometer (Bhatti *et al.,* 2016).

Statistical Analysis

The data collected were expressed as mean ± standard deviation of the mean. The significance of the differences between groups were determined using one-way analysis of variance (ANOVA) and Duncan multiple test were used for comparison, $P<0.05$ were considered as significant.

Results and Discussion

The results of primarily phytochemical screening of waterlily is presented in Table 1, were by for the aqueous extract, the leave shows the present of alkaloid, flavonoid, terpenoid, tannins, glycoside, steroid while sterol was absence, also for the ethyl-acetate it shows the presence of alkaloid, flavonoid, terpenoid, tannin, saponin, glycoside with the absence of steroid and sterol.

Also for the root of *Nymphaea lotus* the aqueous extract shows the present of alkaloid, flavonoid, terpenoid, tannins and glycoside while saponin, steroid and sterol were absence and for ethyl acetate it shows the presence of all the secondary metabolites. Therefore, for the leave extract it shows the presence of the secondary metabolites in aqueous extract than in ethyl acetate extract while for the root, it shows the presence of secondary metabolites in ethyl acetate extract than in aqueous extract.

Table 1. Phytochemical Screening of Waterlily

Plant part	**Leaf**		**Root**	
Solvent	**Aqueous**	**Ethyl acetate**	**aqueous**	**Ethyl acetate**
Alkaloid	+	+	+	+
Flavonoid	+	+	+	+
Terpenoid	+	+	+	+
Tannin	+	+	+	+
saponin	+	+	-	+
Glycoside	+	+	+	+
Steroid	+	-	+	-
Sterol	-	-	+	-

Key: (+) = Present, (-) =Absent.

For the aqueous extract of the leaf, the result reveals the present of all the secondary metabolite with the exception of steroid, and also the root of aqueous extract reveal the presence of the secondary metabolite with the absence of saponin, sterol and steroid which is also similar to the work of (Prasad and Savithramma, 2016) on screening of phytochemical constituents of *Nymphaea caerulea*, an aquatic plant resource for Drug Development which reveal the presence of secondary metabolite in the aqueous extracts. Also, for the ethyl acetate extract of the leave it reveals the presence of secondary metabolite with the absence of sterol and steroid and also for the root it shows the presence of all secondary metabolite which is related to the work of (Longbap *et al*., 2018) on Phytochemical Screening and Quantitative Determination of

Phytochemicals in Leaf Extracts of *Hannoa undulata*, which reveal the presence of the secondary metabolite in the ethyl acetate extracts.

The phytochemical presence in these plant can influence different body processes, *Nymphaea lotus* can be used as an analgesic, anaesthetic and as social drugs in view of the fact that it contains alkaloids. The alkaloid has been reported to possess various pharmacological activities including antihypertensive effects, antiarrhythmic effect, antimalarial and anticancer activity. Flavonoid have antioxidants and free radical scavengers which prevent oxidative cell damage and also strong anticancer activity and inhibit tumor growth.for the saponins are precursors of important therapeutic drugs such as cortisone and contraceptive estrogens (Saxena *et al*., 2013).

The presence of tannins in the plant extract confers the plant to be a good source for the treatment of wounds emanating from varicose ulcers and hemorrhoids (Njoku and Akumufula, 2017). Saponins possess antioxidant, antitumor, and anti-mutagenic activities and may also reduce the incidence of human cancers by inhibiting the growth of cancer cells (Simon, 2012).

Pharmacologically, glycosides have been found to be useful in treatment of several illnesses for instance cardiac glycoside have long been employed as important ingredient for arrow poisons and drugs. The presence of terpenoids that have carboxylic acid groups could also be responsible for the activity of the organic extracts. Plants produce volatile terpenes either to attract specific insects for pollination or otherwise to expel certain preys which consume these plants as food. In addition, terpenoids possess medicinal properties such as anticarcinogenic, antimalarial, anti-ulcer, antimicrobial or diuretic activity (Dudareva *et al.*, 2004). The presence of secondary metabolite in one solvent extract and absence in other solvent extract is due to the presence of solvent polarity were by like dissolve like.

Table 2. Mineral analysis of *Nymphaea lotus* (mg/kg)

Sample	Sample A (leaf)	Sample B (root)
Na	3.56±0.09	6.73± 0.25
K	6.61±0.11	3.22±0.02
Ca	6.60±0.38	0.87±0.31
Fe	2.77±0.07	3.93±0.06
Cu	0.70±0.01	0.70±0.01
Mg	0.65±0.02	0.28±0.01

The values are Mean ± Standard deviation of the Mean of the three replicate

Table 2 show the results for mineral constituents of *Nymphaea lotus*, the root showed high content of sodium (6.73± 0.25 m/kg) than the leave (3.56±0.09 mg/kg),potassium show high content in leave (6.61±0.11 mg/kg) than the root(3.22±0.02 mg/kg), calcium show high content in leave (6.60±0.38mg/kg) than the root (0.87±0.31mg/kg),for iron it show high content in root (3.93±0.06mg/kg) than leave (2.77±0.07mg/kg), copper show the same content in both the leave and the root(0.70±0.01mg/kg) and lastly for the magnesiumS it show high content in leave (0.65±0.02mg/kg) while for the root(0.28±0.01mg/kg). Lalitha and Vijayalakshmi (2018), researched on proximate composition, nutritional evaluation and mineral analysis in the leaves of an indigenous medicinal plant, *Alternanthera sessilis* which reveal the presence of Cu, Fe, Na, K, Zn, Mn, Mg, Cr, Ca and P the result showed similar analysis with the sample under studies. According to (Ayoola *et al.*, 2010), Zn, Fe and Cu in medicinal plants make them exhibit antidiabetic, antianaemic and antihelmintic properties respectively.

Minerals are biological components of diet that perform biochemical and physiological

function in living cell through synergetic interaction. The *Nymphaea lotus* could be of interest in complementary food formulation and pharmaceutical industries. The sodium is found to be lower in leave sample than in root sample. The low level of sodium in leaf is a characteristic of plant product, the low sodium content coupled with high potassium content make them a good food source for patients with hypertension and also increasing dietary potassium lowers blood pressure in humans and reduces the risk of stroke (Tapan, 2011). The calcium content was high in leave than root sample. Calcium is an important mineral required for strong bones and teeth, provide structural rigidity of the body and involved in blood clotting and cellular permeability (Indrayan *et al*., 2005). Calcium in conjunction with magnesium are involved in the formation of bones, it also plays important role in blood clotting and co-ordination of inorganic elements present in the body (Bargagli, 1998).

Copper is needed as a part of many enzymes during normal metabolic processes, copper is found to be in the same level for both the leave and the root sample. The availability of Cu in the medicinal plants plays a number of roles in treatment of ailments. In the treatment of bone related ailments such as arthritis, paining and swollen joints (Abullude, 2007). The magnesium s found to be high in leave than root of the plant sample was by magnesium is an antioxidant micronutrient and its presence may boost the immune system and aid in removing magnesium deficiencies which could lead to severe metabolic disorders and compromise the health of the organism (Hassan *et al.*, 2007).

Iron plays important role in blood clotting and co-ordination of inorganic elements present in the body Iron also helps regulate cell growth and cell differentiation. Iron is found high in the root than the leave. It helps keep muscles and nerves functioning normally and also helps to regulate heartbeat, supports the immune system and keeps the bones strong.

Conclusion

The Phytochemical and mineral constituents present in *Nymphaea lotus* plant act as a potential source of useful medicine for the treatment of various diseases, it has the potential to improve the lives of both urban and rural dwellers. This study indicates that some of these plants accumulate certain elements, and this property is exploited by the use of these plants for medicinal purposes in addition to their bioactive secondary metabolites constituents. The elucidation of element specification in these plants helps interpret the therapeutic actions claimed by traditional medicine practitioners and served as the basis for their uses in medication. So there is need for further research to isolate, purify and identify the structure of the active components of the plants which will likely lead to new drug.

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