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Original Research Article

Some Heavy Metals, Nutritional Values and Phytochemicals of Water Lily Plant Found in Girei Local Government of Adamawa State, Nigeria

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Abstract

The nutritional composition, phytochemicals and heavy metal contents of Nymphaea lotus in the rhizome, stem leaves and seeds were determined. The powdered plant samples were extracted successively with methanol using Soxhlet apparatus at 55-85 °C for 8-10 hours. Phytochemical Screening was carried out in order to determine the qualitative and quantitative analysis of saponins, tannins, alkaloids and flavonoids. Nutritional composition analysis such as Moisture content, fat composition, ash content, carbohydrate, crude fiber and protein were determined using standard methods whilst, mineral composition was determined using atomic absorption spectrophotometer. The plant parts such as leaf, stem, and rhizome were qualitatively and quantitatively screened for phytochemicals such as tannins, saponins, alkaloids and flavonoids. The leaf sample had the highest amounts of tannins (29.12 mg/g) and saponins (17.26 mg/g), which made the plant good for medicinal purposes. Analysis of elemental components such as Cd, Mn, Pb and Zn were carried out on the plant parts using Atomic Absorption Spectrophotometer (AAS). Pb was of a higher concentration in all the four parts of the plant than the other elements. The proximate composition in the seed showed crude protein content to have (2.16±0.141 %), crude fat (15.01±0.100 %), crude fiber (11.50±0.100 %), ash content (35.18±0.045 %), moisture (4.76±0.000 %) and carbohydrate (31.40±0.529 %). Oil was extracted from the seed with 6.5 % yield and was quantified for crude protein to have 6.83 %. Anti-nutritional analysis of the seed sample showed tannins to contain (3.210±0.100 mg/100 g), phytate (0.216±0.118 mg/100 g), saponins (0.010±0.00 mg/100 g) as oxalate was not detected. The anti-nutrients were at a permissible limit of WHO/FOA.It was observed that the biochemical composition of Nymphaea lotus seed is beneficial to both humans and animals for proper growth and maintenance while the anti-nutritional contents were reasonably low and may be used in chemical and pharmaceutical industries and the presence of phytochemicals are of great medicinal importance.

Keywords: Heavy metals, Nutrients, Phytochemicals, Water lily

INTRODUCTION

The bioactive molecules found in plants, such as antioxidants, antibacterial agents, anti-hypertensive agents, and anti-inflammatory agents, among others, are abundant sources of health-promoting properties. Aquatic plant growth is occurring all around the world in lakes, ponds, slow streams, and ditches, which may have either positive or bad consequences on the water bodies. It is tough to regulate them and appears impossible to

eradicate them. Asexual parts of the water lily plant (*Nymphaea lotus*), which is an aquatic plant with roots in shallow water, emerge above the water's surface. It is commonly found in freshwater environments such as ponds, lakes and slow-moving waters (Liu et al., 2019).
The plant has broad leaves that float horizontally on the water's surface. It has a greenish hue because photosynthesis is aided by this. They come in a variety of shapes and hues; some are edible, while others are not. The plant produces seed from mature flowers that contain numerous brown, crimson, or black seeds that are typically nut- or berry-like in shape. The seeds have a globular, stiff, spongy, and leafy green outer covering. According to the eaters, they can be eaten raw or fried and have a hint of paper with oil content.

The plant serves a significant purpose in water filtration in addition to being very attractive. Poisonous compounds including lead, mercury, phenol, and others can be absorbed by its root. It filters the microorganisms in the water as well as decontaminates the water and improves landscaping and afforestation. Some folks frequently wrap their meals in the leaves. In addition to serving as food packaging, leaves are crucial for giving fishes a cool, shaded environment. The essence of the flowers is frequently used in fragrances (Brunetti et al., 2018).

Phytochemicals are bioactive nutrients in plantsfound in seeds, vegetables, grains and other plants. They help resist fungi, bacteria et cetra to reduce risk of major chronic diseases (Liu et al., 2019). Secondary metabolites generated by plants, including terpenoids, phenolic metabolites, and alkaloids, are regarded to be where plants obtain their therapeutic effects. Water lily seeds contain phytochemicals like tannins, phytate, saponins, and lycopene (Chukwuma et al., 2017). From this plant, numerous bioactive and pharmacologically significant substances have been isolated and applied as medicines. Major groups of phytochemicals under study include carotenoids and polyphenols, which comprise phenolic acids, flavonoids, and stilbenes/lignans (Heneman and Zidenberg-Cherr, 2008). Based on their similar chemical structures, flavonoids can be categorized into categories, such as anthocyanins, flavones, flavanones, isoflavones, and flavanols. Flavanols are further divided into three groups: proantho-cyanins, epicatechins, and catechins (Heneman and Zidenberg-Cherr, 2008). Some of these phytochemicals may shield cells from harm that can cause cancer. Over 25,000 phytochemicals have been identified in total, and the majority of these compounds are found in high concentrations in the colorful portions of plants, such as seeds, vegetables, nuts, legumes, whole grains, et cetra (Falcone Ferreyra et al., 2012).

The nutritional value of the water lily plant is determined by its nutrient content, medicinal uses, and tea made from the roots, which is useful for mouth and throat irritation or inflammation. It can also be used as lotion, to heal sores, and to soften the skin. Traditional treatments for pharyngoplasty, pectoralgia, sperma-torrhagia, fever, cholera, etc. include the use of the entire plant (stem, leaves, blossom, and roots) as an anxiolytic agent. Saponins and sugars are the two main secondary metabolites found in the seed. Some locals claim that the seed may be used to cure eczema, as well as diabetic patients when it is cooked with rice and for nerve disorders (Falcone Ferreyra et al., 2012; Andualem and Gessesse, 2014).

In recent years, there has been an increase in heavy metals, which has led to issues all over the world. Although some are present naturally, they are frequently concentrated in agricultural soils as a result of the use of heavy metal-containing manures, commercial fertilizers, and sewage sludge (Zhuang et al., 2009). Due to their inability to be broken down, which also causes bioaccumulation, heavy metals can be toxic. Chronic sickness in humans may result from this buildup in food. Long-term exposure to low concentrations of metals may result in cumulative effects of metals (Atayese et al., 2008). The water lily has a better capacity for absorbing heavy metals, according to a study on the contents of heavy metals in water lily plants that had been done. The plants exhibit a strong propensity to preferentially bioaccumulate Zn and Pb over Cd and Fe. In another study Atayese et al. (2008) found that the water lily's roots had the highest concentrations of heavy metals, which were then found in the stems and leaves. Zn and Pb had the highest concentrations, followed by As, Cu, Ni, and Hg (Sneddon et al., 2006). Two essential micronutrients (Cu and Zn) and two non-essential elements (Pb and Cd) were considered in order to determine the concentrations in the water lily due to the potential for oxidative damage, impairment of photo-synthesis, impairment of nutrient uptake, and impeding of root growth in plants caused by Cadmium, as well as the impairment of plant metabolic processes, impairment of chlorophyll synthesis, and inhibition of enzyme activity.

The consumption and usage of *Nymphaea lotus* is on the increase without many knowing its composition and health benefit. This research work will help identify the phytochemicals, nutritional, anti-nutritional and heavy metal composition in the plant to help acquire a proper knowledge of its usage for proper consumption and treatment of some ailments seeing that recent researches determined that many phytochemicals can also protect human against diseases.

Experimental procedures

Materials

The following equipment/reagents were employed at the course of this research: weighing balance, laboratory glass wares, heating mantle, Soxhlet extractor, distillation

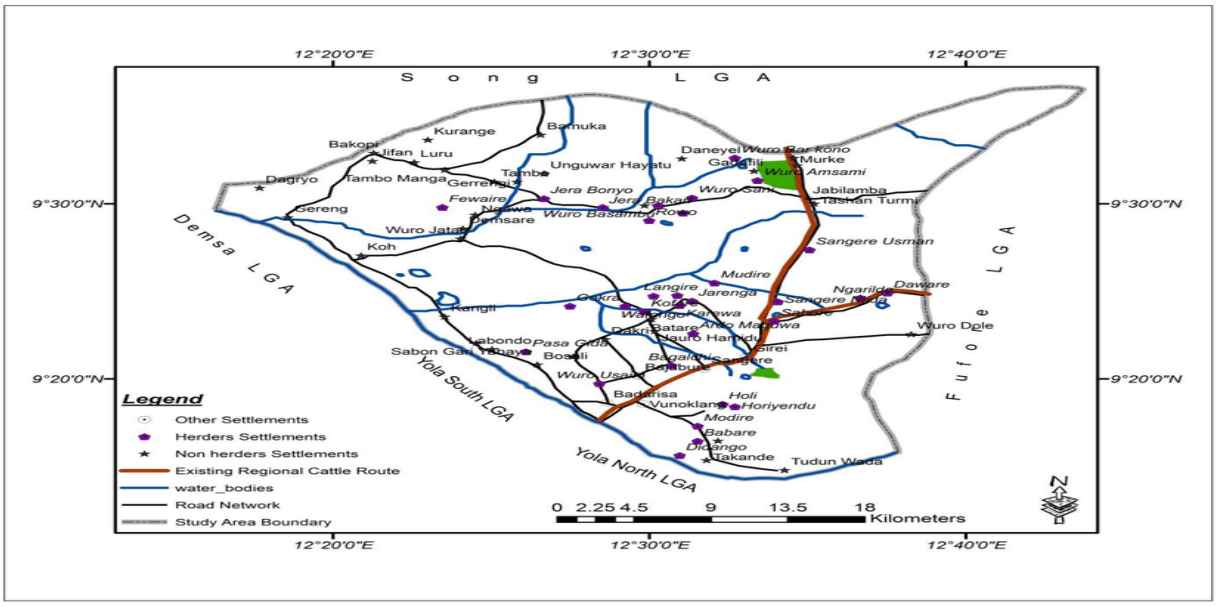

Figure 1. Sample site

Figure 2. *Nymphaea lotus* plant **(a)**, *Nymphaea lotus* seed**(b)**

set, water bath, beaker, Buchner funnel, filter paper, Whatmann number 1 paper, volumetric flask, oven, conical flask and atomic absorption spectrometer. Hydrochloric acid (HCl), hydrogen trioxonitrate (HNO_3), concentrated sulphuric acid (H_2SO_4), glacial acetic acid, ferric chloride, lead acetate, sodium hydroxide, potassium mercuric chloride solution, distilled water, hydrogen peroxide (H_2O_2), ammonium thiocyanate (NH_4SCN), potassium permanganate ($KMnO_4$).

METHODS

Area of the study

This research work was carried out in Girei Local Government Area of Adamawa State. It has an estimated population of 149,738 inhabitants (Figure 1).

Collection and Preparation of Sample

The leaf, stem, rhizome and seeds of Nymphaea lotus plant were collected from Bulirga dam, Fadama 11 project, and Labondo location of Girei local government area of Adamawa State Nigeria, where it was put in a polythene bag, labeled and conveyed to the laboratory for analysis, Figure 2 shows the Nymphaea lotus collection site and its seed.

Sample preparation

The Nymphaea lotus samples were collected, washed, and then shade dried in the lab. After drying, it was homogeneously ground into a fine powder using a wooden mortar and pestle. To eliminate any leftover debris, the powdered material was run through a fine

(2 mm mash) sieve. In order to analyze the finely powdered samples and ascertain their biological contents, they were subsequently placed into labeled plastic containers using (Wasagu et al., 2015) standard methodologies.

Extraction procedure

Method of (Elgorashi and Van Staden, 2004; Wakirwa et al., 2013) were employed; To extract the polar and non-polar chemicals, the powdered plant samples (50 g) were repeatedly extracted with methanol using a Soxhlet equipment at 55-85 °C for 8–10 hours. The powdered pack material was air dried before use for each solvent extraction.

Phytochemical Screening

Phytochemical Screening was carried out in order to determine the qualitative and quantitative analysis of saponins, tannins, alkaloids and flavonoids. The plant materials were screened for phytochemicals using standard methods as described by (Kubmarawa et al., 2007).

Qualitative Phytochemical screening

Qualitative phytochemical screening was carried out to investigate the various classes of natural compounds present in the extracts such as; test for saponins - 20 ml of distilled water was made from 5 g of extract. For 15 minutes, the suspension was stirred in a graduated cylinder. Two cm-thick layers of foam indicative of saponins were produced; to test for tannins, 2 g of the extract was diluted in 2 ml of distilled water. There were a few drops of a 5% ferric chloride solution that was neutral. The presence of tannins was indicated by a dark green color; to test for flavonoids, the extract was mixed with a few drops of sodium hydroxide solution.Flavonoids appeared as a strong yellow color that goes colorless when diluted acid is added; alkaloids were detected by adding two drops of Mayer's reagent (potassium mercuric iodide solution) to 2 g of plant sample extract. The presence of alkaloids is indicated by the appearance of a white, creamy precipitateas described by (Kubmarawa et al., 2007).

Quantitative phytochemical screening

By utilizing protocols for total tannins, the phytochemicals in the aqueous extracts were measured, and the results are as follows: 5 g of the sample were weighed into a 50 ml beaker and shaken mechanically. A 50 ml addition of distilled water was stirred for an hour. After filtering, it was produced to the proper volume in a 50 ml volumetric flask. Then, 2 ml of 0.1 M FeCl3 in 0.I N HCl and 0.008 M potassium ferrocyanide were added to 5 ml of the filtrate in a test tube. Using a UV-visible spectrophotometer, the absorbance reached 120 nm in about 10 minutes. Equation (1) was used to compute the total tannin.

$$\text{Tannic acid} = (\text{mg}/100\ \text{g}) = \frac{C\ x\ Extract\ volume\ x\ 100}{Aliquot + volume\ x\ weight\ of\ sample}$$

Equation ... (1)

The total flavonoids (Millaleo et al., 2010) was determined by weighing 10 grams of the plant sample and extracted repeatedly with 100 ml of 80 % aqueous methanol at room temperature.Filter paper No. 42 (125 mm) by Whatman was used to remove the entire solution. The filtrate was then put into a crucible, dried over a water bath, and weighed until it reached a steady weight. The method outlined by Makkar et al. (2007) was used to calculate the total saponins, and it goes as follows: 5 g of the dried powdered plant sample was obtained and added to 200 ml of a 20% ethanol solution. The suspension was cooked at around 55–60°C over a hot water bath for 3–4 hours while being constantly stirred. After filtering the mixture, 200 ml of a 20% ethanol solution was used to extract the plant powder once more. To get the volume down to around 40 ml, the two mixed solutions were evaporated over a water bath at about 80–90°C. 20 ml of diethyl ether was added to the concentrated solution in a 250 ml separating funnel, and the mixture was forcefully shaken to eliminate contaminants from the starting solution.

The ether layer with impurities was discarded, and the aqueous layer was recovered for further extraction. Then, 60 ml of n-butanol was twice poured after the purification procedure was completed once more. Two times, 20 ml of 5% aqueous sodium chloride were used to wash the combined n-butanol solutions (120 ml). The leftover aqueous solution was transferred to a porcelain crucible that had been dried and pre-weighed before being dried at 60 °C to a consistent weight. Equation (2) was used to calculate the saponin product, which is the residual residue.

$$\text{Saponin \%} = \frac{Final\ weight\ of\ residue}{Initial\ weight\ of\ the\ sample}\ \text{x } 100$$

Equation ... (2)

The procedure outlined by (Brunetti et al., 2018) was used to calculate the total alkaloids, and it goes as follows: 5 g of the sample was weighed into a 250 ml beaker, 200 ml of 10% acetic acid in ethanol was added, and the mixture was covered and let to stand for 4 hours. A percent of the original volume of the extract was concentrated on a water bath after it had been filtered. The extract received dropwise additions of concentrated ammonium hydroxide until the precipitation was finished.

After allowing the entire solution to settle, the precipitate was collected, cleaned with diluted ammonium hydroxide, and then filtered. The alkaloid, which was dried and weighed, is the residual. Equation (3) was used to determine the alkaloid.

$$\text{Alkaloid \%} = \frac{Final\ weight\ of\ residue}{Initial\ weight\ of\ the\ samp} \times 100$$

Equation ... (3)

Nutritional composition analysis

Chemical analysis to determine proximate composition of sample was carried out using standard procedures. Moisture content was determined by air drying, fat composition by Soxhlet extraction, ash content by incineration, carbohydrate calculated by difference, crude fiber by incineration after acid and base digestion, mineral element composition using the AAS after acid digestion of the samples and protein by the Kjedahl method (Afolayan et al., 2013) and the determination of crude protein (Ide et al., 2016) which follows that after carefully weighing 2 g of the sample, it was moved to a Kjedahl flask, where one catalyst tablet, 12 ml of concentrated H_2SO_4 (98%) acid, and 3 ml of H_2O_2 were gently added. The tubes will be put in a block digester that has been preheated to 410 °C after the reaction is finished. Continuous digestion was carried out until the mixture was clear, at which point the tubes were taken out and allowed to cool for 10 minutes at room temperature (Ide et al., 2016). Using equation (4), the crude protein percentage was determined. The sample's nitrogen content was estimated, and the nitrogen's conversion to protein was done using a factor of 6.25.

$$\%\ \text{Nitrogen content} = \frac{\text{Sample titre} - \text{Blank titre} \times \text{Normality of HCl} \times 14 \times \text{volume made of digest}}{\text{Aliquot of the digest taken} \times \text{Weight of sample} \times 1000} \times 100$$

$$\%\ \text{Protein content} = \%\ \text{Nitrogen} \times 6.25 \quad \text{Equation … (4)}$$

Anti-nutritional values

Anti-nutritional determination was evaluated thus for Oxalate (Agbaire, 2011), it follows that 2 g of the sample was dissolved in 100 ml of 0.75M H_2S0_4. The solution was carefully stirred with a magnetic stirrer for 1hr and filtered. 25 ml of the filtrate pipetted and titrated (80—90^0C) against 0.1M $KMnO_4$ to an end point of a faint pink colour that persisted for more than 30 seconds. Result was calculated using equation (5)

T x constant (0.225) Equation … (5)
where T = Titre value.

It follows that 4 g of sample was steeped in 100 ml of 2% HCl for 5 hours before filtering for the purpose of determining phytate (Kubmarawa et al., 2007). A conical flask was filled with 25 ml of the filtrate and 5 ml of a 0.3% ammonium thiocyanate (NH_4SCN) solution. A brownish yellow color was obtained at the end point after the mixture was titrated against 0.1M $FeCl_3$ and remained for 5 minutes. Eq. (6) was used to calculate the result.

T x constant (0.1635) Equation … (6)
where T = Titre value.

Tannin was measured using the procedure described by (Adejumo and Victoria, 2019), which stated that: 5 g of the material was weighed into a 50 ml plastic container in a mechanical shaker. A 50 ml addition of distilled water was agitated for an hour. After filtering, it was produced to the proper volume in a 50 ml volumetric flask. Then, 2 ml of 0.1 M FeCl3 in 0.l N HCl and 0.008 M potassium ferrocyanide were added to 5 ml of the filtrate in a test tube. Using a UV visible Spectrometer, the absorbance at 120 nm was determined in less than 10 minutes.

By weighing 5 g of the sample powder, 100 ml of 20% aqueous ethanol, and the sample powder were used to determine the presence of saponin (Ugbaja et al., 2017). The sample was heated to roughly 55°C over a hot water bath for 4 hours while being constantly stirred. Filtering the mixture allowed the residue to be extracted again using 200 ml of 20% ethanol. Over a water bath at roughly 90^0C, the combined extract was reduced to 40 ml. 20 ml of diethyl ether was added to the concentrate in a 250 ml separating funnel, agitated rapidly, and then added.The ether layer was discarded while the aqueous layer was removed. When 60 ml of n-butanol was added, the purification procedure was repeated. Double-washing the combined n-butanol extract with 10 ml of 5% aqueous sodium chloride was done. In a water bath, the residual solution was warmed. After evaporation, the sample was dried in an oven to a fixed weight, and the percentage of saponin was determined.

Instrumentation

An acid digest of each sample species was prepared and analysis of cadmium (Cd), lead (Pb), Zinc (Zn), Copper (Cu), and Cadmium (Cd) was determined using Atomic Absorption Spectrophotometer (AAS) as adopted by (Younes and Fawzi, 2016).

RESULT AND DISCUSSION

Qualitative phytochemical screening

The result of the qualitative phytochemical screening in the leaf, stem and rhizome of nymphaea lotus plant

Table 1. Qualitative analysis of the leaf, stem and rhizome of Nymphaea lotus

Parameters	WL	WS	WR
Saponins	+	+	+
Tannins	+	+	+
Alkaloids	-	+	+
Flavonoids	-	-	-

Key: + indicates presence and – indicates absence
WL = Water lily leaf WS = Water lily stem WR = Water lily rhizome

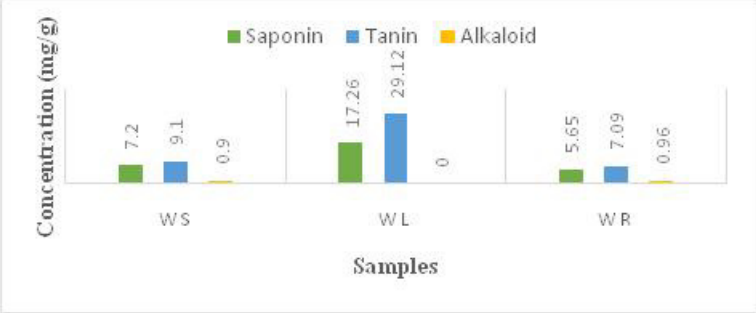

Figure 3. Quantitative analysis phytochemicals of the leaf, stem and rhizome of *Nymphaea lotus*
WS = Water Lilly Stem
WL = Water Lilly Leaf
WR = Water Lilly Rhizome

revealed the presence of saponins, alkaloids and tannins. Saponins and tannins were present in the leaf, stem and rhizome. Alkaloid was present in the stem and rhizome but absent in the leaf. There was absence of flavonoids in the leaf, stem and rhizome which could be attributed to genetic variation; different plant species and even varieties within the same species can exhibit variation in flavonoid production. Additionally, certain species may lack specific enzymes or regulatory factors necessary for flavonoid biosynthesis, resulting in their absence in the leaf tissue (Falcone Ferreyra et al., 2012).

Environmental conditions can also play a crucial role in flavonoid production in plants. Suboptimal environmental factors such as insufficient light, extreme temperatures, water stress, or nutrient deficiencies can significantly affect flavonoid synthesis and accumulation. In such conditions, plants may divert their resources towards other essential processes, leading to reduced or no flavonoid production in the leaves (Falcone Ferreyra et al., 2012).

Furthermore, the developmental stage of leaves is known to influence flavonoid content. Flavonoid synthesis varies throughout the growth and maturation of leaves, with highest accumulations often occurring during their early development stages. Thus, during senescence or leaf abscission, the levels of flavonoids may decline or become absent (Falcone Ferreyra et al., 2012). The presence of saponins, tannins and alkaloids agree with the reports of (Afolayan et al., 2013; Adegoke et al., 2010) with the exception of alkaloids that was absent in the leaf sample as reported by (Adegoke et al., 2010) whose difference could be as a result of microhabitat variation. The findings of (Com et al., 2016), agrees with this research work in regards to flavonoid as it is absent in the methanol extract.

Nymphaea lotus contains phytochemicals, which is a sign that they are highly important medicinally (Wasagu et al., 2015). The qualitative screening of phytochemicals in all of the Nymphaea lotus plant's sections is displayed in Table 1. All of the components are claimed to contain saponins, which are said to increase antioxidant qualities and give rise to pharmacological potential including anti-inflammation, anti-tumor, and sedative characteristics (Helaluddin et al., 2016). Since the dawn of time, alkaloids found in the plant have been known to have pharmacological effects like sedative and cytotoxic potentials. This helps to provide relaxing and sedative effects on the neurological system and is beneficial in the treatment of illnesses like insomnia, anxiety, and others (Helaluddin et al., 2016). Plant-based phytochemicals have been linked to both human and animal health benefits (Khan, 2018).

Quantitative analysis of the leaf, stem and rhizome of Nymphaea lotus

The quantitative screening of the presence of phytochemicals such as saponins, tannins and alkaloids were determined and presented in Figure 3. The saponin

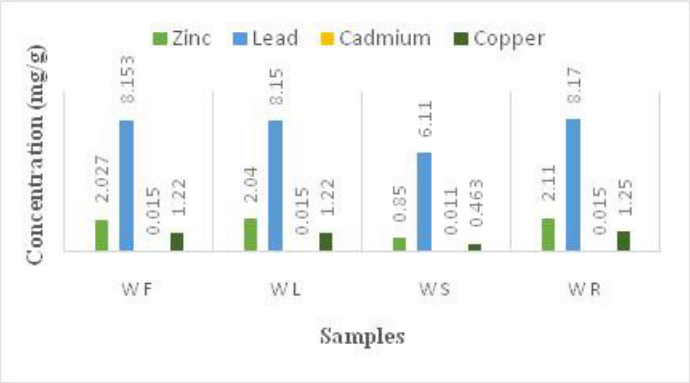

Figure 4. Heavy metal contents in *Nymphaea lotus* plant
WF = Water Lilly Fruit

content was of higher value in the leaf (17.26 mg/100 g), second to it was the stem having (7.20 mg/100 g) and finally the rhizome (5.65 mg/100 g). Tannin has the highest amount in the leaf content (29.12 mg/100 g), the stem (9.10 mg/100 g), then rhizome (7.09 mg/100 g). Alkaloid has its highest amount in the rhizome (0.96 mg/100 g), and the stem has (0.90 mg/100 g), while there was no alkaloid found in the leaf. Flavonoid was also absent in the leaf sample. The quantities of tannins in the leaf and stem sample were significantly higher ($p<0.05$) compared to the rhizome sample, while the saponin content in the leaf sample was significantly higher ($p<0.005$) in the leaf sample than the stem and rhizome. There were no significant differences ($p>0.005$) in the alkaloids present in stem and rhizome. From Figure 3, it shows that *Nymphaea lotus* is higher in tannin content, which shows that, they would be highly required in pharmaceutical industries as it exhibits various pharmacological properties such as anti-toxic, anti-cancerous, anti-inflammatory, anti-helmintic, anti-microbial, anti-viral, healing of wounds, curing of dysentery, et cetra (Ghosh, 2015). Tannins help to build up vitamin stores and strengthen blood vessels. The idea that including seeds in diabetics' diets might be beneficial is supported by the presence of these phytochemicals. These extra details will encourage the use of Nymphaea lotus seeds, at least in endemic regions (Moore et al., 2009).

Heavy metals analysis of Nymphaea lotus

The concentrations of heavy metals such as Cadmium, Copper, Lead and Zinc in Nymphaea lotus were analyzed. Figure 4 shows the result of the heavy metals present. Lead (Pb) has the highest amount compared to zinc (Zn), cadmium (Cd) and copper (Cu) in all parts of the plant. The rhizome has the highest amount of lead (8.170±0.140 mg/kg) followed by the seed (8.153±0.114 mg/kg), the stem (8.150±0.000 mg/kg) and the lowest amount is found in the stem (6.110±0.100 mg/kg) and this could be attributed to factors such as their morphology, physiological processes, and environmental conditions and it has been suggested that the uptake and translocation mechanisms associated with the plant structures can lead to the accumulation of heavy metals (Alaboudi et al., 2018). Zinc is second after lead with the leaf having the highest amount (2.040±0.141 mg/kg), followed by the seed having (2.027±0.037 mg/kg), the rhizome has (2.110±0.141 mg/kg), and the lowest amount of zinc is found in the stem (0.850±0.000 mg/kg). Compared to other metal ions with similar chemical properties, zinc is considered to be relatively non-toxic to humans. It is only exposure to high doses of zinc that has toxic effects, making acute zinc intoxication a rare event. Zinc deficiency is widespread and has a detrimental impact on growth, neuronal development, and immunity, and in severe cases its consequences are lethal.

The amount of copper in the plant is lower than that of lead and zinc, this could be attributed to differences in chemical availability, plant uptake mechanisms, and plant physiological processesand copper been an essential element in plants, plays a key role in various physiological processes such as photosynthesis, enzyme activation, electron transport, cell wall formation, and defense against oxidative stress (Alaboudi et al., 2018).

The rhizome has (1.250±0.001 mg/kg) amount of copper, the seed and leaf are almost of the same amount with (1.220±0.000 mg/kg) and (1.220±0.147 mg/kg) respectively. The stem has the lowest amount of copper with (0.463±0.083 mg/kg). Both too much and too little copper can affect how the brain works. Impairments have been linked with Menkes, Wilson's, and Alzheimer's disease (Ugbaja et al., 2017).

Cadmium has the lowest content in *Nymphaea lotus* compared to the other elements. The amount of cadmium present in the seed and leaf are of the same values (0.015±0.003 mg/kg), as the rhizome is not also far from the seed and leaf with (0.015±0.000 mg/kg). The stem has the lowest amount of cadmium (0.011±0.003 mg/kg).

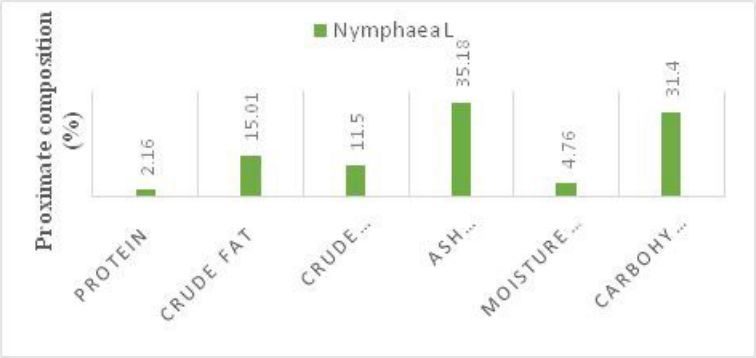

Figure 5. Proximate composition of *Nymphaea lotus* seed

The result obtained from (Wasagu et al., 2015) from the cadmium content in the leaf (0.010±0.0001 mg/kg) is not very far from the one obtained in the cadmium content of the leaf sample as (0.015±0.003 mg/kg). This study shows that the rhizome, leaf and fruit samples had statistical ($p>0.005$) higher concentration of lead than the stem sample but with lower cadmium content.

Proximate analysis of the seed of Nymphaea lotus

The proximate composition of *Nymphaea lotus* bulb was determined to know the crude protein, crude fat, crude fiber, ash, moisture and carbohydrate contents of the seed as presented in Figure 5. Protein content was (2.16±0.141 %), crude fat content (15.01±0.100 %), crude fiber content (11.50±0.100 %), ash content (35.18±0.045 %), moisture content (4.76±0.000 %) and carbohydrate content as (31.40±0.529 %). Statistical results however show no significant difference between the various samples under study at ($p>0.05$). It can be seen that the seed sample had higher amount of ash and carbohydrate than crude fat and crude fiber, according to (Andualem and Gessesse, 2014), seed samples typically have higher amounts of ash and carbohydrates compared to crude fat and crude fiber. This is likely because ash represents the inorganic mineral content of the seed, while carbohydrates serve as the main energy source. On the other hand, crude fat and crude fiber are present in smaller quantities, as fat is used for energy storage and fiber provides structural support.

The protein and moisture content were moderate.The protein content of the bulb obtained in this research work was 2.16±0.141 % which was closely related to that reported by (Wasagu et al., 2015) to be 2.67±0.290 %, and not far from the findings of Musa *et al.* (2012) to be 3.09±0.08 %. However, there is a large difference with the result obtained from (Liu et al., 2019) as 21.66±0.014 %.Protein is an important feed ingredient in fish feed formulation, especially those obtained from *Nymphaea lotus* bulb (Adegoke et al., 2010)>

Protein is important in the repair of worn out tissues, hormones, enzymes, antibodies and other substances, with the synthesis of new cells. They are required for healthy functioning and development of the body and its protection (Afolayan et al., 2013). Crude protein is an important feed ingredient normally used as major ingredient in fish feed formulation (Chukwuma et al., 2017).

Crude fat value obtained was 15.01±0.100 % in the bulb which was higher than 5.07±0.014 % as reported by (Chukwuma et al., 2017) and 9.33±0.29 % by (Wasagu et al., 2015). The fat content is important to the body as it serves as energy storage to the body besides their roles in maintaining healthy skin and hair.

Crude fiber content 11.50±0.100 % obtained in this work was lower compared to the value obtained by (Ugbaja et al., 2017) to be 13.30±0.021 % and higher than that of (Musa et al., 2012) as 6.17±0.581 %. A function of fiber is to help speed up the passage of food through the digestive system which facilitates regularity. It reduces the onset risk of metabolic syndrome and diabetes (Wasagu et al., 2015). Past studies have been linked with low fiber contents in diet with health problems like heart disorder, bowel cancer and appendicitis. Therefore, *Nymphaea lotus* bulb when consumed will prevent health problems linked with bowel cancer, heart disorder and appendicitis.

The ash content 35.18±0.045 % in the bulb of *Nymphaea lotus* is of high value. It is higher compared to those reported by (Wasagu et al., 2015) 2.67±0.29 %, (Musa et al., 2012) as 2.33±0.29 % and 2.81±0.498 % (Nkafamiya et al., 2010).

The moisture content was (4.76±0.000 %). It is lower than the value reported by (Ugbaja et al., 2017) as 48.66±02.9%. The low moisture content indicates that the bulb can be processed and used as animal feeds. Therefore, low moisture content helps in retaining the nutritional components in the bulb of *Nymphaea lotus* as high moisture content promotes susceptibility in microbial growth and enzyme activity (Feigl et al., 2013).

The carbohydrate content (31.40 ± 0.529 %) in

Figure 6. Anti-nutritional contents *Nymphaea lotus* seed

Nymphaea lotus bulb was the second highest in proximate contents. Considerably, this high amount of carbohydrate in the bulb of Nymphaea lotus shows they are good sources of energy. Previous findings from (Chukwuma et al., 2017; Wasagu et al., 2015) were reported to have carbohydrate with the highest percentage amongst the other nutrients. This shows that *Nymphaea lotus* bulb is rich in carbohydrate. Carbohydrates serve as the storage form of energy (glycogen) to meet the immediate energy demands of the body and provide necessary calories in the diet and promotion of the utilization of dietary fats (Brown et al., 2001).

Extracted oil from Nymphaea lotus seed

200 ml of water was added to 65 g of the grounded sample in a container. Hot water floatation method was used to extract oil from the seed (Kalu et al., 2023) as 6.5 % oil yield was obtained. Similarly, (Aliyu et al., 2017) carried out an extraction on *Nymphaea lotus* seed using n-hexane as his solvent. The % oil yield obtained was 13.23 % from 50 grams of the grounded sample and 300 ml of n-hexane.

The oil extracted was then analyzed for crude protein with % N as 6.83 %. According to (Aliyu et al., 2017) when the percentage for crude protein is low, the bacteria responsible for digestion cannot sustain adequate levels to forage. Food manufacturers use crude protein to calculate the amount of carbohydrate in food. Crude protein determines the economic value of the food product and can impact the economic feasibility of new industries for alternate protein production. Evaluating this is important since certain oil seeds contain toxic and or anti-nutritional factors.

Anti-nutritional composition

Anti-nutritional composition of phytate, oxalate, saponins and tannins were determined. The results shown in figure 6 indicates that tannin has the highest concentration which is moderately low as 3.210±0.100 mg/100 g, followed by phytate with a low amount 0.216±0.118 mg/100 g, then saponin at a very low content to0.010±0.000 mg/100 g with the absence of oxalate. Figure 6 also shows the anti-nutritional content of the seed. According to (Wasagu et al., 2015; (Chukwuma et al., 2017) tannins content is the highest in the anti-nutritional content present which is in line with this finding. The compositions here are lower compared to those obtained from (Schlemmer et al., 2009). This shows that the anti-nutrients contents in Nymphaea lotus seed are obtained in low quantities.

Foods rich in tannins are said to be of low nutritional value. Tannins are plant polyphenols which have theability to form complexes with metal ions and with macromolecules such as proteins and polysaccharides (Godswill and Otuosorochi, 2020) decreases or prevents the absorption of Fe, Mg, Zn and other minerals by the body (Schlemmer et al., 2009). According to (Majeed et al., 2020) phytate should be lowered as much as possible, ideally to 25 mg or less per 100 grams. Therefore, the phytate content in this study is permissible since the content is low.

Saponins cause a reduction of blood cholesterol by preventing its reabsorption which makes it useful in cardiovascular disease.

CONCLUSION

This study revealed the phytochemical constituents, heavy metal content, nutritional and anti-nutritional contents of *Nymphaea lotus* plant obtained in Girei Local Government of Adamawa State. This research showed quantification of *Nymphaea lotus* seed with biochemical composition such as carbohydrate, fat, moisture, protein and ash needed by both humans and animals for proper growth and maintenance. The anti-nutritional contents in its seed such as phytate, tannins and saponins concentrations were reasonably low and may be used in chemical and pharmaceutical industries. It contains phytochemicals such as saponins, tannins and alkaloids which are of high medicinal importance. This shows that

the plant can be used for treatment of several diseases. Its low moisture content also makes it a good source of animal feeds. It was also established that lead had the highest concentration in the rhizome which could disrupt plant metabolism, impair chlorophyll synthesis, inhibit enzyme activity, and affect nutrient uptake in the *Nymphaea lotus* plant and it can besuggested that phytoremediation through phytoextraction which an emerging cost-effective remediation technology for the removal of heavy metals in plants, can be adopted for further research.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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REFERENCES

Adegoke A. A., D. Akinpelu, O. A. Aiyegoro, and C. Mboto (2010). "Studies on phytochemical screening and antimicrobial potentials of Phyllanthus amarus against multiple antibiotic resistant bacteria,". [Online]. Available: https://www.researchgate.net/ publication/45258233

Adejumo T. and O. Victoria Akinmoladun (2019). "Molecular diagnostics of fungal contaminants and determination of mycotoxins in cereals-maize, sorghum and rice View project Biopesticides View project," [Online]. Available: http:// www.ijcmas.com

Afolayan A. J., O. J. Sharaibi, and M. I. Kazeem (2013). "Phytochemical analysis and in vitro antioxidant activity of Nymphaea lotus L," International Journal of Pharmacology, vol. 9, no. 5, pp. 297–304, , doi: 10.3923/ijp.2013.297.304.

Agbaire P. O. (2011). "Nutritional and Anti-nutritional Levels of Some Local Vegetables (Vernomiaanydalira, Manihot esculenta, Teiferiaoccidentalis, Talinum triangulare, Amaranthus spinosus) from Delta State, Nigeria," J. Appl. Sci. Environ. Manage, vol. 15, no. 4, pp. 625–628, [Online]. Available: www.bioline.org.br/ja

Alaboudi K. A., B. Ahmed, and G. Brodie (2018). "Phytoremediation of Pb and Cd contaminated soils by using sunflower (Helianthus annuus) plant," Annals of Agricultural Sciences, vol. 63, no. 1, pp. 123–127, Jun. 2018, doi: 10.1016/j.aoas..05.007.

Aliyu M., M. Atiku Kano, N. Abdullahi, I. Aliyu Kankara, S. Ismail Ibrahim, and I. Abdullahi Abdulkadir (2017). "Extraction, Characterization and Fatty Acids Profiles of Nymphaea Lotus and Nymphaea Pubescens Seed Oils," BiosciBiotechnol Res Asia, vol. 14, no. 4, pp. 1299–1307, Dec., doi: 10.13005/bbra/2573.

Andualem B, A. Gessesse (2014). "Proximate composition, mineral content and antinutritional factors of Brebra (Millettiaferruginea) seed flour as well as physicochemical characterization of its seed oil,". [Online]. Available: http://www.springerplus.com/content/ 3/1/298

Atayese MO, A. I. Eigbadon, K. A. Oluwa, and J. K. Adesodun (2008). "Heavy metal contamination of amaranthus grown along major highways in Lagos, Nigeria."

Brown K. H., S. E. Wuehler, and J. M. Peerson (2001). "The importance of zinc in human nutrition and estimation of the global prevalence of zinc deficiency,".

Brunetti C., A. Fini, F. Sebastiani, A. Gori, and M. Tattini (2018). "Modulation of phytohormone signaling: A primary function of flavonoids in plant–environment interactions," Frontiers in Plant Science, vol. 9. Frontiers Media S.A., Jul. 20,. doi: 10.3389/fpls.2018.01042.

Chukwuma Stephen E., C. Imo, A. Kayode Adebisi, I. Chinedu, and A. Attah Samuel (2017). "Chemical Composition of Water Lily (Nymphaea lotus) Bulbs Citation Storability of Sliced Dehydrated Tomato. View project Foods and food additives View project Chemical Composition of Water Lily (Nymphaea lotus) Bulbs Citation," Chemical Composition of Water Lily (Nymphaea lotus) Bulbs. American Journal of Food Science and Nutrition, vol. 4, no. 2, pp. 7–12, [Online]. Available: http://www.aascit.org/ journal/ ajfsn

Com W. O., K. S. Prasad, and N. Savithramma (2016), "American Journal of Advanced Drug Delivery Screening of Phytochemical Constituents of Nymphaea Caeruleasavigny. An Aquatic Plant Resource for Drug Development." [Online]. Available: www. ojadd.com

Elgorashi E. E, J. Van Staden (2004). "Pharmacological screening of six Amaryllidaceae species," J Ethnopharmacol, vol. 90, no. 1, pp. 27–32, , doi: 10.1016/j.jep.2003.09.012.

Falcone Ferreyra M. L., S. P. Rius, P. Casati (2012). "Flavonoids: Biosynthesis, biological functions, and biotechnological applications," Frontiers in Plant Science, vol. 3, no. SEP. Frontiers Research Foundation, Sep. 28,. doi: 10.3389/fpls.2012.00222.

Feigl et al., G (2013). "Physiological and morphological responses of the root system of Indian mustard (Brassica juncea L. Czern.) and rapeseed (Brassica napus L.) to copper stress," Ecotoxicol Environ Saf, vol. 94, pp. 179–189, Aug., doi: 10.1016/j.ecoenv. 2013.04.029.

Ghosh D (2015). "Tannins from Foods to Combat Diseases," Int J Pharma Res Rev, vol. 4, no. 5, p. 40,.

Godswill Awuchi C. and A. Otuosorochi Ikechukwu (2020). "The Biochemistry, Toxicology, and Uses of the Pharmacologically Active Phytochemicals: Alkaloids, Terpenes, Polyphenols, and Glycosides,", doi: 10.5281/zenodo.3967809.

Helaluddin A. B. M., R. S. Khalid, M. Alaama, and S. A. Abbas (2016). "Main analytical techniques used for elemental analysis in various matrices," Tropical Journal of Pharmaceutical Research, vol. 15, no. 2. University of Benin, pp. 427–434, Feb. 01,. doi: 10.4314/tjpr.v15i2.29.

Heneman K, S. Zidenberg-Cherr (2008). Nutrition and Health Info Sheet: Phytochemicals. University of California, Agriculture and Natural Resources,. doi: 10.3733/ucanr.8313.

Ide M., N. Njila, S. H. Mandengue, N. M. I. Ngaha, I. Dahlan, and A. A. Yusuf (2016). "Comparative Proximate Analysis of Leaves and Bark of AlchorneaCordifolia (Euphorbiaceae)," Journal of Agriculture and Environmental Sciences, vol. 5, no. 1, pp. 2334–2412, doi: 10.15640/jaes.v5n1a21.

Kalu et al., K. M (2023). "Extraction, Synthesis and Characterization of an Alkyd Resin from Sesamum indicum Seed Oil," OAlib, vol. 10, no. 07, pp. 1–18, , doi: 10.4236/oalib.1110447.

Khan A (2018). "Phytochemicals and their role in curing fatal diseases: A Review," Pure and Applied Biology, vol. 7, no. 4, Dec., doi: 10.19045/bspab.2018.700193.

Kubmarawa D., G. A. Ajoku, N. M. Enwerem, and D. A. Okorie (2007). "Preliminary phytochemical and antimicrobial screening

of 50 medicinal plants from Nigeria," Afr J Biotechnol, vol. 6, no. 14, pp. 1690–1696, [Online]. Available: http://www.academicjournals.org/AJB

Liu J. et al., (2019). "Extraction of Polysaccharide from Dendrobium nobileLindl. By Subcritical Water Extraction," ACS Omega, vol. 4, no. 24, pp. 20586–20594, Dec. doi: 10.1021/acsomega.9b02550.

Majeed et al., S. "Corn-Expressed Phytase Influence on Broiler Growth Performance."

Makkar H. P. S., G. Francis, and K. Becker (2007). "Bioactivity of phytochemicals in some lesser-known plants and their effects and potential applications in livestock and aquaculture production systems," Animal, vol. 1, no. 9. pp. 1371–1391, doi: 10.1017/S1751731107000298.

Millaleo R., M. Reyes-Díaz, A. G. Ivanov, M. L. Mora, and M. Alberdi (2010). "Manganese as essential and toxic element for plants: Transport, accumulation and resistance mechanisms," J Soil Sci Plant Nutr, vol. 10, no. 4, pp. 476–494, , doi: 10.4067/s0718-95162010000200008.

Moore F., G. Forghani, A. Qishlaqi, F. Moore, G. Forghani, and A. Qishlaqi (2009). "A1 Printed in the Islamic Republic of Iran,". [Online]. Available: https://www.researchgate.net/publication/285964692

Musa A. et al., (2012). "Proximate composition and mineral analysis of Nymphaea lotus seeds Effect of heat processing on lycopene View project Biodiesel Production from Terminalia catappa Seed Oil using CaO derived from Snail Shell as Catalyst. View project Proximate composition and mineral analysis of Nymphaea lotus seeds,". [Online]. Available: https://www.researchgate.net/publication/264942963

Nkafamiya I. I., S. A. Osemeahon, I. I. Nkafamiya, U. U. Modibbo, and A. Aminu (2010). "Nutritional status of non-conventional leafy vegetables, Ficusasperifolia and Ficussycomorus medicinal chemistry View project development of urea formaldehyde and polystyrene waste as copolymer binder for emulsion paint formulation View project Nutritional status of non-conventional leafy vegetables, Ficusasperifolia and Ficussycomorus," Afr. J. Food Sci. vol. 4, no. 3, pp. 104–108, , [Online]. Available: http://www.academicjournals.org/ajfs

Schlemmer U., W. Frølich, R. M. Prieto, and F. Grases (2009). "Phytate in foods and significance for humans: Food sources, intake, processing, bioavailability, protective role and analysis," Molecular Nutrition and Food Research, vol. 53, no. SUPPL. 2. Wiley-VCH Verlag, pp. S330–S375, doi: 10.1002/mnfr.200900099.

Sneddon J., C. Hardaway, K. K. Bobbadi, and A. K. Reddy (2006). "Sample preparation of solid samples for metal determination by atomic spectroscopy - An overview and selected recent applications," Appl Spectrosc Rev, vol. 41, no. 1, pp. 1–14, Mar., doi: 10.1080/05704920500385445.

Ugbaja C. C., O. O. Fawibe, A. S. Oyelakin, I. O. Fadimu, A. A. Ajiboye, and D. A. Agboola (2017). "Comparative Phytochemical and Nutritional Composition of <i>Trichosanthe scucumerina</i> (L.) and Some<i> Solanum lycopersicum</i>(L.) Cultivars in Nigeria," Am J Plant Sci, vol. 08, no. 02, pp. 297–309, 2017, doi: 10.4236/ajps..82021.

Wakirwa J. H., U. E. Yawate, S. G. Zakama, J. Muazu, and S. J. Madu (2013). "Phytochemical and antibacterial screening of the methanol leaf extract of mitragyna inermis (wild o. Ktze rubiaceae),".

Wasagu R., M. Lawal, L. Galadima, and A. Aliero (2015). "Nutritional composition, antinutritional factors and elemental analysis of Nymphaea lotus (water lily)," Bayero Journal of Pure and Applied Sciences, vol. 8, no. 1, p. 1, Nov., doi: 10.4314/bajopas.v8i1.1.

Younes Ibrahim S. and M. M Fawzi (2016). "Determination of Heavy Metals and Other Toxic Ingredients in Henna (Lawsoniainermis)," J Environ Anal Toxicol, vol. 06, no. 03, doi: 10.4172/2161-0525.1000364.

Zhuang P., M. B. McBride, H. Xia, N. Li, and Z. Li (2009). "Health risk from heavy metals via consumption of food crops in the vicinity of Dabaoshan mine, South China," Science of the Total Environment, vol. 407, no. 5, pp. 1551–1561, Feb. doi: 10.1016/j.scitotenv.2008.10.061.