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Characterization of water lily (*Nymphaea lotus*) for nutrients, anti-nutrients, phytochemicals, and antioxidant capacities

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ABSTRACT

Global food problems have challenged organizations to explore the possibility of using neglected aquatic plants as supplementary food. This study was aimed to characterize nutrients, antinutrients, phytochemicals, and antioxidant capacities of water lily (*Nymphaea lotus*). Ethnobotanical survey identified four macrophytes are edible by the local communities. The lethal dose of *N. lotus* extract was greater than 5000 mg/kg. The highest protein content (23.6%) was found in rhizome of *N. lotus*. Higher amount of potassium was found in stem of *N. lotus* (30400 mg/kg) and rhizome of *Arundo donax* (16600 mg/kg). Leucine was the most abundant essential amino acid in all of the samples while methionine was found in the low concentration. The protein and essential amino acids contents make *N. lotus* a useful food. The total phenolic content ranged from 0.77 to 5.77 mg GAE/g. The presence of phytochemicals in these edible freshwater macrophyte are great medicinal importance. A higher half inhibition concentration (IC_{50}) of DPPH (24.48 μg/mL), and ABTS (0.4 μg/mL) free radicals scavenging was reported from *Typha latifolia*. It was concluded that, these edible freshwaters are safe for consumption as the median lethal dose is higher than 5000 mg/kg, and trace metals are negligible.

1. Introduction

Nymphaea lotus which is commonly known as water lily is among the floating leaves aquatic plants that is consumed by people during food scarcity and famine time (www.purdue.edu). *N. lotus* is widely distributed in freshwater ecosystems and were reported to be consumed during times of food shortage in Nigerian, Senegal, Ghana, Guinea, Malawi, Sudan, Bangladesh, India, Pakistan, Finland, Kenya, and Ethiopia. Archaeobotanical studies involving biomarkers extracted from human dental calculus indicated the rhizome and seed of freshwater aquatic plants were chewed and most probably ingested in the Bronze Age, early Middle Ages, Mesolithic, and Neolithic periods across Europe (Buckley et al., 2023). The water lily (*Nymphaea lotus*), which grows abundantly along the shore of Lake Ziway, Ethiopia, is a non-conventional edible aquatic plant. *Nymphaea lotus* is a rooted aquatic macrophyte with submerged rhizomes, and long, spongy roots that firmly anchor the plant to the sediment, and serrated floating leaves. *Nymphaea lotus* plants grow in Lake Ziway at a water depth from 50 to 111 cm, light penetration from 17 to 21 cm depth, slightly alkaline water pH ranging from 8.03 to 8.92, and water temperature from 21.54 to 24.77°C. Locally, its leaf is known as 'Balbate', while the edible rhizome is known as 'Kinta', 'Moche', or 'Kurumbo' (Abelti et al., 2023). Moreover, around Lake Ziway, *Typha latifola, Arundo donax,* and *Nymphaea lotus* were reported to be the edible aquatic plants harvested as food (Merga, 2021).

Water lilies have food, nutritional and health benefits. Fifteen different water lily species were found to contain good nutritional value and phytochemical compounds (Abelti et al., 2023). The rhizome of *N. lotus* is a good source of amino acid for an adult protein supplement (Chinelo & Jega, 2019). The dried parts of *N. lotus* could be mixed with other ingredients to produce various products. The addition of 25% petal of *N. lotus* and 3% pollen of *N. nucifera* improve antioxidant activity and a total phenolic content of gummy jelly (Chuchird & Pattarathitiwat, 2021). *Nymphaea lotus* mixed with cassava starch could be used to treat indigestion, dysentery, and dyspepsia (Morya et al., 2024). Additionally, *N. lotus* infused with wine shows to have therapeutic benefits for the management of inflammation and diabetes (Morya et al., 2024). The extracts of *N. caerulea* can be used as sleep aid, and anxiety reliever (Schimpf et al., 2023). Numerous tribes used the root juice,

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decoction, and powdered roots of *N. odorata to* treat colds and coughs (Meuninck, 2023).

The uses of *N. lotus* as food, nutrition, and health benefits are restricted to particular parts of the world due to traditional knowledge, economic conditions, acceptance as major food or alternative food. Information regarding the nutritional, anti-nutritional, phytochemicals, and antioxidant capacities of *N. lotus* is meager. Food composition studies are essential in establishing the nutritional value of aquatic plants for future use in different food applications. The aim of this study was to characterize ethnobotany, acute toxicity, proximate, anti-nutrient, phytochemicals, antioxidant capacities, elements, and amino acids of *Nymphaea lotus*, compare with *Arundo donax,* and *Typha latifolia* harvested from Lake Ziway, Ethiopia.

2. Materials and methods

2.1. Reagents and chemicals

Chemicals used for quantification of phytochemicals and antioxidant capacities like Tannic acid, sodium phytate, Quercetin, Folin-ciocalteu, Gallic acid, 6-hydroxy-2,5,7,8- tetra methylchroman 2-carboxylic acid, 2,2-Diphenyl-1-Picrylhydrazyl, 2,4,6-tri-2-pyridyl-s-triazine, and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) were purchased from Sigma-Aldrich, Hamburg, Germany. Chemicals used as a standard during the anti-nutritional factors determination like Sulfosalyslic acid, ferric chloride, L-ascorbic acid, Dichlormethane, and Methanol were purchased from Fine Chemicals Ltd, Addis Ababa, Ethiopia.

2.2. Ethnobotany of edible macrophytes

The ethnobotany data of edible freshwater macrophytes from Lake Ziway was collected using semi-structured questionnaires, focus group discussions, key informant interviews, observation, excursions to the lakeshore, and preference rating (Anbessa et al., 2024). The plant's common name, family name, local name, habitat, and ethnobotanical uses were tabulated. The relative frequency of citation was calculated as follows:

$$RFC = \frac{FC}{N}$$

Whereas frequency citation (FC) is the total number of respondents who cited a particular species and N is the total number of the respondents.

The sampling area coordinates, water temperature, and pH were measured using a multiparameter (Hanna multiparameter, Model: HI9829, Hanna Instruments Ltd, Eden Way, Pages Industrial Park, Leighton Bussard, Bedfordshire LU7 4AD, England), water depth was measured using a handheld digital depth sounder (Vexilar LPS-1 Handheld Digital Depth Sounder, LCD portable sounder, Minneapolis, MN 55438, USA) and Secchi disc was measured using a black and white disc with rope and presented in Table 1. At its mature stages, fruits (75) and rhizome (75) of *N. lotus* from each area were collected and composited in equal proportions (Fig. 1). The leaf and stem of *N. lotus,* additionally the rhizome of *Typha latifolia* and *Arundo donax* were collected. The rhizome and seed were dried for 3 day at 60°C (Danhassan et al., 2018). The dried rhizomes and seeds were ground to a fine powder using an electric miller (High speed multi-functional crusher Al Marwani for spice, Model:400A, Shanghai, China), sieved using a 2 mm mesh sieve and then stored in zip-lock plastic bags for further analysis.

2.3. Extraction of bioactive compounds

Bioactive compounds were extracted from the rhizome and seed of *N. lotus* using the procedure of Murtala et al. (2019). The powder samples of *N. lotus* rhizome (5 g) and *N. lotus* seed (5 g) were separately macerated with 50 mL each of aqueous, dichloromethane, and methanol for 24 h at room temperature on an orbital shaker (Benchtop incubator shaker, Model:ZHWY-103B, Shanghai ZHICHENG Analytical Instruments Manufacturing Co.Ltd, 395 Nan Ting Road Fenxian District, Shanghai, China). The extracts were filtered using Whatman No. 1 filter paper. The aqueous extract was concentrated to dryness (40°C) on a water bath (Adebayo et al., 2023). The methanol and dichloromethane solvents were removed from the extracts using a rotary evaporator (Biby Scientif RE300DB Rotary Evaporator, Keison Products, Chelmsford, Essex, CM1 3UP, England).

2.4. Acute toxicity test

Acute toxicity tests of aqueous, dichloromethane, and methanol extracts of *N. lotus* rhizomes and seeds were determined using the method of Bello, Maiha, & Anuka, 2016. Twenty-eight days old female Wistar albino rats weighing from 26.8 to 29.16 g were housed in hygienic standard conditions in clean polyacrylic cages. Food in the form of dry pellets and water was made available *ad libitum*. The study was designed in two phases, in phase I, four groups of three rats per group were administered distilled water, 10, 100, and 1000 mg/kg of extracts. The second phase was continued as no death was reported, a higher dose of the extract (distilled water, 1600, 2900, and 5000 mg/kg) was given to each rat. The geometric mean of the lowest toxic dose and the highest tolerated dose was used to compute the oral median lethal dose (LD_{50}) as follows:

$$LD50 = \sqrt{(D0 \text{ x } D100)}$$

Where D_0 is the highest dose that gave no mortality, D_{100} the lowest dose that produced 100% mortality.

Table 1
Coordinates and ecosystem conditions where samples of *N. lotus*, *Typha latifola*, and *Arundo donex* were collected.

Parts of the Lake	Coordinates	Water depth	Light penetration	pH	Temperature
Eastern part	7°95′32.5″N, 8°88′89.9″E	111 cm	20 cm	8.54	21.8°C
	7°95′76.1″N, 8°89′14.3″E	50 cm	24 cm	8.21	24.7°C
	7°95′56.7″N, 8°89′09.4″E	82 cm	20 cm	8.92	23.3°C
	7°95′37.3″N, 8°88′85.0″E	81 cm	21 cm	8.55	23.2°C
Northern part	8°05′92.8″N, 8°86′16.0″E	95 cm	22 cm	8.52	22.2°C
	8°06′02.5″N, 8°85′72.0″E	67 cm	20 cm	8.03	23.2°C
	8°05′92.8″N, 8°86′30.7″E	81 cm	21 cm	8.27	22.7°C
	8°05′97.7″N, 8°85′96.5″E	88 cm	21 cm	8.15	22.9°C
Western part	7°56′53.9″N, 38°43′32.3″E	75 cm	20 cm	8.55	22.5°C
	7°56′53.3″N, 8°43′33.5″E	105 cm	20 cm	8.44	22.5°C
	7°56′50.9″N, 38°43′31.8″E	64 cm	21 cm	8.35	23.2°C
	7°56′58.1″N, 38°43′34.1″E	55 cm	21 cm	8.18	24.6°C
Southern part	7°53′54.3″N, 8°44′31.7″E	76 cm	19 cm	8.36	21.6°C
	7°53′53.8″N, 38°44′32.2″E	49 cm	21 cm	8.61	21. 5°C
	7°54′29.5″N,38°44′19.7″E	91 cm	17 cm	8.42	22.7°C
	7°53′53.4″N, 38°44′31.7″E	64 cm	20 cm	8.63	21.7°C

Fig. 1. *Nymphaea lotus* leaves (a), stems (b), peeled rhizomes (c), and dried seeds (d).

2.5. Proximate compositions

The moisture content of the rhizome, stem, leaf, and seed of *N. lotus,* as well as the rhizome of *Typha latifolia*, and *Arundo donex,* was determined according to AOAC (2015) Official Method number 925.09 by oven drying method (Advantage-Lab's laboratory drying oven, Model:2030, Jachthoornlaan 8, 2970 Schilde, Belgium). The percentage of moisture content was calculated using the following equation:

$$\text{Moisture content} = \frac{W2 - W1}{W2 - W} \text{ X } 100$$

Where W_1 is the weight of petridish + dried sample, W_2 is the weight of petridish + wet sample and W is the weight of empty petridish.

The protein content was determined using Kjeldahl methods involving digestion, distillation, neutralization and titration AOAC (2015) Official Method number 920.87. The percentage of Nitrogen was calculated using the following equation:

$$\text{Percent N} = \text{N HCl X} \frac{(\text{Volume of sample} - \text{volume of blank})\text{L}}{\text{gram of sample}} \text{X} \frac{14\,\text{g}}{\text{mole}} \text{X} 100$$

$$\text{Tannin (mg / g)} = \frac{((\text{Absorbance of sample} - \text{Absorbance of blank}) - \text{Intercept}) * 10}{\text{Slope} * \text{sample weight} * 0.791}$$

$$\text{Crude protein} = 6.25 \text{ X percent N}$$

Crude fat was determined by semi continuous solvent extraction methods (Soxhlet method) according to Official Method number 922.06. The crude fat in the initial sample was calculated using the following equation:

$$\text{Fat content} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \text{ X } 100$$

$$\text{Phytate } (\mu g / 100\ g) = \frac{((\text{Blank absorbance} - \text{Sample absorbance}) * \text{Intercept}) * 10}{\text{Slope} * \text{Sample weight} * 3}$$

The ash content was determined by igniting the sample for 4 h at 550°C in a furnace (Controller, Model:B400/B4100, Nabertherm GmbH, Germany) according to AOAC (2015) Official Method 942.05. The amount of ash was calculated using the following equation:

$$\text{Ash content} = \frac{W2 - W1}{W3} \text{ X } 100$$

Where: W_1 is the mass of empty crucible, W_2 is the mass of crucible with ash and W_3 is the mass of sample.

Crude fiber content was determined using sequential acid and alkali extraction methods according to Official Method number 978.10.

2.6. Anti-nutritional factors (ANF)

2.6.1. Tannin

Tannin was determined according the method of Parimala and Shoba (2013). The sample (1 g) was mixed with 20 mL of methanol, vortexed, filtered and combined with 20 mL of distilled water, 2.5 mL Folin's phenol reagent, and 10 mL of sodium carbonate (17%). Absorbance was measured at 760 nm a spectrophotometer (PerkinElmer LAMBDA 950 UV-VIS/NIR Spectrophotometer, PerkinElmer, 710 Bridgeport Avenue Shelton, USA). Regression equation ($y = 0.2927x + 0.0105$, $R^2 = 0.9936$) was derived from calibration curves, result was expressed as equivalents of gallic acid per gram (mg GAE/g) of extract.

2.6.2. Phytate

The phytate content was determined according to Teka et al. (2020). The sample (0.03 g) was centrifuged (Table top centrifuge, Model: PLC-Gemmy industrial corp., Taiwan) for 15 min after being extracted for 1 h at room temperature using 10 mL of 0.2 N HCl. Sample extracts (3 mL) were combined with 2 mL of wade reagent (0.03% $FeCl_3.6H_2O$ and 0.3% Sulfosalyslic acid). Regression equation ($y = -0.0092x + 0.3884$, $R^2 = 0.9935$) was derived from calibration curves, result was expressed as mg/L of phytate.

2.6.3. Oxalate

Oxalate was determined using permanganate titration method as described in Anand et al. (2019). Briefly, a 100 mL conical flask containing 1 g of dried sample was weighed. Next, 75 mL of 3 mol/L of H_2SO_4 was added, the mixture was agitated for 1 h and filtered. The

filtrate (25 mL) was titrated against a hot (80–90°C) 0.1 N $KMnO_4$ solution until a light pink color appeared. The amount of oxalate in each sample was calculated using the following equation:

1 mL 0.1 N permanganate = 0.006303 oxalate

2.6.4. Alkaloid

Alkaloid content was determined gravimetrically as described by Akinjogunla et al. (2020). The dried sample (5 g) was extracted with 200 mL of 10% acetic acid in ethanol solution and concentrated to quarter of its original volume. Concentrated ammonium hydroxide was added drop by drop until the precipitate was fully completed, the precipitate was quantitively recovered and measured.

2.7. Determination of macro, micro, and trace elements

2.7.1. Sample digestion

Samples were digested using closed microwave (Ultrawave, Milestone, Italy) digestion Accurately, a sample of 0.15 g was weighed using an electronic balance (AX205, Mettler Toledo, Belgium) and put into polytetrafluoroethylene (PTFE) microwave tube. Concentrated nitric acid (5 mL) was added, and the following microwave program was used: in the first step the temperature was increased to 240°C over 30 min and then held at 240°C for 20 min. Afterwards, the content of the microwave tube was rinsed with ultrapure water in a 50 mL volumetric flask.

2.7.2. Determination of elements

The elemental contents of water lily parts, *A. donax,* and *T. latifolia* were determined using inductively coupled plasma-mass spectroscopy and inductively coupled plasma-atomic emission spectroscopy. ICP-MS (iCAP Q, ThermoScientific, US, Waltham) was used to determine the trace elements (Hg and Se) and ICP-AES (iCAP 7000, ThermoScientific, US, Waltham) was used to determine macro and micro elements (Na, K, Ca, Mg, P, Fe, Zn, B, Cu, Mn, As, Cd, Co, Cr, Mo, Ni, Pb, Sn, and V) using the method of Laboratorium Chemische Analyse, Universiteit Gent, LCA method, Meting met ICP-MS/ICP-AES: ISO 11885. Every sample was measured in radial modus when using ICP-AES. An overview of the selected emission detection wavelength of each element can be found in the Supplementary materials. ICP-MS was used to determine ^{201}Hg and ^{80}Se. ^{193}Ir and ^{72}Ge were used as internal standards. All analytes were analyzed in kinetic energy discrimination (KED) mode with He-gas to remove polyatomic interferences. The instrument software of both ICP-MS and ICP-AES automatically provided standard curve and calculated content of each element in the sample according to the regression equation generated from the standard curve. An overview of the highest/lowest calibration solution that were used and the LOD of each element is found in the Supplementary materials. Content of macro and micro elements in the sample were calculated in mg/kg. Standard solutions of all elements and perchloric acids were obtained from Certipur® Supelco and ultrapure water was obtained from Arium pro from Sartorius.

2.8. Amino acids

Amino acid (AA) profiles of water lily (*N. lotus*) was determined using Liquid Chromatography. Prior to chromatographic analysis, samples underwent acid hydrolysis using 6M HCl to release the AA from their proteinaceous matrix, followed by the HPLC-DAD (HPLC-DAD, Model:1290 Infinity II LC, Agilent Technologies, US, Santa Clara) analysis. The AA identification and quantification were done applying a standard operation procedure (SOP) of Agilent Technologies using *ortho*-phthalaldehyde (OPA)/9-fluorenylmethyl chloroformate (FMOC) online derivatization in an Agilent 1290 Infinity II LC system. The AA were first converted into OPA and FMOC derivatives using the 1260 Infinity II Vialsampler (Agilent, USA), after which separation was performed on an InfinityLab Poroshell 120 HPH-C18 column (4.6 mm × 100 mm × 2.7 μm; Agilent, USA). The mobile phases, at a flow rate of 2 mL/min, consisted of 10 mM Na_2HPO_4, 10 mM $Na_2B_4O_7$, 0.5 mM NaN_3 at pH 8.2 (eluent A) and acetonitrile/methanol/mQ in a ratio of 45/45/10 (v/v/v) (eluent B), and followed the gradient in the SOP. The absorbance was measured at 262 nm for FMOC-amino acids (Pro and Hyp) and at 338 nm for OPA-amino acids (others). Calibration AA standard solutions ranging from 22.5 to 900 μM and internal standards norvaline and sarcosine (both 0.5 mM) were used for quantification. Tryptophan (Trp) was not determined because acid hydrolysis results in the complete destruction of tryptophan.

2.9. Phytochemicals

The dried powder (5 g) of samples were macerated with 50 mL of methanol for 24 h at room temperature on orbital shaker (Incubator shaker, Shanghai ZHICHENG Analytical Instruments Manufacturing Co. Ltd, ZHWY-103B, Shanghai, China). The extracts were filtered using Whatman No.1, then transferred to a bottom round flask that had been pre-weighed and dried using a rotary vapor (Biby Scientif RE300DB Rotary Evaporator, Keison Products, Chelmsford, Essex, CM1 3UP, England) at 55°C (Murtala et al., 2019). The percentage of the extract was then determined by deducting the round bottle's initial empty weight from the round bottle that contain the extracts. The extract was recovered from the bottom round flask by adding calculated amounts of methanol to a round bottle to achieve 50 mg/mL and then expressed as percentage yield of extraction.

$$\text{Percentage yield}\,(\%) = \frac{\text{Weight of the dry concentrated crude extract}}{\text{Weight of the powdered sample used}} \text{X}100\%$$

2.9.1. Total phenolic content

Total phenolic content was quantified using Folin-Ciocalteu method according to Afrin et al. (2024). In brief, five μL of plant extracts (50 mg/mL), 100 μL of Folin-Ciocalteu reagent (0.5N), and 80 μL of sodium carbonate (7.5% w/v) were mixed, and the absorbance was measured at 765 nm spectrophotometer. Regression equation ($y = 0.0084x + 0.2188$, $R^2 = 0.9418$) derived from calibration curves, and results were expressed as gallic acid equivalent per gram (mg GAE/g).

2.9.2. Total flavonoid content

The total flavonoid content was quantified using the aluminum trichloride method (Pokhrel et al., 2022). Briefly, 10 μL of extract (50 mg/mL), 110 μL of distilled water, 60 μL of methanol, 5 μL of potassium acetate (1 M), and 100 μL of $AlCl_3$(10%) were mixed, and the absorbance was measured at 415 nm using spectrophotometer. Regression equation ($y = 0.0079x + 0.2573$, $R^2 = 0.9943$) derived from calibration curves, and results were expressed as equivalents of quercetin per gram (mg QE/g) of extract.

2.10. Antioxidant capacities

2.10.1. DPPH

The DPPH half inhibition concentration of the extract was determined using the method of Rumpf et al. (2023). Briefly, a 3.7 mL of DPPH radical solution (0.004%), methanol, and (5–200 μL) extracts of samples (50 mg/mL) were mixed, vortexed, incubated, and absorbance was measured at 517 nm using spectrophotometer. The IC_{50} value (sample concentration that can neutralize 50% of DPPH free radicals) was determined by plotting the percentage radical scavenging ability against the sample concentration.

$$\text{RSA}\,(\%)\text{ of DPPH} = \left(1 - \left(\frac{\text{Absorbance of sample}}{\text{Absorbance of blank}}\right)\right) * 100\%$$

2.10.2. ABTS

The free radical scavenging activity was measured by mixing plants' extract (5–200 μL) with 2.7 mL of ABTS radical cation, and absorbance was measured at 734 nm using spectrophotometer (Rumpf et al., 2023). A calibration curve with six Trolox (6-hydroxy-2,5,7,8-tetra methyl chroman 2- carboxylic acid) standard was measured in the range of (5–200 ppm). The radical scavenging activity (RSA) was calculated as:

$$\text{RSA (\%) of ABTS} = \left(1 - \left(\frac{\text{Absorbance of sample}}{\text{Absorbance of blank}}\right)\right) * 100\%$$

2.10.3. FRAP

The FRAP antioxidant capacity was measured according to the method of Rumpf et al. (2023). The freshly prepared FRAP reagent (3 mL) was mixed with 10 μL of extracts (50 mg/mL), incubated and absorbance was measured at 595 nm by spectrophotometer. Regression equation ($y = 0.0099x + 0.1748$, $R^2 = 0.9918$) derived from calibration curves, result was expressed as μg/mL of FRAP.

2.11. Statistical analysis

The data of proximate compositions, anti-nutritional factors, total phenolic content, and total flavonoid content were compared using One-way ANOVA of Statistical Package for Social Sciences software. The results were presented as mean ± standard error and mean was separated using Tukey test where significance difference ($p < 0.05$) was detected.

3. Results

3.1. Ethnobotany of edible macrophytes

The ethnobotany of edible freshwater macrophytes from Lake Ziway is presented in Table 2. The survey covered eleven Kebeles of three districts surrounding Lake Ziway, Ethiopia. All respondents were male of age from 15 to 70 years. The communities that were interviewed include fish traders, farmers, fishermen, students, and unemployed youth. The educational level of the respondents was illiterate (23.3%), primary education (47.7%), secondary and high school (26.6%), and college graduate (2.22%). The ethnobotanical study indicated that the rhizomes of four edible freshwater macrophytes are consumed during times of food scarcity. These are *Nymphaea lotus* (Bal-bate), *Typha latifola* (Aware), *Arundo donax* (Kawa), and *Echinochloa stagnina* (Chufo). According to the respondents eating these aquatic plants is safe and healthful (93.3%), useless (5.55%), and do not know (1.11%). Ethnobotanical studies on edible freshwater macrophytes revealed that people use aquatic plants to treat various ailments. The rhizome of *N. lotus* is used to treat dyspepsia or indigestion problem around Kontola kebele of Lake Ziway.

3.2. Acute toxicity test

The acute toxicity of *N. lotus* rhizome and seed extracts is presented in Table 3. The crude methanol extract of *N. lotus* rhizome and seed was 13.2% and 16.8%, respectively. A single dose administration of these extracts did not bring changes in body weight, food consumption, or water intake. The lethal dose (LD_{50}) of all the extracts exceed 5000 mg/kg. Notably, all the rats were alive, no death recorded and showed no signs of toxicity at these levels (Fig. 2). Similarly, there was no death throughout the 14-day observation period, nor were there any toxic indications such as fatigue, weakness, convulsions, hyperactivity, dullness, diarrhea, or diuresis.

3.3. Proximate compositions

The moisture, protein, fat, ash, fiber, and carbohydrate contents of edible freshwater macrophytes are presented in Table 4. The moisture content of *N. lotus* rhizome and seed was significantly ($p < 0.05$) higher than *N. lotus* leaf, stem, Arundo, and Typha. The moisture content of rhizome and seed of *N. lotus* was not significant ($p > 0.05$). There was statistically significant ($p < 0.05$) in protein content of *N. lotus* rhizome (23.6%), seed (15.2%), leaf (18.2%) and stem (13.5%). There was no statistical significance difference ($p > 0.05$) in protein content of *A. donax* and *T. latifolia*. The rhizome of *N. lotus* can be considered as good protein supplement. The crude fat content was highest in the *N. lotus* leaf (3.7%) and not detected in the rhizome of *N. lotus* (Table 4). The ash content of *N. lotus* stem (17.4%) was significantly higher as compared to rhizome and seed of *N. lotus*. The fiber content of *A. donax* (31.6%) was significantly higher as compared to other parts. The fiber content of *N. lotus* rhizome was (4.9%), and seed was (17.1%). The highest carbohydrate content was found in rhizome of *N. lotus* (61.7%), whereas the lowest was from rhizome of *A. donax* (51.3%).

3.4. Anti-nutritional factors

The anti-nutritional factors (ANF) of edible freshwater macrophytes was different ($p < 0.05$) in the tannin, phytate, oxalate, and alkaloid content of different edible freshwater macrophytes (Table 5). The highest tannin content (5699.9 mg/100 g) was found in the seeds of *N. lotus*, followed by the rhizome of *A. donax* (3922.6 mg/100 g), and the least tannin content was found in the rhizome of *N. lotus* (392.8 mg/100 g). The phytate content of edible freshwater macrophytes was significantly higher in rhizome of *A. donax* (3596.3 μg/g) as compared to other edible freshwater plants. There was no significance difference ($p > 0.05$) in oxalate content among the parts of *N. lotus* leaf, stem and rhizome of *A. donax*. The alkaloid content of leaf of *N. lotus* (3.6 mg/100 g) was significantly higher ($p < 0.05$).

3.5. Macro, micro, and trace elements

A total of six macro-elements (sodium, potassium, calcium, magnesium, phosphorus and manganese), seven micro-elements (iron, zinc, boron, copper, vanadium, selenium and tin) and eight trace and potentially toxic elements except cobalt (arsenic, cadmium, cobalt, chromium, molybdenum, nickel, lead and mercury) were identified and quantified from edible freshwater macrophytes (Table 6). The highest sodium (37100 mg/kg) was found in the stem of *N. lotus* and lowest (356 mg/kg) was found in the seed of *N. lotus*. Potassium was the most abundant macro-elements and highest was recorded in the stem of *N. lotus* (30400 mg/kg) and lowest was for the seed of *N. lotus* (4690 mg/kg). Iron, zinc and boron were the most abundant microelements found in *N. lotus*. The edible macrophytes contained virtually nil amounts of

Table 2
Edible freshwater macrophytes grown in Lake Ziway, Scientific name, family, local name, habitat and their relative frequency of citation.

No.	Scientific name	Common name	Family name	Local name	Habitat	Ethnobotanical uses	RFC
1	*Nymphaea lotus*	White Egyptian lotus	Nymphaeaceaea	Bal Bate	Rooted leaf-floating	To treat indigestion problem (dyspepsia)	0.94
2	*Typha latifolia*	Broad leaf cattail	Typhaceae	Aware	Emergent	No known medicinal value	0.97
3	*Arundo donax*	Giant reed	Poaceae	Kawa	Emergent	No known medicinal value	0.88
4	*Echinochloa stagnina*	Hippopotamus grass	Poaceae	Chufo	Emergent	No known medicinal value	0.08

Where RCF is relative frequency of citation.

Table 3
Acute toxicity studies of aqueous, methanol and dichloromethane extracts of *N. lotus* rhizome and seed administered to albino mice.

Phases	Dose (mg/kg)	Number of dead mice after administration of rhizome of *N. lotus* extracts			Number of dead mice after administration of seed of *N. lotus* extracts		
		Aqueous	Methanol	Dichloromethane	Aqueous	Methanol	Dichloromethane
Phase I	DW	0/3	0/3	0/3	0/3	0/3	0/3
	10	0/3	0/3	0/3	0/3	0/3	0/3
	100	0/3	0/3	0/3	0/3	0/3	0/3
	1000	0/3	0/3	0/3	0/3	0/3	0/3
Phase II	DW	0/3	0/3	0/3	0/3	0/3	0/3
	1600	0/3	0/3	0/3	0/3	0/3	0/3
	2900	0/3	0/3	0/3	0/3	0/3	0/3
	5000	0/3	0/3	0/3	0/3	0/3	0/3

Fig. 2. Acute toxicity tests of seed and rhizome of *N. lotus* extracts.

Table 4
Proximate composition of edible aquatic plants from Lake Ziway.

Parts of plants	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Fiber (%)	Carbohydrate (%)
Rhizome of *N. lotus*	9.3 ± 0.1^{a}	23.6 ± 0.17^{a}	0.0 ± 0.0^{d}	0.4 ± 0.0^{d}	4.9 ± 0.02^{e}	61.7 ± 0.25^{a}
Seed of *N. lotus*	9.2 ± 0.0^{a}	15.2 ± 0.17^{c}	1.7 ± 0.2^{bc}	0.4 ± 0.0^{d}	17.7 ± 0.08^{b}	55.7 ± 0.51^{c}
Leaf of *N. lotus*	5.2 ± 0.2^{b}	18.2 ± 0.35^{d}	3.7 ± 0.2^{a}	1.4 ± 0.2^{c}	15.9 ± 0.07^{c}	60.3 ± 0.05^{b}
Stem of *N. lotus*	4.8 ± 0.0^{c}	13.5 ± 0.17^{b}	$1.2 \pm 0.2c$	17.4 ± 0.2^{a}	5.9 ± 0.05^{d}	52.4 ± 0.04^{d}
Rhizome of *A. donax*	4.1 ± 0.1^{c}	11.4 ± 0.17^{e}	0.5 ± 0.0^{d}	1.0 ± 0.2^{c}	31.6 ± 0.31^{a}	51.3 ± 0.04^{d}
Rhizome of *T. latifolia*	4.8 ± 0.0^{d}	12.1 ± 0.17^{e}	2.2 ± 0.2^{b}	2.8 ± 0.0^{b}	17.8 ± 0.17^{b}	60.3 ± 0.6^{b}

Where: means in a column followed by different letters are significantly different ($p < 0.05$).

Table 5
Anti-nutritional factors of edible freshwater macrophytes.

Parts of aquatic plants	Tannins (mg/100 g)	Phytate (μg/g)	Oxalate (mg/100 g)	Alkaloid (mg/g)
Rhizome of *N. lotus*	392.8 ± 14.0^{e}	1248.5 ± 2.9^{b}	0.22 ± 0.03^{c}	1.3 ± 0.05^{d}
Seed of *N. lotus*	5699.9 ± 79.6^{a}	1088.7 ± 18.1^{b}	0.22 ± 0.03^{c}	2.9 ± 0.11^{b}
Leaf of *N. lotus*	2379.8 ± 2.5^{c}	626.0 ± 107.2^{b}	1.48 ± 0.09^{a}	3.6 ± 0.21^{a}
Stem of *N. lotus*	925.1 ± 15.5^{d}	409.9 ± 75.1^{b}	1.54 ± 0.03^{a}	2.1 ± 0.22^{c}
Rhizome of *A. donax*	3922.6 ± 28.5^{b}	3596.3 ± 636.9^{a}	1.63 ± 0.06^{a}	1.3 ± 0.07^{d}
Rhizome of *T. latifolia*	3882.9 ± 116.6^{b}	575.1 ± 49.0^{b}	0.69 ± 0.06^{b}	3.2 ± 0.01^{ab}

Where: means in a column followed by different letters are significantly different ($p < 0.05$).

Table 6
Macro and micro elemental contents (mg/kg) of edible freshwater macrophytes.

Elements	Rhizome of *N. lotus*	Seed of *N. lotus*	Leaf of *N. lotus*	Stem of *N. lotus*	Rhizome of *A. donax*
Sodium	378	356	378	37100	1060
Potassium	5210	4690	12900	30400	16600
Calcium	1350	523	9820	6440	246
Magnesium	1570	931	3890	3000	472
Phosphorus	2580	1910	1900	1350	840
Manganese	17	14.1	48	129	16.6
Iron	28.5	97.7	588	544	156
Zinc	23.5	14.8	18.5	11.4	7.73
Boron	22.8	7.13	22.8	30	2.73
Copper	2.88	4.3	2.79	2.29	1.23
Vanadium	3.51	2.15	11.6	10	1.04
Selenium	0.908	0.585	2.7	2.99	3.55
Tin	<2.28	<2.87	<2.7	<2.36	<2.88
Arsenic	<0.323	<0.407	1.42	<0.334	<0.439
Cadmium	<0.169	<0.212	<0.2	<0.175	<0.213
Cobalt	<0.761	<0.957	<0.901	<0.787	<0.96
Chromium	<0.464	<0.584	1.01	3.77	<0.585
Molybdenum	<0.236	<0.297	<0.28	<0.245	<0.298
Nickel	<0.3	<0.378	2.74	3.04	1.35
Lead	<0.723	<0.93	<0.856	<0.748	<0.912
Mercury	0.0255	<0.00625	<0.00588	<0.00514	<0.00627

trace metals like mercury from <0.00514 to 0.0255 mg/kg and cadmium from <0.169 to <0.213 mg/kg.

3.6. Amino acids

The amino acids profiles, sum of essential, non-essential, and total amino acids is presented in Table 7. Among the essential amino acids required to be taken through diet, eight amino acids (leucine, isoleucine, histidine, lysine, methionine, phenylalanine, threonine, and valine) were identified from *N. lotus*. The essential amino acid tryptophan was

Table 7
The amino acids composition of *N. lotus* (g/kg of protein).

Amino acids	Rhizome of *N. lotus*	Seed of *N. lotus*	Leaf of *N. lotus*	Stem of *N. lotus*
Leucine	5.7	6.7	8.1	2.5
Lysine	3.4	2.4	5.4	1.6
Isoleucine	3.4	4.2	5.0	1.5
Phenylalanine	4.1	3.9	5.2	1.6
Valine	4.0	4.9	6.8	2.3
Metheonine	1.4	2.0	1.6	0.1
Histidine	2.0	2.1	2.2	0.8
Threonine	2.6	2.8	4.0	1.3
Total EAA	**26.6**	**29.0**	**38.3**	**11.7**
Arginine	6.1	9.4	6.0	1.6
Proline	4.1	6.4	7.8	1.7
Tyrosine	3.2	2.7	3.8	1.0
Alanine	4.2	4.5	5.8	2.0
Glutamic acid	7.2	14.3	11.7	3.9
Serine	3.7	4.5	4.0	1.5
Glycine	3.6	2.7	5.4	2.1
Aspartic acid	12.3	6.3	11.0	3.6
Hydroxyproline	5.8	7.9	9.3	3.1
Total Non-EAA	**50.2**	**58.7**	**64.8**	**20.5**
Total amino acids	**76.8**	**87.7**	**103.1**	**32.2**

Table 8
Extract yield and phytochemical content of edible freshwater macrophytes.

Parts of aquatic plants	Extract yield (g)	Methanol (mL)	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)
Rhizome of *N. lotus*	0.66 g	13.2	3.10 ± 0.31^{c}	4.77 ± 0.16^{c}
Seed of *N. lotus*	0.84 g	16.8	1.28 ± 0.04^{d}	3.97 ± 0.33^{c}
Leaf of *N. lotus*	0.44 g	8.8	4.10 ± 0.20^{b}	10.27 ± 0.24^{a}
Stem of *N. lotus*	0.80 g	16	5.77 ± 0.02^{a}	6.26 ± 0.10^{b}
Rhizome of *A. donax*	1.40 g	28	0.77 ± 0.10^{d}	3.90 ± 0.76^{c}
Rhizome of *T. latifola*	1.97 g	39.4	0.79 ± 0.10^{d}	2.45 ± 0.74^{d}

not determined in the analysis because it undergoes chemical breakdown during acid hydrolysis, making it a difficult amino acid to determine in proteins and peptides. Leucine was the most abundantly found essential amino acid in all samples, with valine following. In all the samples, methionine was found in the lowest concentration.

The ability of a protein to meet requirements for nitrogen and essential amino acids determines its nutritional value. As compared to other non-essential amino acids, glutamic acid was found in a higher concentration in seed (14.3 mg/g), leaf (11.7 mg/g) and stem (3.9 mg/g) of *N. lotus*. Aspartic acid was found in a higher concentration in rhizome (12.3 mg/g) of *N. lotus*.

3.7. Phytochemicals

3.7.1. Total phenolic content (TPC)

The TPC of edible aquatic plants are presented in Table 8. The methanolic extract of *N. lotus* was brownish green, oily, and sticky. The extractable yield from different edible freshwater macrophytes varies based on species (Table 8). The TPC was highest in the stem of *N. lotus* (5.77 mg GAE/g) and the lowest (0.77 mg GAE/g) was recorded form the rhizome of *A. donax*.

3.7.2. Total flavonoid content (TFC)

The TFC of edible freshwater macrophytes are presented in Table 8. There is variation in the TFC attributed to parts of plants, species, and solvents used to extract bioactive compounds. The TFC was highest in the leaf of *N. lotus* and the least was for rhizome of *T. latifola*.

3.8. Antioxidant capacity

3.8.1. DPPH

The DPPH free radical scavenging activities of edible freshwater macrophytes and the half inhibition concentration (IC_{50}) are presented in Table 9. The lower the IC_{50} value, the higher the free radical scavenging activity of the extracts. Hence, the IC_{50} was strongest for rhizome of *T. latifolia* followed by leaf of *N. lotus*, rhizome of *A. donax*, seed of *N. lotus*, rhizome of *N. lotus* and seed of *N. lotus*.

3.8.2. ABTS

The ABTS free radical scavenging activities of edible freshwater macrophytes and IC_{50} are presented in Table 10. Except for stem of *N. lotus*, all possessed strongest IC_{50} of ABTS free radical scavenging as compared to the standard antioxidant Trolox evaluated.

3.8.3. FRAP

The FRAP antioxidant capacity of edible freshwater macrophytes are presented in Table 11. There was a significant ($p < 0.05$) difference among the antioxidant of the rhizome of *N. lotus*, seed of *N. lotus*, leaf of *N. lotus*, stem of *N. lotus*, rhizome of *A. donax*, and rhizome of *T. latifolia*. The antioxidant capacity of the assayed samples using FRAP range from 32.47 μg/mL (rhizome of *A. donax*) to 144.55 μg/mL (seed of *N. lotus*).

4. Discussion

4.1. Ethnobotany of edible macrophytes

Ethnobotanical studies on edible freshwater macrophytes revealed that people use four different wild edible macrophytes from Lake Ziway. According to Merga (2021), three edible freshwater plants like *Typha latifolia*, *Arundo donax*, and *Nymphaea lotus* are harvested for food around Lake Ziway, Ethiopia. According to Mohammed et al. (2013), people in northeastern Nigeria consume and commercialized water lily seeds. The concoctions of leaves, petiole, roots, and seeds of *N. lotus* used to treat various ailments. The whole *N. lotus* is used to treat piles, gonorrhea, bilharziasis, jaundice, and vaginal discharge in Yobe state,

Table 9
The free radical (DPPH) scavenging activities of edible freshwater macrophytes.

Concentration (ppm)	Percentage of inhibition						
	Ascorbic acid	Rhizome	Seed	Stem	Leaf	*A. donax*	*T. latifola*
5	27.35	7.49	3.47	2.30	19.66	21.15	26.48
25	96.56	12.92	21.54	4.76	43.04	34.10	48.55
50	96.65	30.47	44.35	6.87	76.07	56.50	70.92
100	96.89	55.56	84.26	17.31	94.77	77.30	88.88
150	96.69	72.19	94.92	33.82	95.25	88.08	90.62
200	96.65	92.42	94.93	38.32	95.27	90.54	90.41
IC_{50}(μg/mL)	6.72	99.18	73.58	250.96	30.66	56.92	24.48

Table 10
The free radical (ABTS) scavenging activities of *N. lotus* rhizome and seed.

Concentration (ppm)	Percentage of inhibition						
	Trolox	Rhizome	Seed	Stem	Leaf	*A. donax*	*T. latifola*
5	19.38	15.62	37.95	5.64	13.29	25.21	64.21
25	29.32	29.56	47.23	9.58	53.75	78.14	92.02
50	29.32	53.49	80.04	22.69	96.35	98.15	99.44
100	76.53	93.16	99.43	39.17	99.84	99.56	99.22
150	99.63	99.74	99.81	57.17	99.86	98.86	97.99
200	99.87	99.89	97.47	77.10	99.77	95.35	95.35
IC50(μg/mL)	69.22	54.95	12.55	128.35	17.44	9.66	0.40

Table 11
FRAP antioxidant activity of different edible freshwater macrophytes.

Parts of aquatic plants	FRAP (μg/mL)
Rhizome of *N. lotus*	111.90 ± 1.09^{b}
Seed of *N. lotus*	144.55 ± 1.46^{a}
Leaf of *N. lotus*	95.25 ± 2.58^{c}
Stem of *N. lotus*	74.33 ± 0.68^{d}
Rhizome of *A. donax*	32.47 ± 3.90^{f}
Rhizome of *T. latifolia*	58.18 ± 8.24^{e}

Nigeria (Dunoma et al., 2023). According to Anbessa et al. (2024), wild edible plants support the local population's livelihoods in a variety of ways, including food security, agriculture, energy production, building, medicine, ecological services, aesthetics, income generation, and domestic utensils.

4.2. Acute toxicity

The results showed that orally administered extracts had no harmful effects on rats. Similar results were reported by Bello, Maiha, & Anuka, 2016, who found LD_{50} higher than 5000 mg/kg, and observed that oral administration of the methanol rhizome extract of *N. lotus* did not result in any adverse responses or fatalities. Several studies reported that the LD_{50} of *N. lotus* extract was higher than 5000 mg/kg (John-Africa et al., 2012; Murtala et al., 2019; Poumeni et al., 2017; Rege et al., 2021).

4.3. Proximate composition

The moisture content of all the edible freshwater macrophytes were low, which could indicate possibility for longer shelf life. Products that have a moisture content of more than 14.5% are susceptible to sprouting, mold growth, heat, and insect damage (Fleurat-Lessard, 2016).

The protein content of *N. lotus* seed was reported as 18.7% (Keak et al., 2022), 3.27% (Mohammed et al., 2013), and 16.3% (Danhassan et al., 2018). The whole part of *N. lotus* was reported to contain 17.2% (Adelakun et al., 2016), and 16.3% (Idowu et al., 2019). It was reported that the protein content of *N. lotus* flower as 15.6% (Aung et al., 2020), tubers of *N. nouchali* as 8.5% (Afrin et al., 2024), rhizome of *N. lotus* as 19.5% (Mohammed et al., 2013), and leaves of *N. lotus* 11.7% (Mohammed et al., 2013). The protein content of *N. pubescens* rhizome and *N. rubra* were reported as 10.34% and 9.68% (Tresina et al., 2020). Similarly, Mohan and Kalidass (2010) reported the protein content of *N. pubescens* and *N. rubra* as 9.62% and 8.31% respectively. The protein content of *N. lotus* rhizome (23.6%) was slightly higher as compared to literature which could be attributed to growing environment, developmental stages of the plants, sample preparation techniques, and analytical methods used to determine protein. The rhizome of the *N. lotus* has a higher protein content than other protein sources, such as beans, peas, and groundnuts, and this shows it can serve as a supplement to potentially reduce malnutrition and food insecurity, particularly during dry seasons and in arid regions where basic protein sources may be expensive and limited (Aliyu et al., 2017). In comparison to eleven wild edible corms, rhizomes, and tubers, that are consumed by the Palliyar and Kanikkar tribes in, India; the rhizome of *N. pubescens* has the highest protein content (Tresina et al., 2020). Wild vegetable can be considered a good protein supplement (Zakaria, Ramaiya, Bidin, Syed, & Bujang, 2023).

The crude fat content was highest in the *N. lotus* leaf followed by 3.7%, followed by rhizome of *T. latifolia* (2.2%) and there was no fat in rhizome of *N. lotus*. Other studies revealed that the crude fat content for *N. lotus* seed meal was 1.19% (Keak et al., 2022), *N. lotus* seed 3.7% (Danhassan et al., 2018), *N. lotus* leave 24.5% (Usman et al., 2018), and *N. lotus* 2.8% (Afrin et al., 2024). According to reports from Mohammed et al. (2013), the fat contents of *N. lotus* leaves, rhizomes, and seeds were found to be 4.8%, 2.8%, and 9.9%, respectively. The fat content of *N. pubescens* and *N. rubra* were reported as 3.96%, and 5.36% respectively (Tresina et al., 2020). Similar results to the present study was reported by Mohan and Kalidass (2010) as 2.98% and 5.05%. Plants-based fat are low in saturated fat, hence they are beneficial in several health aspects, such as heart healthy and helpful in preventing obesity.

The presence of a higher amount of ash indicates the presence of high minerals in edible freshwater macrophytes (Zakaria et al., 2023). The findings from other researchers showed that the ash content of *N. lotus* seed was 1.8% (Danhassan et al., 2018), bulb of *N. lotus* 35.1% (Asose, Kalu, & Emmanuel, 2022), leaf of *N. lotus* 20% (Usman et al., 2018), and tuber of *N. lotus* 8.04% (Afrin et al., 2024). The fiber content of water lily seed was reported as 4.9% (Keak et al., 2022), bulb of *N. lotus* as 11.5% (Asose et al., 2022), leaf of *N. lotus* as 4.0% (Usman et al., 2018), *N. lotus* plant as 9.0% (Adelakun et al., 2016), tubers of *N. nouchali* as 9.78% (Afrin et al., 2024). One of the most important components of eating vegetables is fiber. Dietary fiber is a component of a general health diet that lowers blood cholesterol, lowers the risk of heart disease, and reduces obesity. Consuming fiber in the diet is essential for proper digestion and waste removal. Fiber prevents constipation by stimulating the contraction of the muscular walls of the digestive tract (Mohan & Kalidass, 2010; Tresina et al., 2020).

Carbohydrate is the major composition plants. The rhizome of the present study was lower as compared to the carbohydrate content of *N. pubescens* (78.25%) and *N. rubra* (77.72%) tubers (Tresina et al., 2020). Literature reported the carbohydrate content of bulb of *N. lotus* as 31.4% (Asose et al., 2022), leaf of *N. lotus* as 41.2% (Usman et al., 2018), leaves of *N. lotus* as 41% (Usman et al., 2018), leaves of *N. lotus* as 34.9% (Adelakun et al., 2016), flower of *N. lotus* as 59.8% (Aung et al., 2020), tubers of *N. nouchali* as 67.7% (Afrin et al., 2024). All the studied edible freshwater macrophytes had lower fat and higher carbohydrate contents. The content in the rhizome of *N. lotus* is categorically can be represented as carbohydrate > protein > moisture > fiber > ash. Whereas, for the seed the content follows carbohydrate > fiber > protein > moisture > fat > ash.

4.4. Antinutritional factors (ANF)

The tannin content of some water lily species had been reported as 0.26 g/100 g in *N. pubescens* (Tresina et al., 2020), 0.2 g/100 g in

N. rubra (Tresina et al., 2020), 90 mg/g in the rhizome of *N. lotus* (Murtala et al., 2019), 9.1 mg/g in the stem (Asose et al., 2022), 29.12 mg/g in the leaf of *N. lotus* (Asose et al., 2022), and 3.21 mg/g in the seed of *N. lotus* (Asose et al., 2022). Tannin is plant polyphenols that can form complexes with metal ions, proteins and polysaccharides. These complexes can prevent the absorption of iron, magnesium, and zinc (Asose et al., 2022). The higher tannin content of seed of *N. lotus* would be useful in the pharmaceutical industry due to its pharmacological properties, which include anti-toxic, anticancer, antiviral, anti-inflammatory, anti-helmintic, antimicrobial, wound healing, and dysentery curing (Fraga-Corral et al., 2021). When compared to previous literature, the tannin content of the rhizomes, seeds, stems, and leaves under investigation was found to be higher. Since tannins are known to impair the activity of digestive enzymes, having even trace amounts of tannin is undesirable from a nutritional standpoint. Therefore, processing methods must be applied before the utilization of these aquatic plants. Different authors reported the phytate content in *N. lotus* seed as 0.21% (Danhassan et al., 2018), in rhizome of *N. nouchali* as 149.86 mg/100 g (Anand et al., 2019), in seed of *N. lotus* seed as 0.21 (Asose et al., 2022). The oxalate content of *N. nouchali* rhizome was reported as 24.97 mg/100 g (Anand et al., 2019), and the seed of *N. lotus* as 0.01 mg/100 g (Asose et al., 2022). Other finding indicated that, total oxalate in *N. pubescens* and *N. rubra* was reported as 0.26 g/100 g and 0.21 g/100 g (Tresina et al., 2020). It is important to note that consuming foods high in oxalate increases the risk of kidney stones developing from the formation of crystals. The alkaloid content of stem of *N. lotus* was reported as 0.9 mg/g, and rhizome of *N. lotus* as 0.96 mg/g (Asose et al., 2022). Alkaloids which taste bitter to humans are naturally occurring toxic amines produced by plants. The presence of high amounts of alkaloids can disturb central nervous system, digestive processes, reproduction, and immune system (Mohan et al., 2016).

4.5. Macro, micro and trace elements

The presence of a higher amount of potassium in edible freshwater macrophytes help to prevent atherosclerosis and hypertension as it lowers blood pressure. Potassium and sodium are necessary for controlling body fluids and electrical impulse transmission. The ratio of Na/K less than one is recommended in the body for prevention of high blood pressure. The present study demonstrates that the rhizome and seed of *N. lotus* are beneficial to human health as it likely reduces hypertension, as seen by the Na/K ratio of approximately 0.07. The rhizome of *A. donax* contained a high potassium content when compared to the Recommended Dietary Allowances for children and infants. Consumption of food containing high in potassium is recommended for individuals who use diuretics for hypertension as potassium excretion is high through bodily fluids. According to Tresina et al. (2020), the concentrations of macro and micro elements such as Na, K, Ca, Mg, P, Zn, Mn, Fe, and Cu in *N. rubra* were reported to be 48.5846.4, 366.7, 126.3, 98.4, 1.78, 1.56, 32.0, and 1.1 mg/100 g, respectively. The levels of macro and micro elements in *N. pubescens*, such as Na, K, Ca, Mg, P, Zn, Mn, Fe, and Cu, have been reported as 66.0, 968.3, 336.1, 114.7, 66.7, 1.33, 1.28, 36.1, and 1.3 mg/100 g, respectively, in other water lily species (Tresina et al., 2020).

The amounts of macro elements, micro elements, and trace elements (mg/100 g) in the seed of *N. lotus* were reported as Na (11), K (191), Ca (127), Mg (10.68), Pb (0.85), Cd (not detected), Cr (3.65), Co (5.02), Ni (11.02), Mn (1.94), Fe (16.29), Cu (0.82), Zn (8.78), P (0.38) (Musa, Birnin-Yauri, Muhammad, & Umar, 2012). The macro-elements (mg/kg) of *N. lotus* were reported (N'guessan et al., 2021) as Mg (11433), P (2959), K (21500), Ca (13800), Fe (5859), Zn (33), and Cd (16). Microelements are crucial components of structural proteins like hormones used to treat cancer, play significant roles in cellular metabolism, and act as co-factors in a wide range of enzymatic activities. It has been suggested that zinc and copper cascade enzyme systems for elimination of free radicals. Consequently, the presence of microelements in edible freshwater macrophytes account for the antioxidant, anti-inflammatory, and cytotoxic properties of *N. lotus* as well as justify its traditional usage as a medicinal plant against cancer (N'guessan et al., 2021).

4.6. Amino acids

Similar studies reported the highest and lowest concentrations of essential amino acids as leucine and methionine respectively in seeds of *N. lotus* (Aliyu et al., 2017). The *N. lotus* can contribute significant toward the essential amino acids requirement for children. Another finding by Danhassan et al. (2018) indicated that *N. lotus* seeds can supply essential amino acids required for growth and development in similar or even superior pattern other proteinous foods.

In the seed of *N. lotus*, the two most abundant non-essential amino acids were glutamic acids and aspartic acid (Danhassan et al., 2018). Glutame is an essential constituent of glutathione and a precursor of the neurotransmitter γ-amino butryic acid (GABA). The human brain and other tissues depend on asparagine for healthy functioning, maintenance, and chemical balance. Haroon (2010) reported the total, essential, and non-essential amino acids of *N. lotus* stem as 67.1, 23.7, and 43.5%, respectively. The two limiting amino acids in cereals are lysine and tryptophan. The amino acids of *N. lotus* may be used as an additional source to enhance the nutritional value of grain proteins. The leaf of *N. lotus* has a greater lysine content (5.4 g/kg) than other parts of the plant. The proteins of *N. lotus* could be used to develop useful dietary supplements for grain diets, particularly in developing nations (Haroon, 2010).

4.7. Phytochemicals

4.7.1. Total phenolic content (TPC)

The study conducted by Pokhrel et al. (2022) reported the TPC of *N. lotus* as 194.87 mg GAE/g. The TPC of *N. lotus* leaves extracted using aqueous and acetone was 189.94 and 96.31 mg GAE/g, respectively (Afolayan et al., 2013). The ethanol extract of *N. rubra* was reported to contain 42.12 mg GAE/g (Naznin et al., 2024). Thirty-three varieties of water lily flowers were found to have TPC between 0.77 and 43.01 mg/GAE (Yin et al., 2015). According to Gueye et al. (2022), the TPC of *N. lotus* (black seed) and *N. lotus* (red seed) was 1.95 and 1.39 mg GAE/g, respectively. Afrin et al. (2024) reported 0.188 mg GAE/g of TPC in water lily tuber. The TPC of *N. pubescens* and *N. rubra* was reported as 0.26 and 0.21 g/100 g respectively (Tresina et al., 2020). Phenols are known to donate hydrogen atoms to free radicals readily. The phenolic compounds are capable of inhibiting the deleterious effects of oxidants to the cell and hence phenolic compounds found in *N. lotus* extracts are helpful in this regard.

4.7.2. Total flavonoid content (TFC)

There have been suggestions that phenolics have functional qualities associated to health, including antiviral, antibacterial, anti-inflammatory, hypotensive, and antioxidant action. The methanolic extract of *N. lotus* was reported highest TFC in the leaf (0.97 mg QE/g) as compared to from flower (0.85 mg QE/g), fruit (0.7 mg QE/g), stem (0.08 mg QE/g) and root (0.15 mg QE/g) (Cudalbeanu et al., 2018) The leaves of *Nymphaea rubra* was reported to contain 44.5 mg CAE/g (Naznin et al., 2024). The TFC of *N. micrantha, N. lotus* (black), and *N. lotus* (red seed) was reported as 27.03, 24.05, and 31. 97 mg QE/g, respectively (Gueye et al., 2022). The ethanol leaves extract of *Nymphaea rubra* can be used in food, nutraceutical, and pharmaceutical industries (Naznin et al., 2024). The TFC of *N. pubescens* was reported as 34.6 mg QE/g (Pokhrel et al., 2022) The difference in total flavonoid content could be attributed to genetic variation of plants; different plant species and even varieties within the same species.

4.8. Antioxidant capacity

4.8.1. DPPH

The IC_{50} DPPH inhibition concentrations of different water lily species and their parts were reported as 0.29 μg/mL for the boiled tuber of *N. nouchali* (Anand et al., 2019), and 42.82 μg/mL for the seed of *N. nouchali* (Parimala & Shoba, 2013). The IC_{50} values of the aqueous and acetone extracts of the leaves of *N. lotus* was 25 and 16 μg/mL, respectively (Afolayan et al., 2013). According to Cudalbeanu et al. (2018), the IC_{50} of the methanolic extract of *N. rubra* leaf, flower, fruit, stem, and rhizome was 17, 17, 20, 25, and 19 μg/mL, respectively. The IC_{50} DPPH value of *N. pubescens* of crude extract (10 μg/mL), ethyl acetate fraction (1.43 μg/mL), water fraction (14.3 μg/mL) (Pokhrel et al., 2022). Among the *N. lotus* plant parts, the strongest IC_{50} observed for leaf was most probably related to its high TFC and TPC.

4.8.2. ABTS

The IC_{50} of *N. nouchali* tuber, seed, and L-ascorbic acid was 6.4, 6.3, and 3.88 μg/mL, respectively (Priyanka et al., 2016). Cudalbeanu et al. (2018) reported IC_{50} values of 13, 15, 12, 12, and 10, μg/mL for the leaf, flower, fruit, stem, and root of the methanolic extract of *N. rubra*, respectively. The IC_{50} value of the ABTS assay of lotus rhizome in the present study was lower than DPPH assay. This is attributed to the different reaction of ABTS assay and DPPH assay that; the DPPH radicals can only react with hydrophilic antioxidants, but ABTS cation radicals can react with both lipophilic and hydrophilic antioxidants.

4.8.3. FRAP

Similar studies have shown the aqueous, ethyl, acetate, chloroform, petroleum ether, and ethanolic extracts of leaves of *N. lotus* as 97.27, 86.6, 128.8, 248.92, and 115.43 μg/mL, respectively (Mahmud et al., 2020). The study showed that the antioxidant capacity (DPPH, ABTS and FRAP) evaluated, strong was observed for rhizome of *T. latifolia* and rhizome of *A. donax* as compared to the different plant parts of *N. lotus*.

5. Conclusion

The ethnobotanical data, acute toxicity, proximate composition, ANF, macro, micro and trace elements, amino acids profile, TPC and TFC, and antioxidant capacities of wild freshwater macrophytes from Lake Ziway, Ethiopia, were characterized. This nutritional characterization of wild edible macrophytes is extremely important as it lead to the discover of essentially edible food sources and pave the way for exploitation. Rhizome and seed of *N. lotus* are good sources of protein, essential amino acids, macro, micro and trace elements. The presence of phenolic and flavonoid in these aquatic plants suggest for their importance in traditional medicine and pharmaceuticals. The ANF present in these aquatic plants were slightly higher suggesting the need for traditional processing before the utilization. The lethal death (>5000 mg/kg) and virtually nil potentially toxic trace elements in rhizome and seed makes it safe for consumption. It can be recommended that the rhizome and seed of *N. lotus* can be included as ingredient in food product development. Furthermore, fractionations of protein, phenolic, and flavonoids is important before including this macrophytes into the food systems.

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CRediT authorship contribution statement

Alemu Lema Abelti: Writing – review & editing, Writing – original draft, Software, Methodology, Funding acquisition, Conceptualization. **Tilahun A. Teka:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Formal analysis, Conceptualization. **Geremew Bultosa:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis, Conceptualization. **Pieter Vermeir:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis.

Declaration of competing interest

We have disclosed no conflicts of interest, and the work we have submitted is our original, full-length research article that has not been submitted anywhere else.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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