NUTRITIONAL COMPOSITION, PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY OF STEM OF (*Nymphaea nouchali*) AND (*Nymphaea rubra*)

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Roll No. 0118/17

Registration No. 558

Session: 2018-2019

A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Applied Human Nutrition and Dietetics

Department of Applied Food Science and Nutrition

Faculty of Food Science and Technology

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Chattogram-4225, Bangladesh

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Md. Akram Hossain Khan

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects and that all revisions required by the thesis examination committee have been made

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DEDICATED TO MY BELOVED FAMILY & TEACHERS

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The Author

December, 2019

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Abbreviation

%	:Percentage
&	:And
ANOVA	:Analysis of variance
AOAC	:Association of Official Analytical Chemists
AUC	:Area under the curve
℃	:Degree Celsius
CHO	:Carbohydrate
dl	:Deciliter
DPPH	:2,2-diphenyl-1-picrylhydrazyl
et al	:Et alii/ et aliae/ et alia
etc	:Et cetera
g	:Gram
GAE	:Gallic acid equivalent
Kg	:Kilogramme
mg	:Miligram
NS	:Not significant
NC	:Normal control
N.	: *Nymphaea*
PPM	:Parts per Million
PS	:Powdered Stem
QE	:Quercetin Equivalent
SD	:Standard Drugs
SPSS	:Statistical Package for Social Science
SDM	:Standard Deviation of Mean

Abstract

Currently, all over the world, the developing nations are facing the problem of malnutrition. In developing countries, the conventional aquatic plant has been providing an economically favorable alternative source of protein and energy in lieu of animal protein. *Nymphaea nouchali* (water lily) is our national flower and people prefer to consume water lily stems as a vegetable in curry. This study aimed to evaluate the nutritional composition including major and trace minerals, antioxidants and phytochemical activity to priorities their edibility and the medicinal uses of two *Nymphaeaceae* species such as *N. nochali* (white verity) and *N. rubra* (red verity). Both species serve as a good source of fiber, carbohydrate, minerals and bioactive compounds, assayed by following the Association of Official Analytical Chemist (AOAC-2005) methods. The stems were rich in essential minerals (Na, K, Ca, Mg, P) and sufficient trace elements (Fe, Zn, Cu). Antioxidant activity, IC_{50} values were found 36.67 and 28.48 μg/ml *N. nochali* and *N. rubra* respectively where the value for standard ascorbic acid was 9.56μg/ml. Quantitative analysis of total phenol compounds were 16.51 and 15.48mg GAE /g in *N. rubra* and *N. rubra* respectively and total flavonoid contains of both species were nearly similar 7.6 mg QE/g. From the above biochemical quantification, it can be suggested that both species of aquatic plants can be incorporated as a good source of nutrients in our regular diet.

Keywords: Water lily, Phytochemical, Antioxidant, DPPH

Chapter 1: Introduction

Water Lily is an aquatic plant of the genus *Nymphaea* which has large, disk-like, floating leaves and showy flowers found all over the world. About 50 more species are growing in different countries, in Bangladesh, there are two species *N. nouchali* (white variety) and *N. rubra* (red variety). Both species grow abundantly as a mixed population in almost all shallow natural water bodies, but the latter is more frequent and popular in Bangladesh and has been designated as the national flower. Water lily stem may be eaten raw. In Bangladesh and India, they are cut open, and the seeds removed. The seeds are fried in ghee or oil until they are popped, like amaranth or quinoa. They are mixed with melted sugar, formed into small balls, and are consumed as a snack. The seeds may also be boiled or ground into flour, which is then used for bread (Pond, 2015) Water lily stem is eaten as food in Africa and India. Usually, children's are gather the seeds, stems, and flowers all of which are edible. The seeds and stems of the water lily may be found in some traditional medicines. In Nigeria, Ghana, and parts of India, they are considered to be cooling and are used to treat fevers and skin conditions like eczema (Swapna et al., 2011).

It is a perennial aquatic plant used in the traditional medicine as an aphrodisiac, anodyne, astringent, cardiotonic, sedative, analgesic and as an anti-inflammatory agent. As well as various research studies also revealed that a variety of species under the genus *Nymphaea* possess several health benefits (Tsay and Agrawal, 2005). The chemical composition of water lily stems revealed high protein, fiber, and mineral. Both the raw and processed roots and petals are an excellent source of energy, protein, and essential minerals. In Ayurvedic medicine, stems, roots, and petals are also used as a remedy for diabetes mellitus (Mohammed et al., 2010).

A compound called nymphayol is thought to be behind most water lily health benefits. This alkaloid compound works to regulate insulin levels in the blood, thus helping prevent both hyperglycemia and hypoglycemia (too much and too little blood sugar), which leads to steadier body functions and may help in the treatment and prevention of diabetes and other metabolic disorders. The compounds responsible for water lily hepatoprotective properties have not yet been identified but researchers are studying that alkaloids, polysaccharides, glycosides, steroids, flavonoids, tannin and saponin effects (Lestari et al., 2016).

Polyphenols are a group of chemicals that naturally occur in plants and there are more than 500 exclusive polyphenols. Collectively, these chemicals are known as phytochemicals. Phytochemicals are plant chemicals that have self-protective or disease preventive properties (Shui and Peng, 2004). They are non-essential nutrients, meaning that they are not essential by the human body for sustaining life. It is well-known that plants produce these chemicals to defend themselves but recent researches prove that they can also protect human diseases. There are more than a thousand known phytochemicals. Some of the well-known phytochemicals are flavonoid, phenol, tannin, and saponin, etc (Sindhu and Puri, 2016).

An antioxidant is a molecule that resists the oxidation of other molecules. Oxidation is a chemical reaction that can generate free radicals, leading to chain reactions that may damage cells. A constituent that reduces damage due to oxygen, such as that caused by free radicals well-known antioxidants contain enzymes and additional constituents, such as vitamin C, vitamin E, and beta carotene, which are capable of counteracting the damaging effects of oxidation (Yakovleva et al., 2004). Antioxidants are also universally added on the way to food products such as vegetable oils and prepared foods to inhibit or delay their deterioration from the action of air (Wojdylo et al., 2007).

Antioxidants may reduce the risks of cancer and slow the progression of age-related macular degeneration. Free radicals are highly unstable molecules that are naturally formed when you exercise and when your body converts food into energy. Your body can also be bare to free radicals from a variety of environmental sources, such as cigarette smoke, air pollution, and sunlight. Free radicals can cause oxidative stress a process that can generate cell damage (Tsay and Agrawal 2005).

Oxidative pressure is thought to play a role in a variety of diseases including age related muscular degeneration, Parkinson's disease, Alzheimer's diseases, cancer, cardiovascular diseases, diabetes. There is a study to be hundreds and possibly thousands of substances that can act as antioxidants. Each has its proper role and can interact with others to help the body work effectively (Park et al., 2014). Illustrations of antioxidants that come from outside the body include vitamin A, vitamin C, vitamin E, beta-carotene, lycopene, lutein, selenium, manganese, zeaxanthin. Flavonoids, flavones, catechins, polyphenols, and phytoestrogens are all types of antioxidants and phytonutrients, and they all originate in plant-based foods. Each antioxidant serves a different function and is not exchangeable with another. This is why it is significant to have a varied diet (Halliwell, 1987).

However, there are only limited studies focuses on nutritional composition, antioxidant and phytochemical activity of water lily stems. Most of the previous researches focus mainly on water lily bulbs and seeds. Though raw and cooked water lily stems are consumed in many areas of Bangladesh. Therefore, the present study was conducted to determine the nutritional composition, phytochemical and antioxidant activity of stems extract from the water lily.

Aims and Objectives

Even though, people in Bangladesh eat the water lily as a vegetable without knowing its nutritional and medicinal value. That's why the main aim of this study to let know the people about the nutritional and medicinal importance of water lily.

- To evaluate the Nutritional composition of *Nymphaea nouchali* (white variety) and *Nymphaea rubra* (red variety).
- To analyze the antioxidant activities of two species of *Nymphaea*.
- To Phytochemical screening of both species.

Chapter 2: Review of Literature

2.1 Overview of water lily

Nymphaeaceae species is a long-lived aquatic herb, with rootstock rooting in the mud. Leaves are long-stalked and leathery, floating on the surface of the water, egg-shaped to almost circular, prominently toothed, slightly peltate, 12 to 15 centimeters across, with the base deeply heart-shaped, and densely hairy beneath. Petioles are long, slender and submerged. Flowers are fragrant, white or red, about 8 cm in diameter, borne on long peduncles. Petals are linear-oblong to lanceolate. Stems are globular, with longitudinally numerous, striated seeds (Mohammed and Awodoyin, 2008). *N. nochali* and *N. rubra* are widely distributed in Bangladesh especially after the rainy season. The plant is locally known as Shada shapla and lal shapla. It is marketed as a vegetable in chattogram city to all other markets in Bangladesh. *Nymphaea nochali* is a national flower and icon of Bangladesh and is a delicious item of food for Bangladeshi people. Bangladeshi people purchase it as vegetables because the whole plant especially stems can be eaten as a vegetable after cooking (Tunan and Asif, 2012).

2.1.1 Nymphaeaceae Spp. Family

Nymphaeaceae is a family of flowering plants. Associates of this family are frequently called water lilies and live in freshwater areas in temperate and tropical climates around the world. The family holds eight genera. Water lilies are divided into two main groups: hardy and tropical. The tropical water lilies can bloom either during the day or at night hardy but water lilies bloom only all through the day and are the only group to hold blue-flowered plants (Songpanich and Hongtrakul, 2010). The Nymphaeaceae are aquatic, rhizomatous herbs. The family is further considered by the frequent presence of latex, usually with distinct, stellate-branched sclereids prominent into the air canals and dispersed vascular bundles in the stems. Hairs are simple, usually creating mucilage. Leaves are another and spiral, opposite or seldom whorled, simple, peltate or nearly so, entire to toothed or dissected, short to long Petiole (botanyiolate), with blade underwater, floating or emergent, with palmate to pinnate venation. Stipules are either present or absent and flowers are solitary, bisexual, redial, with a long pedicel and frequently floating or raised above the surface of the water, with girdling vascular packs in the receptacle. Fibers are well differentiated from anthers to laminar and poorly differentiated from anthers, pollen grains usually lacking apertures and separate, free

or adnate to petaloid staminodes, slender and carpels are 3 to numerous, distinct or connate. The stems is combined with nuts, a berry or an irregularly dehiscent fleshy capsule. Seeds are less lacking endosperm or often arillate (Mohammed and Awodoyin 2008).

Taxonomy

Domain: *Eukaryota*
Kingdom: *Plantae*
Subkingdom: *Viridaeplantae*
Phylum: *Tracheophyta*
Subphylum: *Euphyllophytina*
Infraphylum: *Rediatopses*
Class: *Magnoliopsida*
Subclass: *Nymphaeidae*
Superorder: *Nymphaeanae*
Order: *Nymphaeales*
Family: *Nymphaeaceae*
Subfamily: *Nymphaeoideae*
Tribe: *Nymphaeeae*
Genus: *Nymphaea*

2.1.2 *Nymphaea nouchali* (White water lily)

N. nouchali is a day blooming no viviparous plant with submerged roots and stems. Part of the leaves is submerged, while others rise slightly above the surface. The leaves are round and green on top, they usually have a darker underside. The floating leaves have undulating edges that give them a crenelated appearance. Their size is about 20–23 cm and their spread are 0.9 to 1.8 m. This water lily has a beautiful flower which is usually violet-blue with reddish edges. Some varieties have white, purple, mauve, or fuchsia-colored flowers, hence its name red and blue water lily. The flower has four or five sepals and 13-15 petals that have an angular appearance, making the flower look star-shaped from above. The cup-like sepal has a diameter of 11–14 cm (Pond, 2015).

2.1.3 *Nymphaea rubra* (Red water lily)

Nymphaea rubra belongs to the family of the *Nymphaceae*. It is native to Bangladesh, India, and Srilanka. In Europe, it is cultivated as an aquarist plant. The leaves spectacle so called heterophyllous, which means that the leaves that are under the water surface look different than those which are on the water surface. First of all the reddish leaves under the water surface develop (Chase et al., 2009). They are petiole grows nearly in the middle of the lamina. The leaves on the water surface are first reddish than dark green dentate at the leaf edge. The flowers are red with 15 cm in diameter. If one does not like to have flowers one has to remove the leaves on the surface. The plant then builds more leaves under the surface. This occurs only in 4 days. The flowers show protogyny which means that first of all the female organs get fertile and on the next day the pollen is released. This inhibits self-fertilization and promotes outcrossing (Pond, 2015).

2.2 Nutritional value and medicinal properties of water lily

Water lily stem contains carbohydrates, fats, protein, and fiber. They contain minerals like calcium, niacin, potassium, and magnesium (Bakare et al., 2010). The seeds have been found to antimicrobial properties, and can potentially help with reducing infections. Rice cooked with the seeds is said to be good for diabetics. In Ayurvedic medicine in India, they are considered to be cooling and are used to treat fevers. The rhizome of the red-flowered plant is given for blood dysentery and rhizome juice is prescribed against leucorrhoea. Powdered rhizome with honey is given for piles, dysentery, and dyspepsia and root juice is drunk to keep abdominal cool and to get relief from burning sensation during urination. Root paste of the red-flowered plant is given for menorrhagia (Oyelade et al., 2003).

The rhizome (root) of this plant is antiseptic as well as astringent. To use the rhizome for this purpose you will need a small non-aluminum pot. Fill it with about a pint of water and a small handful of very well crushed up rhizome to the water. It is important to not crush up the rhizome until you are ready to place it in the water. Do not add so much heat that the water boils. Let the water simmer like this until about 1/4 of it has evaporated. Flower petal decoction is given against diarrhea. Stems paste is prescribed as a cardiac tonic and also in fever and liver ailments. Dried seed powder is taken along with fresh cow milk against headache. Young seed paste is used externally as a cooling

medicine for skin disease. The powdered root is taken for expelling ringworms and roots paste in lemon juice is taken for the treatment of piles (Lestari et al., 2016).

2.3 Phytochemical properties of water lily

The different classes of phytomolecules such as alkaloids, glycosides, flavonoids glycosides, hydrolysable tannins, lignans, phytosterols, and triterpene saponins are found to be present in the various species of the genus in *Nymphaea* (Premier, 2002). The alkaloids such as nupharidinand apomorphine based compounds were reported from the flowers. Nupharin and nymphaeinewere reported from the flowers. Two phenolic base alkaloids coclaurine reported from the aerial parts of *Nymphaea stellate* species. The cardiac glycoside nymphalin is reported from the alcoholic flower extract (Selvakumari et al., 2016). The flavonoids such as anthocyanins, flavonols and flavones were reported and present as flavonoid glycoside with various glycone moiety among the various species of the genus *Nymphaea*. Nymphayol (25, 26-dinorcholest-5-en-3b-ol), a new sterol has been isolated from the successive chloroform extract of the flower. Protein, pentosan, mucilage, and tannins are reported in the seeds. Astragalin, corilagin, gallic acid, gallic acid methyl ester, isokaempferide, kaempferol, quercetin-3-methyl ether, quercetin, 2,3,4,6-tetrao-galloyl dextroglucose, and 3-o-methylquercetin-3'-o-beta dextroxylopyranoside have been identified in the flowers. The HPTLC method for the quantitative determination of gallic acid from hydroalcoholic dried flower extract has been reported (Evans, 2009). The leaves and shoots of Nymphaea nouchalli have been studied for their chemical composition. The proximate analysis showed dry matter -8.4%, crude protein-16.8%, ash-18.7%, crude fat-2.8%, crude fiber-26.3% for *N. nouchali*. Mineral content showed sodium-1.19, potassium-2.23, calcium-0.52, phosphorus-0.32, and calcium phosphorus ratio 1.63 for *N. nouchalli* (Singh and Jain, 2017).

2.5 Antioxidant properties of water lily

Reactive oxygen species (ROS) are chemically responsive molecules containing oxygen. Instances include peroxides, superoxides, hydroxyl radicals, and singlet oxygen. In a biological framework, ROS is designed as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis. They move everywhere in the body and have a deleterious effect on cells. This may result in major damage to cell structures (Siadat et al., 2012)

An antioxidant is a molecule that prevents the oxidation of other molecules and to solidity, the oxidative state, animals, and plant maintain complex systems of overlapping antioxidants, such as glutathione and enzymes produced internally or vitamins A, C and E obtained by ingestion. Phytochemicals such as phenolic acids, polyphenols and flavonoids can scavenge free radicals and result in inhibition of oxidative mechanisms that are liable for many diseases in humans. Antioxidants are widely used in dietary complements and have been investigated for the prevention of diseases such as cancer or coronary heart disease (Tosun et al., 2012).

A polyphenol is a type of antioxidant containing a polyphenolic substructure and numbering over 4,000 distinct species, many of these compounds have antioxidant activity in vitro, but are unlikely to have antioxidant roles in vivo (Wasagu et al., 2015).

Chapter 3: Materials and Methods

3.1 Study period

The study period was seven months from March 2019 to November 2019. All tasks were conducted in Dept. of Applied Food Science and Nutrition, Dept. of Food Processing and Engineering, Dept. of physiology, Biochemistry and Pharmacology and Poultry Research and Training Center (PRTC) at Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh.

3.2 Sample Collection

Fresh stems of water lily *Nymphaea nouchli* (white variety) and *Nymphaea rubra* (red variety) were collected from Follatoli market, Halishahar, Chattogram on July 10th, 2019 at exactly 7:30 am and the second sample was also collected on 14 July 2019. The stems were carefully rinsed with clean water, air-dried for two weeks in the laboratory.

3.3 Sample Preparation

The water lily parts were thoroughly washed to remove sand and other particles. Then the stem was cutting 4 to 5 inches and drying in cabinet dryer at 60°C around 24 hours. The samples were ground using a blending machine until powder obtained to ensure homogeneity. The powdered sample was passed through a fine (2mm mesh) sieve to remove any remaining residue. The finely powdered sample was then stored into labeled plastic containers before use.

3.4 Nutritional composition of the sample

3.4.1 Protein analysis

Principle: The Kjeldahl method is used to determine the nitrogen content in organic and inorganic samples. Kjeldahl method has been used for the determination of nitrogen in a wide range of samples. The determination of Kjeldahl nitrogen is made in foods and drinks, meat, feeds, cereals and forages for the calculation of the protein content. Also, the Kjeldahl method is used for the nitrogen determination in wastewaters, soils and other samples. It is an official method and it is described in different normative such as (AOAC, 2005), USEPA, ISO, DIN, Pharmacopeias.

Calculations: The calculations for % nitrogen or % protein must take into account which type of receiving solution was used and any dilution factors used during the distillation process. In the equations below, “N” represents normality. “ml blank” refers

to the milliliters of base needed to back titrate a reagent blank if standard acid is the receiving solution, or refers to milliliters of standard acid needed to titrate a reagent blank if boric acid is the receiving solution. When boric acid is used as the receiving solution the equation is

$$\% \text{ Nitrogen} = \frac{(\text{ml standard acid - ml blank}) \times \text{N of acid} \times 1.4007}{\text{Weight of sample in gram}}$$

3.4.2 Determination of Moisture content

Principle: Moisture determination is one of the most important and most widely used measurements in the processing and testing of foods. Since the amount of dry matter in a portion of food is inversely related to the amount of moisture it contains, the moisture content is of direct economic importance to the processor and the consumer. Of even greater significance, however, is the effect of moisture on the stability and quality of foods. Moisture content was determined by using the standard procedure of the Association of Official Analytical Chemists (AOAC, 2005).

Calculation: The percent of moisture was calculated as follow

$$\text{Moisture \%} = \frac{\text{Initial weight - Final weight}}{\text{Sample weight}} \times 100$$

3.4.3 Determination of ash content

Principle: The ash fraction contains all the mineral elements jumbled together. This method performs oxidization of all organic matter by incineration and determines the weight of remaining ash.

Calculation: The ash content was calculated by the following expression.

$$\text{Ash \%} = \frac{\text{The amount of the ash supplied sample}}{\text{Sample weight}} \times 100$$

3.4.4 Crude fat determination

Principle: Fat is estimated by dissolving food samples into organic solvents (chloroform: methanol) separating the filtrate by filtration. Placing the filtrate into separating funnels and then separated mixture is then dried to measure the extract and finally, the percentage of fat is estimated. AOAC (2005) methods using a soxhlet apparatus were used to determine the crude fat content of the samples.

Calculation: The percent of crude fat was expressed as follows expression.

$$\text{Fat \%} = \frac{\text{Weight of the extract}}{\text{Weight of the sample}} \times 100$$

3.4.5 Crude Fiber Determination

Principle: Crude fiber is the water-insoluble fraction of carbohydrate consists mainly of cellulose, hemicellulose, and lignin. It is estimated through digestion of fat-free known amount of food sample by boiling it in a weak solution of acid (1.25% H2SO4) for 30 minutes followed by boiling in a weak solution of alkali (1.25% NaOH) for 30 minutes at constant volume and then deducting ash from the residue obtained. The crude fiber was determined according to the AOAC method (2005).

Calculation: The loss in weight represents the crude fiber

$$\text{Crude Fiber \%} = \frac{\text{Weight of residue with crucible - Weight of ash with crucible}}{\text{Weight of the sample(moisture and fat free)}}$$

3.4.6 Determination of total carbohydrate

The carbohydrate content was determined by calculating the difference between the Nitrogen Free Extractive (NFE). It was given as the difference between 100 and a total of the other proximate components.

Calculation: Hence it was calculated using the formula below

$$\text{\% CHO} = 100\% - \text{\% (Protein + Fat + Fibre + Ash + Moisture content).}$$

3.5 Mineral analysis

Mineral contents were determined by using a biochemical analyzer (Humalyzer 3000). Commercially available biochemical kit (Randox®) was used for biochemical assay. For sample preparation, 5 g of powdered sample was taken into a conical flask. After that, 7.5 ml HNO_3 and 2.5 ml HCL was added into the conical flask. Then it heated over an induction cooker at 200W until complete digestion. Then it was cooled. Finally, deionized water was added up to 100ml. The results were expressed as mg/100g after conversion from mg/dl (Feng et al., 2013).

3.6 Determination of Phytochemical

3.6.1 Total flavonoid content determination

Flavonoid content in samples was measured by the aluminum chloride colorimetric method as described by (Shah and Hossain, 2014).

Preparation of standard quercetin solution: About 10 mg of quercetin was dissolved into 10 ml of distilled water. So the concentration of the solution was 1mg/ml. This is called a stock solution. Then serial dilution was performed to prepare a different concentrated solution (6 ppm, 12ppm, 24ppm, 48ppm, 96ppm). The figure shows the standard quercetin standard curve.

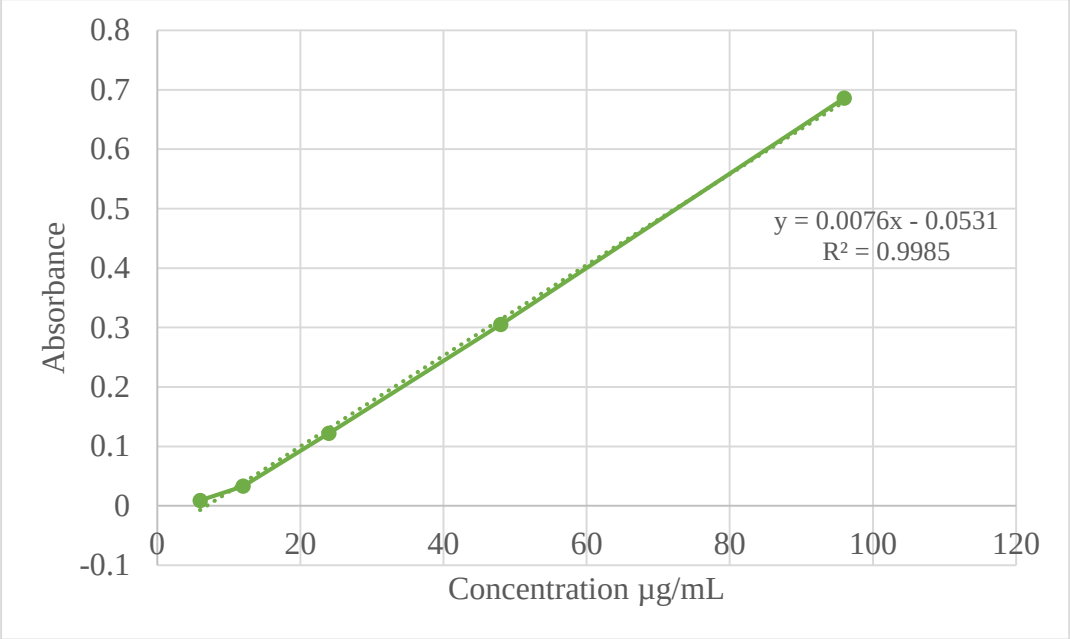

Figure 3.1: Standard curve of quercetin.

Procedure

At first 0.9815g of potassium acetate was dissolved in 10 ml water to prepare a 1M potassium acetate solution. 1g $AlCl_3$ was dissolved in 10 ml water to prepare a 10% $AlCl_3$ solution. About 1ml of sample or standard at different concentration solution was taken in a test tube. After that, 0.2 ml of 10% aluminum chloride, 0.2 ml of 1 M potassium acetate, and 8.6 ml of distilled water were added. The reaction mixture was then incubated at room temperature for 30 min to complete the reaction. The absorbance of the mixture was measured at 420 nm. Quercetin was used to make the calibration curve. The calculation of total flavonoids content in the extracts was carried out in triplicate and the results were averaged. The final result was expressed as mg of

quercetin equivalent (QE) per gram of dried weight. All determinations were performed in triplicate (n = 3).

3.6.2 Determination of total phenol content

Preparation of standard gallic acid solution

About 10 mg of gallic acid was dissolved into 10 ml of distilled water. So the concentration of the solution was 1mg/ml. This is called a stock solution. Then serial dilution was performed to prepare a different concentrated solution (2 ppm, 4 ppm, 8 ppm, 16 ppm, 32 ppm). **Figure 3.2** shows the gallic acid standard curve.

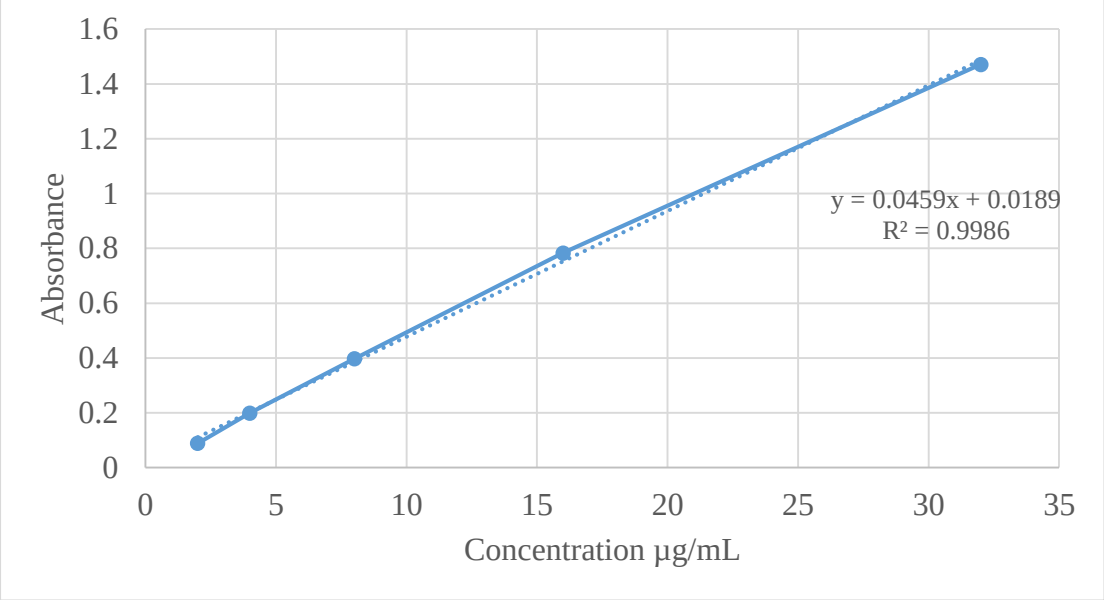

Figure 3.2: Standard curve of Gallic acid

Procedure

The total phenol content of extracts was evaluated by the Folin-ciocalteu method as described by (Wojdylo et al., 2007). About 1ml of sample extracts or standard at different concentrations were mixed with 2 ml of Folin–Ciocalteu reagent (10 times diluted), and incubated at room temperature for 3 min. After that, 10 ml of 20% sodium carbonate was added to the mixture and left for incubation at room temperature for an hour. The absorbance of the mixture was measured at 765 nm with a Shimadzu UV–VIS-2600 spectrophotometer against a blank solution. The blank solution contained all the reagent mixture without extract or standard sample. Gallic acid standard curve was used to quantify total phenolic contents and the results were expressed as mg of gallic acid equivalent (GAE) per gram of dried weight. All determinations were performed in triplicate.

3.6.3 Test for Saponin

Procedure

Take a small amount of powder extracted from water lily stem was dilute with distilled water and boiling the mixture about 15 minutes. After boiling filter the content of the biker through the normal filter paper to remove the residue and collect the aqueous extracted bellow into the biker. After filtration now takes 2ml the aqueous extracted of water lily stem into a test tube. Then shake the test tube and formation of foam indicate the presence of saponin in the extracted (Astuti et al., 2011).

3.6.4 Test for Tannin

Procedure

Each fraction 0.5g powder sample was taken in a biker and added a sufficient amount of water in it. Boiling the mixture of powder sample and water a few minutes to prepared agues exerted of water lily stem. After boiling filter the content of the biker through the normal filter paper to remove the residue and collect the aqueous extracted bellow into the biker. After filtration now takes 2ml the aqueous extracted of water lily stem into a test tube. The same amount of the aqueous extracted taken another test tube for comparison of the color change. Then one test tube few drops of 5% fierce chloride solution if the color of the extracted change to dark green color than it contains condensed tannin and dark blue color it is hydrolyzable tannin (Buzzini et al., 2008).

3.7 Determination of Antioxidant activity

DPPH radical scavenging abilities of the test samples were determined by following the method as described by (Nariya et al., 2013)

At first 4 mg of DPPH was dissolved in 100 ml of methanol (95%) in a dark condition. 10 mg of ascorbic acid was dissolved in 10 ml of distilled water. So, the concentration of the solution became 1mg/ml. This is called a stock solution. Then serial dilution was performed to prepare a different concentrated solution (2 ppm, 4 ppm, 8 ppm, 16 ppm, and 32 ppm). **Figure 3.3** showed the standard ascorbic acid curve.

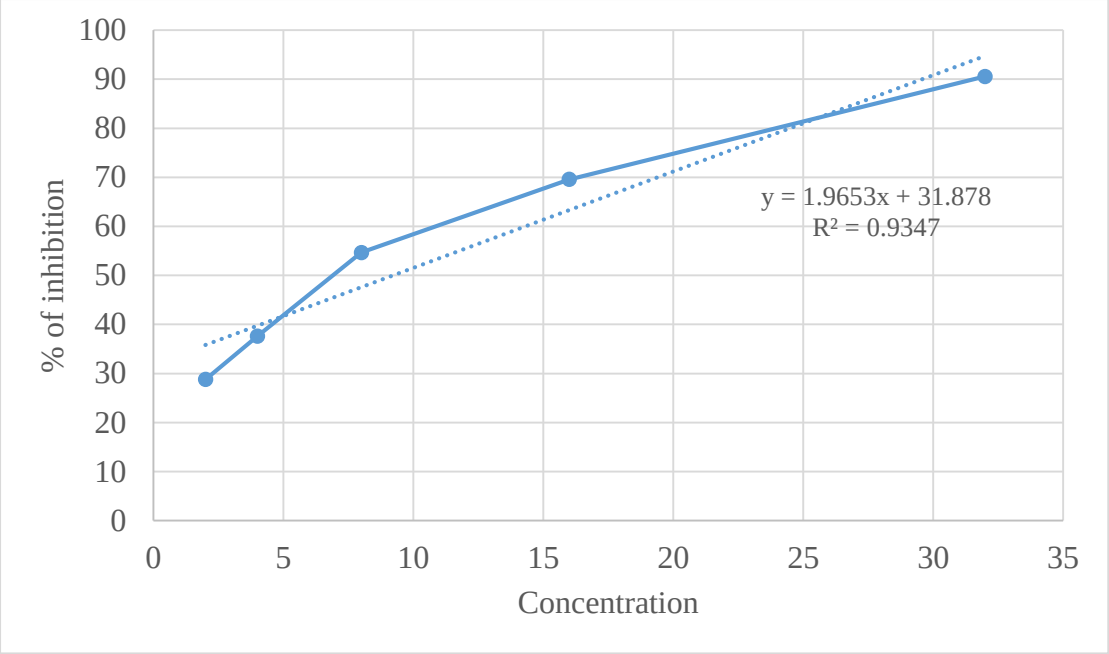

Figure 3.3: Standard curve of ascorbic acid

Procedure

Serial dilution was performed to prepare a different concentrated solution (2 ppm, 4 ppm, 8 ppm, 16 ppm, 32 ppm). About 4 ml of DPPH solution was added to 1 ml of sample extracts or standards at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance of the solution was measured at 517 nm using a UV-Vis spectrophotometer against blank. The control sample was prepared to contain the same volume without any extract and reference ascorbic acid. Methanol was used as blank. IC_{50} was calculated from % inhibition. Scavenging of the DPPH free radical was measured using the following equation:

$$\% \text{ of inhibition} = \frac{\text{Blank absorbance - Sample absorbance}}{\text{Blank absorbance}} \times 100$$

3.8 Statistical analysis

All statistical analysis was done using statistical package for social sciences (SPSS) version 25. Independent T-test was used to evaluate the data. Data are presented as the mean± (Standard deviation Mean) SDM. P values <0.05 were considered significant

Chapter 4: Results

4.1 Nutritive value of water lily

Nutritive value of water lily stems are shown in **Table 4.1**, both species contain an almost similar nutritive value. The result showed that crude fiber (18.78%) of white water lily slightly higher than red water lily (17.30%). The lower amount of crude fat (1.5 %) was found in the water lily of both species.

Table 4.1: Nutritional composition of water lily

Sample	Moisture %	Ash %	Protein %	Crude fiber %	Crude fat %	CHO %
White lily	12.83±0.3	16.16±0.1	8.05±0.02	18.78±0.1	1.47±0.07	42.71±0.06
Red lily	12.85±0.1	17.36±0.06	8.40±0.1	17.30±0.1	1.50±0.1	42.60±0.4
Significant	NS	***	***	***	NS	NS

Legends: All samples were replicate three times and the value represented (ME±SD), and ***=significant (P< 0.05), NS= Not significant (P> 0.05).

4.2 Mineral content of water lily

Figure 4.1 shows the highest amount of macro-mineral contents in both species of water lily were potassium 870.91 and 981.29mg/100g of white and red water lily respectively. Micro-minerals such as zinc and copper were the negligible amount in both species.

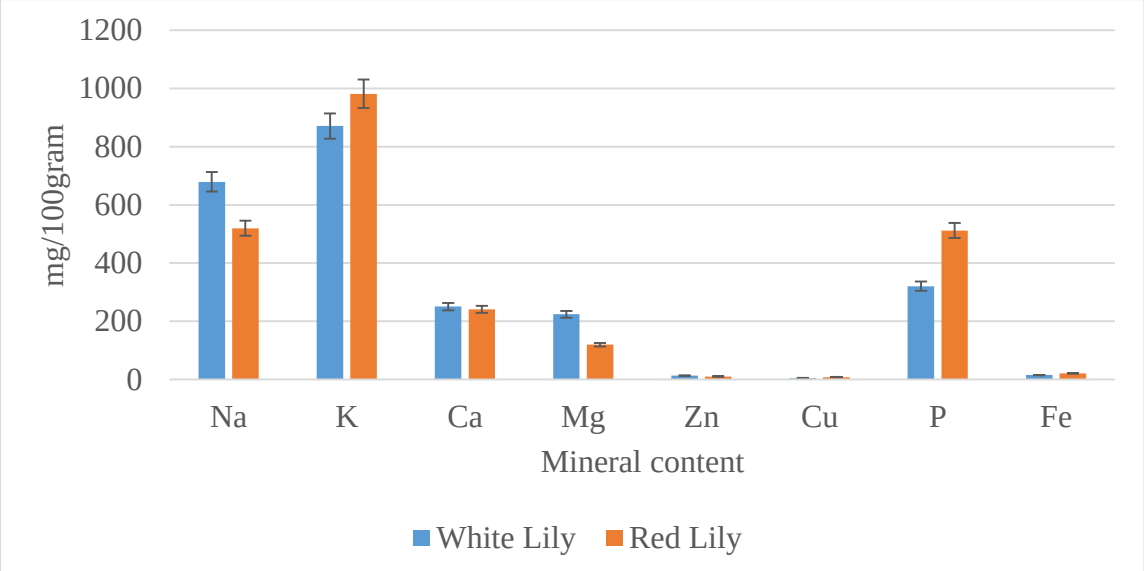

Figure 4.1: Mineral content of water lily

4.3 Phytochemical composition of water lily

4.3.1 Total phenol content

Table 4.2 shows the total phenol content of two types of water lily where both species values were slightly similar to 16.5 and 15.4mg Galic acid equivalent per gram in white and red respectively.

Table 4.2: Total phenol content water lily

	Sample solution	Weight of the dry extract per ml(g)	Absorbance	GAE conc. C µg/ml	GAEE conc. C mg/ml	TPC as QE A= (c.v)/m	Mean	P value
White water lily	1000	0.001	0.779	16.563	0.016563	16.563	16.51±0.05	NS
	1000	0.001	0.774	16.454	0.016454	16.454		
	1000	0.001	0.777	16.523	0.016523	16.523		
Red water lily	1000	0.001	0.718	15.249	0.015249	15.249	15.48±0.2	NS
	1000	0.001	0.733	15.570	0.015570	15.570		
	1000	0.001	0.736	15.640	0.015640	15.640		

Legends: All samples were replicate three times and the value represented (ME±SD), and ***= significant (P<0.05), NS= Not significant (P>0.05).

4.3.2. Total flavonoid content

Table 4.3 shows the total flavonoid content of water lily stems. Both species were content around an average of 7.8mg Quercetin equivalent/g.

Table 4.3: Total flavonoid content water

	Sample solution	Weight of the dry extract per ml(gm)	Absorbance	QE conc. C µg/ml	QE conc. C mg/ml	TFC as QE A= (c.v)/m	Mean	P value
White water lily	1000	0.001	0.003	7.325	0.007325	7.325		
	1000	0.001	0.006	7.676	0.007676	7.676	7.476±0.1	NS
	1000	0.001	0.004	7.427	0.007427	7.427		
Red water lily	1000	0.001	0.008	8.001	0.008001	8.001		
	1000	0.001	0.007	7.825	0.007825	7.825	7.846±0.1	NS
	1000	0.001	0.007	7.802	0.007802	7.802		

Legends: All samples were replicate three times and the value represented (ME±SD), and ***=significant (P< 0.05), NS= Not significant (P> 0.05).

4.3.3 Saponin and Tannin screening

Water lily of two species were given a positive result throughout the screening of saponin and tannin.

Table 4.4: Saponin and Tannin screening of water lily

Sample	Tanin	Saponin
Nymphaea nouchali (White lily)	+ve	+ve
Nymphaea rubra (Red water lily)	+ve	+ve

Legends: (+ve) indicate present while (–ve) indicate absent.

4.4 Antioxidant activity

4.4.1 DPPH activity of water lily

Results for the DPPH free radical scavenging activity of methanolic extracts of white and red water lily are shown in **Table 4.5**. The half inhibition concentration (IC_{50}) value of ascorbic acid was 9.22 µg/ml. In contrast, the IC_{50} value of free radicals achieved was 36.67 and 28.48 µg/ml for white and red lily respectively.

Table 4.5: DPPH radical scavenging activity of white water lily, red water lily, and ascorbic acid

Concentration (µg/ml)	%inhibition of Ascorbic acid	%inhibition of white water lily	%inhibition of red water lily
2	28.78	23.00	24.92
4	37.63	25.89	27.81
8	54.67	34.55	39.36
16	69.59	40.32	44.17
32	90.57	44.17	49.95
IC50 (µg/ml)	9.22	36.67	28.48

Figure 4.2 depicted y=0.731x+27.407 where y indicate % inhibition of white lily, x indicates concentration and R^2= 0.8233 describes the strength of a correlation between two variables.

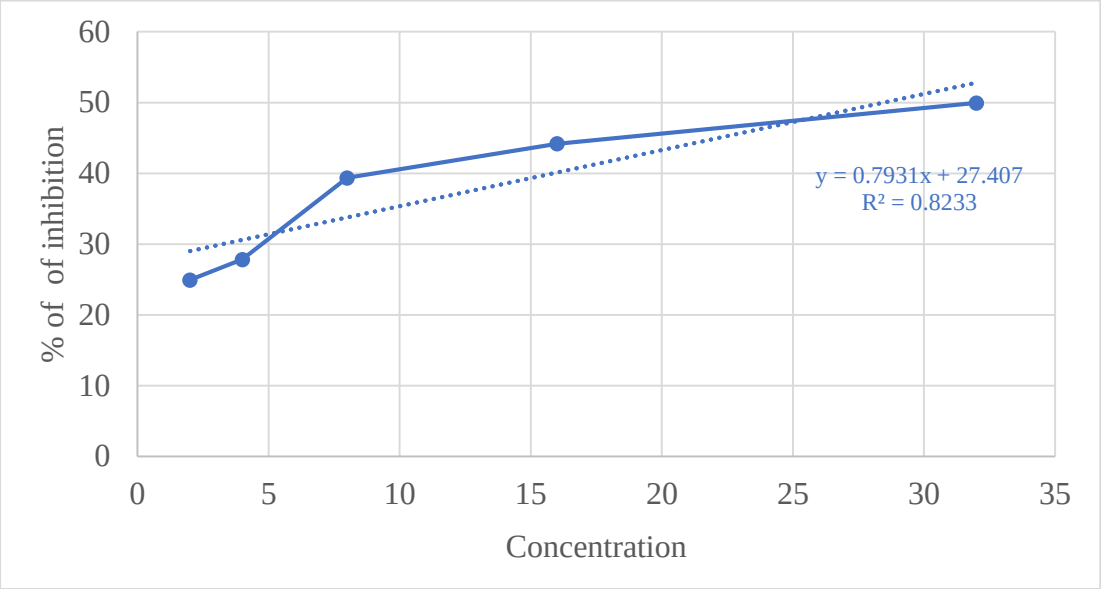

Figure 4.2: DPPH test curve of white water lily

Figure 4.3 depicted y=0.6759x+25.209 where y indicate % inhibition, x indicates the concentration of red lily and R^2= 0.8246 describes the strength of a correlation between two variables.

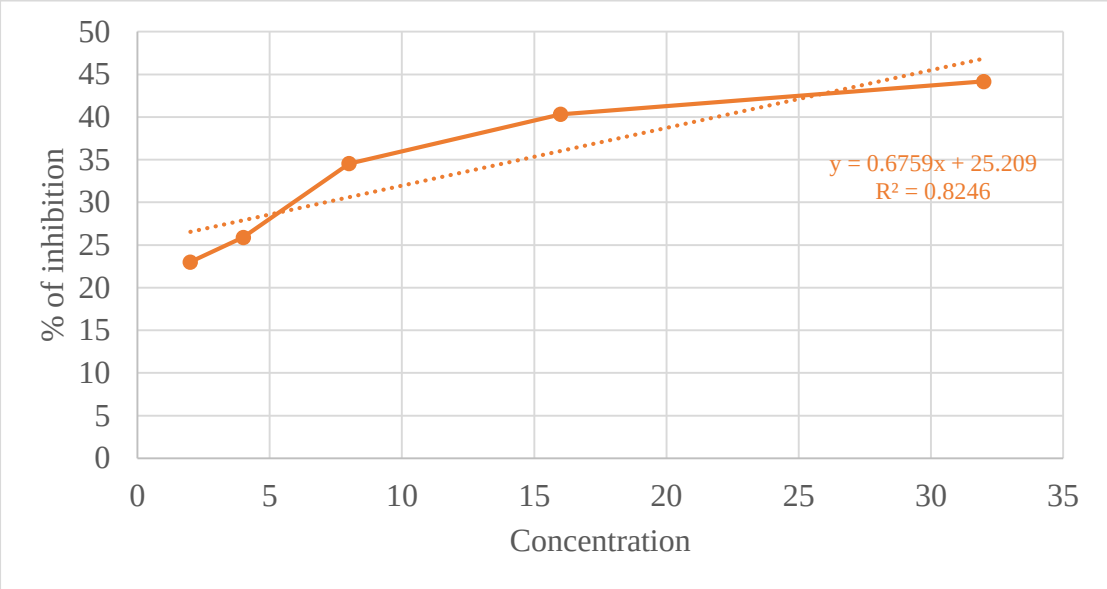

Figure 4.3: DPPH test curve of red water lily

Figure 4.4 showed the comparison among the standard ascorbic acid and two species of water lily DPPH scavenging activity.

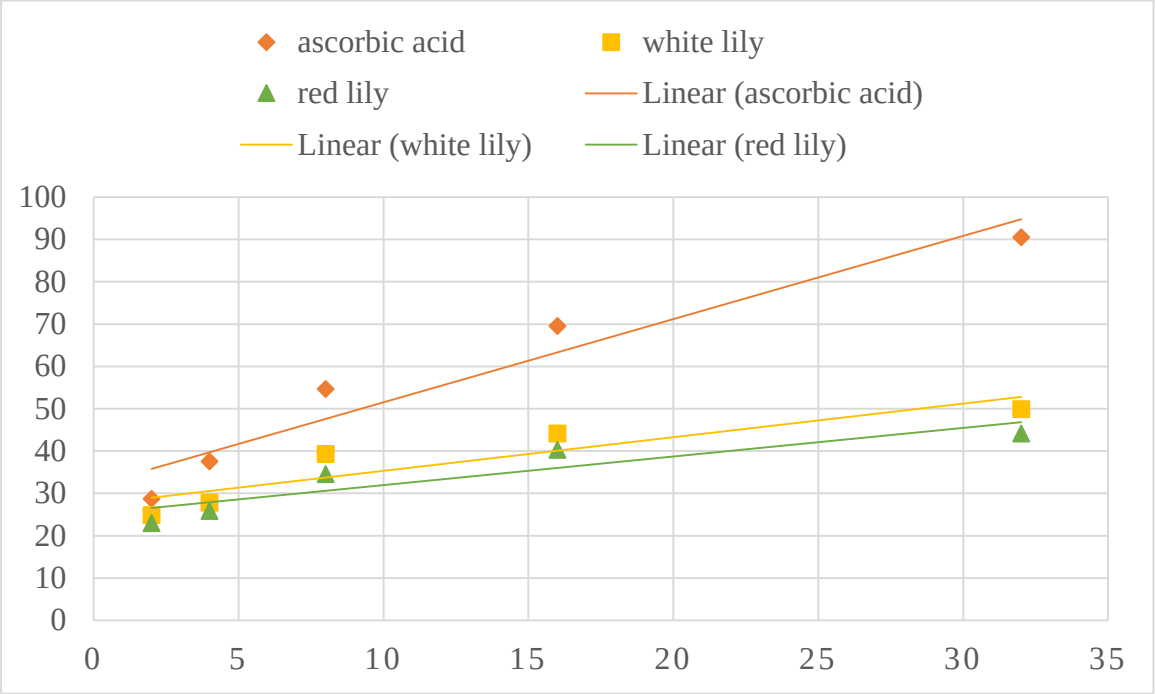

Figure 4.4: DPPH scavenging radical activity

Chapter 5: Discussions

5.1 Nutritional composition of water lily

In this present study, the moisture content of *N. nouchali* and *N. rubra species* was found an average of 12.8% quite higher to compare the other study found in *N. lotus* species was 9% (Stephen et al., 2017). The variation in moisture percentage may be due to different drying processes, regional production or weather condition of the production area. The crude protein found in both species in this research was almost similar which was about 8% but the value is lower than the *N. lotus*, 21.66% (Stephen et al., 2017). This may be due to microhabitat variation in the study area or different species of water lily. In both species, the fat percentage was found 1.5% on an average where another study displayed 5%, for *N. lotus* which is three times higher than the present research. Besides, another researcher mention that fat percentage 1.83% enough for promoting the fat-soluble vitamin absorption (Muhammed and Awodoyin, 2008). Low fat-containing food can thus be recommended as part of the weight-reducing diet since low-fat food reduce the level of cholesterol and obesity (Chinelo and Jega, 2019). The carbohydrate of *N. lotus* was found around 41.92% and in this research, the carbohydrate content of both water lily species was slightly similar to the previous study and the value was about 42% on an average. The high amount of carbohydrate in the stem indicates that it can be used as a good source of energy where the energy value of the plant bulb is 353.13 KCalorie/100g (Stephen et al., 2017). The crude fiber in both species was found about 18% on an average where *N. lotus* showed a lower amount of crude fiber 13.3%. Past studies have linked crude fiber ability to prevent or relieve constipation and proved another health benefit as well as helping to maintain a healthy weight and lowering the risk of diabetes, heart disease and so many types of cancer (Wasagu et al., 2015).

5.2 Mineral content of water lily

The present research demonstrates that water lily stem has a decent source of macro and micro mineral. The potassium content of water lily was 870.91 and 981.2mg/100g in *N. nouchali* and *N. rubra* respectively. Which are higher than other species of water lily such as *N. lotus* was 742.89mg/100g (Adelakun et al., 2016). A study found that the nervous system and muscle activities are attributable to the presence of high potassium and help to maintain the correct water balance in the cells (Nadia, 2014). The

sodium contents of both species were 679.16 and 519.62 mg/100g in white and red species advanced than the *N. lotus* 431.53mg/100g. This variation may occur due to the salinity difference of water where it has grown up. Potassium and sodium are very crucial electrolytes for the human body to regulate the normal blood volume as well as to maintain normal blood pressure. Increase potassium intake and low sodium intake reduce the risk of cardiovascular diseases (Lavid et al., 2001).

This study showed a high amount of calcium and magnesium content in both species where calcium was 250mg/100g and magnesium was 220mg/100g on an average. Both calcium and magnesium level are three times higher than the calcium and magnesium found in species of *N. lotus*. According to Adelakun et al., 2016 the phosphorus content of *N. lotus* was 635mg/100g that was so higher than the present study where the phosphorus was 320, 511mg/100g in *N. nouchali* and *N rubra* respectively.

Iron is another important mineral for us to maintain hemoglobin levels and to reduce the number of anemic patient especially pregnant mothers in developing countries like Bangladesh. Iron is an essential part of many enzymes and the proteins and helps red blood cells transport oxygen to all the parts of the human body. Iron also helps to regulate cell growth and cell differentiation. It helps keep muscles and nerves functioning normally and also helps to regulate heartbeat, supports the immune system and keeps the bones strong (Weinblatt, 2016). The water lily content a moderate amount of iron 15.25 and 20.95mg/100g in white and red species which are higher than some other vegetables consume by rural people as a curry such as cauliflower, cabbage, okra, pumpkin, etc. The copper content (2.75mg/100g) of red water lily was about double than white lily (1.29 mg/100g)) and zinc levels were 13.58 and 10.41mg/100g in *N. nouchali* and *N rubra* respectively where Adelakun et al., 2016 reported that *N. lotus* content 8.16mg/100g of zinc that is lower than this research.

5.3 Phytochemical compound of water lily

Phytochemicals give the plant color, aroma, and flavor, but when we eat them, they work with other phytochemicals and nutrients to fend off cancer, heart disease, age-related eye disease and more (Premier, 2002). Some phytochemicals stimulate the immune system and others slow the growth of cancer cells or prevent DNA damage (Adhami et al., 2008). In this study, some important phytochemicals such as phenol, flavonoid, tannins, and saponins were analyzed. The total phenols of water lily were 16.51 and 15.48 mg GAE/g respectively in white and red species that is higher than reported 4.8 mg GAE/g in *N. pubescens* species (Daffodil and Mohan, 2013).

According to multiple reports in the literature, phenolic compounds exhibit free radical inhibition, peroxide decomposition, metal inactivation or oxygen scavenging in biological systems and prevent oxidative disease burden (Bendary et al., 2013).

Both species of water lily content a similar amount of flavonoids which was around 7.5 mg Quercetin equivalent/g on an average. According to the study reported by (Daffodil and Mohan, 2013) *N. pubescens* species content higher amount of flavonoid was 9.8 mg QE/g.

Flavonoids are also important for human health. Like vitamins, these compounds are not produced endogenously by the body and must be supplied either through the diet or nutritional supplements (Sakihama et al., 2002). Tannin shows an outstanding array of biochemical and pharmacological actions including anti-inflammatory, antioxidant, anti-allergic and anti-carcinogenic activities. Plant-based foods are also rich in saponin that cannot be found in meat and dairy. Studies have illustrated the beneficial effects on blood cholesterol levels, cancer, and stimulation of the immune system (Baehaki et al., 2015). Both species of water lily showed a positive screening test for tannin and saponin which are denatured during the heating process.

5.4 Antioxidant activity of water lily

The effects of antioxidants on DPPH radical scavenging thought to be due to their hydrogen-donating ability (Binsan et al., 2008). Antioxidants inhibit the lipid peroxidation, metal ion chelation, or a combination of these properties (Sarmadi and Ismail, 2010). Antioxidant actions might defend biological systems against damage related to oxidative stress in human disease conditions (Misra et al., 2009). Scavenging radical activity of *N. nouchali* and *N.rubra* stem express as IC50 value was 36.67 and 28.48 µg/ml respectively. The value of IC50 for Standard ascorbic acid was 9.22µg/ml. The values of the IC50 showed that *N. nouchali* and *N. rubra* stems are rich sources of antioxidants because low IC50 value indicates high antioxidant activity. In contrast according to Baehaki et al., 2015, the *N. stellate* and *Nelumbo nucifera* species of water lily were showed IC50 value 43.21 and 139.84 µg/ml respectively that are so higher than this study. So both species of water lily could be used as a rich source of antioxidants in the therapeutic diet as well as in medicinal industries

Chapter 6: Conclusion

Bangladesh is located in the tropical monsoon region and its climate is characterized by high temperature, heavy rainfall, often excessive humidity due to which a lot of water lilies are available here. In this present study, it can be concluded that it is an abundant source of carbohydrate and crude fiber in terms of nutritional value, as well as a great source of the macromineral such as potassium and the micro mineral iron. This research also found that the water lily had higher antioxidant activity than the other aquatic plants. It also showed that there is some phytochemicals test were positive in the stems of water lily which are applied to use in chemical and pharmaceutical industries. On the other side tannin and saponin found in both species of water lily that's why these phytochemicals may hamper absorption of the nutrient. So stems should be processed first by either cooking or any other methods before consumption. Finally, this study suggested that water lily stem can be used as an alternative source of nutrient as well as medicinal purposes more frequently.

Chapter 7: Recommendation and Further Perspective

Nowadays, more than half of the people suffer malnutrition in our country, in these situations water lilies could be a good source of the nutrients and energy as these are available in rural areas of Bangladesh. As water lily are high in fiber content so these can be used in constipation and other gastrointestinal related health problems. Fiber and mineral content suggest that it has potential food value and could be recommended as a functional food ingredient. Moreover as water lily are rich sources of antioxidant that's why it can be used to prevent ageing process, depression, and other free radical related health conditions. Furthermore, as both species are given tannin and saponin positive, so water lily should be consumed after apply the thermal process. Further work could be conducted to evaluate the essential vitamins and other phytochemical compounds of water lily. Apart from this more analysis are recommended for other parts of water lily including petals, roots, and seeds. Finally, a thorough study could be conducted to understand the beneficial effects of water lilies on the human subject.

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Appendix A: Photo Gallery

Fresh Water Lily

Cutting and Drying

Powder Preparation

Extract Preparation

DPPH Solution Preparation

Laboratory Work

Stock Solution Preparation

Laboratory Work

Brief Biography

Md. Akram Hossain Khan passed the Secondary School Certificate Examination in 2009 from Hathazari Parbotti High School, Chattogram, and then Higher Secondary Certificate Examination in 2012 from Hathazari College, Chattogram. He obtained her B.Sc. (Honors) in Food Science and Technology from the Faculty of Food Science and Technology at Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. Now, he is a candidate for the degree of Master of Science in Applied Human Nutrition and Dietetics under the Department of Applied Food Science and Nutrition, Chattogram Veterinary and Animal Sciences University (CVASU). He has an immense interest to work in improving the health status of people through proper guidance and suggestions and to create awareness among people about food safety and nutrition.