NUTRACEUTICAL STUDY AND POTENTIAL ANTIDIABETIC ACTIVITY (*in-vitro*) OF TWO SPECIES OF TROPICAL WATER LILY (*Nymphaea nouchali & Nymphaea rubra*)

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Roll No.: 0119/07
Registration No.: 665
Session: January-June (2019-2020)

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
Chattogram Veterinary and Animal Sciences University
Chattogram-4225, Bangladesh

DECEMBER, 2020

Authorization

I, Kanij Fatema Nishan, hereby declare that the dissertation entitled "Nutraceutical Study and Potential Antidiabetic Activity (*in-vitro*) of two species of Tropical Water Lily (*Nymphaea nouchali* & *Nymphaea rubra*)", submitted by me in partial fulfillment of the requirements for the award of 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 is a complete record of original research work carried out by me during the period of March, 2021-Novembar, 2021 under the supervision and guidance of Mrs. Kazi Nazira Sharmin, Associate Professor, Department of Applied Food Science and Nutrition, CVASU and I am the sole author of the thesis.

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DECEMBER, 2020

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

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DECEMBER, 2020

DEDICATED TO

MY BELOVED PARENTS, SIBLINGS

AND TEACHERS

Acknowledgements

All praises to Allah and His blessing for the completion of this thesis. I thank God for all the opportunities, trials and strength that have been showered on me to finish writing the thesis successfully 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. I experienced so much during this process, not only from the academic aspect but also from the aspect of personality. Throughout the writing of this dissertation I have received a great deal of support and assistance from countless people.

At this moment of accomplishment, I express my sincere thanks to **Professor Dr. Goutam Budha Das**, Vice Chancellor and Dean, Faculty of Food Science and Technology, Chittagong Veterinary and Animal Sciences University, for all his support, dedications and sincere co-operation.

I wish to express my deep sense of gratitude to my supervisor **Mrs. Kazi Nazira Sharmin**, Associate Professor, Department of Applied Food Science and Nutrition, CVASU for her supervision and guidance in successful completion of this work. She has provided positive encouragement and a warm spirit to finish this thesis. It has been a great pleasure and honor to have her as my supervisor and it was impossible to complete the dissertation without her constructive direction and supervision.

I would like to thank my Co-supervisor **Mohammad Mozibul Haque**, Assistant Professor, Department of Applied Food Science and Nutrition, CVASU for his guidance and invaluable support in all stages of my research. I cannot quantify the contributions and supports of my respected teacher **Md. Altaf Hossain**. Even though he is no longer in CVASU due to PhD leave, his supervision by correspondence has left no vacuum. I feel much pleasure to convey my profound gratitude to him.

It is my privilege to acknowledge to Head of the Department **Dr. S. K. M. Azizul Islam**, Professor, Department of Physiology, Biochemistry and Pharmacology for lab support and cooperation next ended to me during the course of investigation. I thank

all the teachers of faculty of Food Science and Technology for their valuable suggestions and support during the research program. I sincerely thank to all members of Department of Applied Food Science and Nutrition, FST; Department of Food Processing and Engineering, FST; Department of Fishing and Post-Harvest Technology, FOF and Department of Physiology, Biochemistry and Pharmacology, FVM; for their constant inspiration and kind co-operation in analyzing my samples for the research activities precisely.

I must not fail to sincerely appreciate and acknowledge the financial support in form of research fellow under the National Science and Technology (NST) fellowship program by Ministry of Science and Technology, Bangladesh and Advanced Studies & Research, CVASU under which I carried out all my research.

Finally, I sincerely and genuinely express my deepest sense of gratitude, cordial respect of feelings to my dear parents and siblings for their supports and the roles they played on my behalf. I owe my wholehearted thanks and appreciation to all my beloved friends who were with me and support me through thick and thin.

The Author
DECEMBER, 2020

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List of abbreviations

%	**:** Percentage
&	**:** And
°C	**:** Degree Celsius
AOAC	**:** Association of Official Analytical Chemists
CAB	**:** Centre for Agriculture and Bioscience
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
GRIN	**:** Germplasm Resources Information Network
IC	**:** Inhibition concentration
mg	**:** milligram
ppm	**:** Parts per million
PS	**:** Petal sample
QE	**:** Quercetin equivalent
spp.	**:** Species
SPSS	**:** Statistical Package for Social Science
SS	**:** Seed sample
TS	**:** Tuber sample
TFC	**:** Total flavonoid content
TPC	**:** Total phenolic content
USDA	**:** U. S. Department of Agriculture
WHO	**:** World Health Organization
µg	**:** microgram

Abstracts

Plant extracts, different parts of plant even the whole plants of water lily act as a source of remedies are widely used for the prevention or treatment of many diseases apart from their nutritional value. The present work was aimed to evaluate the nutraceutical value of three parts (Petal- PS1, PS2; Seed- SS1, SS2; Tuber- TS1, TS2) of Nymphaea nouchali and Nymphaea rubra respectively by means of assessing nutritional composition (Moisture, Ash, crude fat, crude fiber, crude protein, CHO and vitamin C), mineral content (Na, K, Mg, Ca, P, Fe, Cu, Zn), bioactive phytochemical content (Total phenol, total flavonoid, tannin, saponin, alkaloids) using standard method. In vitro DPPH assay and α-amylase inhibition assay were conducted to identify their potential antioxidant and antidiabetic activity respectively. The result show that tuber sample (TS1, TS2) had higher moisture and ash content, while seed sample (SS1, SS2) had higher crude fat and carbohydrate content. Petal sample (PS1, PS2) had higher crude protein and crude fiber content. Vitamin C content is higher in tuber sample than other water lily parts. Macro and micro minerals (K, Ca, P, Fe, Cu and Zn) were higher in petal sample, Na and Mg were higher in seed (SS1) and tuber (TS1) respectively. Phytochemical analysis revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids. Total phenolic content (5.597±0.01 mg GAE/100g) and flavonoid content (794.315±0.86 mg QE /100gm) were found to be higher in petal sample (PS1) of N. nouchali than N. rubra. Lower IC50 value found in petal sample (PS1: 6.91 μg/mL and PS2: 12.70 μg/mL) indicate that ethanolic extracts of petal sample possess higher antioxidant activity that can scavenge more DPPH free radicals. Nymphaea nouchali and Nymphaea rubra inhibited the activity of α-amylase with an IC50 value of 13.25 μg/mL and 27.21 μg/mL in petal; 60.69 μg/mL and 55.24 μg/mL in seed; 59.71 μg/mL and 50.89 μg/mL in tuber respectively. Whereas, the positive control Acarbose used in this study, shows an IC50 value of 22.52 μg/mL. Nutritional composition obtained in this study suggest that petal, seed and tuber of both N. nouchali and N. rubra have potential food value and could be recommended as a functional food ingredients. They are also a promising source of potential antioxidants and may be efficient for the prevention of diabetes. However, the strength of existing data is not enough. Further in vivo studies will be needed to determine the main active ingredient behind its efficiency.

Keywords: Nymphaea, Bioactive, Antioxidant, DPPH, α-amylase.

Chapter 1: Introduction

Biologically active molecules present in food or any edible parts of plants are often known as "Nutraceuticals" since they have characteristics similar to both nutrients and pharmaceuticals. Nutraceuticals include lipids, vitamins, carbohydrates, proteins, minerals, or other necessary nutrients. Those bio-active or chemical compounds which apart from having a nutritional role, provide health-promoting, disease curing or prevention properties. Sachdeva *et al.* (2020) discussed an overview of various bioactive ingredients that act as nutraceuticals (carbohydrates, lipids, edible flowers, alkaloids, medicinal plants, *etc*.) and their role in health benefits, has been discussed. Further applications of nutraceuticals in the prevention of various diseases have also been discussed. According to other investigations, nutraceuticals have been found to be effective in the treatment of a variety of conditions, including cardiovascular disease (Sosnowska *et al*., 2017), diabetes (Nimesh *et al*., 2018), atherosclerosis (Aquila *et al.*, 2019), cancer (McClements, 2019), and neurological disorders (Sarris *et al.*, 2019). Recently published articles addressing distinct aspects of nutraceuticals as alternatives to pharmaceuticals were searched using scientific sites such as Medline, PubMed, and Google Scholar because the recent research has shown promising effects in various difficulties. Nutraceutical and allergy, Alzheimer's, cardiovascular, cancer, diabetes, anti-oxidative, anti-inflammatory, eye, immune, inflammatory or Parkinson's were among the phrases mentioned (Nasir *et al.*, 2014).

Different plant parts (*viz.* roots, leaves, barks, fruits, seeds ,tubers and flowers) have been effectively utilized to cure and prevent diseases since ancient times (Raskin *et al.*, 2002). Nowadays, Plants have been included in all major systems of medicine because of their ubiquitous usefulness in curing diseases, regardless of the philosophical assumptions that explain them and have received much attention due to their possible nutritional, safety, and therapeutic benefits in both developing and developed countries. According to the World Health Organization (WHO), traditional medicine is used by 80% of the world's population for basic health care and the majority of this therapy involves the use of plant extracts and their active components (Krishnaiah *et al*., 2011). Such non-conventional medicinal plant with great nutritional value and disease curing or prevention properties is water lily. The water lily belongs to genus *Nymphaea*, is the largest and most widely spread aquatic plants

of the Nymphaeaceae family , with perennial or annual rhizomes, floating or submerged leaves and solitary, differently colored spectacular flowers (Wiersema, 1988) which has a worldwide distribution and more than 50 species (Borsch *et al*., 2011). The plant bears flowers of different colours, depending on which it is known as the blue, red or white water lily. In Bangladesh, the water lily is commonly known as Shapla and the plant depending on blue, red or white flowers is known as neel shapla, laal shapla or shada shapla (Rahmatullah *et al.*, 2015). Shada shapla (*Nymphaea nouchali* Burm. f.) is Bangladesh's national flower and an edible delicacy. People in Bangladesh buy it as a vegetable since the entire plant, particularly the flower stalk, seeds and tuber can be cooked, roasted and eaten.

Nymphaea nouchali and *Nymphaea rubra* are tropical water lily in *Nymphaea* genus belonging to the Nymphaeaceae family in Bangladesh. They can be found in abundance as a mixed population in almost all shoals and natural water bodies in Bangladesh (Alam *et al*., 2018) such as bills, ponds, lakes, and ditches. Previous studies has reported that extracts of various parts of Nymphaeacea are considered as a rich source of starch, protein, fibers (Pareek and Kumar, 2014). Some of the recent study on *Nymphaea nouchali* tuber (Anand *et al*., 2019), *Nymphaea lotus* and *Nymphaea pubescens* seed (Aliyu *et al*., 2017) revealed that apart from macronutrients like carbohydrates, protein, fat, sugar, it is also a rich source of micronutrients such as ascorbic acid, niacin, riboflavin, thiamin, calcium, iron and zinc. The presence of significant high percentage of fiber content in the root of *Nymphaea lotus* rather than in seed and leaves was also investigated by Wasagu *et al*. (2015).

Nymphaeacea family also have many pharmacological and biological properties such as antioxidant, antidiabetic, anti-hyperlipidemic and anti-obesity (Bhardwaj and Modi, 2016). Many studies have shown that extracts from *Nymphaea nouchali* Burm. f. and *Nymphaea rubra* have effective anti-diabetic properties in animal model (Rahuja *et al*., 2013; Parimala and Shoba, 2014). Diarrhoea, dysentery, eruptive fevers, and infections all can be treated with this plant (Hossain *et al*., 2014; 2015).Many researchers proficiently investigated a good number of secondary metabolites like sterols (Nymphayol, isolated from flower, Verma *et al*., 2012), phenolics (Sundaram *et al*., 2020), alkaloids, saponins, tannins, and flavonoids from

different species of this plant (Raja *et al*., 2010) that are responsible for significant antioxidant activity (Daffodil and Mohan, 2014; Shajeela *et al*., 2012) and traditionally claimed antidiabetic effect (Raja *et al*., 2010; Shajeela *et al*., 2012; Rahuja *et al*., 2013).

Nowadays, the food industry is required to produce "clean label" products as per consumers' concern about natural food products with significant health benefits. Taking this into consideration, producing functional food by the inclusion of medicinal plants in food products must be an effective strategy. As the components of medicinal plants can be the natural substitutes to synthetic additives in foods, medicinal plants provide positive health benefits over and above the normal nutritional benefits and have acquired massive attraction of many researchers and food industrialists. Researchers in many countries are enthusiastically studying the nutritional quality, phytochemical content, antioxidant and antidiabetic activity of different edible parts of water lily to elucidate its physiological benefits. But to the very best of my knowledge, little research has been conducted on water lilies in Bangladesh. Formerly, Sikder *et al*. (2012) studied the bioactivities of *Nymphaea nouchali* Burm. f. the national flower of Bangladesh. Recently, anticancer pathways associated with the potential bioactive phytochemicals and antioxidant properties of *Nymphaea nouchali* tuber extracts was investigated by Uddin *et al*. (2020).

In view of the aforementioned nutritional, therapeutic and medicinal properties, the present study was conducted to determine the nutritional content, phytochemical ingredients, antioxidant activities and antidiabetic activity in order to verify the health benefits and nutraceutical efficacy. Moreover, most of the people in Bangladesh are lower middle class that they live from hand to mouth. When three staple meals a day is not guaranteed, nutritious and healthy food become a luxury to them that they cannot afford. In this context, the present study was designed to disseminate nutritional and nutraceutical information about *Nymphaea nouchali* and *Nymphaea rubra* to nutritionists, industrialists, researchers, policy makers and development agencies so that different parts of tropical water lily of Bangladesh become a part of the normal diet for people of all financial categories. As a consequence, the misconception of considering water lily as 'famine food' or 'poor people's food' will be rectified.

Aim and Objectives:

Considering the high potential and low cultivation costs, it can be concluded that tropical water lilies (*Nymphaea nouchali* and *Nymphaea rubra*) are still underappreciated in Bangladesh. So, the aim of this study is to evaluate the nutraceutical value of *Nymphaea nouchali* and *Nymphaea rubra* and to spread the nutritional information to nutritionists, industrialists, researchers, policy makers and development agencies. The objectives of the present study include:

i. To evaluate the nutritional and phytochemical properties of water lily
ii. To evaluate the nutraceutical effectiveness of water lily against diabetes

Chapter 2: Review of Literature

2.1 History

The name Nymphaea means ‘water lily’ is a direct translation of a Greek word ‘*nymphaia’* and were inspired by the nymph of Greek. Europeans, Asians, and Africans used to eat water lily seeds and tubers as emergency food many years ago. Egyptians adore and eat *Nymphaea nouchali* and *Nymphaea lotus*. Egyptians eat the rhizome, flowers, and leaves, and the buds are commonly shown on ancient monuments, furniture, and murals. During Egyptian civilization, water lilies were also used in religious ceremonies. The Egyptian royal family also thinks that the magnificent water lily blossoms represent purity and longevity. South Africans ate the rootstock of blue water lily either raw or cooked in curry recipes in the early 1800s. The white water lily (*Nymphaea nouchali*) becomes Bangladesh's national flower and the state flower of Andhra Pradesh, India, while the blue water lily becomes Sri Lanka's national flower. For someone born in July, the water lily is a metaphorical character (Singh and Jain, 2017).

2.1.1 Taxonomy

The taxonomic rank of *Nymphaea* varied between species and varieties in many of the cases. According to CAB International datasheet of 2019, the general taxonomic hierarchy of *Nymphaea* genus is:

Domain: Eukaryota
Kingdom: Plantae
Phylum: Spermatophyta
Subphylum: Angiospermae
Class: Dicotyledonae
Order: Nymphaeales
Family: Nymphaeaceae
Genus: *Nymphaea*

2.1.2 Habitant and distribution

The water lily was initially identified in Portugal during the early Cretaceous period. From Alaska to Newfoundland and south to northeastern Mexico, the genus *Nuphar* is found in temperate regions of North America. *Euryale ferox* is found in Asia,

specifically China, India, and Japan. The genus *Nymphaea* has a wide range that includes North America, Africa, Europe, and Asia. For ages, Southeast Asia has grown *Nymphaea nouchali*, particularly near temples. It is also grown in Sri Lanka and harvested from dried ponds in India for the rhizomes, which are used as food and animal fodder. It is used to relieve indigestion in Ayurvedic medicine. *Nymphaea nouchali* var. cyanea has medium-sized pale to deep blue flowers, and *Nymphaea nouchali* var. versicolor is commonly exported in the form of tubers from Sri Lanka to Europe and the United States for use in aquariums; the tubers grow quickly after exposure to warm water, making a "instant" aquarium plant (Singh and Jain, 2017). Another species, *Nymphaea rubra*, is often cultivated in ponds and tanks as an ornamental plant which produces several solitary flowers with long peduncles that blooms above the water surface.

2.2 Brief summary of Nymphaeaceae spp. Family

Because the Nymphaeaceae family lacks genuine stems, the rhizome is big, thick, and black. The rhizome produces the leaves directly. The leaves have huge, flat structures that are spherical or oval in shape. Alternate, floating, pinnately veined, long-petiolate, or cordate are all possible structure of Nymphaeacea family. Because the leaves' margins are somewhat folded inwards toward the topmost side, they play a crucial role in keeping the plant buoyant. Furthermore, the leaves' undersides are always damp, which helps to keep the structure dry. Apart from that, the leaf can be submerged or float on the surface of the water. Large flowers, several ovules in each carpel, a stem that is not free floating and leaves attached by rhizome are all characteristics of Nymphaeaceae. Water lily blossoms are big and axillary or solitary in appearance. Since the flowers have both pistils and stamen on the same plant, it is a bisexual plant. The flower opens in the early morning and closes completely in the late afternoon. During the night, it will be closed. The flower has a lovely scent (Bhattacharjee, 2005) that can entice insects to come and visit. Water lily fruits are dicotyledon, with a berry-like structure and a spongy texture. Aril or no aril will be present in the seed (Singh and Jain, 2017). A lot of synonyms occur for species of *Nymphaea* genus for a variety of reasons. To date there is a controversy among botanists regarding the synonymy and the varieties. Among all the species of *Nymphaea* genus *Nymphaea nouchali* Burm. f. and *Nymphaea rubra* are two common

species of Bangladesh. The work of Hossain *et al.* (2000) and Ahmed (2008) confirmed the identity of the Bangladeshi species.

2.2.1 *Nymphaea nouchali* Burm f.

Because of its magnificent blossoms, *Nymphaea nouchali* is utilized as an ornamental plant (Fig 2.1). This water lily is sometimes referred to as the "Indian blue lotus", however it is not a lotus (Slocum, 2005; Dezhi, 2015). It is the national flower of Sri Lanka and Bangladesh and it is native to the southern and eastern areas of Asia. In India, it's widely spread; it's common and locally dominant in both permanent and temporary bodies of water. *Nymphaea nouchali*, sometimes known as blue lotus or by its synonym *Nymphaea stellata*, is a perennial aquatic herb belonging to the Nymphaeaceae family (USDA GRIN Taxonomy, 2015). The blue star water lily, also known as star lotus, red and blue water lily, is a water flower of *Nymphaea* genus. According to Verdcourt, *Nymphaea nouchali* should be considered a synonym for *Nymphaea stellata*, not *Nymphaea pubescens*, as some have claimed (Verdcourt *et al*., 1989; Simmonds and Howes, 2006). However, *Nymphaea stellata* and *Nymphaea nouchali* have been distinguished as two different species in various literature and books (Singh and Sandhu, 2003).

Source: https://en.wikipedia.org/wiki/Nymphaea_nouchali

Fig 2.1: *Nymphaea nouchali*

A big aquatic herb with tuberous rhizome and peltate leaves, solitary, fragrant blooms that range in color from deep red to pure white and a spongy berry as the fruit. Some of the leaves are submerged, while others float just above the water's surface. The top of the leaves are spherical and green, with a darker underside. The undulating edges of the floating leaves give them a crenellate appearance. Their size ranges from 20–24 cm, with a spread of 0.8–1.9 m. In times of scarcity, all portions of the plant are consumed. *Nymphaea nouchali* is a nonviviparous, day-blooming plant with submerged roots and stems. There has been a surge in interest in the study of medicinal plants in recent decades, as ethnopharmacology, with its holistic system approach and experiential grounding, can deliver safer and cheaper medicines (Patwardhan, 2005).

2.2.2 *Nymphaea rubra*

Shapla in Bengali, Kokaa in Hindi and Kumuda in Sanskrit are all names for the hairy water lily. The official name of a species in the genus *Nymphaea* (family Nymphaeaceae) is *Nymphaea rubra* Roxb. ex Andrews. It is also known by a variety of other names such as *Nymphaea pubescens* Willd., locally Red water lily. A gorgeous floating plant endemic to India is the Red Water Lily. The Red Water Lily can be found all over India. The most common of these is *Nymphaea rubra*, which is marketed commercially as Red Water Lily because of its reddish variety (Fig 2.2). It frequently has purplish leaves. In temperate and tropical Asia, this plant can be found in shallow lakes and ponds: India, Bangladesh, Sri Lanka, Yunnan, Taiwan, Philippines, Cambodia, Laos, Thailand, Myanmar, Vietnam, Malaysia and Indonesia. In other nations, it is commonly grown. Leaves are present, finely serrated on the underside and downy. The flowers are a deep red or rose color. Sepals are normally four in number and petals are numerous. The leaves' lobes slant away from one another. There are 10-20 rays in a stigma (Pareek and Kumar, 2016).

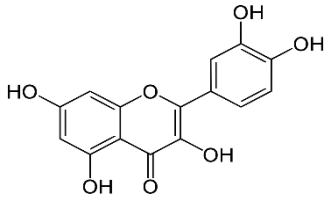
Source: https://www.pinterest.com/pin/24769866672131549/

Fig 2.2: *Nymphaea rubra*

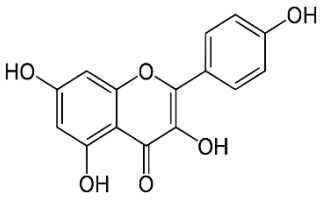
2.3 Phytochemistry

Different solvent extracts of the entire plant have shown the presence of different classes of phytochemical such as phytosterols, alkaloids, glycosides, triterpene saponins, hydrolysable tannins, lignans and flavonoids in various species of *Nymphaea*.

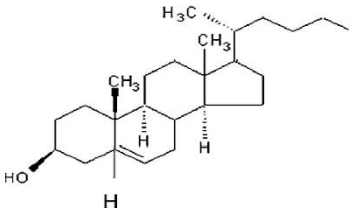
Fig 2.3: Structure of some phytoconstituents from the genus *Nymphaea.*



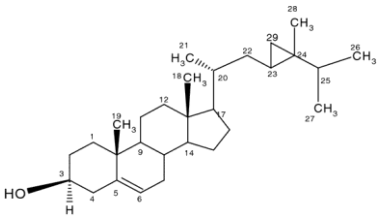
Quercetin



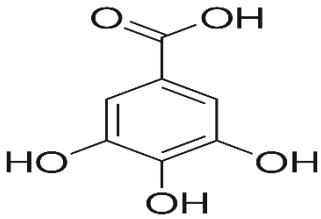
Kaempferol



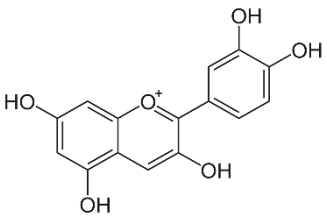
Nymphayol



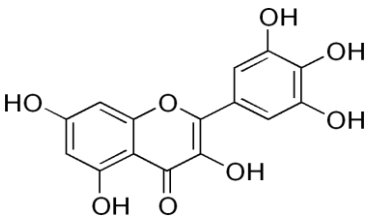
Nymphasterol



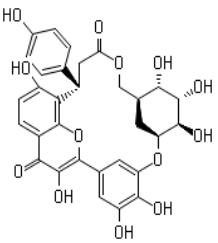
Gallic acid



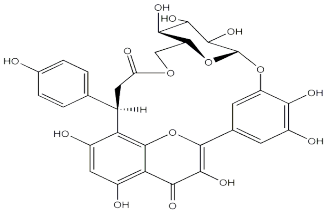
Cyanidin



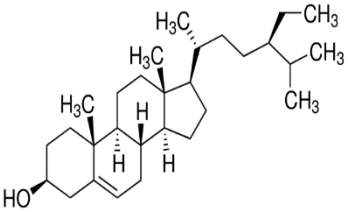
Myrecitin



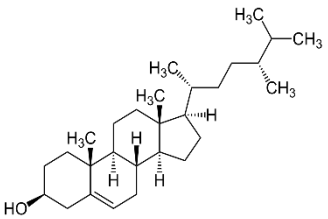
Nympholide A



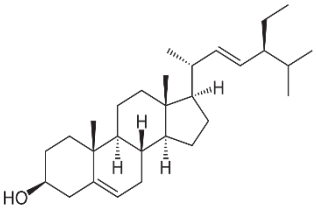
Nympholide B



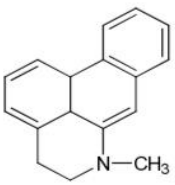
β-sitosterol



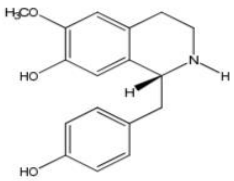
Campesterol



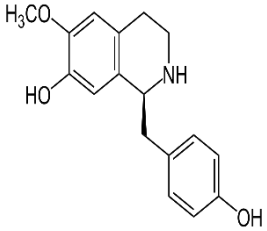
Sigmaesterol



Nupharin and Amorphin based compounds



Nupharin and Nymphaeine



Coclaurine

The alkaloids such as Nupharidin and Apomorphine based compounds were reported from the flowers of *Nymphaea ampla* (Emboden, 1982). Nupharin and Nymphaeine were reported from the flowers of *Nymphaea alba* (Joshi *et al*., 1974). Two phenolic base alkaloids Coclaurine reported from the aerial parts of *Nymphaea stellata*

(Rastogi and Mehrotra, 1995). 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 is reported from *Nymphaea stellata* (Raja *et al*., 2010), Nymphasterol is reported from the seeds of *Nymphaea stellata* (Verma *et al*., 2012).

2.4 Traditional uses of different parts of *Nymphaea*

Whole plant: In Ayurveda, it's used to cure liver problems. The leaves, roots, and flowers are used as a cardiotonic, emollient, diuretic, narcotics, stimulant, and aphrodisiac for diabetes, bolled diseases, antifertility, heart issues, diarrhea, eruptive fevers and indigestion problems (Tirkey *et al*., 2001; Nadkarni, 1982; Kritikar, 2001; Cardiland & Koonin, 2001; Deutschlander *et al*., 2009). The flowers and roots have minor sedative qualities and are used to alter one's state of mind. In Kashmir, the entire plant is utilized as an anti-periodic and cardiac stimulant (Lawrence, 1991; Kaul, 1997; Tyagi, 2005).

Flower: Pipasa daha (burning thurst), Raktapitta (bile-blood), Chardi (vomiting), Murchha (fainting), Hrdraoga (heart sickness), Mutra kechhra (painful discharge of urine affections) and Jvaratisara are all treated with 3-6 mg of the medicine from flower (diarrhoea with fever). The flowers are utilized in the Ayurvedic and Sidhha systems of medicine to treat diabetes mellitus (Madhumeha) and liver diseases. The acrid, bitter-sweet flavor of the blossoms eliminates impurities from the blood, cools and relieves cough, is used as an aphrodisiac for biliousness, vomiting, giddiness, worm infestation and skin burning. The flower's decoction is used to treat heart palpitations and as a narcotic, while the flower's syrup is used to treat high fever, apoplexy, inflammatory brain disease and dysuria. Plant filaments are utilized as an astringent and a cooling agent in cases of body burning, bleeding piles and menorrhagia (Watt & Breyer-Brandwijk, 1962; Nadkarni, 1982; Satyavati, 1987; Kirtikar, 2001; Anonymous, 2001; Manjunatha *et al*., 2004).

Rootstock: Dyspepsia, diarrhea and piles are all treated with rootstock powder (Satyavati, 1987; Kirtikar, 2001).

Root: The roots are used as emollients and diuretics in the treatment of diabetes, blenorrhagia, urinary tract infections and infertility (Watt & Breyer-Brandwijk, 1962; Arnold & Gulumian, 1984; Simmonds and Howes, 2006). *Nymphaea rubra* root and *Ficus religiosa* stilt roots are ground together to produce a paste. The paste is then used to prepare peels. To heal excessive bleeding, three peels are taken in three days, one peel once a day (Chatterjee & Mukherjee, 2015).

Leaf and flower: Ceylonese prepare curries from the tender leaves and flower peduncles (Soyza, 1936).

Rhizome and stem: An infusion is used to treat blennorrhagia and urinary tract disorders and is considered an emollient and diuretic (Satyavati, 1987; Kirtikar, 2001).

Flower and rhizome: The flower and rhizomes are considered as astringent, demulcent, moderate sedative, spasmolytic and antiseptic which are used in an infusion for chronic diarrhea, as a douche for leucorrhea and vaginitis, as a gargle for sore throat and for prostate problems (Ngugi, 1999; Sarma *et al*., 2008).

Leaf: In erysipelas, the leaves are administered topically, while in eruptive fever, the macerated leaves are used as a lotion (Satyavati, 1987; Kirtikar, 2001; Wiart, 2006).

Seed: The seeds are stomachic and regenerative, according to belief. In the Ayurvedic system of medicine, seeds are advised as a diet for diabetes mellitus (Watt & Breyer-Brandwijk, 1962; Satyavati, 1987; Achariya *et al*., 1996; Kirtikar, 2001; Subbulakshmi & Naik, 2001; Singh *et al*., 2007).

Rhizome: It is frequently consumed after being roasted over hot flames. Menstrual problems are treated with rhizome paste. The rhizomes are utilized in the treatment of gastrointestinal problems (Crevost *et al*., 1917; Irvine, 1953; Watt & Breyer-Brandwijk, 1962; Partha & Enayet, 2007). The rhizome is used to cure cystitis, nephritis, enteritis, fevers, and insomnia (Jayaweera, 1981).

Petiole: Excessive menstrual discharge is treated with petioles paste, a pinch of salt along with *Cuminum cyminum* seed powder, butter and a few drops of honey. During pregnancy, strips along with roots of *Pinus longifolia* are used to treat fever, diarrhea, nausea, cough, vertigo, discomfort and bleeding (Singh and Sandhu, 2003). In Africa, different species of *Nymphaea* are used to treat cancer (Sowemimo *et al.*, 2007).

2.5 Nutritional value

The early study on proximate analysis showed dry matter -8.4%, crude protein-16.8%, ash-18.7%, crude fat-2.8%, crude fiber-26.3% and nitrogen free extract-35.4% for *Nymphaea nouchali*; and dry matter-7%, crude protein-16.7%, ash-14.1%, crude fat-2.6%, crude fiber-24% and nitrogen free extract-42.6% for *Nymphaea stellata*, respectively. Mineral content showed sodium-1.19, potassium-2.23, calcium-0.52, phosphorus-0.32, and calcium /phosphorus ratio 1.63 for *Nymphaea nouchali*; and sodium-0.93, potassium-1.30, calcium-0.95, phosphorus-0.21, and calcium/ phosphorus ratio-4.52 for *Nymphaea stellata*. Alkaloids had been detected in fraction A (extracted with chloroform from an ammonical solution) for both, while *Nymphaea nouchali* and *Nymphaea stellata* differed in their nitrate content with 2% and 0.9%, respectively. *Nymphaea nouchali* showed polyphenols total-8.7%, free-5.9% and bound-2.8%; *Nymphaea stellata* showed polyphenols total-10.2%; free-9.3% and bound-0.9% (Banerjee and Matai, 1990).

The study on the nutritional potential of the plant parts (leaves, petiole, root, rhizome and seeds) of Water lily were evaluated through proximate compositions and analyzed in percentages. Moisture content was highest in the rhizome (20.40±1.241%) while the seeds gave the lowest value of (4.18±0.176%). The highest value for Ash content was observed in the root (27.36±1.261%) and the seeds gave the lowest (2.81±0.498%). Highest value for the Crude fat was obtained from the seeds (9.95±0.637%) while the petiole gave the lowest value of (2.27±0.377%). The crude protein and crude fibre values were highest in the leaves 19.54±0.782% and 15.53±0.448%, respectively while the lowest was obtained in the seeds 3.27±0.104% and 1.60±0.200%, respectively (Mohammed *et al*., 2013).

Another comparative study on the seed of *Nymphaea lotus* and *Nymphaea pubescens* showed moisture 5.31%, ash 1.33%, proteins 4.92%, lipids 13.23%, fiber 5.17%, Carbohydrates 75.35% for *Nymphaea lotus* seeds and moisture 6.2%, ash 3%, proteins 4.14%, lipids 9.28%, fiber 5%, Carbohydrates 78.58% for *Nymphaea pubescens* seeds (Aliyu *et al*., 2017).

At the same time a quantitative study published in American Journal of Food Science and Nutrition (AJFSN) revealed that the result of the proximate analysis of water lily bulbs showed the following composition: crude fat (5.07 ± 0.01%), crude fiber (13.30 ± 0.02%), crude protein (21.66 ± 0.01%), ash (8.34 ± 0.01%), moisture (9.72 ± 0.00%) and carbohydrate (41.92 ± 0.06%). This study also showed that *Nymphaea lotus* bulb contains some vital phytochemicals: phytate (3.68 ± 0.01 mg/100g), tannin (13.63 ± 0.01mg/100g), saponins (4.89 ± 0.01mg/100g) and lycopene (1.96 ± 0.01mg/100g) and very rich in minerals: phosphorus (635.39 ± 0.01 mg/100g), potassium (742.89 ± 0.04), sodium (431.53 ± 0.01), zinc (8.16 ± 0.01) and magnesium (87.46 ± 0.01). Other minerals obtained in this research include: cadmium, lead and copper in trace quantity. Vitamin A (51.36 ± 0.02 mg/100g) and C (24.65 ± 0.02) were found in moderate quantities (Stephen *et al*., 2017).

2.6 Medicinal properties

Nymphaea is not only widely utilized for traditional and cultural celebrations. It was mostly prescribed for curing diseases also. It's used to treat diabetes, inflammation, liver disorders, urinary disorders, menorrhagia, blenorrhagia and menstrual problems, as an aphrodisiac and as a bitter tonic, among other things (Raja *et al*., 2010). Modern approaches have proved hepatoprotective, anti-inflammatory, antioxidative and especially anti-diabetic properties of water lily. Nymphayol, a steroid extracted from the flowers, has been scientifically proven to be responsible for the traditionally stated antidiabetic effect; it restores damaged endocrine tissue and increases insulin secretion in -cells. Sterols, alkaloids, saponins, tannins and flavonoids have all been found in various portions of the plant. A novel steroid, nymphasterol, was recently extracted and discovered from the seeds (Verma *et al*., 2012).

2.6.1 Antioxidative property

The antioxidant properties of ethanol and chloroform extracts of *Nymphaea nouchali* leaves were examined by Noor *et al.* (2013). In this study, DPPH (1, 1-diphenyl-2-picrylhydrazyl) scavenging assay, total phenol, total flavonoid were determined to indicate the possession of antioxidant capacity of samples. The extracts' free radical scavenging activity was measured using the DPPH test at 517 nm and compared to that of ascorbic acid. The IC_{50} of *Nymphaea nouchali* ethanolic extract was 10.102±0.23 µg/mL and the IC_{50} of *Nymphaea nouchali* chloroform extract was 13.11±0.11 µg/mL, whereas the IC_{50} of ascorbic acid was 19.89±0.22 µg/mL. Folin-Ciocalteu assay was used to determine phenolic content, which is given as Gallic acid equivalents (GAE). The antioxidant activity of *Nymphaea nouchali* ethanol extract (6.53±0.26 mg/g GAE) is higher than that of *Nymphaea nouchali* chloroform extract (5.55±0.06 mg/g GAE), and flavonoid contents were found to be 4.58±0.19 mg/g quercetin equivalent for chloroform extract and 5.99±0.33 mg/g quercetin equivalent for ethanol extract, respectively. However, the overall antioxidant capacity of both extracts is nearly same, with 2.75±0.12 mg/g ascorbic acid equivalent (AAE) for ethanolic extract and 2.69±0.09 mg/g AAE for chloroformic extract. The medicinal herb *Nymphaea nouchali* is a rich source of antioxidants, according to the findings of this study (Noor *et al*., 2013). The antioxidant potential of an ethanolic extract of *Nymphaea alba* rhizome is examined using DPPH, nitric oxide, and superoxide anion radical scavenging activities. The IC_{50} values for DPPH, NO and superoxide anion radical scavenging activity were 63.9, 49.21 and 79.56 respectively. The presence of tannins and phenolic chemicals may be responsible for the reported antioxidant action (Bose *et al*., 2012).

Ethanol and chloroform extracts of *Nymphaea nouchali* leaves were investigated to evaluate their antioxidant property by Noor *et al*. (2013). The experimental data of all the tests extracts reveal that although there are small differences among the values of total phenol content, total flavonoid content, total antioxidant capacity and DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity; but both of the ethanolic and chloroformic extracts possess strong antioxidant activity.

Bhaskara *et al*. (2014) examined *Nymphaea nouchali* leaves for phytochemical profile, in vitro antioxidant and hemolytic activities. The bioactive extract exhibited

the presence of carbohydrates, phenolic compounds, alkaloids and tannins. The extract showed potent reducing power and total antioxidant activities, while it has also shown 94% of DPPH radical scavenging activity and 88% of metal chelating activity with IC_{50} values of 42µg/mL and 28µg/mL respectively. The extract did not show harmful effect towards human erythrocytes through the test performed.

2.6.2 Antidiabetic property

In normal and Alloxan-induced diabetic rats, hydroethanolic extracts of *Nymphaea stellata* flowers were tested for antidiabetic efficacy. The effect of 30 days of oral administration of the hydro ethanolic extract on blood glucose, glycosylated hemoglobin, total cholesterol, triglycerides, phospholipids, low density lipoprotein, very low density lipoprotein, high density lipoprotein, hexokinase, lactate dehydrogenase and glucose-6-phosphatase in normal and alloxan-induced diabetic rats was investigated. The hydro-ethanolic extract reduced elevated blood glucose, glycosylated hemoglobin, cholesterol, triglycerides, phospholipids, LDL, VLDL, hexokinase while showed an increasing level of liver glycogen, insulin, glucose-6-phosphatase and HDL levels. In diabetic rats, *Nymphaea stellata* flowers have a promising anti-diabetic effect (Rajagopal and Sasikala, 2008).

Another study testing antidiabetic activity showed that when alloxan-induced diabetic rats were given 400 mg/kg of ethanolic and aqueous extracts of *Nymphaea pubescens* Willd, there was a considerable drop in blood glucose levels, with percentage reductions of 21.97% and 19.94%, respectively (Karthiyayini *et al*., 2011). In alloxan-induced diabetic rats, the ethanolic extracts of *Nymphaea pubescens* were screened for antidiabetic, hypolipidaemic and antioxidant activity (Selvakumari and Shantha, 2010), screened for in vitro antidiabetic activity using the α-amylase and α-glucosidase inhibition assay (Rushender *et al.*, 2012). In diabetic rats, oral administration of Nymphayol isolated from *Nymphaea stellata* for 45 days significantly reduced blood glucose levels and effectively increased insulin content (Subash *et al*., 2009).

2.7 Miscellaneous

The Nymphaeaceae family is important in both aspects of ecology and economy. Since water lily is easy to grow, it has been a popular water garden plant. Moreover,

the attractive colour variation of water lily enhances the beauty of the pond. Water lily is not only an ornamental plant but also an important water purification plant also. Because the roots of water lily can absorb the poisonous substances like mercury, lead, phenol etc. and filter the microorganism in water. It plays an important role in decontaminating water, afforesting and landscaping (Li *et al*., 2005; Shi *et al*., 2009).

Water lily family serves as good habitat for aquatic organisms such as fish, crabs and others. The blooming flowers will attract insects to pollinate them, while frogs favour to inhabit on water lily pads and wait for their prey. Besides that, the presence of water lily in the pond or lake could control the growth of algae in the pond as their leaves block the penetration of sunlight. Other organisms as well as humans consume these plants as food. So that, water lily is important for maintaining energy flow throughout the ecosystem. Humans, in addition to animals, consume the carbohydrate-rich rootstalk that the First Nations people used to eat. The water lily is able to absorb nutrients from the surrounding water, allowing it to maintain clear and clean water. They also provide oxygen to aquatic organisms. Asians and Native Americans eat water lilies, particularly the shoots and leaves, for their socioeconomic values. Seed, fruits and rhizomes of *Euryale* spp., for example, give nourishment to the residents in an Asian country (Singh and Jain, 2017).

In addition to their ecological benefits water lilies are incredibly nutritious, which people may not know. The Herb Guru Brand wrote in one of their cleanse articles about them, "Starch and glucose are abundant in the seeds of the yellow water lily. The lilies' nutritional characteristics can be obtained by boiling and eating the roots and leaves. The blooms can also be used to make jam and spread on toast. It is necessary to determine the species of lilies before eating them because there are many dangerous lily species." To get healthy hair, one can boil its roots a bear and can use it as hair rinse. It truly provides us with gorgeous, healthy and lustrous hair. The seed can be used as a coffee substitute. (Comedis *et al.*, 2017).

Some researchers in Bangladesh have started an attempt to create paper from water hyacinth stems. The fiber of the water hyacinth does not form very good paper on its own, but when combined with waste paper or jute, it produces a high-quality paper (Hossain *et al.*, 2020).Rope can be made from the fiber of the water hyacinth plant's

stems. The plant's stem is shredded lengthwise to reveal the fibers, then dried for several days. In Dhaka, they developed a fiber board that can be used for a variety of purposes, including low-cost roofing. There are a lot of phases to the procedure and it takes a lot of time.

The water hyacinth is dried in the Philippines and India and used to make baskets and matting for houses and the local tourism trade. Water hyacinth can be utilized fresh from the field or dried and used as mulch or soil amendment. It decomposes quickly and can be blended with ash, other soils and animal dung to boost soil fertility and crop productivity. Trays, chests, bags, traditional bags, hampers, cabinets, lamp shades of all shapes and sizes and even dining tables are all constructed from water hyacinths as well as slippers. (Comedis *et al.*, 2017). These researches may also be valid for tropical water lilies (*Nymphaea nouchali* and *Nymphaea rubra*) of Bangladesh.

Chapter 3: Materials and Methods

3.1 Study Period

The study was conducted over a six-month period, from May to October, 2020. The experiments were carried out in the laboratories of the following departments at Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh:

- Dept. of Applied Food Science and Nutrition, FFST.
- Dept. of Food Processing and Engineering, FFST.
- Dept. of Animal Science and Nutrition, FVM.
- Dept. of Physiology, Biochemistry and Pharmacology, FVM.
- Dept. of Fishing and Post Harvesting, FOF.

3.2 Layout of Experiment

First, two different types of water lilies were collected from different areas based on their availability. After collecting the samples, they were thoroughly washed to remove sand, clay and other particles. The samples were categorized into three parts: petals, seeds and tubers. The samples from three different parts of the water lilies were then dried and ground into powder form. The powder sample was then analyzed to determine its proximate composition (moisture, ash, crude fat, protein, crude fiber and carbohydrate), mineral contents (Sodium, Potassium, Calcium, Magnesium, Phosphorus, Iron, Zinc, Copper), bioactive compounds (Total phenol, Total Flavonoids, tannin, saponin, alkaloids), *in vitro* antioxidant activity and *in vitro* anti-diabetic activity (Figure 3.1).

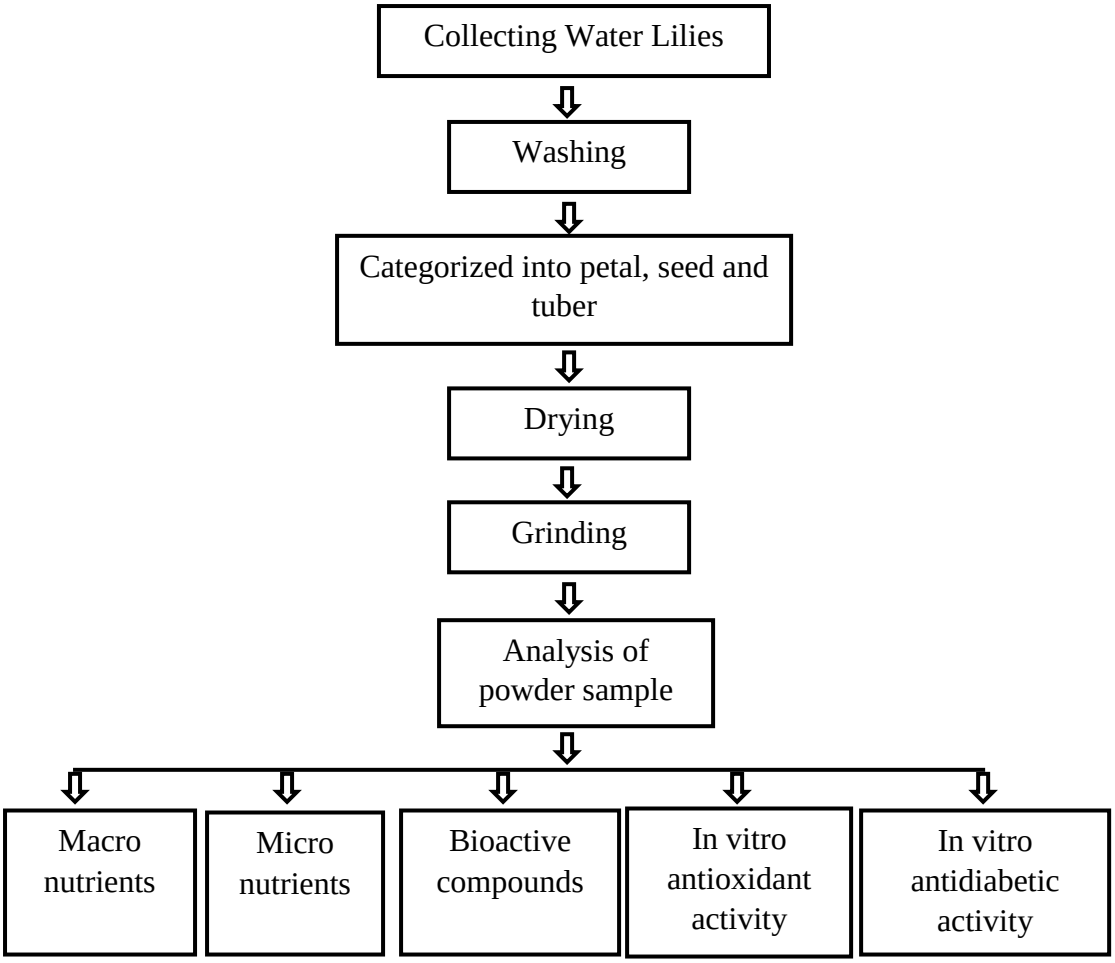


Figure 3.1: Experimental design

3.3 Collection and identification of sample

The whole plant, along with tuberous rhizome *Nymphaea nouchali* (white verity) and *Nymphaea rubra* (red verity) were obtained randomly from different ponds and canals of Chakaria Upazila, Chattogram, Bangladesh (Figure 3.2) in the month of May, 2020. The samples were taxonomically identified and authenticated by consulting with an expert: Professor Dr. Shaikh Bokhtear Uddin, Department of Botany, University of Chittagong, Bangladesh and through several literature sources. The identified plant species were further compared with the "*Dictionary of Plant Names of Bangladesh* (vascular plants)" (Pasha and Uddin, 2013) for justification of correct scientific names and author citations.

Figure 3.2: Location map of study area.

Source: https://en.wikipedia.org/wiki/Chakaria_Upazila

Source: https://en.banglapedia.org/index.php/Chakaria_Upazila

3.4 Preperation of sample

Collected water lily samples of both *Nymphaea nouchali* and *Nymphaea rubra* were washed under running tap water to remove mud and separated into three parts, such as: petals of *Nymphaea nouchali* (PS1), petals of *Nymphaea rubra* (PS2), seeds of *Nymphaea nouchali* (SS1), seeds of *Nymphaea rubra* (SS2), tubers of *Nymphaea nouchali* (TS1) and tubers of *Nymphaea rubra* (TS2). Following that, each of the samples was shade dried at room temperature. Dried samples were ground using a grinder until the powder was homogenous. To remove any remaining residue, the powder samples were passed through a fine (2mm mash) sieve. Prior to use, the fine powder samples were stored in labeled, airtight plastic containers.

3.5 Methods of analysis

The macronutrient components of the samples including crude protein, total carbohydrates, crude fat and micronutrients such as vitamin C, minerals (Na, K, Mg, Ca, P, Fe, Cu, Zn), crude fiber, bioactive compounds (total phenol, total flavonoid, tannin, saponin , alkaloids), antioxidant property and anti-diabetic activity, were estimated using standard methods of chemical analysis on a moisture-free basis.

3.5.1 Proximate analysis

AOAC (2016) methods were used to measure the moisture, ash, crude protein, crude fat and crude fiber content of samples in triplicate. The moisture content was determined by oven drying to a constant weight at 105°C. The Kjeldahl technique (crude protein for plant origin: 5.85×N) was used to determine the crude protein concentration. Crude lipid was extracted using the Soxhlet device. In a muffle furnace, ash was measured gravimetrically by heating to a constant weight at 550°C.

3.5.1.1 Determination of crude protein (AOAC, 2016)

Apparatus: Kjeldahl flask, Condenser, Kjeldahl digestion unit

Reagents used: Concentrated H_2SO_4 (98% pure), Catalyst (Potassium sulphate: Copper sulphate=9:1), Boric acid solution (4%), Sodium hydroxide (35%), Mixed indicator solution (Bromocresol green, methyl red), Standard HCl (0.2N).

For estimation of protein, the steps were followed:

Digestion: 0.3g sample, 4g catalyst and 5 ml H_2SO_4 was taken in a kjeldahl digestion tube. It was placed in the digestion unit and digested for at 320°C for 30 minutes. The digestion was completed when the color of the substance was pale yellow.

Distillation: After cooling the digestion tube at room temperature 25ml distilled water, 25 ml 35% NaOH and glass blitz were added to kjeldahl flask which containing about 10 ml 4% boric acid and 2-3 drops mixed indicator. Cooled tube and receiving solution were placed into the distillation unit. 25ml of 35% NaOH was automatically filled into the tube. The distillation process takes place for 3 minutes. The receiving solution turned green at the end of the process.

Titration: The solution collected was titrated with 0.2N HCl solution and titer value was recorded.

Calculation: The calculation of the percent of protein in the plant sample using protein factor 5.85 is:

$$\% \text{ Nitrogen} = \frac{\text{ml of titrant} \times \text{Normality of acid (0.2N)} \times \text{Equivalent of Nitrogen}}{\text{Weight of sample (gm)}} \times 100$$

Where, Equivalent of Nitrogen (N_2) = 0.014

$$\% \text{ Protein} = \% \text{ Nitrogen} \times 5.85 \text{ (for plant origin)}$$

3.5.1.2 Determination of crude fat (AOAC, 2016)

Apparatus: Porcelain, gas burner, muffle furnace

Procedure: The dried sample remaining after moisture determination was transferred to a thimble and plugged the top of the thimble with a wad of fat free cotton. The thimble was dropped into the fat extraction tube attached to a Soxhlet flask. Approximately 75ml or more of anhydrous ether was poured into a flask. The top of the fat extraction tube was attached to the condenser. The sample was extracted for 16hrs or longer on a water bath at 80°C. At the end of the extraction period, the thimble was removed from the apparatus and distilled off most of the ether by allowing it or collected in Soxhlet tube. The ether was poured off when the tube was nearly full. When the ether reached a small volume, it was poured into a small, dry

beaker through a small funnel containing a plug of cotton. The flask was rinsed and filtered thoroughly, using ether. The ether was evaporated on a steam bath at low heat; it was then dried at 1000°C for 1hr, cooled and weighed. The difference in the weights gave the ether soluble material present in the sample**.**

Calculation: The presence of fat was expressed as follows:

$$\% \text{ Crude fat} = \frac{\text{Loss of ether soluble materials}}{\text{Weight of sample}} \times 100$$

3.5.1.3 Determination of moisture content (AOAC, 2016)

Apparatus: Crucible, hot air oven, desiccator, weighing balance

Procedure: At first weight of empty crucibles were dried and 5gm of sample was placed on it. Then the crucible was placed in an air oven (thermostatically controlled) and dried at temperature of 105°C for 24 hrs. After drying, the crucible was removed from the oven and cooled in desiccator. It was then weighed with cover glass. The crucible was again placed in the oven, dried for 30 minutes, took out of the dryer, cooled in desiccator and weighed. Drying, cooling and weighing were repeated until the two consecutive weights were same. Calculation: From these weights, the percentage of moisture in food samples was calculated as follows:

$$\% \text{ Moisture} = \frac{\text{Loss of weight of sample}}{\text{Initial weight of sample}} \times 100$$

3.5.1.4 Determination of ash content (AOAC, 2016)

Apparatus: Porcelain, gas burner, muffle furnace

Procedure: The ash content of the samples was determined by the standard AOAC method (AOAC, 2016). In this method, an empty crucible was cleaned properly and dried in a hot air oven. It was placed in desiccators and cooled then the weight was recorded. 3 gm of the sample was weighed and placed in the crucible. It was allowed to burn upto no smoke. The crucible was cooled and transferred to the muffle furnace at 550°C for 5 hours. The process ends when formation of white ash accomplished. It was cooled at 150°C and then placed to desiccator. When it cooled to mild warm the weight was recorded.

Calculation: Ash content was calculated using the following formula.

$$\% \text{ Ash} = \frac{w\text{-}w1}{w2} \times 100$$

Where, W= weight of the crucible with ash; W_1= weight of the empty crucible; W_2= weight of the sample.

3.5.1.5 Determination of crude fiber (AOAC, 2016)

Apparatus: Liebig condenser, Reflux condenser, Gooch crucible

Reagent used: 0.255N sulphuric acid solution, 10.0% Potassium sulphate solution, Asbestos- Gooch grade.

Procedure: Crude fiber was determined according to AOAC method (2016). At first 2 gm of the sample was weighed and then taken into a beaker. Then 125ml 0f 1.25% sulfuric acid solution and 3-4 drops of n-octanol were added into the same beaker. N-octanol was using as an antifoaming agent. The beaker was boiled for 30 minutes at constant volume. After that, the sample was washed three times to remove the acid. After washing 125ml of 1.25% sodium hydroxide and 3-5 drops of antifoam were added. It was again boiled for another 30 minutes at constant volume. The mixture was filtrated and again washed the residue like before. It was washed again with 1% HCL solution in order to remove the acid. Then the residue was dried in a hot air oven at 105°C until a constant weight was found out. It was placed in a desiccator for cooling and the weight was recorded. Finally, the residue was burned up to smoke and ignited in the muffle furnace at 550-660°C for about 3-4 hours until that turned into white ash.

Calculation: The ash particles were weighed and calculated to determine the crude fiber content of the sample.

$$\% \text{ Crude Fiber} = \frac{w\text{-}w1}{w2} \times 100$$

Where, W= weight of crucible containing crude fiber and ash; W1= weight of crucible containing ash; W= weight of the sample.

3.5.1.6 Estimation of total carbohydrate

The available carbohydrate content was determined by subtracting the sum of the values of moisture, ash, protein and fat from 100/100gm (AOAC, 2016). Hence, it was calculated using the formula below:

% Carbohydrate = 100 − (Moisture %+Ash%+Protein%+Fat %+Fiber %)

3.5.2 Determination of Vitamin C content

Principle: Chemically assay of the Vitamin C depends on the market reducing properties of the Vitamin C. Generally, Vitamin C is determined in plant or animal extract by its reducing action on the dyes stuff 2,6-dichloride phenol indophenols. In this matter, Vitamin C oxidized by the color dye to the dehydroascorbic acid. At the same time, the dye is reduced to the color less compound. S that end point of the reaction can easily determine. Rapid excretion and filtration are desirable as excess may be introduced in plant product by oxidized partially destroying Vitamin C during sampling and grinding. Oxidation is presented by the use of metaphosphoric acid during extraction. Strongly acidic solution will provide most accurate result. The titration should be complete within one minute. The dye has blue color in aqueous solution. Pink in acidic solution and become colorless when completely reduced (AOAC, 2016).

Reagent required:

A. Dye Solution

1. 260 mg of dye (2,6-dichlorophenol indophenols)
2. 210 mg of $NaHCO_3$ dissolved in 100 ml of distilled water.

B. Metaphosphoric acid solution (3%)

1. 7.5mg of Metaphosphoric acid.
2. 20ml of glacial acetic acid dilutes to make 250 ml with distilled water.

C. Standard ascorbic acid solution

25 mg of crystalline ascorbic acid dissolved in 250ml of metaphosphoric acid solution.

Procedure

Dye solution was taken in the burette. Then 5 ml Vitamin C solution was taken in a conical flask. The conical flask was placed under the burette and the dye was added drop wise. Titration was completed when pink color was appeared and stayed for 20 seconds and then disappeared. The reading was taken at least 3 times. The same procedure was performed for ascorbic acid solution of unknown concentration. The result was expressed as milligram percentage (mg %).

3.5.3 Mineral analysis

This method involves the extraction of minerals from the organic food matrix by digestion through wet digestion. The mineral contents such as Sodium, potassium (Schoenfeld, 1964), magnesium (Burtis *et al*., 2012), calcium (Prince *et al*., 2003), phosphorus (Burtis *et al*., 2012), iron, Copper and zinc in the digested compounds was determined by using biochemical analyzer (Humalyzer 3000®). Commercially available biochemical kit (Randox) was used for biochemical assay.

Digestion:

a. **Apparatus:** Beaker, Measuring pipets, Volumetric flask, Analytical balance, Heating mantle or hot plate, Filter paper, Whatman® No. 541.
b. **Required Reagent:** Nitric acid and Perchloric acid
c. **Procedure:**

   One (01) g of dry sample was weighted in a conical flask. For dried samples, 7.5 mL conc. HNO_3, and 2.5mL conc. $HClO_4$ in the ratio of 2:1 was prepared. For wet sample, 5 mL HNO_3 and 1 mL $HClO_4$ was added (HNO_3: $HClO_4$ = 5:1). Then the flask was placed in a hot plate at 200W for 1-2 hours until full digestion. After digestion, it was cooled at room temperature. Then transferred the digested samples into 100 mL volumetric flask and diluted up to 100 mark with Deionized water and mixed well. Later, the solution was filtered through Whatman® filter paper No. 1 and transfer to Eppendorf Tubefor mineral quantification.

3.5.3.1 Determination of Sodium (Na)

Principle: Sodium is precipitated as a triple salt with magnesium and Uranyl acetate. The excess of uranyl ions are reacted with ferrocyanide in an acidic medium to develop a brownish colour.The intensity of the colour produced is inversely proportional to the concentration of sodium in the sample.

Procedure:

Step 1: Precipitation

For the blank sample, 1.0 mL of precipitating reagent (L1) and 20 µl of sodium standard were pipetted into a cuvette. Pipetting 1.0 ml of precipitating reagent (L1) with a 20 µl sample yielded the standard. After thoroughly mixing, the blank and standard were held at room temperature for 5 minutes with intermittent shaking. To get a clean supernatant, they were centrifuged at 2500 to 3000 RPM.

Step 2: Color Development

Corresponding the following table (Table 3.1), reagents were added into the cuvette of blank, standard and sample in the order.

Table 3.1 Determination of sodium (Na).

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Acid Reagent (L2)	1.0 ml	1.0 ml	1.0 ml
Supernatant from step 1.	-	20µl	20µl
Precipitating Reagent(L1)	20µl	-	-
Colour Reagent (L3)	100µl	100µl	100µl

After mixing well, cuvette were incubated at room temperature (25-35°C) for 5-7 minutes. Then the spectrophotometer was set to zero using blank at 530 nm and the absorbance of standards, test sample were measured.

Calculations:

$$\text{Sodium (mmol / L)} = \frac{\text{absorbance of blank} - \text{absorbance of sample}}{\text{absorbance of blank-absorbance of standard}} \times \text{Standard conc.}$$

3.5.3.2 Determination of Calcium (Ca^{++})

Principle: Calcium ions form a violet complex with O-Cresolphthalein complexone in an alkaline medium.

Procedure: Corresponding the following table (Table 3.2), reagents, standards and sample were added into cuvette in the order.

Table 3. 2 Calcium (Ca^{++}) determination

	Pipette into cuvette		
	Reagent blank SO	**Standard SI**	**Sample**
Sample	-	-	25µl
Distilled water	25µl	-	-
Standard	-	25µl	-
Working reagent	1.0 ml	1.0 ml	1.0 ml

After mixing well, cuvette were incubated at room temperature (25-35°C) for 15 minutes. Then the absorbance of standards, test sample were measured using biochemical analyzer (Humalyzer 3000®).

Calculation:

$$\text{Concentration in mg / dl} = \frac{(A)\text{sample}}{(A)\text{standard}} \times \text{Standard conc. (mg/dl)}$$

3.5.3.3 Determination of magnesium (Mg)

Principle: The method is based on the specific binding of calmagite, a metallochromic indicator and magnesium at alkaline pH with the resulting shift in the absorption wavelength of the complex. The intensity of the chromophore formed is proportional to the concentration of magnesium in the sample.

Procedure: Corresponding the following table (Table 3.3), reagents, standards and sample were added into cuvette in the order.

Table 3. 3 Magnesium (Mg) determination

	Pipette into cuvette		
	Blank	**CAL. standard**	**Sample**
Sample	-	-	10µl
CAL. standard	-	10µl	-
R1. reagent	1.0 ml	1.0 ml	1.0 ml

After mixing well, cuvette were incubated at room temperature (25-35°C) for 15 minutes. Then the absorbance of standards, test sample were measured using biochemical analyzer (Humalyzer 3000®).

Calculation:

$$\text{Magnesium (mg /dl)} = \frac{\text{absorbance of sample}}{\text{absorbance of standard}} \times \text{Standard conc. (mg/dl)}$$

3.5.3.4 Determination of phosphorus (P):

Principle: Inorganic phosphate reacts with ammonium molybdate in the presence of sulfuric acid to form a phosphomolybdic complex which is measured at 340nm.

Procedure: Corresponding the following table (Table 3.4), reagents, standards and sample were added into cuvette in the order.

Table 3. 4 Phosphorus (P) determination

	Pipette into cuvette		
	Blank	**CAL. standard**	**Sample**
Sample	-	-	10µl
CAL. standard	-	10µl	-
R1. reagent	1.0 ml	1.0 ml	1.0 ml

After mixing well, cuvette were incubated at room temperature (25-35°C) for 10 minutes. Then the absorbance of standards, test sample were measured using biochemical analyzer (Humalyzer 3000®).

Calculation:

$$\text{Phosphorus concentration (mg / dl)} = \frac{\text{absorbance of sample}}{\text{absorbance of Standard}} \times \text{Standard conc. (mg/dl)}$$

3.5.3.5 Determination of Potassium (K^+)

Principle: Sodium tetraphenyl boron reacts with potassium to produce a fine turbidity of potassium tetraphenyl boron. The intensity of turbidity is directly proportional to the concentration of potassium in the sample.

Procedure: Corresponding the following table (Table 3.5), reagents, standards and sample were added into cuvette in the order.

Table 3. 5 Potassium (K^+) determination

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Sample	-	-	0.02 ml
Deionized water	0.02 ml	-	-
Standard	-	0.02 ml	-
K+ reagent	1.0 ml	1.0 ml	1.0 ml

After mixing well, cuvette were incubated at room temperature (25-35°C) for 5 minutes. Then the absorbance of standards, test sample were measured using biochemical analyzer (Humalyzer 3000®).

Calculation:

$$\text{Potassium in (mmol / L)} = \frac{\text{absorbance of sample}}{\text{absorbance of Standard}} \times \text{Standard conc. (mmol / L)}$$

3.5.3.6 Determination of Iron (Fe)

Principle: The iron is dissociated from transferring-iron complex in weakly acid medium. Liberated iron is reduced into the bivalent form by means of ascorbic acid. Ferrous ions give with FerroZine a colored complex. The intensity of the color formed is proportional to the iron concentration in the sample.

Procedure: Corresponding the following table (Table 3.6), reagents, standards and sample were added into cuvette in the order.

Table 3. 6Iron (Fe) determination

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Sample	-	-	200µl
Standard	-	200µl	-
Reagent	1.0 ml	1.0 ml	1.0 ml

After mixing well, cuvette were incubated at room temperature (25-35°C) for 10 minutes. Then the absorbance of standards, test sample were measured using biochemical analyzer (Humalyzer 3000®).

Calculations:

$$\text{Iron in µg / dl} = \frac{\text{absorbance of sample- absorbance of blank}}{\text{absorbance of Standard}} \times \text{Standard conc. (mg/dl)}$$

3.5.3.7 Determination of Zinc (Zn)

Principle: Zinc in an alkaline medium reacts with Nitro-PAPS to form a purple coloured complex. Intensity of the complex formed is directly proportional to the amount of Zinc present in the sample.

Procedure: Corresponding the following table (Table 3.7), reagents, standards and sample were added into cuvette in the order.

Table 3. 7 Zinc (Zn) determination

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Working reagent	1.0 ml	1.0 ml	1.0 ml
Distilled water	50µl	-	-
Zinc standard	-	50µl	-
Sample	-	-	50µl

After mixing well, cuvette were incubated at room temperature (25-35°C) for 5-6 minutes. Then the absorbance of standards, test sample were measured using biochemical analyzer (Humalyzer 3000®).

Calculations:

$$\text{Zinc in µg/dl} = \frac{\text{absorbance of sample}}{\text{absorbance of Standard}} \times \text{Standard conc. (µg/dl)}$$

3.5.3.8 Determination of Copper (Cu)

Principle: Copper, reacts with Di-Br-PAESA to from a coloured complex. Intensity of the complex formed is directly proportional to the amount of Copper present in the sample.

Procedure: Corresponding the following table (Table 3.8), reagents, standards and sample were added into cuvette in the order.

Table 3.8 Copper (Cu) determination

	Pipette into cuvette		
	Blank	**Standard**	**sample**
Buffer reagent (L1)	500µl	500µl	500µl
Colour reagent (L2)	500µl	500µl	500µl
Distilled water	50µl	-	-
Copper standard	-	50µl	-
Sample	-	-	50µl

After mixing well, cuvette were incubated at room temperature (25-35°C) for 5-6 minutes. Then the absorbance of standards, test sample were measured using biochemical analyzer (Humalyzer 3000®).

Calculations:

$$\text{Copper in } \mu g/dl = \frac{\text{absorbance of sample}}{\text{absorbance of Standard}} \times \text{Standard conc. } (\mu g/dl)$$

3.5.4 Determination of bioactive phytochemical

Solvents

The solvents were selected based on polarity, the extraction with non-polar solvents Hexane: Petroleum ether (1:1) is used to remove chlorophyll and fatty acids, which may have non-specific biological activities. For hydrophilic compounds, bipolar and polar solvents such as ethyl acetate, water, methanol, and ethanol can be used to extract plant material. Dichloromethane can be used for lipophilic compounds (Cos *et al.*, 2006). In this study, ethanol was used as solvent for determining bioactive compounds.

Preparation of extracts

Preparation and identification of phenolic acids were determined according to a modified method described by (Ferreres *et al*., 2008). Dried samples were transferred into respective beakers added with absolute ethanol and left to shake on a shaker for 72 hr at room temperature. The solvent was then separated from residue by straining. The filtrate was collected and stored at room temperature while the residue was re-extracted twice, each time with fresh solvent. Finally, all the filtrates were combined and evaporated under reduced pressure at 60°C using a rotary evaporator to obtain the crude extracts. The crude extracts were weighed and stored at 4°C until further analysis.

3.5.4.1 Total Phenolic Content (TPC)

TPC of the sample extracts will be determined according to the method described with slight modifications (Azizi *et al*., 2010). Stock solutions (1mg/mL) of extracts and standard solutions of gallic acid (1.0, 2.0, 4.0, 6.0, 8.0 mg/mL) will be prepared. Extracts or gallic acid standard solution (0.3 mL) will be pipetted into a cuvette. Diluted FC reagent (1.5 mL) will be then added and mixed. The mixture will be left

for 3 min before adding 1.5 mL of sodium carbonate (75 g/L) solution and left for 60 min. The absorbance was read at wavelength 765 nm using a UV spectrophotometer in triplicate manner and ethanol will be used as the blank. TPC will be calculated and expressed as milligrams of gallic acid equivalents (GAE) per gram of extracts (mg GAE/g).

3.5.4.2 Total flavonoid Content (TFC)

TFC of the (sample) extracts will be determined using the aluminum chloride colorimetric method described by (Chang *et al*., 2002). Stock solution (1 mg/mL) of extracts will be prepared. Quercetin will be dissolved in 80% ethanol to make standard solutions (1.0, 3.0, 5.0, and 7.0 mg/mL) to plot a standard curve. Aliquots of 0.5 mL of diluted extract or standard solution will be mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 mol/L potassium acetate, and 2.8 mL of distilled water in the cuvette. The mixture will be left at room temperature for 30 min. The absorbance will be read at wavelength 415 nm in UV-visible spectrophotometer in triplicate manner. For the blank, 10% aluminum chloride will be substituted with distilled water of the same amount. TFC will be calculated and expressed as milligrams quercetin equivalents (QE) per gram of extract (mg QE/g).

3.5.4.3 Total Tannin

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 for a few minutes. After boiling the content of the biker was filtered through the normal filter paper to remove the residue and the aqueous extract was collected. After filtration, 2ml the aqueous extract was taken into a test tube. Then few drops of 5% fierce chloride solution was added. If the color of the extract change to dark green color than it contains condensed tannin and dark blue color it is hydrolyzable tannin (Buzzini *et al.*, 2008).

3.5.4.4 Total Saponin

Small amount of powder samples were diluted with distilled water and boiled the mixture for 15 minutes. After boiling, the content of the biker was filtered through the normal filter paper to remove the residue and the aqueous extract was collected. After

filtration, 2ml of aqueous extract was taken into a test tube. Then the test tube was shaken and formation of foam indicate the presence of saponin in the sample extract. (Astuti *et al*., 2011).

3.5.4.5 Alkaloids

Hager's test revealed the presence of alkaloids. In a test tube, 3ml of extract was placed and treated with a few drops of Hager's reagent (saturated picric acid solution). The production of a yellow hue precipitate confirmed the presence of alkaloids (Kumar et al., 2012).

3.5.5 Determination of antioxidant activity by DPPH Assay

Antioxidant capacity of the extracts was determined using DPPH assay as described by (Azlim *et al*., 2010) with slight modifications. Stock solution (1 mg/mL) of extract was diluted to concentrations of (0.50, 1.00, 1.50, 2.00, 2.50) mg/mL in methanol. Methanolic DPPH solution was prepared by dissolving 6 mg of DPPH in 100mL methanol. The methanolic DPPH solution (2 mL) was added to 1 mL of each extract solution of different concentrations and the mixture was left for 30 min and the absorbance was read at wavelength 517 nm by using UV-VIS spectrophotometer (UV-2600, Shimadzu Corporation, USA) in triplicate manner. Control was prepared by mixing 1 mL of methanol with 2 mL of DPPH solution. Methanol was used as a blank while ascorbic acid was used as a standard. Antioxidant capacity based on the DPPH free radical scavenging ability of extracts was calculated and expressed as milligrams of Ascorbic acid per gram of extracts (mg/g).The formula used to calculate DPPH free radical scavenging activity is:

$$\%\ \text{inhibition} = \frac{\text{Absorbance of control-absorbance of sample}}{\text{Absorbance of control}} \times 100$$

3.5.6 *In-vitro* Antidiabetic activity by alpha amylase inhibition assay

Preparation of extracts:

For the aqueous extraction 2 gm of powdered plant material was stirred in 40mL of distilled water. It was placed in a rotary shaker for 24 hr. Thereafter it was subjected to centrifugation at 8000 rpm for 10 min. The resultant supernatant was filtered using

Whatman No. 1 filter paper. The crude extracts were stored in a freezer at -20°C until analysis, for no more than 1 week (Pinto *et al*., 2010).

Test for α-Amylase Inhibitory Activity:

The inhibitory effects of aqueous extract upon α-amylase activities were measured according to the method of Kazeem *et al.* (2013) with slight modification. For α-amylase inhibition, the aqueous plant extracts from different plant parts were diluted in buffer (PBS, 20 mM, pH 6.9) to give a final concentration of 25mg/ml, 50mg/ml, 75mg/ml, and 100mg/ml. Subsequently, 1ml of sample was further mixed with 1mL α- amylase (0.5 mg/mL) and followed by incubating for 30 min at room temperature. Then, 1mL of 1% (w/v) soluble potato starch was added and followed by incubating for another 10 min. The reaction was terminated by adding 1 mL of DNS (dinitrosalicylic acid) reagent (12g of sodium potassium tartrate tetrahydrate in 8ml of 2M NaOH (Sodium Hydroxide) and 96mM 3, 5-dinitrosalicylic acid solution). After heating for 5 min at a boiling water bath, an orange red color was developed. The absorbance was read at 540 nm using the spectrophotometer for measuring α-amylase activities in triplicate manner. To eliminate the absorbance produced by plant extract, appropriate extract controls with extract and except the enzyme were also included. Commercial inhibitor acarbose was used as a positive control. As a blank buffer solution was used instead of substrate. The tube with enzyme solution but without plant extracts/acarbose served as the control with total enzyme activity.

Calculation: The enzyme inhibition rate expressed as percentage of inhibition was calculated using the following formula:

$$\text{Inhibition of } \alpha\text{-amylase activity (\%)} = \frac{\text{Absorbance of control-absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Where, the control possess 100 % enzyme activity and the tested sample was plant extract or the standard (acarbose).

3.6 Statistical Analysis

Data were determined and stored in Microsoft Excel 2013 spread sheet to perform statistical analysis. All samples were in three replicates. Descriptive statistics mean and standard deviation were done for proximate composition, mineral composition and vitamin C content. Data were stored, coded and recorded in IBM SPSS Statistics

25 for statistical analysis. The raw data obtained for total phenol and total flavonoid were analyzed by performing t-test. The statistical analysis was conducted at 5% level of significance ($p \leq 0.05$).

Chapter 4: Results

4.1 Nutraceutical Evaluation

The results of the nutraceutical study of the tree different parts (petal, seed and tuber) of *Nymphaea nouchali* and *Nymphaea rubra* comprehended proximate composition, vitamin C content, mineral analysis, total phenolic content, total flavonoid content, qualitative analysis for other bioactive compound (tannin, saponin and alkaloids) as well as Antioxidant activity. Antidiabetic activity was also determine as α- amylase inhibition capacity of the sample extracts.

4.1.1 Nutritional Composition

The mean percentage with standard deviation (ME±SD) of proximate composition value and vitamin C content of both *Nymphaea nouchali* and *Nymphaea rubra* for the three parts consisting petal, seed and tuber are summarized in Table 4.1.

From the table, it is evident that the tuber sample of both *N. nouchali* and *N. rubra* had highest percentage of moisture content (TS1: 13.46±0.344%; TS2: 15.45±0.026%), ash content (TS1: 13.43±0.12%; TS2: 15.45±0.026%) compared to seed and petal samples respectively. Higher percentage of protein content was evident in petal sample (PS1: 23.025±0.001%; PS2: 22.347±0.29%) rather than seed (SS1: 15.132±0.002%; SS2: 15.302±0.380%) and tuber sample (TS1: 11.976±0.855%; TS2: 12.558±0.11%) in both species and also higher amount of lipid content (SS1: 4.92±0.05%; SS2: 5.32±0.141%) and carbohydrate content (SS1: 52.428±0.698%; SS2: 45.838±0.138%) were found to be possessed by the seed sample of both species of water lily. Vitamin C content were higher in the tuber sample (TS1: 24.379±0.035%; TS2: 19.406±0.138%) than petal (PS1: 7.167±0.001%; PS2: 6.94±0.032%) and seed samples (SS1: 3.54±0.02%; SS2: 3.051±0.037%) of *N. nouchali* and *N. rubra* respectively.

Table 4. 1Nutritional Composition of three different parts (petal, seed, tuber) of *Nymphaea nouchali* and *Nymphaea rubra*.

Species	Sample ID	Moisture% (ME±SD)	Ash% (ME±SD)	Crude Fat (%) (ME±SD)	Crude Protein (%) (ME±SD)	Crude Fiber (%) (ME±SD)	CHO (%) (ME±SD)	Vit C (mg %) (ME±SD)
Nymphaea	PS1	12.84±0.026	12.65±0.061	3.04±0.075	23.025±0.001	17.5±0.522	30.945±0.642	7.167±0.001
nouchali	SS1	10.3±0.073	8.26±0.036	4.92±0.05	15.132±0.002	8.96±0.469	52.428±0.698	3.54±0.02
	TS1	13.46±0.344	13.43±0.12	1.09±0.14	11.976±0.855	9.6±0.1	50.444±0.068	24.379±0.035
Nymphaea	PS2	11.00±0.261	12.94±0.425	3.66±0.233	22.347±0.29	12.2±0.299	37.853±0.707	6.94±0.032
rubra	SS2	14.67±0.22	10.21±0.681	5.32±0.141	15.302±0.380	8.66±0.39	45.838±0.138	3.051±0.037
	TS2	15.45±0.026	15.45±0.026	1.123±0.015	12.558±0.11	11.79±0.469	43.629±1.06	19.406±0.138

Legends: ME= Mean; SD= Standard Deviation; PS = Petal Sample; SS = Seed Sample; TS = Tuber Sample

4.1.2 Mineral analysis

Mineral composition of petal, seed and tuber from *N. nouchali* and *N. rubra* are presented in Table 4.2. Almost all the sample contain highest amount of sodium (Na) and potassium (K) than other minerals. Higher amount of Na, K, Mg, Ca, P content were found 2638.83±0.19mg/100g in seed sample (SS1), 2527.92±0.12 mg/100g in petal sample (PS1), 178.07±0.17 mg/100g in tuber sample (TS1), 276.19±0.31mg/100g in petal sample (PS1), 478.627±0.04mg/100g in petal sample (PS1) of *N. nouchali* respectively. Among three micro minerals copper content (PS1: 287.01±0.02mg/100g; PS2: 263.01±0.03mg/100g) are higher in petal sample of both *N. nouchali* and *N. rubra*. Three sample of both species of water lily were moderately rich in Fe and Zn.

Table 4. 2Mineral composition (mg/100g dry basis) of *Nymphaea nouchali* and *Nymphaea rubra*.

Minerals		*N. nouchali*			*N. rubra*		
		PS1(mg/100g)	SS1(mg/100g)	TS1(mg/100g)	PS2(mg/100g)	SS2(mg/100g)	TS2(mg/100g)
Macro-minerals (ME±SD)	Na	1553.84±0.29	2638.83±0.19	1909.01±0.02	1400.19±2.56	1457.23±0.27	1780.96±0.06
	K	2527.92±0.12	1169.84±0.19	2011±0.27	2028.01±0.02	1798.34±0.09	1988.945±0.07
	Mg	167.07±03	60.68±1.14	178.07±0.17	119.998±0.01	112.76±0.06	169.896±0.19
	Ca	276.19±0.31	113.13±0.08	228.58±0.28	262.95±0.06	98.67±0.31	227.36±0.56
	P	478.627±0.04	309.36±0.06	283.43±0.31	499.95±0.05	289.32±0.52	280.18±0.32
Micro-minerals (ME±SD)	Fe	28.674±0.02	10.02±0.02	16.54±0.29	32.28±0.23	9.66±0.03	14.83±0.03
	Cu	287.01±0.02	6.06±0.05	4.85±0.02	263.01±0.03	4.7±0.04	3.80±0.02
	Zn	11.93±0.06	6.49±0.06	10.23±0.02	12.22±0.05	4.85±0.02	9.77±0.33

Legends: ME= Mean; SD= Standard Deviation; PS = Petal Sample; SS = Seed Sample; TS = Tuber Sample

4.1.3 Bioactive Phytochemicals

4.1.3.1 Total phenolic content (TPC)

As shown in Table 4.3, the recorded total phenolic content were 5.597±0.01 mg GAE/100g (PS1), 2.663±0.02 mg GAE/100g (PS2) in petal; 4.256±0.01 mg GAE/100g (SS1), 2.247±0.01 mg GAE/100g (SS2) in seed; 0.497±0.00 mg GAE/100g (TS1); 2.033±0.01 mg GAE/100g (TS2) in tuber of both *N. nouchali* and *N. rubra* respectively.

Table 4. 3 Total phenolic content of *Nymphaea nouchali* and *Nymphaea rubra*.

Sample ID	TPC as mg GAE /100g(ME±SD)		P Value
	Nymphaea nouchali	*Nymphaea rubra*	
PS	5.597±0.01	2.663±0.02	<0.001
SS	4.256±0.01	2.247±0.01	<0.001
TS	0.497±0.00	2.033±0.01	<0.001

Legends: ME= Mean; SD= Standard Deviation; PS = Petal Sample; SS = Seed Sample; TS = Tuber Sample; the mean difference are significant ($p<0.05$).

4.1.3.2 Total flavonoid content (TFC)

Table 4.4 shows the total flavonoid content as mg QE/100g. According to the table total flavonoid content found in *N. nouchali* and *N. rubra* were 794.315±0.86 mg QE /100gm, 464.574±0.33 mg QE /100g in petal sample; 126.273±0.04 mg QE /100g, 32.247±0.48 mg QE /100g in seed sample; 230.112±0.04 mg QE /100g, 57.52±0.06 mg QE /100g in tuber sample respectively.

Table 4. 4 Total flavonoid content of *Nymphaea nouchali* and *Nymphaea rubra*.

Sample ID	TFC as mg QE /100g(ME±SD)		P Value
	Nymphaea nouchali	*Nymphaea rubra*	
PS	794.315±0.86	464.574±0.33	<0.001
SS	126.273±0.04	32.247±0.48	<0.001
TS	230.112±0.04	57.52±0.06	<0.001

Legends: ME= Mean; SD= Standard Deviation; PS = Petal Sample; SS = Seed Sample; TS = Tuber Sample; the mean difference are significant ($p<0.05$).

4.1.3.3 Qualitative analysis of Tannin, Saponin and Alkaloids

The result found by screening test of Tannin, saponin and alkaloids are enlisted in Table 4.5. Three different parts (petal, seed and tuber) of both *N. nouchali* and *N. rubra* found positive result for tannin content by ferric chloride test and alkaloids by the test using Hager's reagent. By foam test saponin was evident to be absent in petal sample while present in seed and tuber sample of both species.

Table 4. 5 Qualitative analysis of Tannin, Saponin and Alkaloids from aqueous extract of different parts of *Nymphaea nouchali* and *Nymphaea rubra*.

Phytochemicals	Chemical test	Sample ID	*Nymphaea nouchali*	*Nymphaea rubra*
Tannin	Ferric Chloride	PS	+	+
		SS	+	+
		TS	+	+
Saponin	Foam test	PS	-	-
		SS	+	+
		TS	+	+
Alkaloids	Hager's reagent	PS	+	+
		SS	+	+
		TS	+	+

Legends: Where, PS = Petal Sample; SS = Seed Sample; TS = Tuber Sample

Note: Symbol in the table represents "+" presence of compound "–" absence of compound

4.1.4 Antioxidant capacity by DPPH assay

The antioxidant activity of the methanolic extract of the petal, seed and tuber sample of *Nymphaea nouchali* and *Nymphaea rubra* were measured using the DPPH assay at concentrations of 2, 4, 8, 16 and 32 µg/ml. Ascorbic acid was used as a standard to determine antioxidant capacity of water lily samples.

At 2, 4, 8, 16 and 32 µg/ml concentrations, the percentage inhibition were ranges from 36.29% to 71.07% and 27.52% to 74.19% in petal sample; 21.57% to 69.56% and

21.77% to 62.30% in seed sample; 16.94% to 57.86% and 17.84 to 53.83% in tuber sample of both water lily sample respectively (Table 4.6). For *N. nouchali* and *N. rubra*, the IC_{50} determined for petal was 6.91 µg/ml (PS1) and 12.70 µg/ml (PS2), for seed was 15.51 µg/ml (SS1) and 17.76 µg/ml (SS2) and for tuber was 22.37 µg/ml (TS1) and 24.98 µg/ml (TS2) respectively.

Table 4. 6 Antioxidant capacity by DPPH assay

SL. No.	Conc. (µg/ml)	% inhibition						
		Ascorbic acid	*Nymphaea nouchali*			*Nymphaea rubra*		
			PS1	SS1	TS1	PS2	SS2	TS2
1	2	25.40	36.29	21.57	16.94	27.52	21.77	17.84
2	4	34.68	46.77	37.40	27.21	38.31	36.90	29.23
3	8	52.52	57.16	42.24	39.31	48.79	46.67	32.96
4	16	68.15	66.94	57.16	47.68	59.07	52.32	47.48
5	32	90.12	71.07	69.56	57.86	74.19	62.30	53.83
IC_{50}(µg/ml)		10.25	6.91	15.51	22.37	12.70	17.76	24.98

Legends: Where, PS = Petal Sample; SS = Seed Sample; TS = Tuber Sample.

In Fig. 4.1, a comparison of DPPH percent inhibition for three different parts (petal: PS1, PS2; seed: SS1, SS2; tuber: TS1, TS2) of *N. nouchali*, *N. rubra* respectively with standard ascorbic acid are presented.

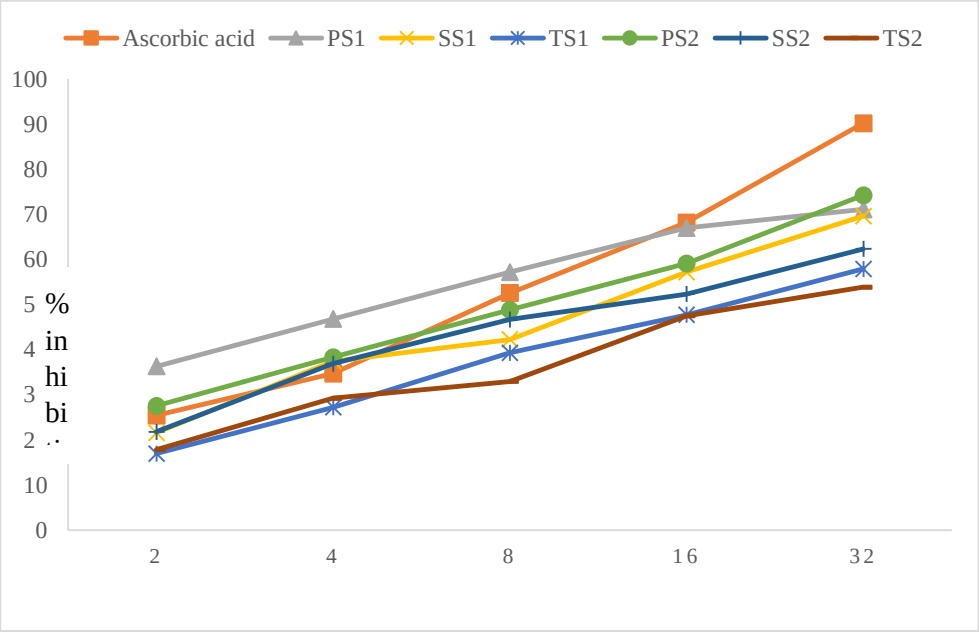


Figure 4.1: DPPH radical scavenging activity.

4.1.5 Antidiabetic Activity by α-amylase inhibition assay

In the present study, aqueous extract of petal, seed and tuber sample of *Nymphaea nouchali* and *Nymphaea rubra* were assessed for inhibition of α-amylase effects on starch break down *in vitro* and showed potent α-amylase inhibitory activity.

Table 4. 7 Antidiabetic activity by α-amylase inhibition assay

SL. No.	Conc. (µg/ml)	% inhibition						
		Acarbose	*Nymphaea nouchali*			*Nymphaea rubra*		
			PS1	SS1	TS1	PS2	SS2	TS2
1	20	48.41	55.29	27.80	34.04	45.14	28.54	34.99
2	40	58.56	59.73	38.58	37.42	56.45	44.40	46.93
3	60	71.25	69.56	49.47	53.70	67.44	57.51	50.85
4	80	77.59	77.59	60.25	57.82	78.22	60.57	66.07
5	100	90.06	89.85	71.99	77.91	83.51	71.04	72.73
IC_{50} (µg/ml)		22.52	13.25	60.69	59.71	27.21	55.24	50.89

Legends: Where, PS = Petal Sample; SS = Seed Sample; TS = Tuber Sample.

The crude aqueous extract of water lily samples at the concentration of 100 µg/mL exhibited 89.85% and 83.51% of inhibition in petal sample; 71.99% and 71.04% of inhibition in seed sample; 77.91% and 72.73% of inhibition in tuber sample repectively (Table 4.7).

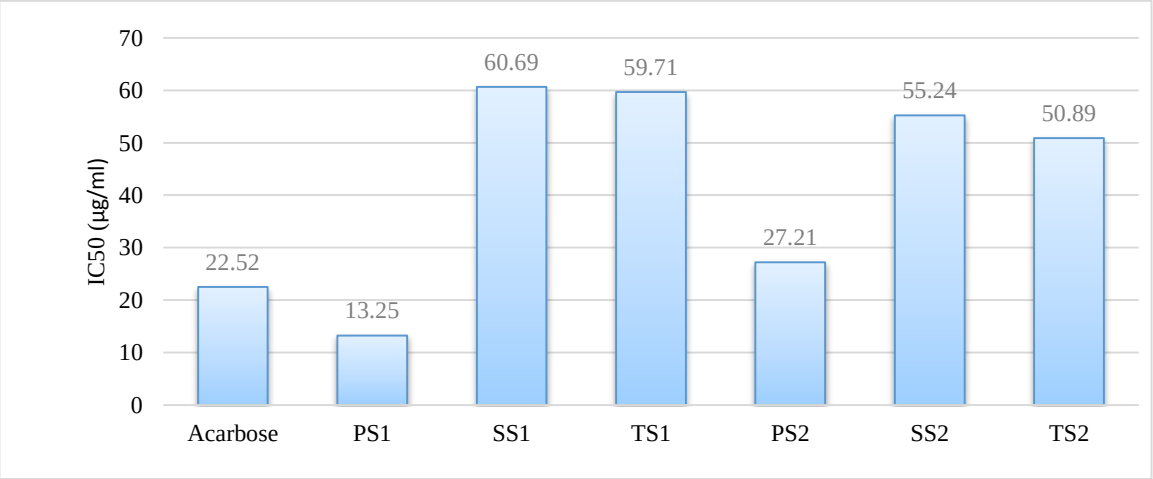


Figure 4.2: IC $_{50}$ values of Acarbose and three different parts (petal, seed and tuber) of *N. nouchali* and *N. rubra*.

The inhibitory activity of aqueous extracts of the water lily samples were explored on the basis of their resulting IC_{50} values. *Nymphaea nouchali* and *Nymphaea rubra* inhibited the activity of α-amylase with an IC50 value of 13.25 and 27.21 μg/mL in petal; 60.69 and 55.24 μg/mL in seed; 59.71 and 50.89 μg/mL in tuber respectively. Whereas, the positive control Acarbose used in this study, shows an IC_{50} value of 22.52 μg/mL (Figure: 4.2).

Chapter 5: Discussion

5.1 Nutritional composition

Macronutrients include fats, carbohydrates and proteins, while micronutrients (micros) refer to essential vitamins and minerals. The body needs both types of nutrients to stay healthy and function correctly, but it requires more macronutrients than micronutrients. Since macronutrients (carbohydrate, fat and protein) are the sources of energy, it is logical to consider energy balance and macronutrient balance together as the opposite side of the same coin (Schutz, 2013).Carbohydrates are one of our body's primary sources of calories and also helps in our body metabolisms. Carbohydrates act as fuel for our brain, heart, kidney, and central nervous system. Along with fats and proteins, carbohydrates are a significant source of our nutrition (Sizer and Whitney, 2008).Protein is essential for life from the beginning of gestation through old age (WHO/FAO/UNU Expert Consultation, 2007). Dietary protein recommendations are based on the requirements for indispensable amino acids, conditionally indispensable amino acids and nitrogen that is needed for syntheses of dispensable amino acids, and other critical nonprotein nitrogen-containing molecules necessary to support growth, tissue repair, and maintenance (Institute of Medicine of the National Academies, 2005).From a nutritional point of view, dietary fats are important for several health related aspects and for optimal functioning of the human body. Dietary fats are not just a source of energy; they function as structural building blocks of the body, carry fat-soluble vitamins, are involved in vital physiological processes in the body, and are indispensable for a number of important biological functions including growth and development (FAO and WHO, 2010).

5.1.1 Moisture

From table 4.1, moisture content of the tuber of *N. nouchali* and *N. rubra* were revealed to be an average of 14.5% which is higher than that of reported value 9.72% and 9.07% of *N. lotus* bulb (Stephen *et al*., 2017) and boiled tuber powder of *N. nouchali* (Anand *et al*., 2019) respectively. Another recent study revealed completely different and high percentage of moisture content (48.83%) for *N. lotus* rhizome (Chinelo and Kabir, 2019). It implies that high percentage of moisture content of rhizome is responsible to have a short storage capacity. The moisture content of

different parts of *Nymphaea lotus* found by Mohammed *et al.*(2013) are the evidence of possessing high moisture percentage of rhizome in compare to other parts such as leaves, petiole, root and seeds. The higher moisture percentage of tuber found in this study than petal and seed samples of both species is sufficient to maintain the nutritional composition of both water lily tubers. In addition, when compared to the moisture level of wheat flour (Chandra and Samsher, 2013), the moisture content of *N. nouchali* and *N. rubra* dry rhizome samples is relatively similar, implying that rhizome can be mixed, processed, and stored for human use.

5.1.2 Ash

The Ash content of three different parts of *N. nouchali* ranged from 8.26% to 13.43% (petal 12.65%; seed 8.26%; tuber 13.43%) and 10.21% to 15.45% for *N. rubra* (petal 12.94%; seed 10.21%; tuber 15.45%) were found in the present study. The average of the ranges of those ash content are within the range reported by Adelakun *et al.* (2016). Among three parts present study revealed to possess higher percentage of ash content in tuber than petal and seed of both species which reflects that more mineral content are stored in tuber. Proper functioning of tissues and bone mechanisms are mostly dependent on minerals content (Saltman & Strause, 2013).

5.1.3 Crude Fat

The highest value crude fat content (4.92±0.05%; 5.32±0.141%) were recorded in seed and very low percentage of crude fat (1.09±0.14%; 1.123±0.015%) were reported in tuber for *N. nouchali* and *N. rubra* respectively while the other research revealed the crude fat percentage of seed ranges from 9% to 13% and of tuber ranges from 1.83% to 5.07% (Mohammed *et al*., 2013; Wasagu *et al*., 2015; Aliyu *et al*., 2017). Highest percentage of crude fat content in seed promotes fat soluble vitamin whereas tuber contain low amount of crude fat among the other two parts (petal, seed) can be served as low fat diet and obviously low fat diet has a great contribution in reducing obesity in some sort of people (Bray *et al*., 2004) by reducing bad blood cholesterol level (Blom *et al*., 2019).

5.1.4 Crude protein

The result found in the present study shows highest percentage of crude protein in petal sample (23.025±0.001%; 22.347±0.29%) while the lowest value found in tuber sample (11.976±0.855%; 12.558±0.11%) of both species respectively. All the crude protein value of different parts of two species of water lily exceed the crude protein value reported from previous study (Mohammed *et al*., 2013; Wasagu *et al.*, 2015; Aliyu *et al*., 2017). As a consequences the petal sample of both species can be suggested as protein supplement as the daily protein requirement of human body. Protein is the main source of some essential amino acid and also serve multiple function including building muscle ,body growth, wound healing and tissue regeneration, functioning as enzymes and hormones , making antibodies , maintaining fluid and electrolyte balance and most importantly proving energy in case of carbohydrate and fat inadequacy.

5.1.5 Crude Fiber

The range of fiber content found in this study varies from 12.2% to 17.5% in petal; 8.96% to 8.66% in seed and 9.6% to 11.79% in tuber of N. *nouchali* and *N. rubra* respectively. The study shows that the petal sample of both species of water lily possess significant higher amount of fiber than tuber and seed sample. However the crude fiber percentage recorded from three different parts of both species of water lily were found to be lower in comparison with other aquatic plants (Adelakun *et al.*, 2016).

5.1.6 Available carbohydrate content

The carbohydrate content of petal, seed and tuber smple of *N. nouchali* and *N. rubra* were lower compared to the studied value in different parts of *N. lotus* ranges from 61% to 81% (Wasagu *et al.*, 2015; Aliyu *et al*., 2017). The variation in carbohydrate content is due to different climate and soil condition but the value found in present study is good enough to prevent ketosis.

5.1.7 Vitamin C content

Vitamin C is a vital nutrient for health. It helps to form and maintain bones, skin, and blood vessels. It is also an antioxidant. Vitamin C content of petal, seed and tuber

sample of *N. nouchali* and *N. rubra* obtained from this study are shown in Table 4.1 also. Among three understudied part of both species of water lily ,the tuber sample possess a high content of vitamin C where the vitamin C content of *N. nouchali* tuber (24.37%) and *N. rubra* tuber (19.406%) are slightly lower than the value represented by Stephen *et al*., 2017. Contemporaneously vitamin C content found in petal and seed sample of *N. nouchali* and *N. rubra* are within the range reported by other researcher (Wasagu *et al*., 2015; Nagavani and Rao, 2010). Heffernan *et al*. (2017) concluded that nonheme iron absorption is promoted by ascorbic acid.

5.2 Mineral composition

Vitamins and trace elements are essential for the human body to function properly and stay healthy. Mineral deficiency in the diet is frequently linked to an increased susceptibility to infectious diseases due to immune system weakness. In the present study mineral content as mg/100g of petal, seed and tuber sample of *N. nouchali* and *N. rubra* were represented in Table 2. Higher value of potassium content found in petal (2527.92±0.12; 20.28.01±0.02), seed (1169.84±0.19; 1798.3±0.09) and tuber (2011±0.27; 1988.945±0.07) sample of both N. *nouchali* and *N. rubra* respectively than all other mineral content. Data obtained for sodium content in this study are considerably higher than the one showed in *N. lotus* bulb sample (Stephen *et al*., 2017). From table 4.2 it is evident that different parts of *N. nouchali* species are the abundant source of trace elements. The comparative evaluation between present study and mineral contents of different parts of *N. alba* conducted by Cudalbeanu *et al*., 2018 indicate that different species and different parts of water lily possess different amount of mineral content based on their ecological variation like growth area, soil type and also environmental variations. Nervous system and muscle activities are facilitated by potassium, phosphorus helps in energy storage, maintaining and repairing cells and tissue, beneficial for healthy bones and teeth also taking parts in acid base balance. Iron facilitate oxygen transportation through red blood cells also supports the immune systems. Copper and magnesium, zinc are necessary for over than 200 enzymatic reactions (Saikia *et al*., 2015; Stephen *et al.*, 2017).

5.3 Bioactive compounds

Plant polyphenols (Pandey and Rizvi, 2009) and constitute an important class of dietary antioxidants. These phytochemicals have emerged as new generation therapeutics for treatment and management of a number of modern lifestyle induced diseases (Tiwari, 2004). Supplementation of dietary antioxidants in moderation has been shown to reduce markers of oxidative stress in geriatric population (Nelson *et al*., 2003).

In this study, the petal (PS1) and seed (SS1) of *Nymphaea nouchali* were found to be significantly higher ($p<0.05$) in phytochemicals such as phenol than other samples (Table 4.3). Petal samples (PS1, PS2) were found to be significantly higher ($p<0.05$) in flavonoids than seed and tuber sample of *Nymphaea nouchali and Nymphaea rubra* according to the findings of our current study (Table 4.4). These sample extracts also contain tannin, saponin and alkaloids (Table 4.5). The presence of certain compounds or interactions between several compounds could explain the observed bio-activities of *Nymphaea nouchali* and *Nymphaea rubra* by many researcher. These new findings suggest that the phytochemical elements in these two species of water lily could be used to prevent or mitigate diseases like diabetes and Alzheimer's disease. More in vivo research is needed to confirm these two water lily species' anti-oxidant and antidiabetic properties. Gallic acid, ellagic acid, quercetin, and rutin are examples of dietary phenolic acids and flavonoids that have been shown to provide a variety of health advantages (Adefegha, 2018). The effects of two classes of phytochemicals (Phenol and Flavonoids) on hypertension, diabetes, and cancer have gotten a lot of attention of many researcher (Huang *et al*., 2013; Kalita *et al*., 2018). Both species of water lily were positive for tannin, saponin, and alkaloids, with the exception of the petal sample, which tested negative for saponin. Baehaki *et al*. (2015) illustrated that these bioactive compounds have beneficial effects on blood cholesterol levels, cancer and stimulation of immune system.

5.4 Antioxidant capacity

Radical scavenging activities are very important due to the deleterious role of the free radicals in biological systems. Free radicals are chemical entities that have one or more unpaired electrons and can exist independently. Free radical production can

result in thousands of reactions, causing significant tissue damage. Lipids, proteins, and DNA are all vulnerable to free radical damage (Sasikumar *et al*., 2010). By scavenging free radicals or supporting antioxidant defense metabolisms, antioxidants may provide resistance to oxidative stress. The efficiency of antioxidants is determined by observing the suppression of oxidation of a suitable substrate.

The synthetic antioxidants have been found to be toxic and mutagenic, necessitating a shift in focus to naturally occurring antioxidants. Natural antioxidants protect cells by removing highly reactive free radicals and reactive oxygen species (ROS) from biological processes (Doughari, 2012).Many plants and plant products have been scientifically discovered and proven as natural antioxidant sources as well as for their nutraceutical properties. According to numerous studies, phenolic compounds and ascorbic acid, as well as flavonoid concentration, play a significant role in antioxidant activity.

The DPPH radical is frequently employed as a model system to study the scavenging activities of a variety of natural substances, such as phenolic or crude plant extracts. At ambient temperature, DPPH is a relatively stable free radical that accepts an electron or hydrogen radical to form a stable diamagnetic molecule (Halliweli and Gutteridge, 2007). DPPH radical is scavenged by antioxidants present in the *N. nouchali* and *N. rubra* ethanolic extract through the donation of proton forming the reduced DPPH. Radical scavenging activity of *N. nouchali* and *N. rubra* ethanolic extract of petal, seed and tuber increased with increasing percentage of the free radical inhibition.

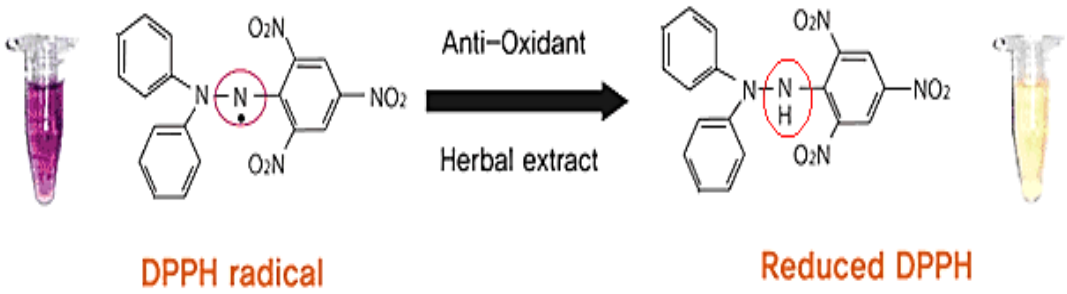


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Fig 5.1: Structural changes of DPPH during oxidation

In this study antioxidant capacity is evaluated by DPPH assay and expressed as IC_{50} value. IC_{50} value implies the concentration of antioxidant required to scavenge 50% of DPPH free radical within specific time period. IC_{50} is inversely proportional to inhibition capacity that means low IC_{50} indicate higher capacity to scavenge free radical (Maisuthisakul *et al*., 2007). From table 4.6, it is elicited that ethanolic extracts of petal (PS1) of *Nymphaea nouchali* exhibit lower IC_{50} which is lower than that of ascorbic acid. When compared amongst the three parts of both species of water lily, the petal sample exhibit lower IC_{50} value which means petal sample can scavenge more free radical than seed and tuber sample. The study about in vitro antioxidant activity of methanolic extract of *N. rubra* rhizome by Mohan and Daffodil (2013) showed IC_{50} value 18.26µg/ml is lower than the IC_{50} value found in this study 22.37 µg/ml (TS1) and 24.98 µg/ml (TS2) for tuber sample. Another study on another *Nymphaea* species (*Nymphaea alba*) found the IC_{50} values for the methanolic *N. alba* extracts were as follows: fruit- (17 µg/mL), flower (17 µg/mL), leaf (20 µg/mL), stem (25 µg/mL), and root (19 µg/mL) (Cudalbeanu *et al*., 2018). According to this study, the fruit (seed) sample possessed lower IC_{50} value means higher antioxidant capacity compared to other parts of *Nymphaea alba*. The IC_{50} value found by Cudalbeanu *et al*. (2018) for seed sample (17µg/mL) is quite similar to the value shown in the present study for seed sample (SS1: 15.51µg/mL and SS2: 17.76µg/mL).These findings indicate that different parts (petal, seed and tuber) of both *Nymphaea nouchali* and *Nymphaea rubra* have the ability to combat oxidative stress in high-stress circumstances.

5.5 Antidiabetic activity

In general, human starch digestion occurs in two phases, commencing with alpha amylase and ending with alpha glucosidase to produce glucose before insulin penetration of the small intestine. High blood glucose levels are caused due to insulin insufficiency and malfunctioning (Boonpisuttinant *et al*., 2019). Alpha amylase catalyzes the hydrolysis of 1, 4-glucosidic bonds in starch, glycogen, and other oligosaccharides into simpler sugars that can be absorbed more easily in the gut. Inhibition of the alpha amylase enzyme in the human digestive tract is thought to be useful in treating diabetes by reducing glucose absorption from starch (Iyer, 2008).All aqueous extracts of petal, seed and tuber sample from *Nymphaea nouchali* and

Nymphaea rubra were investigated anti-diabetic by inhibition of α-amylase activity. All sample extracts could inhibit α-amylase were elicited in Table 4.7.

Amongst the selected plants the aqueous extract of petals of *Nymphaea nouchali* 100μg/mL concentration, had the highest amylase inhibition of 89.85% (PS1) followed by *Nymphaea rubra* with the inhibition of 83.51% (PS2). The concentration of 100 μg/ml of *Nymphaea nouchali* seed extract showed the highest inhibition of 71.99% (SS1), followed by *Nymphaea rubra* which showed the inhibition of 71.04% (SS2) for aqueous seed extract. *Nymphaea nouchali* for its aqueous extracts of the tuber at the concentration of 100μg/mL exhibited the highest inhibition of 77.91% (TS1) compared to the tuber extracts of *Nymphaea rubra* which showed inhibitory activity of 72.73% (TS2) at the similar concentration. IC_{50} value were calculated by plotting concentration vs. percent inhibition graph. IC_{50} is inversely proportional to potential antidiabetic activity that mean low IC_{50} indicate higher capacity to inhibit alpha amylase. From table 4.7 it can be elucidated that aqueous extract of petal (PS1) of *Nymphaea nouchali* exhibit lower IC_{50} which is lower than that of acarbose. When compared amongst the three parts of both species of water lily the petal sample exhibit lower IC_{50} value which means petal sample can inhibit more alpha amylase than seed and tuber sample.

Based on the findings from this present study, it can be inferred that using these plant extracts will help to slow the pace of carbohydrate digestion and absorption, and so contribute to the effective management of diabetes by lowering post-prandial hyperglycemia.

Chapter 6: Conclusion

This study showed the quantification of three different parts (petal, seed and tuber) of *Nymphaea nouchali* and *Nymphaea rubra* with different biochemical compositions such as Carbohydrate, ash, moisture, protein, lipid, fiber and also some essential elements such as sodium, potassium, magnesium, calcium, phosphorus, iron, copper and zinc in addition to anti-oxidant vitamin vit-C. Present study also shows significant amount of bioactive compounds like phenol, flavonoids. Other phytoconstituents such as tannin, saponin and alkaloids are also present in three different parts of both water lily species. A negative screening result was found for saponin in petal sample of both species. This study clearly indicate that all parts of *Nymphaea nouchali* and *Nymphaea rubra* exhibit potential anti- oxidant and anti-diabetic activity by DPPH assay and alpha amylase inhibition assay respectively.

Nutritional composition obtained in this study suggest that petal, seed and tuber of both *Nymphaea nouchali* and *Nymphaea rubra* have potential food value and could be recommended as a functional food ingredients. They are also a promising source of potential antioxidants and may be efficient as preventive agents in the pathogenesis of some diseases. However, the strength of existing data is not enough to suggest a reasonable mood of action for antioxidant effects. Further phytochemical studies are required to isolate and characterize active ingredients that are responsible for their antioxidant activity. Obviously, to confirm the antioxidant activity of all sample extracts, it is necessary to carry out further studies about their in vivo activity and bioavailability. Aqueous extracts of petal, seed and tuber sample of *Nymphaea nouchali* and *Nymphaea rubra* offers a promising therapeutic value in prevention of diabetes. Further studies will be needed in future to determine the main active ingredient having the beneficial antidiabetic effects.

Chapter 7: Recommendation and Future perspectives

Natural products are considered the safest for consumption as compared to other medicinal treatments or clinical candidates derived from synthetic compounds. Over the past few years, some plant extracts, fractions, and compounds were evaluated to check their nutraceutical value as well as anti- oxidant and anti-diabetic activity. From reports that different parts of both *Nymphaea nouchali* and *Nymphaea rubra* appeared as promising agents for many bioactive phytoconstituents. Previous studies demonstrated that the numerous natural products/compounds are employed in the management and prevention of long-term complications of diabetes mellitus and other related diseases.

Despite its valuable significance, there is still a high need to explore their clinical significance, proper physiological attention of inhibitors, and their mode of action to validate their beneficial role as an accompanying treatment in diabetes. Naturally occurring compounds like polyphenols are predisposed by various factors with bioaccessibility, molecular structures, transporters, metabolizing enzymes, etc. and none of the commercially available drugs are free from toxic effect. Therefore, it is extremely important to design theranostic products by using the latest techniques, such as nanotechnology and homogenization that can improve the bioavailability of natural inhibitors including polyphenols (Yallapu *et al*., 2013), vitamins, and herbal plant extracts, so that we can find a treatment technique with less toxicity. We require polyherbal medications for obtaining the appropriate response towards the nonvulnerable treatment options, since synthetic drugs have adverse effects as usual, and diabetic complications are multifactorial.

However, there is a gap in scientific evidence of plant-derived therapeutic benefits that remains unclear. Its chemical constituents improve the condition of oxidative stress, lower the level of cytokines, and inhibit inflammatory pathways for many years. Many researchers showed its importance in the prevalence of the diseases for many years. We conclude that the *Nymphaea* species are the most effective plants with valuable secondary metabolites so that it is a well-known antioxidant. Also, the *Nymphaea* species and its different plant parts may have more space to further explore the valuable chemical constituents against various metabolic disorders and communicable diseases that has no or the least toxic nature or adverse effect.

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Appendices

Appendix A: Photo Gallery

Appendix A1: Picture of Fresh sample

Nymphaea nouchali

Nymphaea rubra

Water lily fruit

Seed

Tuberous rhizome

Appendix A2: Dry sample

Dry Petal

Dry Seed

Dry Tuber

All dry sample in powder form after grinding.

All powder sample were stored in airtight container with proper labeling

Appendix A3: Laboratory work for Nutraceutical study




Extract preparation

Weighing Sample

Moisture

Ash

Sample preparation for protein analysis

Distillation unit

Operating distillation unit

Digestion

Titration

Lipid analysis

Fiber analysis

Acid digestion

Pipetting

Mineral analysis

Reagent for antidiabetic assay

Performing serial dilution

Using UV-vis Spectrophotometer

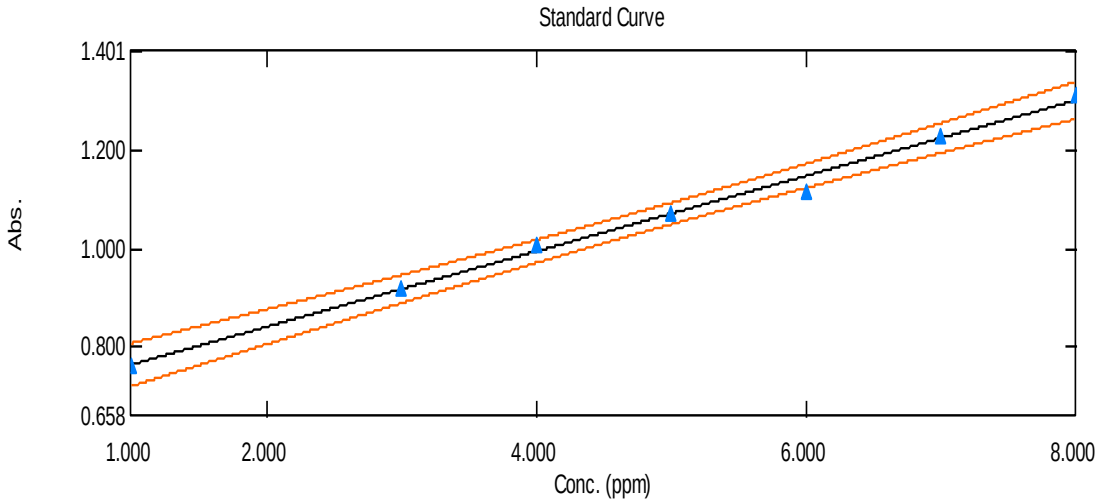
Appendix B: Bioactive compound and antidiabetic activity standard curve

Total Phenolic content (TPC)

Standard table of Gallic acid:

SL. No.	Sample ID	Type	Conc.	WL 760nm	Wgt Factor	Comments
1	STD1	Standard	1.000	0.763	1.000	
2	STD2	Standard	2.000	0.780	1.000	
3	STD3	Standard	3.000	0.920	1.000	
4	STD4	Standard	4.000	1.007	1.000	
5	STD5	Standard	5.000	1.074	1.000	
6	STD6	Standard	6.000	1.115	1.000	
7	STD7	Standard	7.000	1.230	1.000	
8	STD8	Standard	8.000	1.314	1.000	

Standard curve:

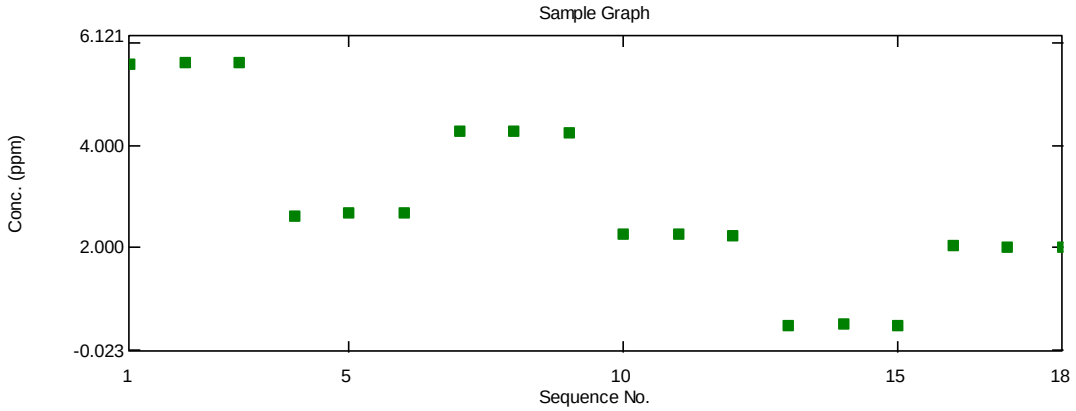


y = 0.0768527 x + 0.687090
r2 = 0.99301

Sample table

Sl. no	Sample ID	Type	Conc. (mg/100g)	WL 760nm
1	PS1.1	Unknown	5.583	1.116
2	PS1.2	Unknown	5.601	1.118
3	PS1.3	Unknown	5.609	1.118
4	PS2.1	Unknown	2.631	0.889
5	PS2.2	Unknown	2.670	0.892
6	PS2.3	Unknown	2.688	0.894
7	SS1.1	Unknown	4.266	1.015
8	SS1.2	Unknown	4.260	1.014
9	SS1.3	Unknown	4.242	1.013
10	SS2.1	Unknown	2.253	0.860
11	SS2.2	Unknown	2.255	0.860
12	SS2.3	Unknown	2.234	0.859
13	TS1.1	Unknown	0.495	0.725
14	TS1.2	Unknown	0.506	0.726
15	TS1.3	Unknown	0.489	0.725
16	TS2.1	Unknown	2.047	0.844
17	TS2.2	Unknown	2.024	0.843
18	TS2.3	Unknown	2.029	0.843

Sample graph

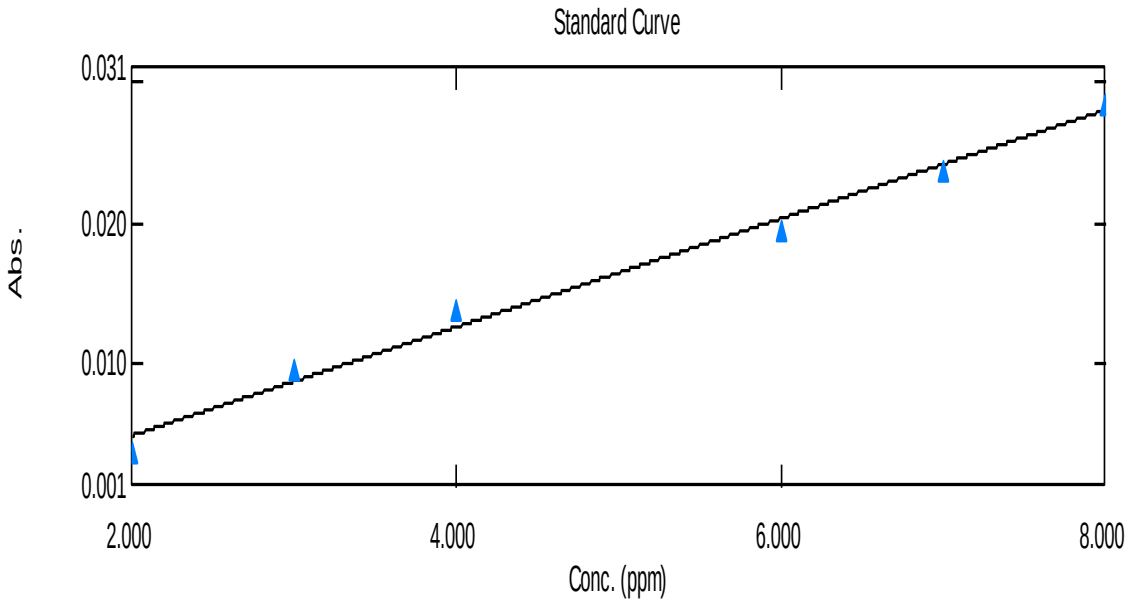


Total Flavonoid content (TFC)

Standard table of Quercetin:

SL. No.	Sample ID	Type	Conc.	WL 415nm	Wgt. Factor	Comments
1	STD1	Standard	2.000	0.004	1.000	Dilution Factor 1
2	STD2	Standard	3.000	0.010	1.000	Dilution Factor 1
3	STD3	Standard	4.000	0.014	1.000	Dilution Factor 1
4	STD4	Standard	6.000	0.020	1.000	Dilution Factor 1
5	STD5	Standard	7.000	0.024	1.000	Dilution Factor 1
6	STD6	Standard	8.000	0.029	1.000	Dilution Factor 1

Standard curve:



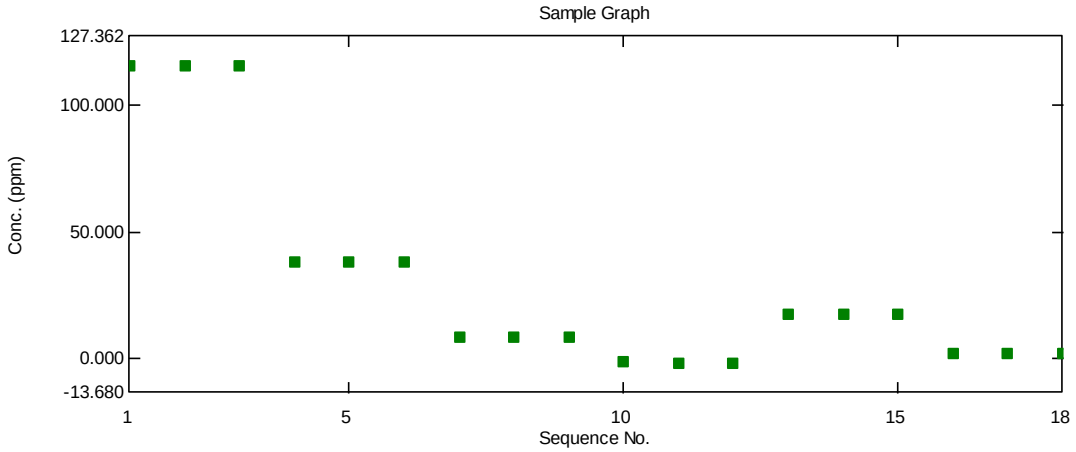
y = 0.00385110 x - 0.00271158

r2 = 0.98868

Sample table

Sl. no	Sample ID	Type	Conc. (mg/100g)	WL 760nm
1	PS1.1	Unknown	794.366	3.056
2	PS1.2	Unknown	795.153	3.060
3	PS1.3	Unknown	793.427	0.053
4	PS2.1	Unknown	464.193	1.785
5	PS2.2	Unknown	464.797	1.787
6	PS2.3	Unknown	464.733	1.787
7	SS1.1	Unknown	126.309	0.484
8	SS1.2	Unknown	126.283	0.484
9	SS1.3	Unknown	126.228	0.483
10	SS2.1	Unknown	32.304	0.122
11	SS2.2	Unknown	32.219	0.121
12	SS2.3	Unknown	32.220	0.121
13	TS1.1	Unknown	230.154	0.884
14	TS1.2	Unknown	230.073	0.883
15	TS1.3	Unknown	230.111	0.883
16	TS2.1	Unknown	57.454	0.219
17	TS2.2	Unknown	57.522	0.219
18	TS2.3	Unknown	57.588	0.219

Sample graph

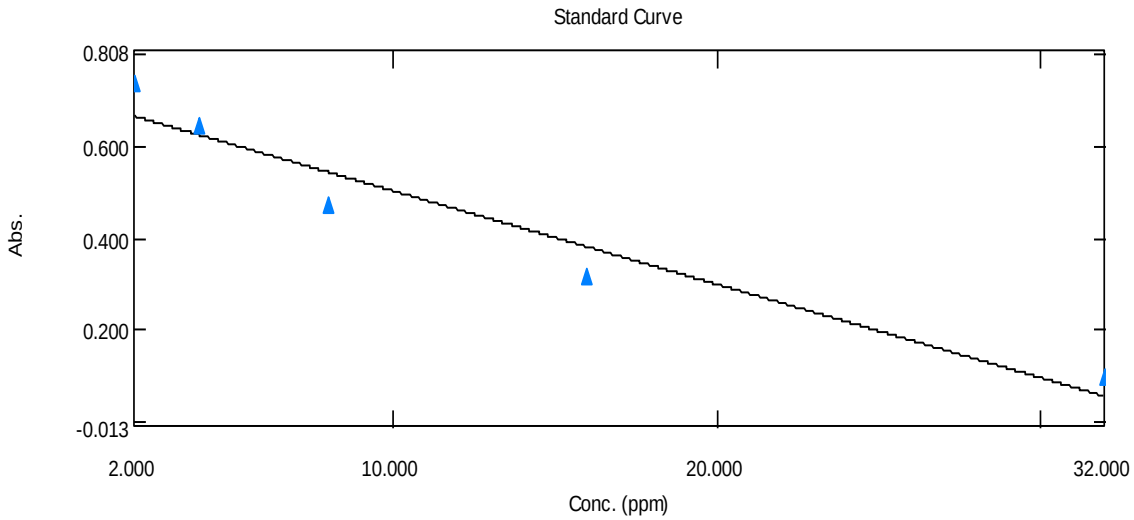


Antioxidant capacity

Standard Table of Ascorbic acid:

SL. No.	Sample ID	Type	Conc.	WL 517nm	Wgt. Factor	Comments
1	STD1	Standard	2.000	0.740	1.000	
2	STD2	Standard	4.000	0.648	1.000	
3	STD3	Standard	8.000	0.471	1.000	
4	STD4	Standard	16.000	0.316	1.000	
5	STD5	Standard	32.000	0.098	1.000	

Standard curve:



y = - 0.0204174 x + 0.707828

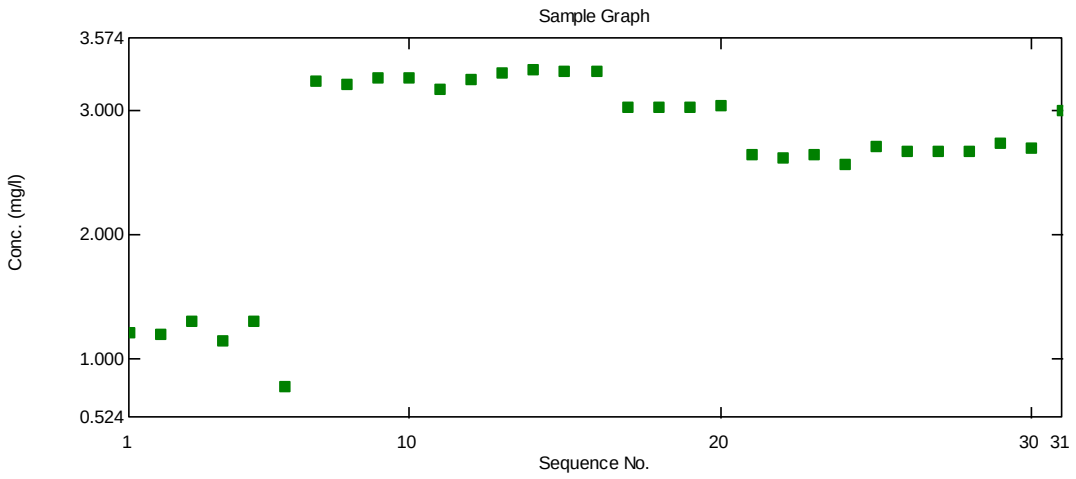
Correlation Coefficient r2 = 0.93522

Sample table

SL. No.	Sample ID	Type	Conc.	WL 517nm
	Control	Unknown	1.309	0.992
1	PS1.1	Unknown	1.189	0.632
2	PS1.2	Unknown	1.306	0.528
3	PS1.3	Unknown	1.143	0.425
4	PS1.4	Unknown	1.299	0.328
5	PS1.5	Unknown	0.778	0.287
6	SS1.1	Unknown	3.237	0.778
7	SS1.2	Unknown	3.207	0.621
8	SS1.3	Unknown	3.258	0.573

9	SS1.4	Unknown	3.264	0.425
10	SS1.5	Unknown	3.163	0.302
11	TS1.1	Unknown	3.246	0.824
12	TS1.2	Unknown	3.298	0.722
13	TS1.3	Unknown	3.320	0.602
14	TS1.4	Unknown	3.308	0.519
15	TS1.5	Unknown	3.312	0.418
16	PS2.1	Unknown	3.024	0.719
17	PS2.2	Unknown	3.025	0.612
18	PS2.3	Unknown	3.016	0.508
19	PS2.4	Unknown	3.033	0.406
20	PS2.5	Unknown	2.644	0.256
21	SS2.1	Unknown	2.609	0.776
22	SS2.2	Unknown	2.635	0.626
23	SS2.3	Unknown	2.566	0.529
24	SS2.4	Unknown	2.703	0.473
25	SS2.5	Unknown	2.663	0.374
26	TS2.1	Unknown	2.672	0.815
27	TS2.2	Unknown	2.671	0.702
28	TS2.3	Unknown	2.737	0.665
29	TS2.4	Unknown	2.699	0.521
30	TS2.5	Unknown	2.996	0.458

Sample graph

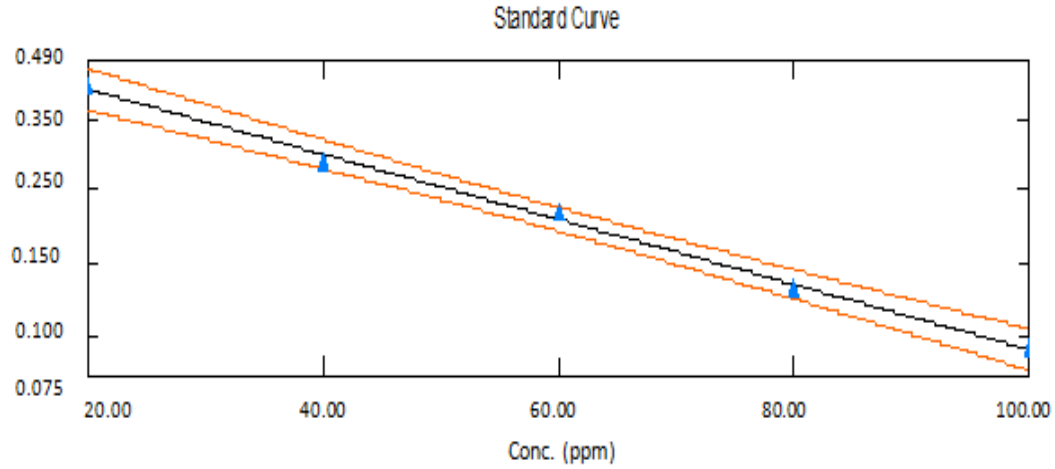


Antidiabetic activity

Acarbose standard table:

SL. No.	Sample ID	Type	Conc.	WL 540nm	Wgt. Factor	Comments
1	STD1	Standard	20.000	0.468	1.000	
2	STD2	Standard	40.000	0.292	1.000	
3	STD3	Standard	60.000	0.172	1.000	
4	STD4	Standard	80.000	0.112	1.000	
5	STD5	Standard	100.00	0.094	1.000	

Standard Curve:



$y = -0.0025x + 0.3273$

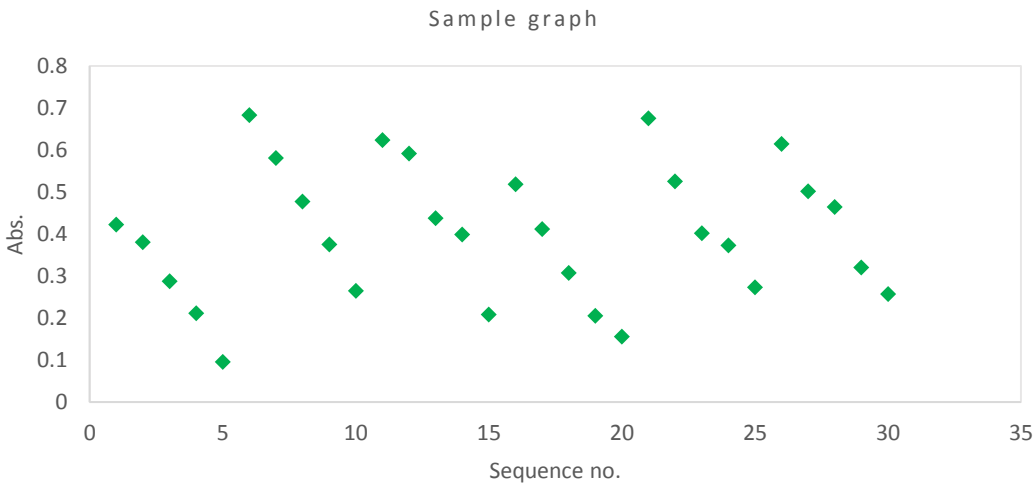
$r^2 = 0.975$

Sample table

SL. No.	Sample ID	Type	Conc.	WL 517nm
	Control	Unknown	***	0.946
1	PS1.1	Unknown	***	0.423
2	PS1.2	Unknown	***	0.381
3	PS1.3	Unknown	***	0.288
4	PS1.4	Unknown	***	0.212
5	PS1.5	Unknown	***	0.096
6	SS1.1	Unknown	***	0.683
7	SS1.2	Unknown	***	0.581

8	SS1.3	Unknown	***	0.478
9	SS1.4	Unknown	***	0.376
10	SS1.5	Unknown	***	0.265
11	TS1.1	Unknown	***	0.624
12	TS1.2	Unknown	***	0.592
13	TS1.3	Unknown	***	0.438
14	TS1.4	Unknown	***	0.399
15	TS1.5	Unknown	***	0.209
16	PS2.1	Unknown	***	0.519
17	PS2.2	Unknown	***	0.412
18	PS2.3	Unknown	***	0.308
19	PS2.4	Unknown	***	0.206
20	PS2.5	Unknown	***	0.156
21	SS2.1	Unknown	***	0.676
22	SS2.2	Unknown	***	0.526
23	SS2.3	Unknown	***	0.402
24	SS2.4	Unknown	***	0.373
25	SS2.5	Unknown	***	0.274
26	TS2.1	Unknown	***	0.615
27	TS2.2	Unknown	***	0.502
28	TS2.3	Unknown	***	0.465
29	TS2.4	Unknown	***	0.321
30	TS2.5	Unknown	***	0.258

Sample graph



Brief Biography

Kanij Fatema Nishan passed the Secondary School Certificate Examination in 2010 from Chakaria Central High School, Chakaria, Cox's Bazar, and then Higher Secondary Certificate Examination in 2013 from Chattogram College, Chattogram. She 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, she 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). She has a great enthusiasm in work for improving the health status of people through proper guidance and suggestions and creating awareness among people about food safety and nutrition.