Research Article

Comparative Analysis of the Antioxidant and Antidiabetic Potential of *Nelumbo nucifera* Gaertn. and *Nymphaea lotus* L. var. *pubescens* (Willd.)

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**Background.** *Nelumbo nucifera* Gaertn. and *Nymphaea lotus* L. var. *pubescens* (Willd.) are both aquatic rhizomatous perennial plants mostly found in the tropical region of Nepal, India, Bangladesh, China, and Eastern Asia. *Nymphaea pubescens* and *Nelumbo nucifera* plants are famous for their different biological activities such as antidiabetic, antioxidant, hepatoprotective, anti-diarrheal, and anti-inflammatory properties. **Objective.** The present study majorly focused on the determination of in vitro antioxidant and antidiabetic properties of *N. nucifera* and *N. pubescens*. Methods. In vitro α-glucosidase inhibition was performed using PNPG as a substrate. Antioxidant property of the plant extract was determined by DPPH free radical scavenging assay. The aluminium trichloride method was done for the estimation of total flavonoid content. Likewise, Folin–Ciocalteau reagent was used for determining total phenolic content. **Results.** The total phenolic content of *N. nucifera* and *N. pubescens* was found to be 172.827 ± 0.41 and 194.87 ± 0.93 mg GAE/g, respectively, while the total flavonoid content was reported 17.12 ± 1.04 and 34.59 ± 1.73 mg QE/g, respectively. The IC₅₀ values of the crude extract and its fractions of *N. nucifera* against the DPPH free radical ranged from 33.46 ± 0.6 to 3.52 ± 0.09 μg/mL, while that of the *N. pubescens* ranged from 14.30 ± 0.43 to 1.43 ± 0.08 μg/mL. Similarly, for the in vitro α-glucosidase inhibition activity, the IC₅₀ of the crude extract and its fractions of *N. nucifera* varied from 349.86 ± 2.91 to 29.06 ± 0.24 μg/mL and that of *N. pubescens* ranged from 224.4 ± 6.85 to 5.29 ± 0.39 μg/mL. **Conclusion.** Both aquatic plants *N. nucifera* and *N. pubescens* show antioxidant properties and can inhibit α-glucosidase in in vitro. Further research is required to identify the inhibiting compounds.

1. Introduction

Natural products have been playing a vital role to cure health problems for decades. Since ancient times, in the field of drug discovery and development, natural products attract the interest of modern researchers [1]. Natural products serve as beneficial biological agents, and a remarkable number of drugs [2] and high-quality food products [3] are extracted from natural sources. It is predicted that more than 10% of the biodiversity is evaluated for potential biological activity [4]. Plants are a good source of bioactive secondary metabolites [5–8]. Secondary metabolites from plants attract the interest of the modern researcher; through in vivo and in vitro studies of plant products, their pharmacological importance is revealed nowadays [9, 10]. More than 25% of prescribed drugs are derived from the plant [11]. *Nelumbo nucifera* Gaertn. and *Nymphaea pubescens* Willd. are aquatic plants having attractive pharmacological properties [12, 13].

*Nelumbo nucifera* Gaertn. belongs to the Nelumbonaceae family and *Nelumbo* genus, commonly known as lotus [14]. Lotus flowers are opened for three days after flower pollination occurs, petals fall, and seeds are developed [15]. China is regarded as the major lotus cultivated country due to its important traditional medicinal value seeds, and
rhizomes of the lotus are consumed as vegetable in South Asia and China [16]. *Nymphaea pubescens* Willd. is the national flower of Bangladesh and belongs to the family Nymphaeaceae. It is commonly known as water lily. Water lilies are aquatic rhizomatous perennial plants with large rounded leaves attached to the tuberous, erect stem through long petiole [17]. Flowers of the water lilies are bloomed for 3-4 consecutive nights, and the size of the flower ranges from 7 to 12 cm wide [17].

Different parts of *Nelumbo nucifera* have attractive pharmacological properties. Seed of lotus are used for cognitive enhancing and neuroprotective purpose [18], antiestrogenic [19], antipyretic [20], antiviral [21], and anti-inflammatory [22]. In vivo study on Wister rats [23], in vitro DPPH scavenging [24], and alpha-glucosidase inhibition assay [25] show the attractive antioxidant and antidiabetic potential of the lotus plant. In China, different parts of the lotus plant such as leaves, flowers, roots, and seeds are used as herbal medicine to cure vaginal discharge, tonify in the kidney, to promote weight loss, and tranquilize the mind [26]. Similarly, *Nymphaea pubescens* is also one of the important traditional medicinal herbs. The crude methanol extract of the lily flower shows a strong β-glucoronidase inhibition activity [27]. In vivo anti-diabetic screening of the ethanol and aquatic extracts of the flowering part of water lily on alloxan-induced diabetic rats reduces the blood glucose level [28]. The leaves, roots, and flowers of the water lily are attractive antioxidants [29]. Beside this, *Nymphaea pubescens* shows immunomodulatory [30], antipyretic [30], anti-inflammation [31], hepatoprotective [32], and anti-diarrhoea [33] activities.

Due to the important pharmacological importance of lotus and water lily in the herbal industry, the current study focused on total phenolic content (TPC), total flavonoid content (TFC), DPPH scavenging activity, and alpha-glucoside inhibition activity of floral parts of lotus and water lily.

### 2. Materials and Methods

#### 2.1. Chemicals and Reagents

α-Glucosidase from *Saccharomyces cerevisiae*, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and p-nitrophenyl-α-D-glucopyranoside were purchased from Sigma-Aldrich.

#### 2.2. Plant Collection and Preparation of Extract

*Nelumbo nucifera* Gaertn. was collected from the Rupandehi district, and *Nymphaea pubescens* Willd. was collected from the Kapilvastu district of Nepal. The collected plants were verified at National Herbarium Center, Lalitpur, Nepal. The flowers of both plants were air-dried and powdered. The powdered form of both plants was macerated for three days in 70% alcohol. It was then filtered, and the filtrate was finally concentrated in a rotary evaporator.

#### 2.3. Total Phenolic Content (TPC)

The color photometric method was applied to estimate the total phenolic content of ethanolic extract of plant sample using Folin–Ciocalteu reagent (FCR) [34]. In short, 20 μL of plant sample, 100 μL FC reagent, and 80 μL of Na₂CO₃ were mixed and kept for 15 minutes in dark. After that, absorbance was taken at 765 nm in a spectrophotometer (Epoch™ 2 Microplate Spectrophotometer, BioTek Instruments, USA). Gallic acid was used as standard, and total phenolic content was expressed in terms of (mg GAE/g) of extract.

#### 2.4. Total Flavonoid Content (TFC)

The aluminium trichloride method was applied for the estimation of total flavonoid content [35]. Briefly, 20 μL of plant sample, 110 μL of distilled water, 60 μL of ethanol, and 5 μL of potassium acetate (1 M) with 5 μL AlCl₃ (10%) were mixed and allowed to stand for 30 minutes, and absorbance was taken at 415 nm using a spectrophotometer. Quercetin was used for standard, and total flavonoid content of the sample extract was expressed in terms of (mg QE/g) of extract.

#### 2.5. DPPH Scavenging Activity

The antioxidant activity of plant extract was determined by using the DPPH assay [36]. In a 96-well plate, 100 μL of different concentrations of sample extract and quercetin (standard reference) were loaded triplicate, initial reading was taken at 517 nm, and then, 100 μL of 0.1 mM DPPH was added to each well. It was then kept in the dark for 30 min. Finally, the final absorbance was taken at 517 nm. Percentage inhibition of free radical scavenging assay was calculated using the following formula.

\[
%\text{inhibition} = \left(\frac{A_c - A_s}{A_c}\right) \times 100,
\]

where \(A_c\) is the absorbance of the control and \(A_s\) is the absorbance of the sample.

The IC50 value was calculated by using GraphPad Prism 8 software (GraphPad Prism, California, USA).

#### 2.6. In Vitro Alpha-Glucosidase Inhibition Activity

The α-glucosidase inhibition of the plant extract was analyzed by using PNPG as a substrate [37]. 20 μL plant extract and acarbose (standard reference) of different concentrations were loaded triplicate, and 10 μL of the α-glucosidase (0.1 unit/mL in 5% DMSO) enzyme was loaded on each well. Similarly, 130 μL buffer was also added to each well. After the initial reading at wavelength 405 nm, the plate was incubated for 15 minutes at 37°C. After incubating the solution, 20 μL of PNPG (0.5 mM) was added to each well. Then, the reaction mixture was incubated again for 15 minutes at 37°C. Finally, 20 μL of Na₂CO₃ (80 mM) was loaded into each well. The yellow color of the para-nitrophenol was determined at 405 nm by using a microplate reader (Epoch2, BioTek Instruments, Inc., USA). The following formula calculated the percentage of α-glucosidase inhibition activity.

\[
\%\text{inhibition} = \left(\frac{A_c - A_s}{A_c}\right) \times 100,
\]

where \(A_c\) is the absorbance of the control and \(A_s\) is the absorbance of the sample.
3. Result

3.1. Total Phenolic and Flavonoid Content. Various gallic acid and quercetin concentrations were plotted against the absorbance to calculate TPC and TFC. The TPC and TFC are expressed as mg GAE/g and mg QE/g, respectively. The TPC and TFC values of both plants are given in Table 1.

3.2. Antioxidant Activity. DPPH scavenging activity of *Nelumbo nucifera* and *Nymphaea pubescens* is significantly attractive. IC\(_{50}\) value of quercetin is 1.199 ± 0.22 μg/mL. The IC\(_{50}\) values of the different solvent fractions of both plants are given in Table 2.

3.3. α-Glucosidase Inhibition Activity. α-Glucosidase inhibition activity of both crude plant extracts was evaluated by diluting the extract from initial concentration 500 μg/mL, and the inhibition percentage was calculated. The IC\(_{50}\) value of the standard acarbose was found to be 5.65 ± 0.209 μg/mL. After screening, both plant extracts were further diluted to determine the IC\(_{50}\) value. The IC\(_{50}\) value of the plant extract was found to be 66.32 ± 4.35 and 5.29 ± 0.39 μg/mL, respectively, for lotus and water lily. α-Glucosidase inhibition activity of different solvent fractions of both plant extracts is given in Table 3.

4. Discussion

*Nelumbo nucifera* and *Nymphaea pubescens* plants have excellent medicinal importance in South Asia and China [38, 39]. Both plant flowers show antipyretic, antidiabetic, antioxidant, anti-inflammatory, hepatoprotective, and antimicrobial activities [38, 40]. In the previous study, TPC and TFC of the *N. pubescens* flower were reported as 69.57 ± 1.77 mg GAE/g and 8.82 ± 0.27 mg QE/g, respectively [41]. In this present study, TPC and TFC of the *N. pubescens* were reported 194.87 ± 0.935 mg GAE/g and 34.60 ± 1.73 mg QE/g, respectively. In this study, the IC\(_{50}\) value for DPPH scavenging activity of the crude extract of *N. pubescens* flower was determined 10.00 ± 0.34 μg/mL, which is attractively higher than the previously reported IC\(_{50}\) value < 100 μg/mL [29]. Similarly, TPC, TFC, and TTC of the ethanol extract of *N. nucifera* were reported 20.9 ± 0.91, 7.19 ± 0.24, 23.85 ± 0.46 mg, respectively [42]. In this study, TPC and TFC of the *N. nucifera* flower were determined 172.82 ± 0.41 mg GAE/g and 17.12 ± 1.04 mg QE/g, respectively. In this present study, polar phenolic compounds are extracted by 70% ethanol; due to the significant amount of phenolics and flavonoid content, flower extract shows potent DPPH scavenging activity. DPPH inhibition activity of the leaf, seed, root, and flower for the similar concentration of 100 μg/mL are 47.8, 42.1, 21.9, and 59.3% inhibition, respectively [43]. In the present findings, the IC\(_{50}\) value of DPPH scavenging activity of *N. nucifera* was found 30.93 ± 2.37 μg/mL.

From in vivo and in silico analysis, it is also reported that the leaf, flower, rhizoids of *N. pubescens* shows potent antidiabetic activity [44, 45]. Ethanolic extract of the *Nelumbo nucifera* extract also showed glucose tolerance in rabbits and rats [46, 47]. Crude extract of *N. nucifera* and *N. pubescens* showed attractive α-glucosidase inhibition activity with IC\(_{50}\) values 66.32 ± 4.35 and 5.29 ± 0.39 μg/mL. Both aquatic plants of Nepalese origin show attractive DPPH scavenging activity and α-glucosidase inhibition activity. From the literature survey and our analysis of TPC, TFC, DPPH assay, and α-glucosidase assay, both plants are good sources of phenolic and flavonoid compounds.

### Table 1: Total phenolic and flavonoid contents of *N. nucifera* and *N. pubescens*.

| Name of sample     | Total phenolic content (mg GAE/g) ± SD | Total flavonoid content (mg QE/g) ± SD |
|--------------------|---------------------------------------|----------------------------------------|
| *Nelumbo nucifera* | 172.82 ± 0.41                         | 17.12 ± 1.04                           |
| *Nymphaea pubescens* | 194.87 ± 0.94                         | 34.60 ± 1.73                           |

Data are expressed as the mean of three different assays ± error mean.

### Table 2: IC\(_{50}\) values of antioxidant activity of *Nelumbo nucifera* and *Nymphaea pubescens*.

|                | *Nelumbo nucifera* (μg/mL) ± SD | *Nymphaea pubescens* (μg/mL) ± SD |
|----------------|---------------------------------|-----------------------------------|
| Crude extract  | 30.93 ± 2.37                    | 10.00 ± 0.34                      |
| DCM fraction   | 8.88 ± 0.26                     | 8.26 ± 0.16                       |
| Ethyl acetate fraction | 3.52 ± 0.09              | 1.43 ± 0.08                       |
| Water fraction | 33.46 ± 0.6                     | 14.30 ± 0.43                      |

Data are expressed as the mean of three different assays ± standard error mean.

### Table 3: IC\(_{50}\) values of in vitro α-glucosidase inhibition of *Nelumbo nucifera* and *Nymphaea pubescens*.

|                | *Nelumbo nucifera* (μg/mL) ± SD | *Nymphaea pubescens* (μg/mL) ± SD |
|----------------|---------------------------------|-----------------------------------|
| Crude extract  | 66.32 ± 4.35                    | 5.29 ± 0.39                       |
| DCM fraction   | 349.86 ± 2.91                   | 224.4 ± 6.85                      |
| Ethyl acetate fraction | 29.06 ± 0.24           | 9.85 ± 1.43                       |
| Water fraction | 109.75 ± 0.32                   | 16.75 ± 0.32                      |

Data are expressed as the mean of three different assays ± standard error mean.
5. Conclusion
The present study indicates that ethanolic extract of flowering parts of N. nucifera and N. pubescens is a good source of phenolics and flavonoids compounds. Ethanolic extract of both flowering aquatic plants shows potent antioxidant and antidiabetic activities. Thus, flowering parts of both aquatic plants can be good sources of herbal medicine for diabetes and problems associated with free radicals.

Data Availability
The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

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