Unveiling the potential antidiabetic, antidyslipidemic, and antimicrobial effects of methanolic root extracts of *Nymphaea pubescens* Willd. plant: Evidence from *in-vitro* and *in-vivo* approaches

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ABSTRACT

This study investigated the therapeutic potential of methanolic root extracts of *Nymphaea pubescens* Willd. with a focus on their antidiabetic, antilipidemic, and antimicrobial properties. The methanolic root extracts of the *N. pubescens* at higher doses (500 mg/kg) exhibited a pronounced serum blood glucose-lowering activity ($p < 0.001$) and also demonstrated remarkable outcomes in body weight loss ($p < 0.01$), closely resembling the effects of the standard metformin. The same experiment displayed a noticeable reduction in low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) levels ($p < 0.001$), along with a notable elevation in high-density lipoprotein cholesterol (HDL-C) levels ($p < 0.01$). Furthermore, the plant extract at 500 mg/kg unveiled significant antimicrobial activities ($p < 0.001$) against both *Staphylococcus aureus* and *Aspergillus niger* and also exhibited significant ($p < 0.01$) inhibition of *Escherichia coli*. In summary, these findings highlight the multifaceted therapeutic potential of *N. pubescens* root extracts, warranting further investigation for their possible development into novel therapeutic agents.

KEYWORDS

Antidiabetic; Antihyperlipidemic; Antimicrobial; *In vitro*; *In vivo*; *Nymphaea pubescens* Willd.; Nymphaeaceae

1. Introduction

Diabetic individuals often experience lipid abnormalities that are characterized by higher LDL-C (low-density lipoprotein cholesterol) concentrations, reduced HDL-C (high-density lipoprotein cholesterol) levels, along with elevated TC (total cholesterol) and triglycerides (TG) levels. While the concentrations of LDL-C may stay steady or slightly elevated in diabetes patients, dyslipidemia associated with this condition is exacerbated due to resistance to insulin or malfunction affecting important enzymes and metabolic pathways for lipid breakdown (Chehade et al., 2013; Taskinen, 2002). Insulin-resistant adipocytes produce a surplus of unrestricted fatty acids, which speeds up the formation of TG and, in turn, release of the very low-density lipoprotein cholesterol (VLDL-C) and apolipoprotein B (ApoB), are both major indicators of cardiovascular disease (Bassalat et al., 2023). The lipid particles found in diabetic individuals suffering from dyslipidemia exhibit a greater propensity for atherogenesis, thus emphasizing a strong co-relationship between dyslipidemia and atherosclerosis (Artha et al., 2019; Marzan et al., 2023).

Natural products having antidiabetic properties have seen a dramatic increase in use due to the lower effectiveness and tolerance of many currently available oral hypoglycemic treatments. These plant-based treatments have recently gained appeal due to their efficiency, affordability, and low risk of unwanted side effects (Chukiatsiri et al., 2023). Therefore, given their manifold recognized pharmacological properties, there has been an increase of interest in utilizing novel natural anti-hyperlipidemic agents, such as flavonoids and phenol-containing compounds derived from plants (Bouhrim et al., 2020).

The rapid rise of multidrug-resistant bacteria presents a severe global health threat, compromising our ability to effectively treat common infectious diseases. Widespread misuse and overuse of commercial antibiotics are widely recognized as major contributors to the development of antimicrobial resistance. Alarming projections from the World Health organization (WHO) indicate that by the year 2050, drug-resistant infections may cause 10 million fatalities annually along with substantial economic losses on a global scale. Urgent action is imperative to combat this escalating crisis and safeguard the future of healthcare (Organization, 2019).

In underdeveloped nations, approximately 80% of individuals rely on plant-based treatments due to their perceived safer profile when compared to synthetic alternatives. The accessibility and affordability of these natural remedies make them a viable option for healthcare in regions with limited resources (Acharjee et al., 2023; Fisher et al., 2020).

The widespread occurrence of biologically active substances in natural resources offers a promising pathway for the exploration of new antimicrobial agents. Among these resources, plants have gathered significant recognition as a valuable reservoir of pharmacologically active secondary metabolites. These substances, such as alkaloids, phenols, terpenoids, saponins, and various other biochemicals, have the ability to combat microbes in addition to having substantial physiological roles in plants (Calheiros et al., 2023).Their unique chemical structures and different ways of working make them promising options for developing new antimicrobial drugs. By exploiting the potential of these plant-derived compounds, researchers can uncover new ways for combating microbial infections and contribute to the ongoing efforts in the field of antimicrobial drug discovery (Calheiros et al., 2023; Ifesan et al., 2013).

Nymphaea pubescens Willd., a plant belonging to the Nymphaeaceae family which encompasses a diverse range of pharmacological and biological properties. Their list of properties is extensive and includes anti-inflammatory, antinociceptive, antiproliferative, hepatoprotective, antibacterial, anti-cancer, antiurolithiatic, anti-obesity, and antidiabetic actions (Selvakumari et al., 2016; Sundaram et al., 2020). Diverse secondary metabolites, notably phenols, alkaloids, flavonoids, saponins, tannins, as well as sterols, are responsible for these extraordinary qualities. The leafy parts and tubers of *N. pubescens* might possess antidiabetic as well as antihyperlipidemic actions; however, the plant's roots remain comparatively underexplored (Angadi et al., 2013; Prodhan & Mridu, 2023; Selvakumari et al., 2016; Shajeela et al., 2012).

The present study assessed the *in vitro* antimicrobial activity of *N. pubescens* root extracts, along with their glucose- and lipid-lowering potentials in experimental mice.

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2. Experimental

2.1 Collection, drying, grinding and extraction of plant part

In October 2022, the roots of *N. pubescens* were collected from various ponds in the Narayanganj region of Bangladesh. Accession number for this plant is 36231, which was assigned by the National Herbarium Institute, Mirpur, Dhaka, Bangladesh. The plant was taxonomically identified and authenticated by Md. Adnan Iqbal who was responsible for plant identification. A photograph of the plant from the sampling site is provided in Fig. 1. Any plant debris and other unnecessary elements were eliminated by a rigorous washing procedure and the desired root parts were left to desiccate for two weeks. Using an appropriate blender (Panasonic MX-GX1521), the cleaned plant pieces were pulverized into a powdery substance, which was stored in hermetic containers in a chilly, shadowy, and arid location. Next, a total of 900 g of the powdery materials were put into a level-bottomed container of amber glass containing 400 mL of 99% methanol for 14 days at ambient temperature and intermittently shaken. The resultant submerged plant substances were filtered employing the Whatman filter paper, and a rotary evaporator was used to condensate the liquid into a brownish-black sticky concentrate. Then, the crude methanolic extract was kept at 4 ºC until analysis. The percentage of extract yield was calculated using the following formula (Eq. 1):

Yield (%) = (Amount of extract yield)/(Amount of plant material soaked) × 100 (Eq. 1)

2.2 Materials

Atorvastatin, metformin, ketoconazole, and streptomycin were purchased from Square Pharmaceuticals Ltd. in Bangladesh. The Pharmacy department of Noakhali Science and Technology University (NSTU), in Bangladesh, supplied all extra chemicals and materials (Ethanol, cholesterol UV test kits, triglyceride UV test kits, HDL UV test kits, LDL UV test kits, chloroform, EDTA tubes, eppendorf tubes, microtips, vinyl gloves etc., needed for the experiment, all of these reagents were of analytical grade. Completely cultured strains of *Aspergillus niger*, *Escherichia coli*, and *Staphylococcus aureus* were collected from the Department of Microbiology at NSTU in Bangladesh.

2.3 Preparation of plant extract and suspension of the reference drug

After carefully measuring the extraction volumes, the extracts were uniformly mixed with a small amount of distilled water and given orally to the mice at doses of 500, 250, and 100 mg/kg of total body weight, respectively. Once the extract had been properly mixed, distilled water was incorporated incrementally to attain the total suspension volume to 5 mL. The precise doses of 250 and 40 mg/kg body weight of metformin and atorvastatin tablets were obtained by grinding them using a pestle and mortar, respectively, and then submerging them in a small amount of distilled water. Water was added gradually until the suspension reached a volume of 5 mL. To achieve stabilization of the suspension, it was vigorously agitated using a vortex mixer (Hussain et al., 2019).

2.4 Phytochemical screening of plant root extract

Multiple studies have outlined the standard testing protocols to detect chemical compounds such as alkaloids, flavonoids, steroids, saponins, carbohydrates, glycosides, proteins, anthraquinones, terpenoids, triterpenoids, tannins, and phenolic substances in root extract from *Nymphaea pubescens* (Akinyemi et al., 2005; Auwal et al., 2014; Banso & Adeyemo, 2006; Hossain et al., 2022; Kancherla et al., 2019).The identification was carried out by visually observing color changes or the formation of a precipitate after adding the appropriate chemicals.

2.5 Experimental animals

The Swiss albino mice employed in the current research were between four- and five-weeks age and weighed approximately 25 ± 5 g. The mice were housed in visible cages made from polypropylene with a moisture content

of 55 ± 10% and an ambient temperature range of 20-25 °C. The ICDDRB-formulated mice food as well as water were provided to the mice on an ad libitum basis. Before the commencement of experimentation, the mice participants were given a 7-day period to acclimatize to the laboratory environment in compliance with the guidelines established by the Institutional Animal Ethics Committee for this research (Mazumder et al., 2021).

2.6 Initiation of type-2 diabetes in mice

A total of thirty healthy mice were fasted for twelve hours before initiating the experiment. All of the mice were injected with streptozotocin (35 mg/kg diluted in 0.1 mol/L citrate buffer; pH 4.5) to trigger hyperglycaemia (Hui et al., 2015). Following streptozotocin administration, serum glucose concentrations were appraised using a glucometer with glucose oxidizing technique after a period of 48 hours. The experimental mice underwent additional procedures with the aim of further investigation after hyperglycemia (serum glucose concentration>250 mg/dL) developed in them.

2.7 Study design

In this experiment, 30 mice were randomly placed into six group of 5 mice each.
Group 1 (control group): Ordinary diets were given to all diabetic mice.
Group 2 (metformin group): All diabetic mice received standard metformin (250 mg/kg body weight) in combination with their regular diet.
Group 3 (atorvastatin group): All mice with diabetes received standard atorvastatin (40 mg/kg total body weight) along with their regular diet.
Group 4 (100/kg mg root extract): In addition to their ordinary diet, all diabetic mice received a methanolic extract of *N. pubescens* root at a dose of 100 mg/kg total body weight.
Group 5 (250 mg/kg root extract): In addition to their ordinary diet, all diabetic mice received a methanolic extract of *N. pubescens* root at a dose of 250 mg/kg total body weight.
Group 6 (500 mg/kg root extract): In addition to their ordinary diet, all diabetic mice received a methanolic extract of *N. pubescens* root at a dose of 500 mg/kg total body weight.
Mice in every group under investigation received the same food and liquids for a week, and daily measurements of their body weight as well as serum glucose levels were made. The changes in body mass as well as glucose levels in each mice were assessed after the 7-day period.

2.8 Assessment of serum TC, TG, HDL-C, LDL-C and VLDL-C level

The treated mice were killed without any pain by using chloroform at the end of the 17th day of treatment, and a syringe was used to take a blood sample from the heart. Centrifugation was employed to separate the blood serum after the blood was drawn. Using the auto-pack kits along with an enzymatic approach, the amounts of TC, TG, HDL-C, LDL-C, and VLDL-C were determined. These substances were prepared in compliance with the guidelines by Human Diagnostics Worldwide, Germany. The level of TC was identified through an enzyme-mediated colorimetric endpoint assay (Hui et al., 2015), while TG levels were quantified with an enzymatic colorimetric GPO-PAP procedure (Mokha et al., 2010). HDL-C levels were determined via the phosphotungstate technique with the Mindray BA-88A Semi-Auto Clinical Chemistry Analyzer (Crown Healthcare, Tanzania). The TG results were used to estimate the VLDL-C level, and Fried Wald's Equation was used to compute the LDL-C level (Salam et al., 2023).
VLDL-C = TG/5 (Eq. 2)
LDL-C = TC – (HDL-C + VLDL-C) (Eq. 3)

2.9 Antimicrobial effect analysis

Kirby-Bauer's disc diffusion approach was employed to assess the antimicrobial effectiveness of the methanolic extract of *N. pubescens* root (Shams-Ghahfarokhi et al., 2006; Sharmin et al., 2018). The root extract was

successfully prepared at concentrations of 500, 250, and 100 mg/mL by dissolving it in the suitable solvent. Fifty microliters of each concentration of root extract were collected individually and dispensed onto sterile, 6-millimeter-diameter disc-shaped substrates, which were then added to plated agar media (Muller-Hinton agar) containing 2×106 colony-forming units per milliliter. Each of these plates was allowed to equilibrate at 4 °C for two hours prior to being incubated at 37 °C for a duration of 24 hours. The zone of inhibition on the plates was measured three times (in millimetres) to assess the antimicrobial effectiveness of the root extract. The discs containing 30 µg/disc of ketoconazole and 30 µg/disc of streptomycin were utilized as the positive controls for antifungal and antibacterial assays, respectively, while blank (solvent) discs served as the negative controls.

2.10 Statistical analysis

The findings were presented as mean (n=5) ± standard error mean (SEM). Simple linear regression analysis, Dunnett's post hoc test, as well as one-way analysis of variance (ANOVA) were carried out to statistically assess the findings. Statistical analyses were performed utilizing SPSS version 27. For statistical significance, $p < 0.05$ was the threshold, while for strong significance, $p < 0.001$. GraphPad Prism version 9.5.0 software was used for preparing graphical presentations.

3. Results and Discussion

3.1 Phytochemical analysis

A total of 7.0 g of brownish-black sticky concentrate was obtained as the final extract, corresponding to a yield of 0.78%. As shown in Table 1, phenols, terpenoids, alkaloids, flavonoids, glycosides, tannins, saponins, anthraquinones, and sterols were all detected, compounds that hold immense potential for therapeutic applications. In contrast, carbohydrates, proteins, and triterpenoids were not detected in the extract based on chemical tests.

Any plant's innate phytochemical elements clarify its pharmacological effectiveness. This current study revealed the presence of a diverse array of potent phytochemical elements in the methanolic root extracts of *N. pubescens*. These include alkaloids, glycosides, sterols, saponins, tannins, phenols, flavonoids, anthraquinones, and terpenoids. Notably, similar bioactive elements have been detected in other Nymphaea species previously and have shown promising antibacterial, antihyperlipidemic, as well as antidiabetic benefits. As *N. pubescens* root extract possesses these phytochemicals, it may have therapeutic uses in the management of infections caused by microbes, diabetes, and lipid regulation. These outcomes emphasize the significance of *N. pubescens* root as a potentially valuable resource of nature for the creation of innovative therapies with a wide range of pharmacological benefits (Arya et al., 2022; Selvakumari et al., 2016).

3.2 Effect on body weight changes

After a duration of seven days of treatment, the experimental groups displayed substantial increases in body weight than the control group (Table 2). In contrast to the other extract groups (100 and 250 mg/kg), the 500 mg/kg extract group of *N. pubescens* exhibited a substantially greater rate of body weight restoration tendency ($p < 0.01$), nearly resembling the standard metformin treatment group (Fig. 2). Interestingly, the 100 mg/kg root extract of *N. pubescens* demonstrated a comparatively lower level of body weight recovery when compared to all other treatment groups. These findings highlight the potent impact of *N. pubescens* extract on promoting body weight restoration, particularly at higher doses, and emphasize its potential as a promising therapeutic intervention for weight management.

In this study, we found that the methanolic root extract of *N. pubescens* at 500 mg/kg dose demonstrated greater efficacy in promoting body weight increase compared to other concentrations tested. Interestingly, the observed impact was similar to the group receiving standard metformin therapy. Our findings align with previous research, which also highlighted a dose-dependent pattern of body weight improvement, indicating that doubling the administered dose led to increased effectiveness (Dodamani et al., 2012). So, these findings reveals that the *N.*

pubescens extract has the potential to facilitate body weight restoration, and supporting its role as a valuable therapeutic option in diabetes management.

3.3 Analysis of serum blood glucose level

Table 3 reveals that the metformin treatment group as well as all *N. pubescens* treatment groups experienced a very significant ($p < 0.001$) decreased trend in serum blood glucose level, while comparing the atorvastatin therapy group with the control group revealed the opposite tendency. The 500 mg/kg root extracts demonstrated a greater degree of serum glucose reduction ability as compared to the 250 and 100 mg/kg extracts, as illustrated in Fig. 3. It is noteworthy that the activity of 500 mg/kg root extracts was almost identical to the metformin. These results highlight the potent antidiabetic potential of *N. pubescens* root extract at the 500 mg/kg dose, suggesting its promising role in glucose management and further supporting its potential as a therapeutic intervention for diabetes.

Antidiabetic efficacy is a highly prevalent happenings, mostly noticed within the Nympheaceae family. Various studies were conducted on a range of species to ascertain antidiabetic efficacy (Arya et al., 2022; Rajagopal & Sasikala, 2008). In the present research, methanolic root extracts of *N. pubescens* at 500 mg/kg stated substantially greater antidiabetic actions than extracts at 250 and 100 mg/kg. Previous research showed the similar serum blood glucose level reduction ability of *N. Pubescens* when high concentrations applied (Angadi et al., 2013). Dodamani et al. (2012) proposed that a heightened concentration was associated with lowered serum glucose levels. This antidiabetic activity of *N. Pubescens* has been attributed to its rich reservoir of flavonoids and phenolic compounds (Pokhrel et al., 2022).The phytochemicals found in the methanolic root extract of *N. pubescens* offer promising therapeutic potential in managing diabetes and related metabolic disorders (Al-Ishaq et al., 2019; Vinayagam et al., 2016). Additional phytochemicals, including alkaloids, saponins, tannins, steroids, anthraquinones that have serum blood glucose reduction ability, also found in the *Nymphaea* species (Gueye et al., 2022; Raja et al., 2010).

3.4 Evaluation of biochemical parameters and the interrelationship between serum blood glucose and lipid profile

The extract groups of *N. pubescens* root with 250 and 500 mg/kg exhibited a notable reduction in TC, TG, LDL-C, and VLDL-C levels than the atorvastatin group (Table 4). It was determined that the group that administered 500 mg/kg of root extracts exhibited the greatest reduction in TC as well as LDL-C levels ($p < 0.001$) as compared to standard atorvastatin. Furthermore, HDL-C levels were considerably ($p < 0.01$) elevated in the 500 mg/kg extract group as opposed to the atorvastatin, 100 mg/kg, and 250 mg/kg extract groups. Interestingly, the metformin treatment group displayed the most remarkable ($p < 0.001$) reduction in TC levels compared to the 500 mg/kg extract group. However, no significant changes in other lipid indices were observed in the metformin group, except for TC levels. These results demonstrate the efficacy of the 500 mg/kg extract of *N. pubescens* root in lowering TC and LDL-C levels as well as improving the lipid profile, and suggest its potential as a therapeutic option for managing dyslipidemia.

Our results suggest a strong and significant association between the 1 unit decrease in serum glucose levels and the decline in TC, TG, VLDL-C, and LDL-C levels. Notably, *N. pubescens* root extract with 500 mg/kg dose demonstrated an extensive and noteworthy tendency to reduce these lipid markers as compared to standard atorvastatin. Furthermore, the 500 mg/kg extract had a lesser impact on decreasing HDL-C units than 100 mg/kg or 250 mg/kg extracts along with the standard metformin, whereas the standard atorvastatin had a greater effect on lowering HDL-C units (Fig. 4). These outcomes imply that *N. pubescens* root extracts at 500 mg/kg may have a potent and distinctive impact on improving lipid profiles, which could make them an efficient therapeutic alternative for managing dyslipidaemia in diabetes.

Our research revealed that, as opposed to inferior concentrations (250 and 100 mg/kg), a greater concentration (500 mg/kg) of the methanolic root extract of *N. pubescens* greatly impacted the levels of TC, TG, VLDL-C, and LDL-C. Notably, the ability to reduce TC as well as LDL-C levels were particularly noticeable. Additionally, the 500 mg/kg extract group showed a significant increase in HDL-C levels compared to the standard atorvastatin group,

while the metformin treatment notably reduced total cholesterol levels. Linear regression analysis indicated that higher concentrations of *N. pubescens* root extract could potentially provide increased cardiovascular protection in diabetic patients by effectively reducing TG, TC, VLDL-C, and LDL-C levels, while also preventing a decline in HDL-C levels. Moreover, the 500 mg/kg root extract exhibited significant serum glucose level-lowering ability compared to standard atorvastatin. These findings suggest the potential of the *N. pubescens* root extract as a valuable intervention for managing lipid abnormalities and promoting cardiovascular health in diabetic patients. Angadi (2013) suggested that increased concentration of *N. pubescens* exhibited more antilipidemic activity.

3.5 Evaluation of antimicrobial efficacy

Fig. 5 depicts the antimicrobial potential of *N. pubescens* extract from roots at different concentrations. When compared with the standard streptomycin, the outcomes reveal that the 500 mg/mL root extract of *N. pubescens* exhibited significantly greater zones of inhibition against gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria than 100 and 250 mg/mL concentrations. Additionally, the antifungal action against *Aspergillus niger* was highly pronounced in the 500 mg/mL extracts of *N. pubescens* when compared with the standard ketoconazole.

Plants having flavonoids have a well-established history of potent antimicrobial actions (Cushnie & Lamb, 2005). Thawabteh et al. (2019) documented that alkaloid demonstrate remarkable effectiveness in combating infections caused by both bacteria and fungi. The outcomes of the current research revealed that the 500 mg/kg root extract of *N. pubescens* showed a more remarkable antimicrobial tendency as compared to standard streptomycin, where a highly significant zone of inhibition for both *Staphylococcus aureus* and *Aspergillus niger* was observed (almost 20 mm). In terms of *Escherichia coli,* a significantly inhibited bacterial colonial zone was observed that was also very close to standard streptomycin when higher concentrations of *N. pubescens* root extract were applied. A similar dose-dependent effect was noticed in different species of Nymphaea, where the activity of the inhibiting zone on Gram-positive as well as Gram-negative bacteria escalated in accordance with the increasing concentration (Akinjogunla et al., 2009).

4. Concluding remarks

The outcomes of our investigation unequivocally demonstrate the remarkable hypoglycemic properties of *N. pubescens* root extract in STZ-induced diabetic mice, especially at higher doses. The extract also effectively prevented abnormalities in lipid profiles and aided in maintaining a healthy body weight in diabetic mice. These results demonstrate the potential therapeutic benefits of *N. pubescens* root as a protectant against the progression and development of atherosclerosis, as well as potential cardiovascular complications associated with diabetes mellitus. Furthermore, the higher concentrations of the root extract exhibited superior antimicrobial activity, could be a potential therapeutic option for patients resistant to commercially available antimicrobial agents including streptomycin, ketoconazole etc. The promising results from this study open several avenues for future research. First, isolating and characterizing the active compounds within *Nymphaea pubescens* root extract is essential to better understand its mechanisms of action. This will not only help in standardizing its use but also in identifying specific bioactive molecules that may be developed into targeted therapies. In addition, further preclinical studies involving larger animal models are necessary to validate its safety and efficacy before progressing to human clinical trials. Given its dual benefits—antidiabetic and antimicrobial—the extract holds strong potential for addressing two major global health challenges: Metabolic disorders and antibiotic resistance. Long-term, the development of *N. pubescens*-based formulations could provide a cost-effective, natural alternative to current pharmaceutical options, particularly in resource-limited settings. This study has several limitations. First, the treatment period was relatively short (7 days), which may have overlooked the long-term effects of *N. pubescens* root extract on glucose and lipid metabolism. Second, the sample size was limited to five mice per group, reducing both statistical power and the generalizability of the findings. Finally, the investigation focused on a single species and a single route of administration, which limits the applicability of the results to other models or potential human use.

Author contribution statement

Conceptualization and literature search were performed by Md. Saddam Hussain, Mohammad Nurul Amin, and Md. Shalahuddin Millat. The experiments were carried out by Sahid Ahmad Santo, Tanmay Deb, and Md. Abdus Salam. The data analysis was performed by Sahid Ahmad Santo, Tanmay Deb, Arafat Miah, and Anamika Datta. The facilities and instruments were prepared by Mohammad Sarowar Uddin, and Mohammad Safiqul Islam. The original draft was written by Md. Abdus Salam. The manuscript was reviewed and edited by Md. Shalahuddin Millat. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgements

The Departments of Pharmacy as well as Microbiology at Noakhali Science and Technology University in Bangladesh are deeply appreciated by the authors for their persistent laboratory assistance and collaboration throughout this research.

Agreement to publication

All authors have read and approved the final version of the manuscript and consent to its submission and publication in this journal.

Ethical approval

The Noakhali Science and Technology University's ethical committee approved this research work with the reference number NSTU/SCI/EC/2022/114.

Accessibility of data and materials

On request, the corresponding author may provide the details that have been utilized to analyze the study's findings.

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Table 1. Phytochemical analysis of the methanolic root extracts of *Nymphaea pubescens*.

Phytochemicals	Chemical test name	Result
Alkaloids	a) Mayer's Test	+
	b) Wagner's Test	+
	c) Hager's Test	+
Carbohydrates	a) Benedict's Test	-
	b) Molisch's Test	-
	c) Fehling's Test	-
Glycosides	a) Keller-Killiani Test	+
	b) Bromine Water Test	+
Sterols	a) Salkowski Test	+
	b) Liberman-burchard test	+
Saponins	a) Foam Test	+
	b) Frothing test	+
Flavonoids	a) Shinoda Test	+
	b) Alkaline Reagent Test	+
Tannin and phenolic Compounds	a) Ferric Chloride Test	+
	b) Lead Tetra Acetic Acid Test	+
	c) Gelatin Test	+
Anthraquinones	a) Borntrager's Test	+
Proteins	a) Biuret Test	-
	b) Ninhydrin Test	-
Terpenoids	a) Salkowski Test	+
Triterpenoids	a) Horizon Test	-
	b) Liebermann Burchard Test	-

Here, the symbol (+) denotes the existence of a chemical constituent in the root extract, whereas the symbol (-) denotes its absence.

Table 2. Effect of *N. pubescens* root extracts on the body weight of mice.

Name of the group	Initial body weight (gm)	Final body weight (gm)
Control	28.60 ± 0.73	32.05 ± 0.21
Metformin (250 mg/kg)	26.84 ± 0.35	28.23 ± 0.43***
Atorvastatin (40 mg/kg)	26.54 ± 0.36	28.98 ± 0.33***
100 mg/kg extract	28.42 ± 0.24	29.14 ± 0.32***
250 mg/kg extract	27.34 ± 0.55	28.25 ± 0.18***
500 mg/kg extract	27.45 ± 0.39	28.55 ± 0.22***

Here, values are expressed as mean ± SEM (n = 5); the level of significance stated as ***$p < 0.001$.
By calculating the weight distinction between the beginning and the end points, the body weight variation was computed as a percentage.

Figure 1. Photograph of the *Nymphaea pubescens* Willd. root.

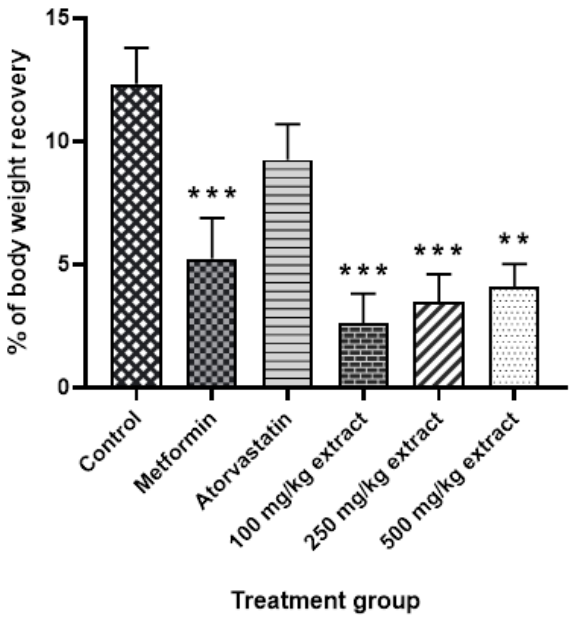

Figure 2. The percentage of body weight recovery tendency of *N. pubescens* Willd. root extract. Values are expressed as mean ± SEM; significant level stated as $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to control.

Table 3. Effect of different concentrations of *N. pubescens* Willd. root extract on the serum blood glucose level.

Group	Baseline reading (before treatment)	Post-treatment Reading (Day 7)
	After 12 hrs. fasting (mg/dl)	**After 12 hrs. fasting (mg/dl)**
Control	381.96 ± 21.93	483.48 ± 12.61
Metformin (250 mg/kg)	344.52 ± 24.31	259.20 ± 2.13***
Atorvastatin (40 mg/kg)	353.16 ± 16.49	434.52 ± 15.89
100 mg/kg extract	347.76 ± 51.93	281.52 ± 7.28***
250 mg/kg extract	358.20 ± 10.31	283.68 ± 2.90***
500 mg/kg extract	369.72 ± 14.60	287.01 ± 2.89***

Here, values are expressed as mean ± SEM (n = 5); the level of significance stated as ***$p < 0.001$.

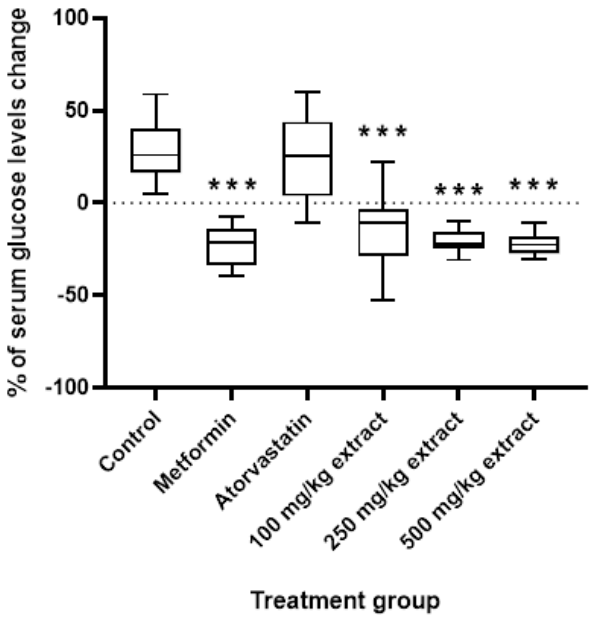

Figure 3. The percentage of serum glucose levels change in different experimental groups. Values are expressed as mean ± SEM; significance level stated as ***$p < 0.001$ compared to control.

Table 4. Analysis of the lipid profiles of different experimental groups.

Group	TC	TG	HDL-C	LDL-C	VLDL-C
Control	143.15 ± 3.23	130.03 ± 2.58	0.51 ± 0.04	116.63 ± 3.41	26.01 ± 0.52
Metformin (250 mg/kg)	68.51 ± 3.96***	108.68 ± 4.44	0.65 ± 0.05	99.54 ± 6.29	21.74 ± 0.89
Atorvastatin (40 mg/kg)	74.78 ± 8.86**	63.93 ± 4.87***	1.03 ± 0.04***	54.69 ± 3.80***	12.79 ± 0.98***
100 mg/kg extract	121.93 ± 5.81	90.0 ± 6.63*	0.76 ± 0.03*	77.45 ± 8.59	18.02 ± 1.33*
250 mg/kg extract	96.18 ± 8.69*	79.76 ± 7.54*	0.85 ± 0.02**	65.75 ± 2.51**	15.95 ± 1.51*
500 mg/kg extract	82.55 ± 2.60***	68.81 ± 8.91*	0.93 ± 0.03**	60.09 ± 7.66***	13.76 ± 1.78*

Here, values are expressed as mean ± SEM (n = 5); the level of significance stated as *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

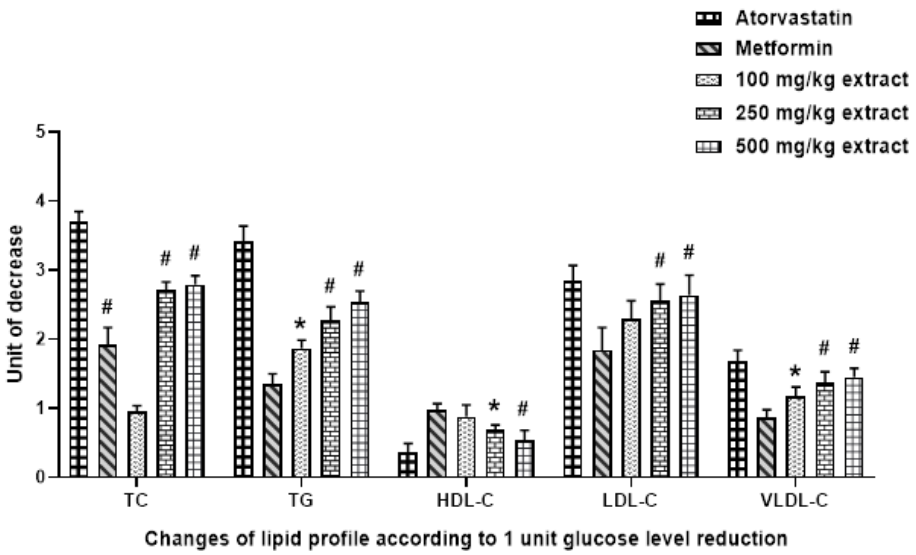

Figure 4. The unit change of lipid profile according to the reduction of 1 unit serum glucose level in different treatment groups of *N. pubescens* Willd. Values are expressed as mean ± SEM; significant level stated as *p < 0.05 and #p < 0.001 compared to atorvastatin.

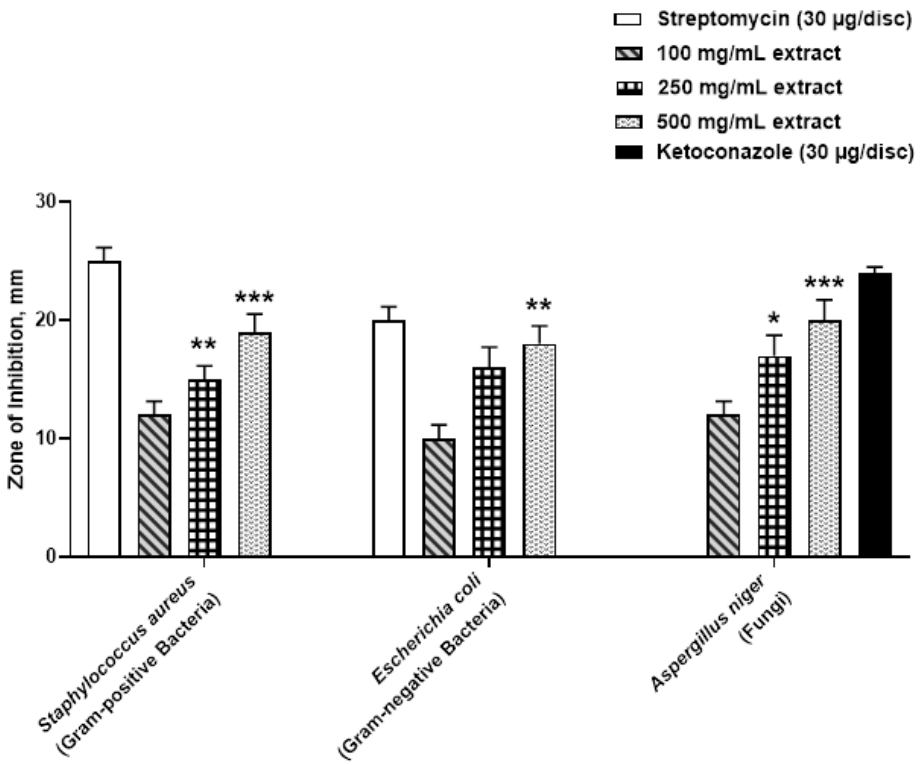

Figure 5. Antimicrobial activity of the methanolic root extract of *Nymphaea pubescens* Willd. Values are expressed as mean ± SEM; significance level stated as $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$.