



# Quantitative Phytochemical Analysis and Antioxidant Profile of *Nymphaea nouchali* Flower

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## Abstract

The objective of the current investigation was to conduct both qualitative and quantitative phytochemical analyses, assess extraction yield, and evaluate the *in-vitro* antioxidant activity of *N. nouchali* flower through standard protocols. The extracts of *N. nouchali* flower were tested for hydrogen peroxide radical scavenging (HPRS), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation scavenging (ABTS) assay, Ferric reducing antioxidant power (FRAP) assay and superoxide anion radical scavenging (SOARS) radical scavenging inhibition using *in-vitro* procedure. Additionally, quantitative phytochemical analysis *e.g.*, Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were performed. The extraction yields indicated that water extract exhibits the highest yield (5.68g/100g), while petroleum ether shows the lowest yield (1.32g/100g). The ethyl alcohol extracts have the highest total phenolic content (19.39±0.07 mg/GAE/g), while the acetone extract shows the lowest value (9.82±0.05 mg/GAE/g). The examination of total flavonoid content revealed that methyl alcohol exhibited the highest concentration (15.34±0.07 mg/QE/g), while petroleum ether demonstrated the lowest concentration (3.19±0.05 mg/QE/g). The extract of the *N. nouchali* flower exhibited significant antioxidant potential. The higher amount of phenolic compounds leads to a stronger ability to neutralize harmful radicals, as shown by the *N. nouchali* flower. This investigation indicates that this plant can prevent and treat many oxidative stress-related diseases.

**Keywords:** *Nymphaea nouchali*; Flower; Total phenolic content; Total flavonoid content; *In-vitro* antioxidant assay.

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## 1. Introduction

Pharmaceuticals for traditional and modern medicine, nutraceuticals, dietary supplements, folk remedies, pharmaceutical intermediates, and chemical entities for synthesised medicines can all be found in large amounts in plant-based medicines. Several studies have shown that medicinal plants and their separate parts have pharmacological properties like the ability to fight inflammation, diabetes, bacteria, viruses, and other microbes.<sup>[1]</sup> The diverse function that plant-based medications play in both traditional and modern healthcare settings highlights the significant contribution that they make to the overall health and welfare of individuals. Not only do these natural remedies function as nutraceuticals and nutritional supplements, but they also play

an important role in the practice of folk medicine all over the globe.<sup>[2]</sup> Furthermore, their relevance extends into the pharmaceutical industry, where they provide important intermediates and chemical entities for the production of medications that have been synthesised. Numerous studies have shown that medicinal plants have the potential to be effective in treating a wide range of health conditions, such as diabetes, inflammation, and infections brought on by bacteria and viruses.<sup>[3]</sup> It is becoming more apparent that plant-based medications are not only relics of the past but rather crucial partners in our continued search for successful medical solutions. This realisation is occurring as we continue to explore the depths of nature's drugstore.<sup>[4]</sup> As a result of pharmacological and metabolic processes, living cells can generate free radicals and other reactive oxygen species (ROS) as byproducts. These free radicals can cause oxidative stress, which leads to damage to lipids, proteins, and DNA. Such damage is associated with the development of various chronic conditions, including cancer, diabetes, age-related disorders, and other long-term health issues. The intake of natural

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antioxidants has been shown to reduce the risk of cancer, cardiovascular diseases, diabetes, and other age-related ailments. Recently, there has been a growing global shift toward the use of natural phytochemicals derived from different parts of plants and leafy vegetables, recognizing their potential health benefits and therapeutic applications.<sup>[5]</sup> These phytochemicals are thought to provide protective benefits primarily due to their antioxidant properties, which help combat oxidative stress and inflammation in the body. Consequently, incorporating these natural compounds into the diet may contribute significantly to improved health and increased longevity.<sup>[6]</sup> An antioxidant is defined as any substance that, when present in small amounts relative to an oxidizable substrate, significantly delays or prevents the oxidation of that substrate, a definition established by the National Institute of Standards and Technology (NIST). In recent years, there has been a marked increase in the demand for flowers, fruits, and their derived products, largely due to their rich content of polyphenols and phytonutrients known for their antioxidant properties.<sup>[7]</sup> Numerous researchers have conducted phytochemical screenings on a wide range of plant species, revealing the presence of various bioactive compounds such as alkaloids, flavonoids, steroids, phenols, glycosides, and saponins. Among these, phenolic compounds represent one of the largest and most widely distributed classes of plant metabolites. Extensive research has focused on the biological activities of phenolic compounds, particularly their roles as antioxidants and free radical scavengers.<sup>[8]</sup> These chemicals have been the subject of interest. The food sector is finding a growing amount of interest in the crude extracts of herbs, spices, and other plant materials that are high in phenolics and flavonoids. This is because these extracts slow lipid oxidative destruction, improving food quality and nutritional content.<sup>[9]</sup> Although molecular oxygen is necessary for the continuation of existence, it may be harmful to living things since it can lead to the production of reactive oxygen species (ROS). Superoxide radicals, hydroxyl radicals, singlet oxygen, and hydrogen peroxide are all examples of reactive oxygen species (ROS). It has been shown that ROS play a significant part in the onset and advancement of a number of illnesses, including atherosclerosis, intestinal damage, malignancies, and heart failure.<sup>[10]</sup> These free radicals are the ones that trigger oxidative stress, which is stabilised by pairing electrons with cellular macromolecules like proteins, lipids, and DNA in healthy human cells. Oxidative stress also causes damage to proteins and DNA, in addition to causing lipid peroxidation. However, organisms possess a variety of defence mechanisms that protect biological components (DNA, RNA, and proteins) against damage caused by reactive oxygen species (ROS). There are repair enzymes like DNA

glycosylases and AP endonucleases in this group, as well as antioxidant enzymes like superoxide dismutase and glutathione peroxidase. There are also antioxidants inside and outside of cells like glutathione, uric acid, ergothioneine, vitamin E, vitamin C, and phenolic substances.<sup>[11]</sup> Nevertheless, when the oxidative stress is persistent, this biotic antioxidant process may not be able to effectively protect the body. As a result of this concept, there has been a major need for curative and chemopreventive antioxidant agents that have a low level of toxicity. These agents are intended to improve the antioxidant function of the body and assist in mitigating harm that is caused by reactive oxygen species (ROS). Antioxidants are a group of substances characterised by their ability to undergo oxidation instead of other molecules. Antioxidants are a broader collective. Antioxidants are chemicals that stop the beginning of oxidation chain reactions, which in turn prevents damage to the cells of the human body.<sup>[12]</sup> Manufactured antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are already marketed; nevertheless, it has been shown that these antioxidants are hazardous to people as well. Researchers have discovered antioxidant properties in certain therapeutic plants, such as polyphenols, in addition to other pharmacological actions. Because of their non-toxic nature, organic antioxidants derived from plants are garnering the greatest attention all over the globe.<sup>[13]</sup>

*Nymphaea nouchali*, a member of the Nymphaeaceae family, is commonly known as the water lily in English and *Shapla* in Bangla. It is also referred to by its synonym *Nymphaea stellata*, a name often used for the red and blue varieties of the water lily. Native to the Indian subcontinent, *N. nouchali* is distributed across a wide geographical range, extending as far as Australia.<sup>[14]</sup> Both Bangladesh and Sri Lanka have designated it as their national flower. *N. nouchali* may be found in its natural condition in aquatic environments that are either stagnant or slow-flowing and have a depth that ranges from shallow to moderate. *N. nouchali* is a plant that blooms during the day and has roots and stems that are submerged. The leaves are wide, the petiole is extremely long, the blooms may be white, rose, or red, and the fruit is typically a globose berry. It is a huge water plant.<sup>[15]</sup> Traditionally, people used the entire plant to treat liver problems and for its aphrodisiac properties. Besides treating kidney disorders, people also use leaves, roots, and flowers as cardiac tonics, bitters, and demulcents. Several studies have shown that flowers possess anti-inflammatory, anti-hepatotoxic, and anti-diabetic properties and leaves were reported for antimicrobial activity.<sup>[16]</sup> The multifaceted benefits of water plant properties reveal a remarkable intersection of aesthetic beauty and medicinal utility. With their wide leaves and long petioles, adorned with striking blooms in hues of white, rose, or red, these plants not only enhance the visual landscape but also serve a pivotal role in traditional herbal medicine. The globose berries they produce are emblematic of their diverse applications, particularly in addressing liver and kidney

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disorders.<sup>[17]</sup> These plants have extensive therapeutic potential, serving as cardiac tonics, bitters, and demulcents that enhance overall health. Also, the fact that their flowers can reduce inflammation, protect against liver damage, and help people with diabetes shows how important these botanical wonders are in modern medicine. Thus, the water plant stands as a testament to nature's ability to provide both beauty and healing, reminding us of the intricate connections between flora and human well-being.<sup>[18]</sup> There are three steroids that have been identified in the stem bark of *N. nouchali*. These steroids include 24-ethyl-5 $\alpha$ -cholestan-3-one, 5 $\alpha$ -stigmast-22-en-3-one, and stigmast-5,22-dien-3-one. These steroids have antibacterial and cytotoxic properties.<sup>[19]</sup> However, according to the Ayurvedic medical system, the seeds are considered to be stomachic and therapeutic, and thus, they are recommended as a dietary supplement for those who suffer from diabetes mellitus.<sup>[20]</sup> Therefore, the flowers of the plant contain flavonoids, gallic acid, astragalin, quercetin, and kaempferol. Moreover, the seeds are a source of proteins, pentosans, mucilage, and other substances. The flowers, on the other hand, have not yet been the subject of a comprehensive scientific examination to determine the antioxidant activity of the blooms.<sup>[21]</sup> The plant and flower of *N. nouchali* is given in Fig. 1.

Several studies have indicated that limited research has been conducted on the antioxidant properties and the qualitative and quantitative phytochemical profiles of *Nymphaea nouchali* flowers, particularly through comparative approaches. In light of this gap, the present study aimed to evaluate the antioxidant potential of the floral parts of *N. nouchali*. To achieve this, preliminary phytochemical screening, extraction yield analysis, various *in-vitro*

antioxidant assays, including hydrogen peroxide radical scavenging (HPRS), ABTS radical cation scavenging, ferric reducing antioxidant power (FRAP), and superoxide anion radical scavenging (SOARS) were employed to identify the components responsible for the plant's high antioxidant activity. Furthermore, quantitative analyses were performed to estimate the total phenolic content (TPC) and total flavonoid content (TFC), focusing on key bioactive constituents such as phenolic acids and flavonoids. These findings were compared to reported values from other parts of the plant, including the tubers, leaves, and seeds. This investigation is expected to offer helpful suggestions for future research on the functional properties of different plant parts. It also lays a foundational understanding that may guide more targeted and comprehensive studies on the therapeutic potential of *N. nouchali*.

## 2. Materials and methodology

### 2.1 Sample collection and plant authentication

Fresh floral specimens of *Nymphaea nouchali* were collected in September 2022 from Mohar area, Paschim Medinipur district, West Bengal, India, following standard ethnobotanical protocols. Sampling sites were selected based on natural habitat suitability to ensure taxonomic accuracy and phytochemical consistency. Ethnomedicinal relevance was cross-validated through literature and consultations with local Vaidyas. Taxonomic authentication was performed by the Botanical Survey of India and confirmed at the Central National Herbarium. A voucher specimen (CVRU/SKB-01) was prepared and deposited, with official validation (Ref: CNH/Tech.II/2023/118; dated 28 July 2023) to ensure reproducibility.

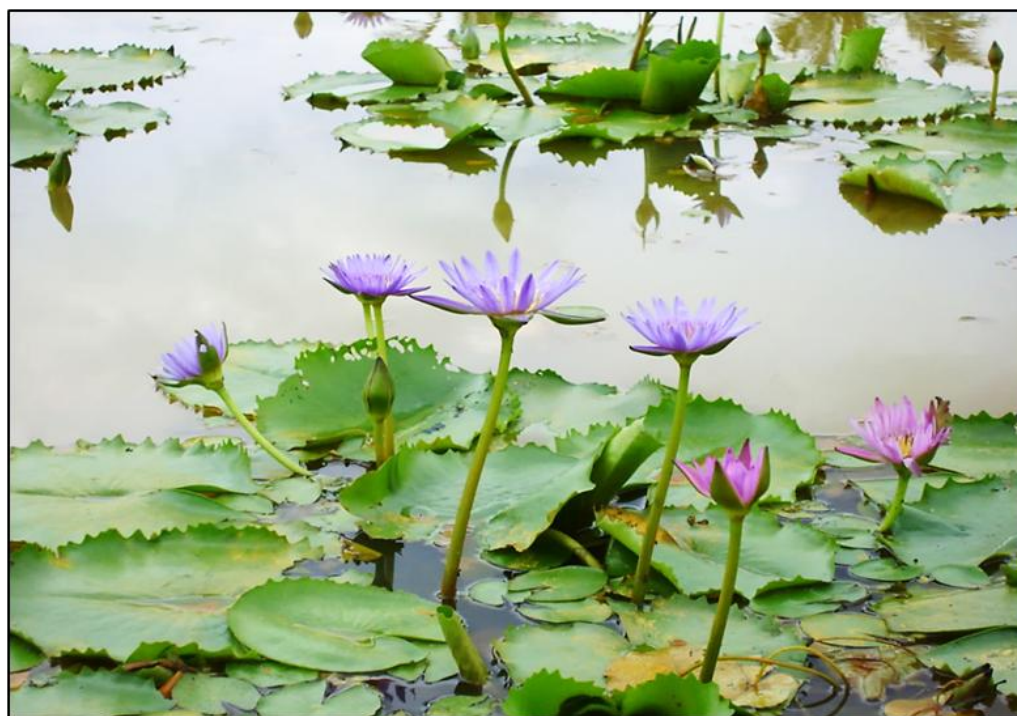
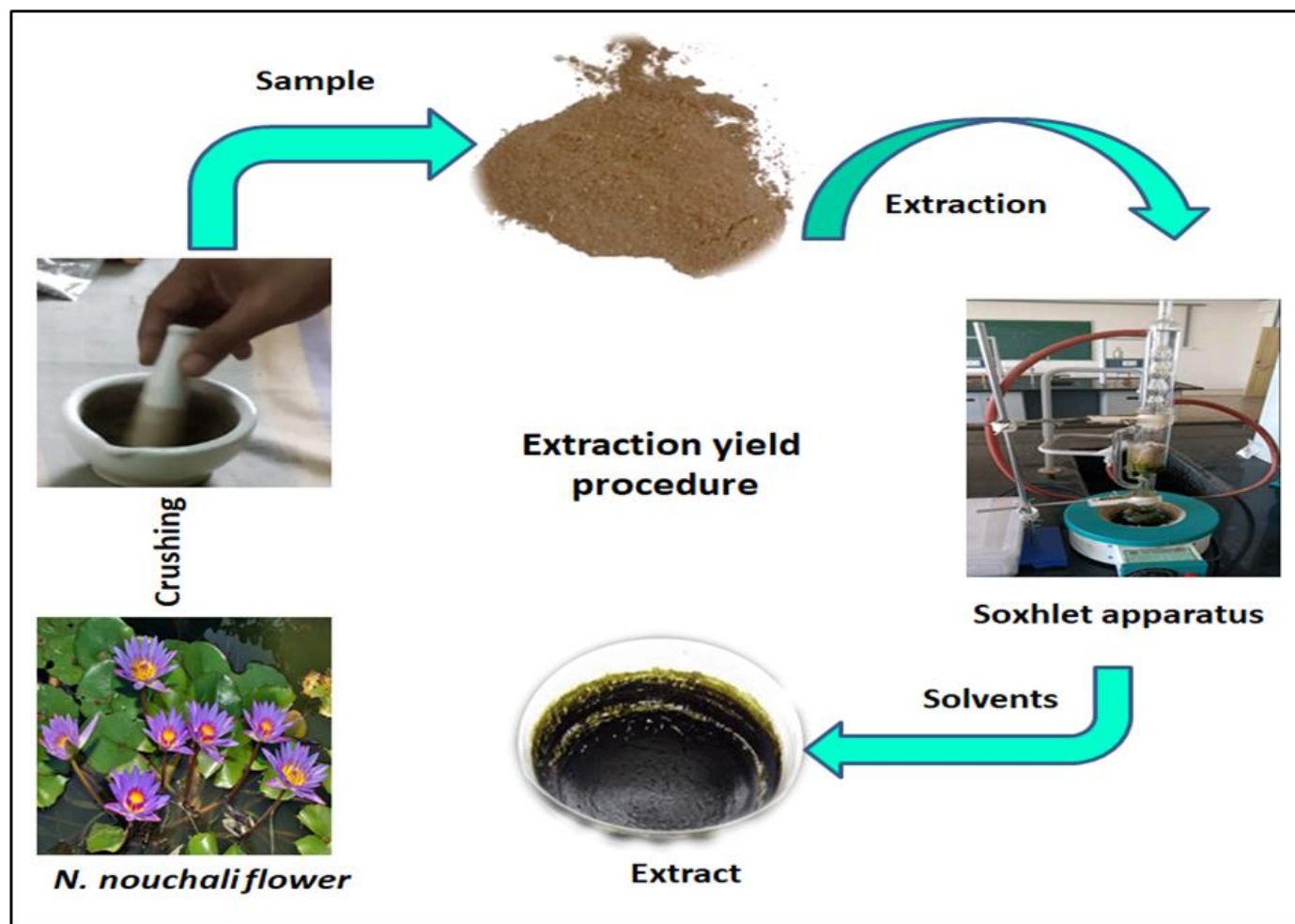


**Fig. 1:** Plant and flower of *N. nouchali*.



**Scheme 1:** Executed quantitative phytochemical extraction yield procedure.

## 2.2 Sample preparation

Collected samples (flowers) were washed with tap and distilled water, air-dried, and shade-dried (5-6 weeks) to preserve bioactive constituents. The dried material was finely powdered using an electric grinder and the powdered sample was stored in airtight containers for further analysis.

## 2.3 Extraction procedure

The Soxhlet solvent extraction technique was applied to make the crude plant extract. 100 gm of coarsely powdered sample were poured into a thimble and sequentially extracted with 250 mL of petroleum ether, dichloromethane (DCM), trichloromethane (TCM), acetone, ethylacetate, Ethanol, Methanol and water were used depending on their polarity nature. The extraction procedure takes approximately 18-24 hours or until the solvents in the extractor's syphon rod turn colourless. The extract was then added to the flask, which was then placed on a heating mantle at 40–50 °C until all of the solvent had been vaporized. Dried crude extracts were preserved in the freezer at 4 °C for further analysis. The crude extracts were then re-dissolved in dimethyl sulfoxide (DMSO) to make a 10 mg/10 mL for each extract, which was then tested for *in-vitro* antioxidant activity. Extraction procedure is outlined in the following [Scheme 1](#).

## 2.4 Qualitative phytochemical analysis

The *N. nouchali* flower extract was the subject of a preliminary phytochemical study. The analysis was carried out in accordance with our previously described methods and standard protocols. The extract underwent screening to ascertain the presence of various bioactive components. The freshly prepared crude extract was qualitatively tested for the presence of active phytochemical constituents. To analyze the different kinds of phyto-materials present in *N. nouchali* flower, it was subjected to qualitative analysis with our previously described protocols and standard analytical procedures.<sup>[22-26]</sup> All of the chemicals used in the investigation were analytical grade, procured from Sigma-Aldrich, Borosil, India, manufactured by all the graduated, calibrated, and carrying-grade glassware. The executed procedure are systematically described below:

**1. Alkaloids Test:** 2mL extract few drops of Wagner's reagent was added and observed for a reddish brown colouration was appeared that confirmed the presence of alkaloids in the samples.

**2. Flavonoids Test:** 2mL extract, little magnesium powder and 2 drops of conc. HCl were mixed and boiled for 5 minutes. Reddish orange color appearance indicates the presence of flavonoids.

**3. Test for Terpenoids:** 2 mL of extract, 2 mL of trichloro methane (TCM) and 2 drops concentrated sulphuric acid were added respectively. A reddish color precipitate obtained that indicates the presence of terpenoids in the sample.

**4. Test for Saponins:** 10 mL of extract 10 mL of Distilled Water (DW) was poured in a test-tube and shaking well, appearance of foams indicates the presence of saponins in the plant extract.

**5. Test for Tannins:** 2 mL of extract, 8% alcoholic ferric chloride solution was poured appearance of bluish/greenish colouration indicates the occurrence of tannins in the plant extracts.

**6. Test for Phenols:** 2 mL of flower extract, 2 mL 10% aqueous FeCl<sub>3</sub> (ferric chloride) solution was poured, appearance of dark green colour indicates the presence of phenol in the samples.

**7. Test for Sterols:** 2 mL of flower extract, 5 drops trichloro methane (TCM), 5 drops of acetone and 2 mL of concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) were added respectively and mixed well, reddish orange colouration indicates the presence of sterols in the plant extracts.

**8. Test for Cardiac glycosides:** 5 mL of extracts, 2 mL glacial acetic acid (GAA) were mixed and 1mL of ferric chloride solutions was added to it. This was carefully underlaid with 1 mL concentrated sulfuric acid. A brownish ring was appeared at the junction indicated the occurrence of deoxysugar characteristic of cardenolides.

**9. Carbohydrates Test:** 2 mL of extracts, 1 mL Molisch's reagent, 1mL concentrated sulphuric acid (by the side of test tube) were added and mixed, a reddish colouration was observed, that indicated the presence of carbohydrates of plant extract.

**10. Test for Proteins:** 2 mL of extract, 5 drops of 1% ninhydrin solutions was added and heated for 1 minute, purple colouration obtained that indicates the presence of amino acid and protein in the sample.

**11. Anthocyanin** Combine 2 mL of plant extract with 2 mL of 2 N concentrated HCl acid. 1 mL of ammonia solution was added. A positive test result appears when the colour changes from magenta to blue-violet.

**12. Test for Phlobatannins:** 10mL of extract and 5 mL 1% aqueous hydro chloric acid was mixed and boiled, red precipitate indicated the occurrence of phlobatannins in the samples.

**12. Quinones Test:** 5 mL extract, 1mL of concentrated hydrochloric acid was added and observed for the appearance of yellow precipitate or colouration that clearly indicate that the presence of quinones in the sample.

**14. Test for Oxalate:** 2 mL extract in test-tube, 2 drop glacial acetic acid (GAA) was poured blackish colouration indicated the occurrence of oxalates in the plant extracts.

## 2.5 Quantitative phytochemical analysis

Quantitative analysis serves as a crucial method for determining the quantity of phytoconstituents found in

plant extracts. The extraction yield, total phenolic content (TPC), and total flavonoid content (TFC) are assessed. Extracts collected from the flowers of the *N. nouchali* were subjected to an estimation of the quantity of extraction yield, TPC and TFC using accepted methods.

### 2.5.1 Extraction yield

The extraction yield represents the percentage of material obtained from the total sample initially used, providing a quantitative measure of the efficiency of the extraction process.<sup>[26-28]</sup> The extraction yield (%) was calculated using the following formula (Eq. 1).

$$\text{Extraction Yield (\%)} = \frac{W_{EX}}{W_s} \times 100 \quad (1)$$

where  $W_{EX}$  is the weight of the dried extract (g) and  $W_s$  is the weight of the initial sample (g). The executed quantitative phytochemical extraction yield procedure for *N. nouchali* flower is given in Scheme 1.

### 2.5.2 Total Phenolic content (TPC)

The total phenolic content (TPC) in *Nymphaea nouchali* flower extracts was determined spectrophotometrically using the Folin-Ciocalteu reagent (FCR), following the method described by Molina-Cortes *et al.* (2020),<sup>[29]</sup> with slight modifications. Briefly, the assay was conducted in a 10 mL volumetric flask by mixing 4 mL of distilled water with 0.4 mL of either the standard solution, the blank, or the extract to be analyzed. Subsequently, 0.4 mL of Folin–Ciocalteu reagent was added, and the mixture was allowed to react for 5 minutes. Following this, 4 mL of 7% sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) solution was added, the mixture was shaken well, and the flask was filled to the mark with distilled water. The solution was then incubated in the dark at 25 °C for 90 minutes. After incubation, absorbance was measured at 730 nm using a UV-Visible spectrophotometer (Jenway Model 7315 UV/Visible Single Beam). Gallic acid (GA) was used as the standard, and distilled water served as the blank. The total phenolic content was expressed in milligrams of gallic acid equivalents per gram of dry matter (mg GAE/g). The following Eq. (2) was used to calculate total phenolic content of extract.

$$\text{TPC \%} = \frac{C_s \times V}{W_{ex}} \times 100 \quad (2)$$

where,  $C_s$  is the concentration of standard solution,  $V$  is the volume of extract in mL, and  $W_{ex}$  is the weight of dried plant extract in gram.

### 2.5.3 Total Flavonoids content (TFC)

The determination of total flavonoid content in *Nymphaea nouchali* flower extract was performed using the aluminum chloride (AlCl<sub>3</sub>) colorimetric method, as previously described by Molina-Cortes *et al.* (2020).<sup>[29]</sup> Briefly, a stock solution was prepared by dissolving 5.0 mg of quercetin in 1.0 mL of methanol. Standard quercetin solutions were then prepared by serially diluting the stock solution with methanol to obtain

concentrations ranging from 5 to 200 µg/mL. For the assay, 0.6 mL of either the diluted quercetin standard or the extract was mixed with 0.6 mL of 2% aluminum chloride solution. The mixtures were incubated at room temperature for one hour. After incubation, the absorbance of each solution was measured at 420 nm using a UV-Visible spectrophotometer (Cary 50 Varian Bio UV-Vis Spectrophotometer), with pure methanol used as the blank. The total flavonoid content in the test samples was calculated using the calibration curve ( $Y = 0.0162x + 0.0044$ ,  $R^2 = 0.999$ ) and expressed as mg quercetin equivalents (QE) per gram of dry matter. Quercetin served as the standard, and all determinations were performed in triplicate to ensure accuracy. The following Eq. (3) was used to calculate total flavonoid content of extract.

$$\text{TFC \%} = \frac{C_s \times V}{W_{ex}} \times 100 \quad (3)$$

where,  $C_s$  is the concentration of standard solution,  $V$  is the volume of extract in mL, and  $W_{ex}$  is the weight of dried plant extract in gram.

## 2.6 In-vitro antioxidant assays

### 2.6.1 Hydrogen peroxide radical scavenging (HPRS) activity

HPRS assay of *N. Nouchali* flower extract were completed using previously described procedure by Al-Amiery et al. (2015),<sup>[30]</sup> with little changes. In brief, make a serial of solutions ranging between 2mL to 10mL. Combining 2.4 mL of 0.1M phosphate buffer with a pH of 7.4, 2 mL of hydrogen peroxide with a concentration of 43 mmol, and 1.0 mL of flower extract consisting of 20–100 µL extract of the plant in ethanol with a concentration of 4 mg/mL, and incubated 30 minutes at dark place. After incubation each test tube was employed to UV-Vis spectrophotometer (Agilent G6860A Cary 60 UV-Vis Spectrophotometer) and recorded the absorbance at 230 nm. Each examination has done at triplicate time. Ascorbic acid was used as a standard solution, and pure methanol was used as a blank solution. Both the blank and the control were made without the addition of hydrogen peroxide. The inhibition percentage of HPRS were calculated by the following formula in Eq. (4):

$$\text{Inhibition \%} = \frac{A_c - A_T}{A_c} \times 100 \quad (4)$$

where,  $A_c$  is the absorbance of control (without extract) and  $A_T$  is the absorbance of the extract with standard solution.

### 2.6.2 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation scavenging (ABTS) activity

The ABTS assay is a robust and sensitive method used to evaluate the radical scavenging activity of antioxidants. The ABTS assay was performed the method following described by Re et al. (1999)<sup>[31]</sup> with minor modifications. It is based on the generation of the ABTS radical cation ( $\text{ABTS}^{+\bullet}$ ), a blue-green chromophore with strong absorption at 734 nm.

This radical is produced by reacting ABTS with an oxidizing agent such as potassium persulfate. To prepare the working solution, ABTS (7 mM) is mixed with potassium persulfate (2.45 mM) and allowed to react in the dark for 12–16 hours at room temperature. The resulting  $\text{ABTS}^{+\bullet}$  solution is diluted with ethanol or phosphate buffer to obtain an absorbance of approximately  $0.70 \pm 0.02$  at 734 nm. A defined volume of sample extract is added to the ABTS solution, and the decrease in absorbance is recorded after 6 minutes of incubation. The reduction in color intensity reflects the antioxidant capacity of the sample. Calculations were made to determine the inhibition percentage of ABTS in given formula using Eq. (5).

$$\text{Inhibition \%} = \frac{A_0 - A_1}{A_0} \times 100 \quad (5)$$

where,  $A_0$  = Absorbance of control,  $A_1$  = Absorbance of sample.

### 2.6.3 Ferric Reducing Antioxidant Power (FRAP) activity

The FRAP assay is a widely accepted method for determining the reducing power of antioxidants based on their ability to convert ferric ions ( $\text{Fe}^{3+}$ ) into ferrous ions ( $\text{Fe}^{2+}$ ). The FRAP assay was performed following the method described by Benzie and Strain (1990)<sup>[32]</sup> with minor modifications. The assay utilizes a ferric-tripyridyltriazine ( $\text{Fe}^{3+}$ -TPTZ) complex, which upon reduction forms a blue-colored ferrous complex ( $\text{Fe}^{2+}$ -TPTZ) measurable at 593 nm using a UV-Visible spectrophotometer. The FRAP reagent is freshly prepared by mixing acetate buffer (pH 3.6), TPTZ solution in HCl, and ferric chloride ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ) in a 10:1:1 ratio. A known volume of sample extract is added to the FRAP reagent and incubated at 37°C for 4–10 minutes. The increase in absorbance indicates the antioxidant potential. The antioxidant capacity is calculated using a standard calibration curve, typically prepared with ferrous sulfate ( $\text{FeSO}_4$ ) or ascorbic acid. Calculations were made to determine the inhibition percentage of FRAP in given formula (Eq. (6)):

$$\text{Inhibition \%} = \frac{\text{Blank}_{\text{Abs}} - \text{Test}_{\text{Abs}}}{\text{Blank}_{\text{Abs}}} \times 100 \quad (6)$$

where,  $\text{Blank}_{\text{Abs}}$  is the absorbance of blank solution, and  $\text{Test}_{\text{Abs}}$  is the absorbance of tested solution.

### 2.6.4 Superoxide anion radical scavenging (SOARS) activity

The measurement of the superoxide anion radical scavenging (SOARS) activity of *Nymphaea nouchali* flower extract was carried out following the method described by Jadhav and Tatke (2024)<sup>[33]</sup> with slight modifications. 156 µM nitroblue tetrazolium (NBT) solution (prepared in 100 mM phosphate buffer, pH 7.4), 1 mL of 468 µM NADH solution (also in 100 mM phosphate buffer, pH 7.4), and 0.1 mL of various concentrations of *N. nouchali* extract and reference compounds (diluted in distilled water) were mixed. The reaction was initiated by adding 100 µL of 60 µM phenazine

methosulfate (PMS) in 100 mM phosphate buffer (pH 7.4). The mixture was incubated at 25 °C for 30 minutes. After incubation, the absorbance of the reaction mixture was measured at 560 nm using a UV-Visible spectrophotometer (Agilent G6860A Cary 60 UV-Vis Spectrophotometer) against a blank. An alkaline DMSO solution served as the blank, and quercetin was used as the standard. All experiments were performed in triplicate, and the results were averaged. The percentage of inhibition was calculated by comparing the absorbance values of control and test samples. A decrease in absorbance indicated an increase in SOARS activity. Calculations were made to determine the inhibition percentage of HPRS in given formula (Eq. (7)):

$$\text{Inhibition \%} = \frac{A_C - A_T}{A_C} \times 100 \quad (7)$$

where,  $A_C$  is the absorbance of control (without extract) and  $A_T$  is the absorbance of the extract with standard solution.

### 3. Results and discussion

The objective of this study was to investigate the phytochemical activities of *Nymphaea nouchali* flower extracts, both qualitatively and quantitatively. This included measuring the total phenolic content (TPC) and total flavonoid content (TFC), as well as evaluating the antioxidant potential through *in-vitro* assays such as HPRS, ABTS, FRAP, and SOARS. Various solvents like petroleum ether, dichloromethane (DCM), trichloromethane (TCM), acetone, ethyl acetate, ethanol, methanol, and water etc. were used to assess percentage extraction yield. The Soxhlet extraction method was employed, with extractions performed in accordance with the polarity of the solvents. Among the solvents, water (5.68 g/100 g) and ethyl acetate (5.49 g/100 g) showed the highest extraction yields. Standard methods were used to validate the *in-vitro* antioxidant activity of the extracts.

The comparative study of solvent systems provided valuable insights into how solvent polarity affects the efficiency of bioactive compound extraction. Soxhlet extraction proved to be a reliable technique, and water and ethyl acetate emerged as the most effective solvents, yielding significant quantities of desired compounds. The positive results from antioxidant assays not only confirmed the effectiveness of the extraction process but also indicated the potential health benefits of these extracts. This comprehensive analysis highlights the importance of solvent selection in maximizing both extraction yield and biological activity, paving the way for future research focused on optimizing extraction methods for enhanced medicinal applications.

#### 3.1 Qualitative phytochemical analysis

In the current examination the subjective investigation of *N. nouchali* flower extricates were done for dried bloom. The fundamental phytochemical screenings on blossom separates were tried in this examination. Different phytochemicals were uncovered to be available in flower concentrate of *N. nouchali*

(Table 1). Every extract of the plant sample contains flavonoids, terpenoids, saponins, phenols, sterols, proteins, and anthocyanins. Conversely, none of the extracts of the *N. nouchali* flower contain phlobatanin, quinone, and oxalate.<sup>[25]</sup> Tannin is exclusively present in extracts made using ethyl alcohol, methyl alcohol, and water, while alkaloids are found in all extracts but are absent from petroleum ether, DCM, and TCM extracts.<sup>[26]</sup> Cardiac glycoside has been found in all of the extracts, but it is not discovered in the petroleum ether extract or the DCM extract. On the other hand, carbohydrates are not present in any of the extracts of the *N. nouchali* flower, except for the petroleum ether extract.<sup>[27]</sup> The analysis of *N. nouchali* flower extracts reveals a fascinating composition that underscores the plant's potential therapeutic properties. Notably, all of the extracts had alkaloids in them. Alkaloids are a group of compounds that have many biological effects. Petroleum ether, dichloromethane, and trichloromethane (TCM) extracts, on the other hand, did not contain any cardiac glycosides.<sup>[28]</sup> The only ones that did were those made from petroleum ether and DCM. This suggests that they may be important in pharmaceutical applications. Interestingly, carbohydrates appeared solely in the petroleum ether extract, suggesting a unique profile that could influence the overall functionality of these extracts. All of these results shed light on the complicated chemical make-up of *N. nouchali* flowers and open the door for more research into their potential medical uses.<sup>[29-30]</sup>

It shows that the plant constituents which may have elevated level of restorative qualities and could be committed for the adaptable supportive properties of the herbal. These optional metabolites are known to be organically dynamic and assume noteworthy functions in bioactivity of therapeutic plants, on the grounds that the restorative estimations of restorative plant lies in these phytochemical mixes which produce a distinct and explicit activity on the human body.<sup>[34-35]</sup> The presence of saponins, phenols and alkaloids could present anti-toxin property on the plant. The characteristic propensity of saponins toward off microorganisms makes them great possibility for treatment of contagious and yeast impurities.<sup>[36]</sup> As known in usually the application of this herbal at vicinity entities as "quit draining and recuperating wounds", analysts were seen during *in-vitro* phytochemical screening the saponin content in the plant may be high in the leaves of this plant. Since, the herbal saponins by and large assistance people to battle contagious contaminations, battle microorganisms and infections and take out some tumor cells, especially lung and blood malignancies.<sup>[37]</sup> It also helps in lowering of cholesterol level in blood, therefore diminishing heart issue at any rate the most animating and marvelous open doors for saponins, in the manner by which they stifle and execute infection cells. Hence, the phytochemical shorting consequence reveals that the occurrence of these phytochemical components supports the usage of water lily herbal in traditional medications and almost certainly, these phytochemicals are subject for the retouching quality of this

**Table 1:** Qualitative phytochemical analysis of *N. nouchali* flower.<sup>[38]</sup>

| S. No. | Phytochemicals    | Pet. Ether | DCM | TCM | ACE | EA | EtOH | MeOH | Water |
|--------|-------------------|------------|-----|-----|-----|----|------|------|-------|
| 1      | Alkaloids         | A          | A   | A   | P   | P  | P    | P    | P     |
| 2      | Flavonoids        | P          | P   | P   | P   | P  | P    | P    | P     |
| 3      | Terpenoids        | P          | P   | P   | P   | P  | P    | P    | P     |
| 4      | Saponin           | P          | P   | P   | P   | P  | P    | P    | P     |
| 5      | Tannins           | A          | A   | A   | A   | A  | P    | P    | P     |
| 6      | Phenol            | P          | P   | P   | P   | P  | P    | P    | P     |
| 7      | Sterol            | P          | P   | P   | P   | P  | P    | P    | P     |
| 8      | Cardiac glycoside | A          | A   | P   | P   | P  | P    | P    | P     |
| 9      | Carbohydrates     | A          | P   | P   | P   | P  | P    | P    | P     |
| 10     | Protein           | P          | P   | P   | P   | P  | P    | P    | P     |
| 11     | Anthocyanine      | P          | P   | P   | P   | P  | P    | P    | P     |
| 12     | Phlobatannins     | A          | A   | A   | A   | A  | A    | A    | A     |
| 13     | Quinone           | A          | A   | A   | A   | A  | A    | A    | A     |
| 14     | Oxalate           | A          | A   | A   | A   | A  | A    | A    | A     |

**Note:** P= present; A= absent; Pet. Ether= petroleum ether; DCM= di-chloro methane; TCM= trichloro methane; ACE= acetone; EA= ethyl acetate; EtOH= ethyl alcohol; MeOH= methyl alcohol.

herbal which have been affirmed by the social orders in the assessment region. It is recommended that the proportion of each phytochemical part ought to be resolved.

### 3.2 Quantitative phytochemical analysis

The pursuit of quantitative phytochemical analysis is a critical endeavor in the fields of plant chemistry and health sciences, providing invaluable knowledge about the complex nature of plant-derived compounds. Through detailed and systematic analysis, researchers can uncover the diverse benefits these phytochemicals provide, thereby expanding their applications in areas such as pharmaceuticals, functional foods, and nutrition. Moreover, this analytical approach is essential for identifying potential health risks linked to specific compounds, ensuring consumer safety and informed use. By bridging botanical research with public health initiatives, quantitative phytochemical analysis not only deepens our understanding of plant bioactives but also reinforces efforts to enhance human well-being in a constantly evolving environmental and healthcare landscape.<sup>[39]</sup>

#### 3.2.1 Extraction yield

The extraction yield analysis of *Nymphaea nouchali* flowers revealed that water produced the highest percentage yield, while petroleum ether yielded the lowest. This suggests that water is a significantly more effective solvent for extracting bioactive compounds from *N. nouchali* flowers compared to petroleum ether. These results demonstrate the value of solvent selection, as it directly impacts the efficiency of the extraction process. In this study, water emerged as the most suitable medium for maximizing the yield of valuable phytochemicals. The extraction yields and their corresponding

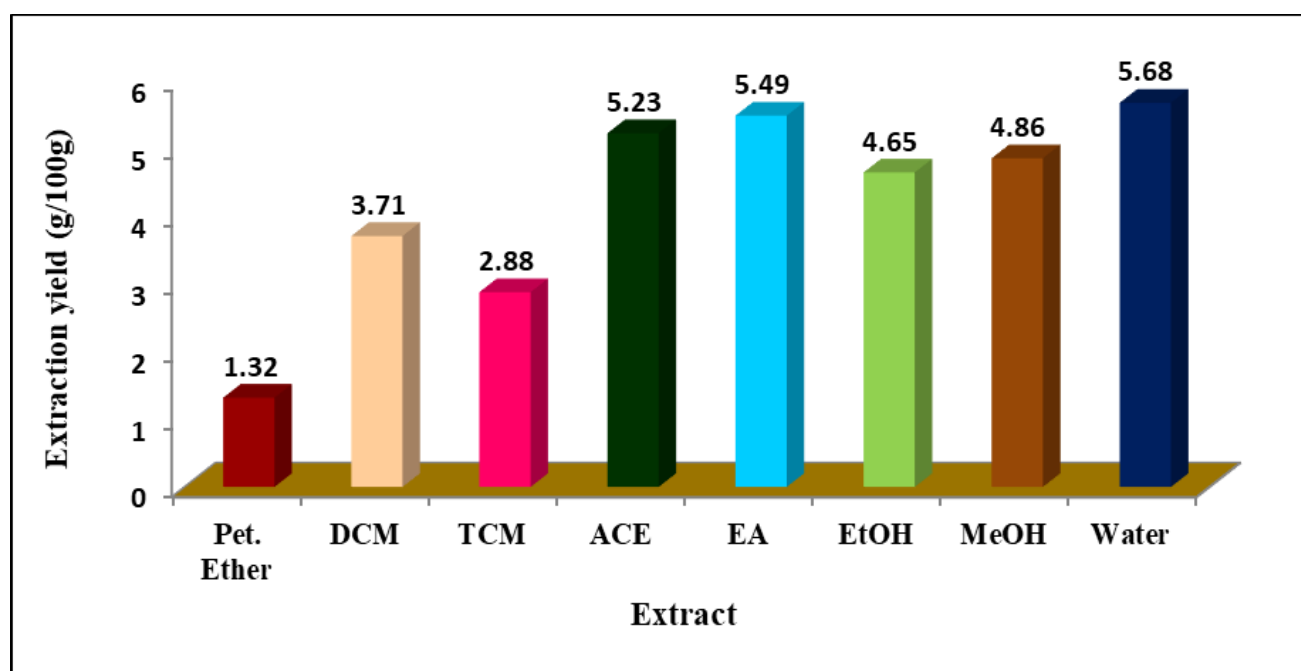
colorations are presented in Table 2 and Fig. 2, respectively.

The significance of extraction yield in plant extracts cannot be overstated, as it directly impacts both the economic viability and the therapeutic potential of these natural products. A high extraction yield means that more bioactive compounds are extracted, which not only makes herbal medicines more effective but also makes the best use of resources. Factors such as solvent choice, extraction methods, and environmental conditions play crucial roles in determining yield levels, necessitating a careful approach to optimize these variables. Moreover, understanding the relationship between extraction yield and the quality of active constituents is vital for researchers and industry professionals alike. As the demand for sustainable and effective natural remedies grows, prioritizing optimal extraction methods will be essential in harnessing the full benefits of plant-derived substances while promoting environmental stewardships. Thus, focusing on maximizing extraction yields is not merely a technical challenge; it is essential for advancing both scientific research and practical applications in health and wellness.<sup>[40]</sup> The evaluation of *N. nouchali* flower extraction yields reveals a compelling narrative about the interplay between solvent choice and extraction efficiency. The analysis clearly demonstrates that water emerges as the most effective solvent, yielding the highest extraction rates, while petroleum ether falls short with the lowest yields. This difference shows how important it is to choose the right solvent to get the best extraction results, emphasizing both the chemical features of solvents and their real-world effects in plant extractions. So, choosing the right solvent is very important for improving the amount we can extract, which helps us use *N. nouchali* more effectively in different uses.

**Table 2:** Extraction yield of *N. nouchali* flower.

| S. No. | Extract    | Coloration    | Extraction yield (g/100g) |
|--------|------------|---------------|---------------------------|
| 1      | Pet. Ether | Light brown   | 1.32                      |
| 2      | DCM        | Maroon        | 3.71                      |
| 3      | TCM        | Light yellow  | 2.88                      |
| 4      | ACE        | Pale Yellow   | 5.23                      |
| 5      | EA         | Earthy        | 5.49                      |
| 6      | EtOH       | Rusty brown   | 4.65                      |
| 7      | MeOH       | Reddish brown | 4.86                      |
| 8      | Water      | Green brown   | 5.68                      |

**Note:** Pet. Ether= petroleum ether; DCM= di-chloro methane; TCM= trichloro methane; ACE= acetone; EA= ethyl acetate; EtOH= ethyl alcohol; MeOH= methyl alcohol.

**Fig. 2:** Extraction yield analysis of *N. nouchali* flower.

### 3.2.2 Total phenolic content

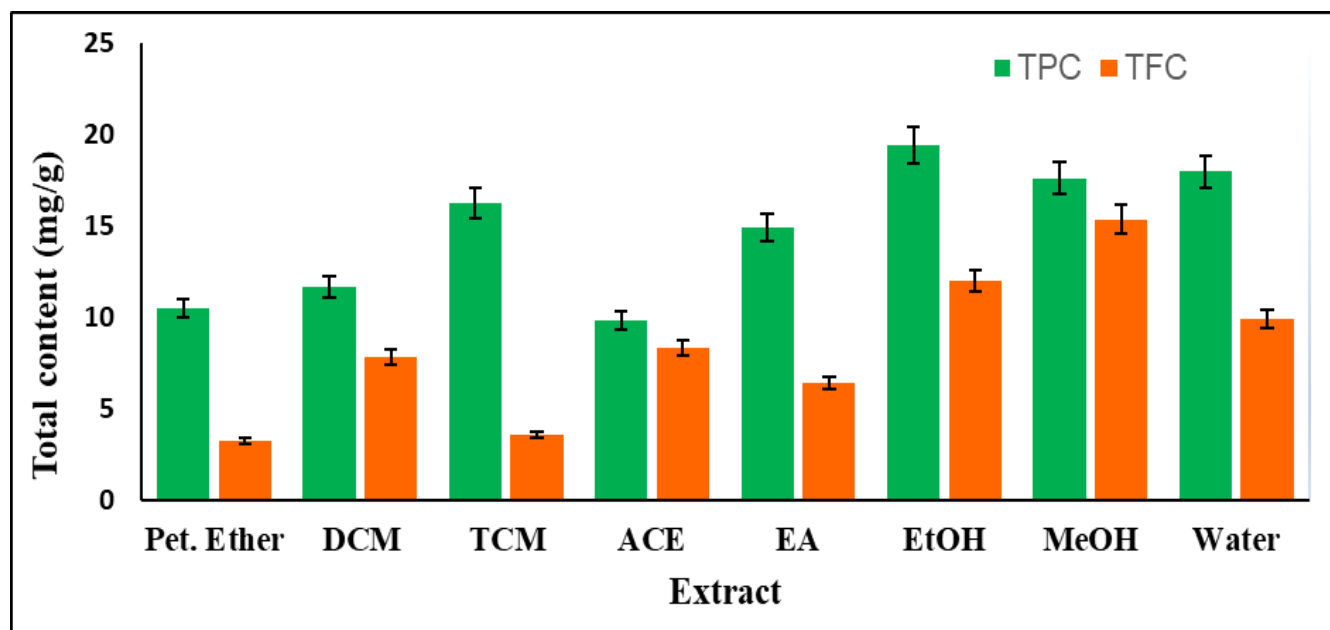
The total phenolic content analysis of *N. nouchali* flower indicated that water extract had the greater amount 17.95mg/GAE/g, whereas acetone extract had the lower amount 9.82mg/GAE/g per extract. This finding suggests that the water extract of *N. nouchali* flowers is more effective in extracting phenolic compounds compared to the acetone extract. Consequently, the higher phenolic content may indicate greater potential health benefits associated with the water extract. The study of phenolic content in the *N. nouchali* flower shows important information about its possible health benefits, especially in its ethyl alcohol extract, which contains a high level of  $19.39 \pm 0.07$  mg/GAE/g, compared to the acetone extract's  $9.82 \pm 0.05$  mg/GAE/g. This notable difference underscores the importance of extraction methods in maximizing the bioactive compounds present in this plant. The higher phenolic content not only highlights *N. nouchali*'s nutritional value but also suggests its promising role in promoting health and wellness. As research continues to explore the myriad ways these compounds can contribute to

human health, *N. nouchali* stands out as a botanical resource worthy of further investigation and appreciation. Total phenolic content results are given in Table 3 and Fig. 3, respectively. The significance of *N. Nouchali* flower's phenolic content extends far beyond its captivating beauty, revealing a treasure trove of health benefits and applications. Rich in antioxidants, these phenolic compounds play a crucial role in combating oxidative stress and may contribute to the prevention of chronic diseases. Furthermore, their potential anti-inflammatory properties highlight the flower's relevance in traditional medicine and modern therapeutic practices alike. As research continues to unveil the myriad ways in which these compounds can enhance human health, it becomes increasingly clear that the *N. Nouchali* flower is not merely a visual delight but a valuable resource deserving of further exploration and appreciation in both botanical and pharmaceutical fields. Embracing its potential could lead to innovative developments that bridge nature and wellness, marking a significant step toward harnessing the power of our natural environment for improved health outcomes.<sup>[41-42]</sup>

**Table 3:** Quantitative phytochemical analysis of *N. nouchali* flower.

| S. No. | Extract    | TPC (mg/GAE/g) | TFC (mg/QE/g) |
|--------|------------|----------------|---------------|
| 1      | Pet. Ether | 10.48±0.07     | 3.19±0.05     |
| 2      | DCM        | 11.67±0.05     | 7.81±0.2      |
| 3      | TCM        | 16.24±0.06     | 3.56±.06      |
| 4      | ACE        | 9.82±0.05      | 8.29±0.09     |
| 5      | EA         | 14.88±0.04     | 6.41±0.09     |
| 6      | EtOH       | 19.39±0.07     | 11.99±0.07    |
| 7      | MeOH       | 17.57±0.06     | 15.34±0.07    |
| 8      | Water      | 17.95±0.06     | 9.89±0.08     |

**Note:** GAE= gallic acid equivalent; QE= quercetin equivalent; mg= milligram, g= gram; Pet. Ether= petroleum ether; DCM= di-chloro methane; TCM= trichloro methane; ACE= acetone; EA= ethyl acetate; EtOH= ethyl alcohol; MeOH= methyl alcohol.

**Fig. 3:** TPC and TFC analysis of *N. nouchali* flower.

### 3.2.3 Total flavonoid content

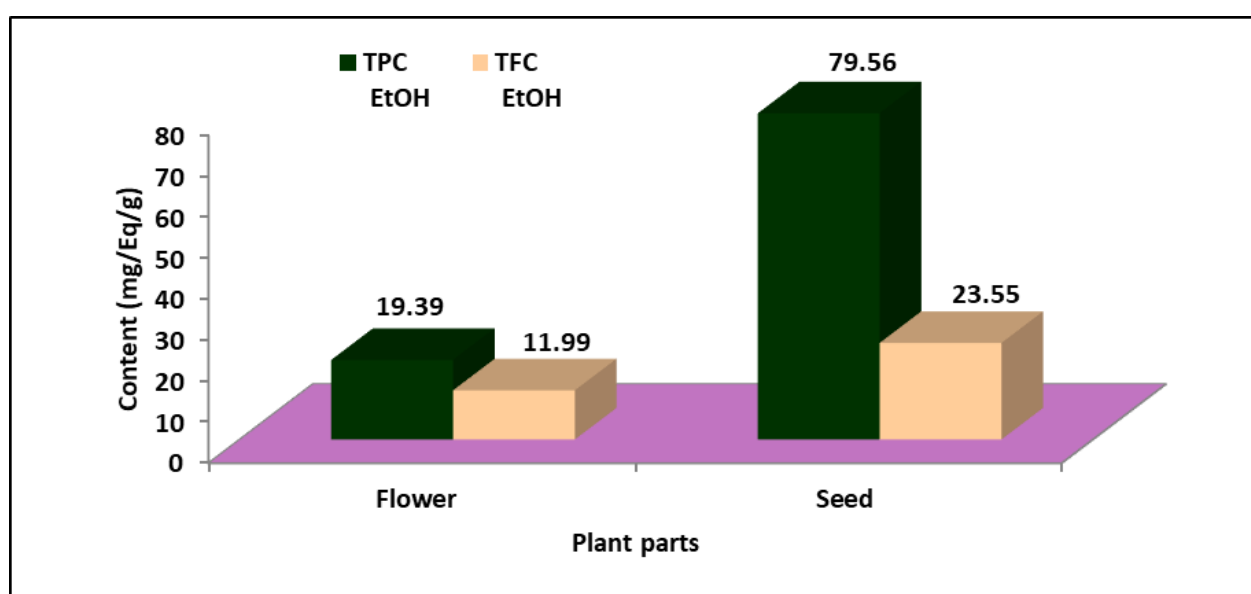
The analysis of total flavonoid content in *Nymphaea nouchali* flower extracts revealed that methanol yielded the highest flavonoid concentration at 15.34±0.07 mg QE/g, whereas petroleum ether yielded the lowest at 3.19±0.05 mg QE/g. This finding demonstrates that methanol is significantly more effective than petroleum ether in extracting flavonoids from *N. nouchali* flowers. As a result, methanol emerges as the preferred solvent for studies aiming to maximize flavonoid yield. These results highlight the critical role of solvent selection in phytochemical research and suggest that future studies should prioritize extraction methods that enhance flavonoid recovery. Such an approach may lead to a deeper understanding of the health-promoting properties and potential applications of flavonoids. Table 3 and Fig. 3 present the detailed flavonoid content data, respectively. The exploration of *N. Nouchali* flower's flavonoid content underscores its vital role in promoting health and well-being, highlighting the flower's potential as a natural source of

antioxidants. The rich array of flavonoids present not only contributes to the flower's vibrant color but also offers significant benefits in combating oxidative stress and inflammation. Additionally, studying its phytochemical properties shows that it could be used in nutraceuticals and functional foods in a beneficial way.<sup>[42]</sup> This leads to the need for more research into environmentally friendly ways to grow it so that these benefits can be fully realized. As awareness grows regarding the importance of plant-based compounds in healthcare, *N. Nouchali* stands out as an extraordinary example of nature's ingenuity, urging us to revive traditional uses while paving the way for innovative approaches in modern medicine. This multifaceted significance emphasizes that valuing biodiversity is essential for unlocking new therapeutic possibilities and enhancing our overall quality of life.<sup>[43,44]</sup>

Our findings were compared with previously reported data from different parts of *Nymphaea nouchali*. The total phenolic content (TPC) and total flavonoid content (TFC) observed in

**Table 4:** Comparative analysis between our finding data and reported data to various another plant parts.

| S. No. | Content        | Extract | Flower (Our findings) | Seed <sup>[45]</sup> | Bulb <sup>[46]</sup> | Leaf <sup>[47]</sup> |
|--------|----------------|---------|-----------------------|----------------------|----------------------|----------------------|
| 1      | TPC (mg/GAE/g) | EtOH    | 19.39±0.07            | 179.56               | -                    | -                    |
|        | TFC (mg/QE/g)  | EtOH    | 11.99±0.07            | 23.55                | -                    | -                    |
| 2      | TPC (mg/GAE/g) | MeOH    | 17.57±0.06            | -                    | 108.23               | -                    |
|        | TFC (mg/QE/g)  | MeOH    | 15.34±0.07            | -                    | 9.34                 | -                    |
| 3      | TPC (mg/GAE/g) | EtOH    | 19.39±0.07            | -                    | -                    | 6.53                 |
|        | TFC (mg/QE/g)  | TCM     | 3.56±0.06             | -                    | -                    | 5.55                 |

**Fig 4:** Comparative analysis between flower and seed of *N. nouchali*.

our study were notably lower than those reported in the seeds. This indicates a variation in phytochemical composition among different plant parts, with seeds containing higher concentrations of these bioactive compounds. Such differences may influence the nutritional and medicinal value of each section, highlighting the importance of plant part selection in phytochemical and therapeutic research (Table 4 and Fig. 4, respectively).

The comparison of bulb and flower data reveals that the bulb's total phenolic content (TPC) value is considerably high at 108 mg/GAE/g, in contrast to our reported value of 17.57±0.06 mg/GAE/g. Conversely, the bulb's total flavonoid content (TFC) value is lower at 9.34 mg/QE/g compared to our reported value of 15.34±0.07 mg/QE/g. This suggests that while the bulb has a significantly higher total phenolic content, its total flavonoid content is comparatively lower. These discrepancies indicate a need for further investigation into the factors that may influence the variations in phytochemical composition (Table 4 and Fig 5, respectively).

A comparison between our findings and previously

reported data on the leaf part of *Nymphaea nouchali* revealed distinct differences in phytochemical content. The flower exhibited a higher total phenolic content (19.39±0.07 mg GAE/g) compared to the leaf (6.53 mg GAE/g). In contrast, the leaf showed a greater total flavonoid content (5.55 mg QE/g) than the flower (3.56±0.06 mg QE/g). These results suggest that the flower is richer in phenolic compounds, while the leaf contains more flavonoids, indicating that different parts of *N. nouchali* have unique phytochemical profiles. Such variation may influence their respective biological activities and potential applications in herbal medicine (Table 4 and Fig. 6, respectively).

### 3.3 In-vitro antioxidant assay

All the *in-vitro* antioxidant activities of *N. nouchali* flower have been completed to follow the standard procedure. These actions have revealed that the *N. nouchali* flowers are very effective and useful plants for curing oxidative cell damage. All the *in-vitro* antioxidant activity analysis findings (IC<sub>50</sub> values) are given in Table 5 and Fig. 7 respectively.

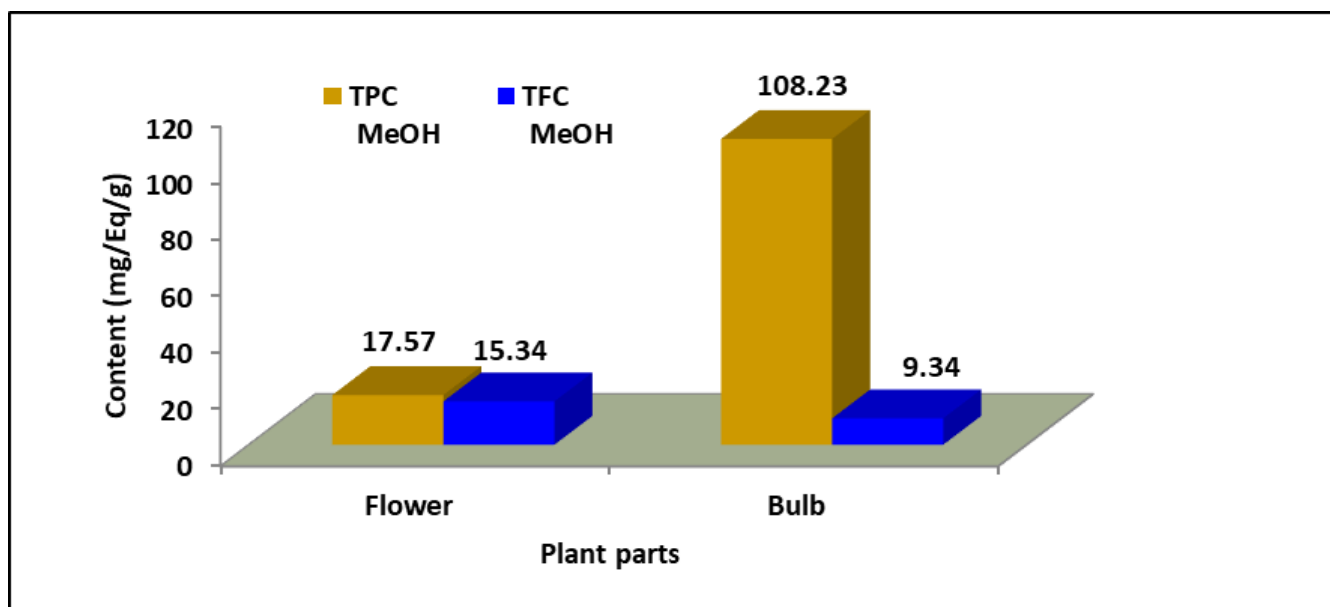


Fig 5: Comparative analysis between flower and bulb of *N. nouchali*.

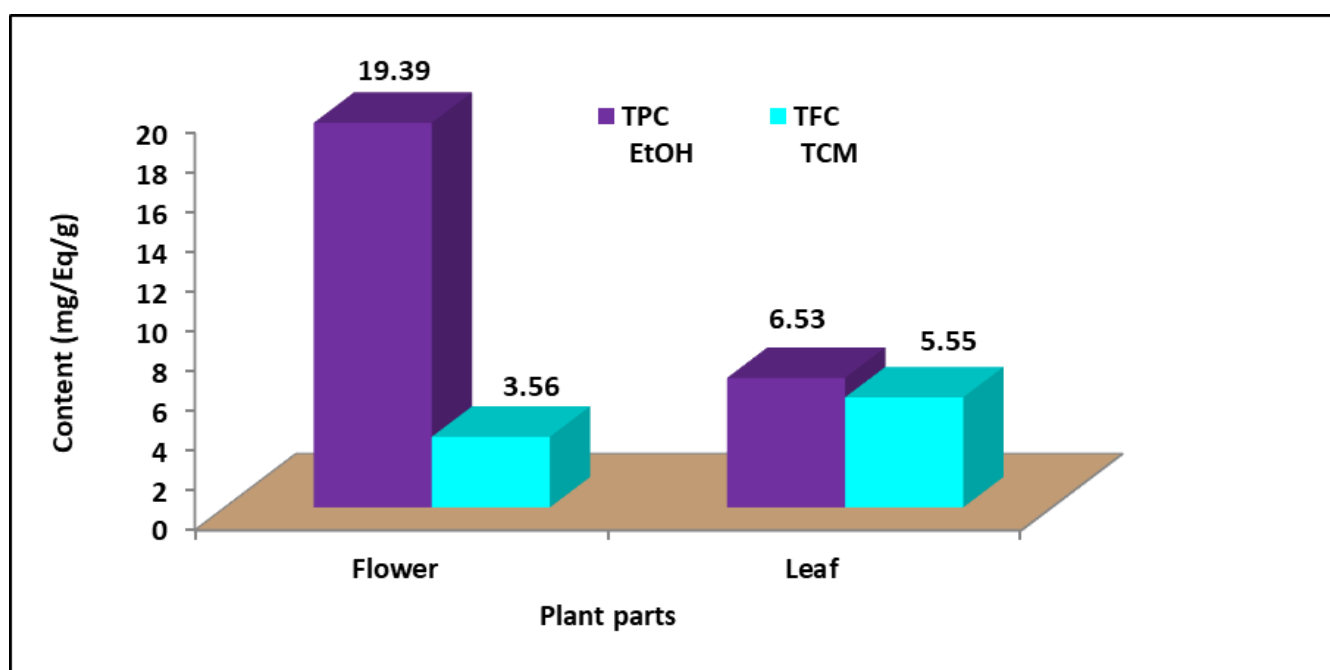


Fig 6: Comparative analysis between flower and leaf of *N. nouchali*.

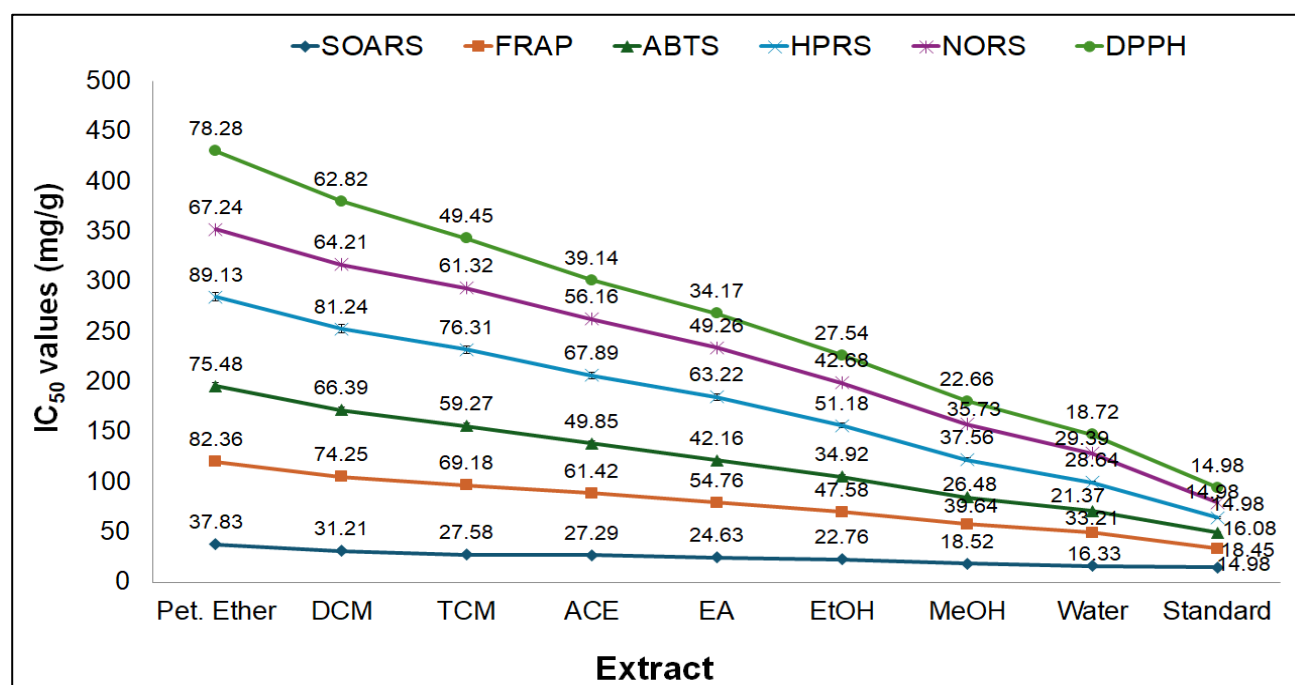
The antioxidant potential of different solvent extracts of *N. nouchali* flower was evaluated using multiple *in-vitro* assays, namely FRAP, HPRS, ABTS, and SOARS, and the results are expressed as  $IC_{50}$  values (mg/g) in Table 5. A lower  $IC_{50}$  value corresponds to higher antioxidant activity. The graphical representation of radical scavenging assays is illustrated in Fig. 7. From Table 5, a clear trend is observed in which antioxidant activity increases with solvent polarity. The petroleum ether extract exhibited the highest  $IC_{50}$  values across all assays (FRAP:  $82.36 \pm 1.12$  mg/g; HPRS:  $89.13 \pm 0.77$  mg/g; ABTS:  $75.48 \pm 1.05$  mg/g; SOARS:  $37.83 \pm 0.67$  mg/g), indicating comparatively weak antioxidant potential. Similarly, dichloromethane (DCM) and trichloromethane (TCM) extracts also showed higher  $IC_{50}$  values, reflecting

moderate activity. In contrast, polar solvent extracts demonstrated significantly improved antioxidant capacity. The ethyl acetate (EA), ethanol (EtOH), methanol (MeOH), and aqueous extracts showed progressively lower  $IC_{50}$  values. Among these, the aqueous extract exhibited strong antioxidant activity (FRAP:  $33.21 \pm 0.94$  mg/g; HPRS:  $28.64 \pm 1.96$  mg/g; ABTS:  $21.37 \pm 0.89$  mg/g; SOARS:  $16.33 \pm 1.97$  mg/g), closely followed by methanol extract. This pattern suggests that bioactive compounds with antioxidant properties, such as phenolics and flavonoids, are more efficiently extracted in polar solvents. The standard sample (SS) showed the lowest  $IC_{50}$  values in all assays (FRAP:  $18.45 \pm 0.52$  mg/g; HPRS:  $14.98 \pm 0.66$  mg/g; ABTS:  $16.08 \pm 0.47$  mg/g; SOARS:  $14.98 \pm 0.66$  mg/g), confirming its strong antioxidant efficacy

**Table 5:** IC<sub>50</sub> values (mg/g) analysis of *N. nouchali* flower.

| S. No. | Extract    | HPRS       | ABTS       | FRAP       | SOARS      | NORS <sup>[38]</sup> | DPPH <sup>[38]</sup> |
|--------|------------|------------|------------|------------|------------|----------------------|----------------------|
| 1      | Pet. Ether | 89.13±0.77 | 75.48±1.05 | 82.36±1.12 | 37.83±0.67 | 67.24±0.06           | 78.28±0.73           |
| 2      | DCM        | 81.24±0.98 | 66.39±1.21 | 74.25±1.34 | 31.21±0.91 | 64.21±0.1            | 62.82±2.54           |
| 3      | TCM        | 76.31±0.78 | 59.27±1.42 | 69.18±1.08 | 27.58±0.5  | 61.32±0.63           | 49.45±1.26           |
| 4      | ACE        | 67.89±1.77 | 49.85±1.88 | 61.42±1.96 | 27.29±1.05 | 56.16±1.07           | 39.14±0.89           |
| 5      | EA         | 63.22±3.04 | 42.16±2.03 | 54.76±2.11 | 24.63±0.97 | 49.26±1.06           | 34.17±0.98           |
| 6      | EtOH       | 51.18±0.63 | 34.92±1.56 | 47.58±1.27 | 22.76±1.01 | 42.68±0.9            | 27.54±1.57           |
| 7      | MeOH       | 37.56±0.39 | 26.48±1.11 | 39.64±1.02 | 18.52±1.98 | 35.73±0.58           | 22.66±1.03           |
| 8      | Water      | 28.64±1.96 | 21.37±0.89 | 33.21±0.94 | 16.33±1.97 | 29.39±0.89           | 18.72±2.55           |
| 9      | SS         | 14.98±0.66 | 16.08±0.47 | 18.45±0.52 | 14.98±0.66 | 14.98±0.66           | 14.98±0.66           |

**Note:** Pet. Ether= petroleum ether; DCM= di-chloro methane; TCM= trichloro methane; ACE= acetone; EA= ethyl acetate; EtOH= ethyl alcohol; MeOH= methyl alcohol; SS= Standard solution. ABTS = 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation scavenging assay; SOARS (Superoxide anion radical scavenging assay); FRAP = (Ferric reducing antioxidant power assay); HPRS = Hydrogen peroxide radical scavenging assay, NORS= Nitric Oxide Radical Scavenging assay; DPPH= 2,2-diphenyl-1-picrylhydrazyl assay.

**Fig. 7:** SOARS, FRAP, ABTS, HPRS, NORS and DPPH analysis of *N. nouchali* flower.

and serving as a reference for comparison. Among the assays, ABTS and SOARS demonstrated relatively lower IC<sub>50</sub> values compared to FRAP and HPRS, indicating higher sensitivity toward radical scavenging activity. This is further supported by Fig. 7, where a consistent decrease in IC<sub>50</sub> values from non-polar to polar extracts is visually evident across all tested models.

We have reported earlier in our previous work about NORS and DPPH antioxidant assay of *N. nouchali*.<sup>[38]</sup> The antioxidant potential observed for *N. nouchali* is comparable with other *Nymphaea* species, where *Nymphaea lotus* exhibits strong DPPH radical scavenging activity with low IC<sub>50</sub> values (8.35-8.96 µg/mL).<sup>[48]</sup> while *Nymphaea alba* also demonstrates significant antioxidant efficacy across multiple assays, including DPPH and hydroxyl radical scavenging

methods.<sup>[49,50]</sup> In comparison, *Nymphaea nouchali* exhibited stronger and more consistent antioxidant activity across multiple assays, including FRAP, ABTS, HPRS, and SOARS, particularly in polar extracts. While FRAP assay showed that *Nymphaea lotus* petal extracts possess considerable ferric reducing antioxidant capacity, with values reported around 1.24-1.31, indicating effective electron-donating potential.<sup>[48]</sup> In contrast, *Nymphaea alba* evaluated through the hydroxyl radical scavenging assay exhibited IC<sub>50</sub> values ranging from 16.21 to 72.25, reflecting moderate to strong radical quenching activity depending on the extract type.<sup>[50]</sup> These results collectively demonstrate that both species exhibit assay-dependent antioxidant behavior, with reducing power prominent in *Nymphaea lotus* and radical scavenging efficiency evident in *Nymphaea alba*.<sup>[48,50]</sup> Overall, the results

indicate that *N. nouchali* flower possesses significant antioxidant potential, particularly in polar extracts, and the activity is assay-dependent but follows a consistent extraction polarity trend. The findings suggest that antioxidant activity is a common characteristic within the *Nymphaea* genus, with variations primarily influenced by solvent polarity and phytochemical composition.

It is important to note that a higher IC<sub>50</sub> value indicates weaker antioxidant activity, as IC<sub>50</sub> is inversely related to the compound's inhibition efficiency. Based on this context, the water extract exhibited the highest antioxidant capacity, whereas petroleum ether showed the weakest. The comparison of antioxidant activities among various extracts clearly demonstrates that water is the most effective solvent for extracting antioxidant-rich compounds from *Nymphaea nouchali* flowers.<sup>[51-52]</sup> This superiority highlights the effectiveness of water as a solvent for extracting beneficial compounds, while the low antioxidant capacity of petroleum ether underscores its limitations in this context. The inverse relationship between IC<sub>50</sub> values and antioxidant effectiveness indicates that higher IC<sub>50</sub> values correspond to weaker free radical scavenging activity. Taken together, these findings emphasize the importance of selecting appropriate extraction methods to maximize health benefits. They also support further research into the potential health-promoting applications of water-based extracts.<sup>[53]</sup> The antioxidant effects of *N. nouchali* flowers in a lab setting opens up a new area of study that could be useful for both science and medicine. It has been shown through careful testing that these flowers have powerful antioxidants that can fight oxidative stress and the health problems that come with it.<sup>[54]</sup> The phytochemical composition identified in the flowers highlights a rich array of bioactive compounds, reinforcing their potential role in promoting health and wellness. As the demand for natural remedies continues to rise, these findings on *Nymphaea nouchali* not only enhance our understanding of plant-based antioxidants but also encourage further investigation into their mechanisms of action and potential benefits for human health. Ultimately, this study lays the groundwork for leveraging nature's resources in developing effective strategies against oxidative stress, emphasizing the value of integrating traditional knowledge with modern scientific approaches.<sup>[55]</sup>

#### 4. Conclusion

The present study provides a systematic phytochemical and antioxidant evaluation of the flower extracts of *Nymphaea nouchali* using a combination of qualitative screening, quantitative phytochemical estimation, and multiple *in-vitro* antioxidant assays. The results confirmed the presence of several biologically active secondary metabolites, including phenolics, flavonoids, terpenoids, saponins, sterols, and alkaloids, indicating the rich phytochemical diversity of the floral matrix. Quantitative analysis revealed that ethanol and methanol extracts possessed comparatively higher total phenolic and flavonoid contents, while aqueous extracts

exhibited the strongest antioxidant performance in ABTS, FRAP, HPRS, and SOARS assays, as reflected by lower IC<sub>50</sub> values. The observed antioxidant activity showed a clear dependence on solvent polarity, suggesting that polar solvents are more efficient in extracting radical-scavenging phytochemicals. A key novelty of this study lies in the comprehensive comparative assessment of solvent-dependent phytochemical yield and multi-assay antioxidant capacity of *N. nouchali* flower extracts, supported by a comparative analysis with previously reported phytochemical data from other plant parts. The findings provide new insight into the biochemical potential of the floral component, which has been relatively less explored compared with other parts of the plant. Overall, the results highlight the potential of *N. nouchali* flowers as a promising natural source of antioxidant compounds with possible applications in nutraceutical, pharmaceutical, and functional food development. Further research involving compound isolation, advanced analytical profiling, and *in-vivo* validation is recommended to explore its potential applications in natural drug development and integrative healthcare.

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#### Conflict of Interest

There is no conflict of interest.

#### Supporting Information

Not applicable.

#### CRedit Statement

**Sanjoy Kumar Bera, Nand Kumar Kashyap, and Tomeshwar Prasad:** Contributed to the methodology, Analysis, Data generation and Data validation, Discussing the results and drafted the paper. **Harishankar Dewangan and Manisha Masih Singh:** Contributed to the analysis, Data generation and data validation and Discussing the results. **Birjesh Singh and Milan Hait:** Conceptualized the research work, Scrutinized the analysis of the results, Supervised the project, Reviewed the findings and Provided critical insights into manuscript preparation, Final editing and Communicating for publication.

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