

ASSESSMENT OF ANTIOXIDANT, ANTIMICROBIAL, ANTIPARASITIC,
AND ANTI-INFLAMMATORY PROPERTIES OF DRIED *NELUMBO*
NUCIFERA

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Abstract

Nelumbo nucifera is an important aquatic medicinal plant known for its rich phytochemical composition and diverse pharmacological properties. The present study aimed to investigate the influence of different post-harvest drying techniques on the phytochemical profile, proximate composition, and biological activities of *N. nucifera*. Fresh plant samples were subjected to various drying methods, including oven drying, sun drying, freeze drying, and shade drying, followed by extraction using methanol, chloroform, and aqueous solvents. Qualitative phytochemical screening revealed the presence of several bioactive secondary metabolites, including phenols, flavonoids, alkaloids, terpenoids, tannins, saponins, glycosides, and steroids, with methanolic extracts showing the

highest phytochemical diversity. Proximate analysis demonstrated significant variations among

drying treatments, where oven-dried samples exhibited the highest protein (34.90%) and fiber contents (24.02%), while freeze-dried samples retained higher carbohydrate (17.49%) and fat contents (8.49%). The antioxidant activity evaluated by the DPPH assay showed concentration-dependent radical scavenging activity, with the highest inhibition ($81.4 \pm 1.8\%$) observed at 500 $\mu\text{g/mL}$. Antiparasitic activity assessed through the brine shrimp lethality assay demonstrated significant dose-dependent mortality, reaching $86.7 \pm 1.7\%$ at 500 $\mu\text{g/mL}$. The methanolic extract also exhibited considerable anti-inflammatory activity by inhibiting protein denaturation, with maximum inhibition of $78.2 \pm 1.9\%$ at 500 $\mu\text{g/mL}$. Furthermore, the extract displayed notable antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*, with greater effectiveness against Gram-positive bacteria. Significant antifungal activity was also observed against *Candida albicans*, *Aspergillus niger*, *Aspergillus flavus*, and *Fusarium solani*. The findings suggest that drying techniques significantly influence the nutritional composition and bioactive potential of *N. nucifera*. Among the evaluated methods, freeze-drying and oven-drying showed superior preservation of nutritional and phytochemical constituents. Overall, *N. nucifera* represents a promising natural source of bioactive compounds with potential pharmaceutical, nutraceutical, antimicrobial, antioxidant, anti-inflammatory, and antiparasitic applications.

1. INTRODUCTION

Aquatic macrophytes are an interesting and little-exploited source of biologically active compounds of high nutritional, pharmaceutical, and environmental value (Márquez-Rangel et al., 2026). In these, *N. nucifera* Gaertn., commonly called lotus, is an important aquatic perennial plant, which is widely distributed in freshwater ecosystems of the tropical and subtropical regions (Kumar et al., 2025). It is a plant of great value in traditional medicine, with a wide range of pharmacological properties and high nutritional value. Leaves, flowers, rhizomes, and seeds of *N. nucifera* have been widely used as herbs to treat inflammation, microbial infections, oxidative stress-induced diseases, and metabolic diseases. Recent studies indicate that *N. nucifera* contains various bioactive secondary metabolites,

including phenolics, flavonoids, alkaloids, tannins, terpenoids, and steroids, which are responsible for its antioxidant, antimicrobial, anti-inflammatory, and cytotoxic properties (Vijayan et al., 2025). Environmental factors, water quality, and post-harvest processing can significantly affect the concentration of these phytochemicals and their biological activity, as their biosynthesis and accumulation depend strongly on these factors. Processing methods, especially drying techniques, are important in preserving the bioactivity, stability, and quality of plant metabolites.

Fresh plant material usually has a high moisture content, making it have a short to long shelf life and high biochemical degradation. The various drying processes (oven drying, air drying, and freeze drying) can cause significant differences in the proximate composition (such as ash, fibre, protein, fat, carbohydrates), which may affect phytochemical retention and extraction (Mina et al., 2025). Freeze-drying is regarded as very successful for the preservation of thermolabile substances, while oven-drying might enhance the release of some bound metabolites by breaking the plant tissues. In addition to phytochemical composition, mineral profiling is essential for evaluating the nutritional and therapeutic value of aquatic plants. Essential macro- and micro-elements such as potassium, magnesium, calcium, phosphorus, iron, and sodium are involved in numerous physiological and metabolic functions. As an aquatic species, *N. nucifera* has the ability to absorb and accumulate minerals from its surrounding environment, making it a potentially valuable source of nutritional elements and an indicator of aquatic ecosystem quality (Elbagory et al., 2025).

Furthermore, the biological activities of *N. nucifera* extracts, particularly antimicrobial and cytotoxic properties, have attracted considerable attention in the search for novel natural therapeutics (Shi et al., 2025). The increasing prevalence of antimicrobial resistance has intensified interest in plant-derived antimicrobial agents, while naturally occurring cytotoxic compounds are being explored for their potential applications in cancer research and parasitic disease management (Al-Arnoot et al., 2025). Despite the medicinal importance and wide distribution of *N. nucifera*, comprehensive studies integrating proximate analysis, phytochemical profiling, mineral composition, and biological activities under different drying conditions remain limited.

Therefore, the present study aims to systematically investigate the effect of different drying methods on the proximate composition, phytochemical profile, and mineral content of *N. nucifera*, along with evaluating its antimicrobial and cytotoxic potential. By integrating chemical characterization with biological assessment, this study provides a comprehensive understanding of the functional properties of *N. nucifera* and highlights its potential as a promising natural source of bioactive compounds for nutritional, pharmaceutical, and biomedical applications.

2. Materials and Methods

2.1. Collecting plant materials

Fresh samples of *N. nucifera* were collected from a natural, disease-free, and uncontaminated freshwater site by choosing healthy and disease-free plants free from external damage. Samples were taken for phytochemical analysis during the active growth phase to obtain sufficient phytochemical content and biological activity. The collected samples were washed with tap water and then with distilled water to remove impurities, soil particles, and any debris. The cleaned plant materials were split into equal parts, with each part being subjected to various drying conditions such as fresh, oven dried, freeze dried, sun dried, and shade dried. Samples were labelled, packaged in sterile polyethylene bags, and cooled for transportation to the laboratory to prevent biochemical degradation before analysis. Sample size and sampling procedures were carried out in accordance with environmental sampling principles to ensure that the extraction was sustainable and would cause minimal ecological disturbance.

2.2. Qualitative Phytochemical Analysis

Qualitative Phytochemical Analysis was performed following previous standard protocol (Kabir et al., 2025). Briefly, about 50 g of each of the powdered leaf materials was macerated separately in methanol, chloroform, and distilled water for 72 h with intermittent shaking. The extracts were filtered on Whatman No. 1 filter paper and concentrated using a rotary evaporator under reduced

pressure. The concentrated extracts were dried and kept at 4°C for further phytochemical analysis. The methanolic, chloroform, and aqueous extracts of *N. nucifera* were subjected to preliminary phytochemical screening for the presence of the major secondary metabolites such as phenols, flavonoids, alkaloids, terpenoids, saponins, glycosides, tannins, and steroids using the standard phytochemical methods. The presence of colour changes or precipitate after certain reagents was taken as a positive reaction.

2.2. Proximate Analysis

The earlier standard protocol of Jabeen et al. (2019) has been used to assess the proximate analysis. The comparative proximate analysis of *N. nucifera* was performed for moisture, ash, crude fibre, protein, fat, and carbohydrate contents of fresh and processed samples using different drying methods (oven drying, sun drying, freeze drying, and shade drying) and standard methods (Fabrice et al., 2018). Various drying methods were used as the nutritional composition, physicochemical properties, and stability of bioactive components of plant materials may be considerably affected by the post-harvest processing. Both fresh and dried powdered samples were analyzed to determine changes in composition resulting from moisture removal and thermal or non-thermal processing conditions. Such comparative evaluation of these drying methods is essential in order to evaluate the effect of each method on the preservation or degradation of any heat-sensitive phytochemicals, mineral stability and biological properties of the foods. Freeze-drying is a method that is commonly used for preservation and can retain native phytochemical and nutritional properties, while sun- and oven-drying can impact some components as a result of the heat, oxidation, and exposure to light. Shade drying is a relatively gentle method that reduces the thermal degradation of the product but allows moisture to evaporate over time. Thus, assessment of these drying techniques will give a complete picture of the effects of post-harvest processing on the proximate composition and potential biological values of *N. nucifera*.

2.3. DPPH Free Radical Scavenging Assay

The antioxidant activity of *N. nucifera* extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay following previous research with slight modifications (Nawaz et al., 2025). Different concentrations of the plant extract (50–500 µg/mL) were prepared in methanol. A 0.1 mM DPPH solution was freshly prepared in methanol and protected from light. Briefly, 1 mL of each extract concentration was mixed with 1 mL of DPPH solution in test tubes. The reaction mixture was incubated in the dark at room temperature for 30 minutes. After incubation, the absorbance was measured at 517 nm using a UV-Visible spectrophotometer. Methanol with DPPH solution served as the control, while ascorbic acid was used as the standard antioxidant.

The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{A_c - A_s}{A_c} \times 100$$

Where:

A_c = absorbance of the control

A_s = absorbance of the sample

2.4. Antiparasitic Activity

The antiparasitic activity of *N. nucifera* extracts was assessed using the brine shrimp lethality bioassay following standard procedures (Nguta et al., 2013). Artificial seawater was prepared by dissolving 38 g of sea salt in 1 L of distilled water. *Artemia salina* eggs were incubated in the prepared seawater under continuous aeration and light for 24–48 h to obtain active nauplii. Plant extracts prepared from different drying treatments were dissolved in dimethyl sulfoxide (DMSO) and diluted to obtain concentrations of 50, 100, and 500 µg/mL. Fifteen nauplii were transferred into each sterile vial

containing 5 mL of seawater and the respective extract concentration. A control group containing seawater and DMSO without extract was also maintained, while potassium dichromate was used as the positive control. After incubation for 24 h at room temperature, surviving nauplii were counted, and percentage mortality was calculated using the following formula:

$$\text{Percentage Mortality (\%)} = \frac{\text{Total number of nauplii} - \text{Number of surviving nauplii}}{\text{Total number of nauplii}} \times 100$$

2.5. Anti-Inflammatory Activity

The anti-inflammatory activity of the methanolic extract of *N. nucifera* was evaluated using the protein denaturation inhibition assay (Gadakh et al., 2025). Different concentrations of the extract (50, 100, and 500 µg/mL) were prepared in methanol. A reaction mixture containing plant extract and bovine serum albumin was incubated at room temperature and then heated at 60–70°C for protein denaturation. After cooling, the absorbance was measured spectrophotometrically at 660 nm. Diclofenac sodium was used as the standard anti-inflammatory drug, while the control contained distilled water instead of the extract. The percentage inhibition of protein denaturation was calculated using the formula:

$$\% \text{ Inhibition} = \frac{A_c - A_s}{A_c} \times 100$$

where A_c is the absorbance of the control and A_s is the absorbance of the sample. All experiments were performed in triplicate, and results were expressed as mean $\pm$ standard deviation.

2.6. Antibacterial Activity

The antibacterial activity of *N. nucifera* methanolic extracts obtained from different drying treatments was evaluated using the agar well diffusion method against selected pathogenic bacterial strains, including *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*. Nutrient agar medium was prepared, sterilized, and poured into sterile Petri plates. Fresh bacterial cultures were uniformly spread over the agar surface using sterile cotton swabs. Wells of approximately 6 mm diameter were

made using a sterile cork borer, and 100 μ L of plant extract (10 mg/mL) was introduced into the respective wells. Dimethyl sulfoxide (DMSO) was used as the negative control, while ciprofloxacin served as the positive control. The inoculated plates were incubated at 37°C for 24 h, after which antibacterial activity was determined by measuring the diameter of inhibition zones (mm) around each well.

2.7. Antifungal activity

Antifungal activity was evaluated against *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans*, and *Fusarium solani* (Elgat et al., 2020). Fungal cultures were maintained on potato dextrose agar (PDA) at 28°C. Antifungal activity was determined by the agar well diffusion method using PDA plates inoculated with fungal suspensions. Wells (6 mm) were filled with 100 μ L of methanolic extract at concentrations of 25, 50, and 100 mg/mL prepared in methanol. Fluconazole (10 μ g/mL) and DMSO served as positive and negative controls, respectively. Plates were incubated at 28°C for 48–72 h, and inhibition zones were measured in mm.

2.8. Statistical Analysis

Each experiment was performed three times, and the results of each experiment were shown as mean $\pm$ standard deviation (SD). Significant differences in the differences between various drying treatments and extract concentration were tested by statistical analysis using the program GraphPad Prism/SPSS software. Significant differences among the groups were identified through one-way analysis of variance (ANOVA) and Tukey's multiple comparison test. Differences were deemed to be statistically significant at $p < 0.05$. Correlation analysis was also carried out to evaluate the relationship between the phytochemical composition and biological activities of extracts of *N. nucifera*. The experimental data were also good, as indicated by the low standard deviation values obtained throughout the study, which meant that they had good reproducibility, consistency, and reliability.

3. Results and discussion

3.1. Qualitative Phytochemical Analysis

The qualitative phytochemical analysis of *N. nucifera* leaf extracts revealed the presence of several bioactive secondary metabolites in different solvent extracts (Table 1). Among the tested extracts, the methanolic extract showed the highest diversity of phytochemicals, followed by the aqueous extract, whereas the chloroform extract exhibited comparatively lower phytochemical abundance. Phenols, flavonoids, alkaloids, and terpenoids were detected in all extracts, while saponins and glycosides were mainly observed in the methanolic and aqueous extracts. Steroids were predominantly present in the methanolic extract.

The higher phytochemical content observed in the methanolic extract may be attributed to the polar nature of methanol, which enhances the extraction efficiency of polar and moderately polar phytoconstituents (Wakeel et al., 2019). Phenolic compounds and flavonoids are widely recognized for their antioxidant and free radical scavenging activities due to their hydrogen-donating properties and ability to stabilize reactive oxygen species (ROS). Similarly, alkaloids and terpenoids are known to possess antimicrobial, anti-inflammatory, and cytotoxic activities through membrane disruption and modulation of cellular pathways (Thawabteh et al., 2019). The presence of tannins and saponins in *N. nucifera* extracts may also contribute to biological activities by interacting with proteins and microbial cell membranes. Previous studies on *N. nucifera* have reported that the plant contains various pharmacologically active compounds such as quercetin, kaempferol, catechin, and nuciferine, which are associated with antioxidant, antimicrobial, anticancer, and anti-inflammatory properties (Tungmunthum et al., 2018). Overall, the present findings suggest that *N. nucifera* leaf extracts are rich in bioactive secondary metabolites, particularly in the methanolic extract, supporting their potential use in further pharmacological and phytochemical investigations.

Table 1: Qualitative Phytochemical Analysis of *N. nucifera* extract in different solvents

Secondary Metabolites	Methanol Extract	Chloroform Extract	Aqueous Extract
Phenols	+	+	+
Flavonoids	+	+	+
Alkaloids	+	+	+
Terpenoids	+	+	+
Saponins	+	—	+
Glycosides	+	—	+
Steroids	+	—	—
Tannins	+	+	+

(+): Present, (—): Absent

3.2. Proximate analysis

The proximate composition of *N. nucifera* exhibited noticeable variations under different drying treatments compared with fresh plant material. Fresh samples showed the highest moisture content (87.94%), reflecting the aquatic nature of the plant, whereas all drying methods markedly reduced moisture levels to 6.32–9.40%, thereby enhancing storage stability and concentrating nutritional constituents. Among the processed samples, oven-dried material exhibited the highest crude fiber (24.02%) and protein content (34.90%), followed closely by shade-dried samples with 34.42% protein. Sun-dried samples also demonstrated relatively high protein content (33.50%) with moderate fiber levels (21.00%), while freeze-dried samples retained comparatively lower protein (31.45%) but the highest carbohydrate (17.49%) and fat contents (8.49%). Ash content remained relatively stable among the dried treatments, with the maximum value recorded in oven-dried

samples (14.80%), indicating increased mineral concentration following moisture removal. In contrast, fresh samples exhibited substantially lower ash (1.21%), fiber (1.32%), protein (5.93%), fat (1.57%), and carbohydrate contents (2.03%) due to dilution by high water content. All these results are tabulated in Table 2.

The findings indicate that drying methods significantly influence the proximate composition of *N. nucifera*. The substantial reduction in moisture content confirms the effectiveness of all drying techniques in stabilizing plant material and concentrating nutritional components. Oven-drying produced the highest fiber and protein contents, possibly due to heat-induced disruption of cellular structures that enhances the release and apparent concentration of structural carbohydrates and nitrogenous compounds (Yang et al., 2026). However, thermal exposure may also affect heat-sensitive metabolites, resulting in slight alterations in fat and carbohydrate contents. Freeze-drying preserved the highest levels of carbohydrates and fats, demonstrating its effectiveness in retaining thermolabile and oxidation-sensitive compounds through low-temperature dehydration under vacuum conditions. Sun-drying and shade-drying produced intermediate values, likely due to prolonged environmental exposure, light-induced oxidation, and residual enzymatic activity affecting biochemical stability (Lin et al, 2025). Overall, the results highlight the importance of post-harvest drying techniques in determining the nutritional quality and biochemical composition of *N. nucifera*.

Table 2: Effect of Drying Methods on the Proximate Nutritional Composition of *N. nucifera*

Physiochemical Contents (%)	Sun-dried	Oven-dried	Freeze-dried	Shade-dried	Fresh Sample
Moisture content	6.91	6.32	7.05	9.40	87.94
Ash content	14.12	14.80	13.44	14.14	1.21
Fiber content	21.00	24.02	22.10	19.40	1.32

Protein content	33.50	34.90	31.45	34.42	5.93
Fat content	7.95	6.92	8.49	8.47	1.57
Carbohydrate content	16.55	12.52	17.49	13.70	2.03

2.3. Biological applications

2.3.1. Antioxidant Activity

The antioxidant property of *N. nucifera* extract was tested using the DPPH free radical scavenging assay at various concentrations (50–500 µg/mL). The results showed that the radical scavenging activity exhibited a dose-dependent increase, signifying that the antioxidant activity of the extract was dose-dependent. The extract showed $28.6 \pm 1.2\%$ DPPH radical inhibition at the lowest tested concentration (50 µg/mL). The percentage inhibition gradually increased to $41.3 \pm 1.5\%$, $57.8 \pm 1.6\%$, and $69.5 \pm 1.4\%$ at concentrations of 100, 200, and 300 µg/mL, respectively. The extract exhibited $81.4 \pm 1.8\%$ inhibition at 500 µg/mL, which had the highest antioxidant activity (Figure 1). The gradual rising of the inhibition percentage indicates that the extract has a highly effective electron/hydrogen-donating property that may neutralize free radicals. The antioxidant activity observed could be due to the presence of bioactive phytochemicals like phenolics and flavonoids in *N. Nucifera* (Tungmunnithum et al., 2022). It is known that these compounds act as natural antioxidants, removing the ROS and preventing the ROS-induced damage (Husain et al., 2012). The standard deviations obtained in the study are small, indicating that the results are reproducible and reliable. The results indicate that *N. nucifera* extract has strong antioxidant activity and can be used as a natural source of antioxidants in pharmaceutical, nutraceutical, and therapeutic products.

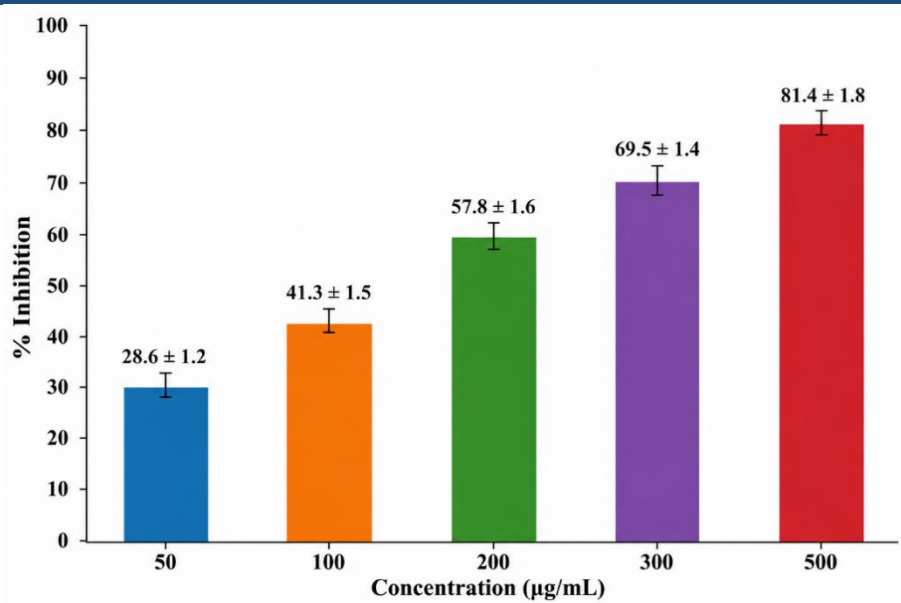


Figure 1. Concentration-Dependent Antioxidant Activity of *N. nucifera* Evaluated by the DPPH Assay

2.3.3. Antiparasitic activity

The antiparasitic activity of *N. nucifera* extracts was determined by the lethality bioassay on *Artemia salina* nauplii in brine shrimp. Results showed that the mortality percentage was increased in a concentration-dependent manner with the increment of extract concentrations. The higher concentrations of the extract showed higher toxicity against the Brine shrimp nauplii, signifying high antiparasitic activity. The extract had a moderate mortality of 33.3 to 46.7 at 50 µg/mL. But the killing effect was significantly higher at 100 µg/mL, reaching up to 66.7%. The highest activity was seen at 500 µg/mL with a mortality percentage of 73.3 to 93.3%. The highest mortality was observed in the positive control (Potassium dichromate 100%) and the lowest in the negative control (Table 3), with negligible mortality.

Based on the present study, it was concluded that the extracts of *N. nucifera* have quite strong antiparasitic activity, which can be seen in the lethality test of brine shrimp. The mortality percentage

in each concentration of plant extract shows that the extract used in this study has a toxic effect on *Artemia salina* nauplii in a dose-dependent manner (Otang et al., 2013). The bioactivity observed could be related to the phytochemical substances like alkaloids, flavonoids, tannins, phenolics, and terpenoids present in the extract. Some of these compounds have been reported to have cytotoxic and antiparasitic activities due to their effects on cellular metabolism, disruption of membrane integrity, and disruption of enzymatic activities (Muthukrishnan et al., 2017). Its high lethal concentration at 500 µg/mL indicates that *N. nucifera* has effective bioactive metabolites which may have pharmaceutical value (Ohikhena et al., 2016). The brine shrimp lethality assay is a quick screening test to assess the cytotoxic and antiparasitic activity of any compounds; thus, the data obtained suggest that the plant has potential for further pharmacological research. The mortality recorded during the activity was important and shows the biological efficacy of the plant extract as per the standard potassium dichromate treatment, although the activity was lower than the standard treatment. Additional purification and characterisation of active compounds, LC50 testing, and in vivo anti-parasitic tests are suggested to confirm its therapeutic potential.

Table 3: Antiparasitic Activity of *N. nucifera* Extract Against *Artemia salina* Nauplii

Treatment/Concentration	Number of Surviving Nauplii	Mortality (%)
Control	14.7 ± 0.3	2.0 ± 0.2
50 µg/mL	10.0 ± 0.5	33.3 ± 1.5
100 µg/mL	7.0 ± 0.4	53.3 ± 1.2
500 µg/mL	2.0 ± 0.3	86.7 ± 1.7
Positive control	0.0 ± 0.0	100 ± 0.0

2.3.4. Anti-Inflammatory Activity

The anti-inflammatory activity of the methanolic extract of *N. nucifera* was assessed by inhibition of protein denaturation at different concentrations. The anti-inflammatory activity of the extract was highly dose-dependent. At 50 µg/mL, the extract exhibited $28.4 \pm 1.3\%$ inhibition, which increased to $49.6 \pm 1.6\%$ at 100 µg/mL. The maximum inhibition ($78.2 \pm 1.9\%$) was found at 500 µg/mL, which suggests that it possesses high anti-inflammatory activity when used in high doses (Figure 2). The standard drug gave an inhibition of $92.5 \pm 1.1\%$ while the control showed almost no inhibition. The anti-inflammatory activity observed could be due to the presence of flavonoids, alkaloids, and phenolic compounds in the methanolic extract of *N. nucifera*.

The methanolic extract of *N. nucifera* showed significant anti-inflammatory activity by showing a dose-dependent effect on the inhibition of protein denaturation. Inhibition percentage was found to be increasing with concentration, and this value of 78.2% was seen at 500 µg/mL, showing high anti-inflammatory properties (Kola et al., 2022). Protein denaturation is a common phenomenon in inflammatory responses, and compounds that prevent protein denaturation can help to stabilize proteins and cellular membranes in inflammatory conditions (Akbar et al., 2023). The anti-inflammatory effect of *N. nucifera* could be due to the presence of phytochemicals with bioactive properties, including flavonoids, phenolic acids, alkaloids, and tannins (Adebayo et al., 2015). They have antioxidant and free radical scavenging activities to counteract oxidative stress and help to inhibit inflammatory mediators. Flavonoids, especially, have been proven to inhibit the production of prostaglandins and other inflammatory cytokines, which help to reduce inflammation. The anti-inflammatory and antioxidant properties of *N. nucifera* extracts were also noted in previous studies, in line with the findings of the present study (Fatema et al., 2024; Zhou et al., 2024). Moreover, the increasing concentration caused the increasing inhibition, indicating that the extract is dose-dependent, like conventional anti-inflammatory medicines. The results suggest that *N. nucifera* may be a good source of natural anti-inflammatory therapeutic agents that have fewer side effects than synthetic ones.

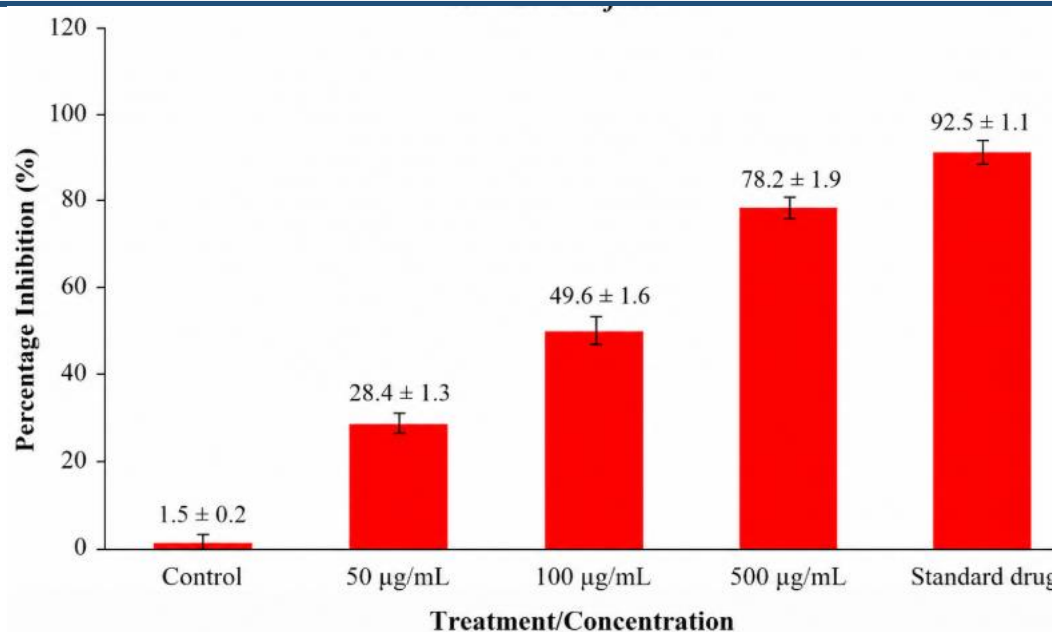


Figure 2. Anti-Inflammatory Activity of *N. nucifera* Methanolic Extract Assessed by Protein Denaturation Inhibition

2.3.5. Antifungal activity

The methanolic extract of *N. nucifera* was found to have significant antifungal activity against all the fungal strains. The inhibition effect was more pronounced with increasing concentration of the extract. *Candida albicans* was the most susceptible, while *Fusarium solani* was comparatively less susceptible among the tested fungi. The extract at a concentration of 100 mg/mL showed the following zones of inhibition: *Candida albicans* showed 18.4 ± 0.5 mm, *Aspergillus niger* showed 16.2 ± 0.4 mm, *Aspergillus flavus* showed 15.7 ± 0.3 mm, and *Fusarium solani* showed 13.5 ± 0.6 mm (Figure 3). Positive control – fluconazole demonstrated more antifungal activity than the plant extract; the negative control did not provide an inhibition zone.

The present study has shown that the methanolic extract of *N. nucifera* has antifungal activity against various pathogenic fungi. The antifungal activity observed could be due to the presence of bioactive phytochemicals like flavonoids, alkaloids, tannins, phenolic compounds, and terpenoids in the

methanolic extract (Khan et al., 2010). They have been shown to have an effect on the integrity of the fungal cell wall, on the permeability of the cell membrane, and on the growth of fungi (Aqil et al., 2010). As the concentration of the extract increases, the zone of inhibition increases, suggesting an antifungal effect in a dose-dependent manner. The extract was found to be most effective on *Candida albicans*, indicating its great activity against yeast-like fungi. However, *Fusarium solani* showed lesser sensitivity, which could be attributed to its relatively less sensitive cell wall structure and adaptability. The methanol used in this study was found to be an effective solvent for extracting polar bioactive components and may account for the potent antifungal properties (Patel et al, 2014). Medicinal plants with phenolic and flavonoid contents showed similar results, with methanolic extracts having wide antifungal activity (Mousavi et al., 2018). The results indicated that the plant extract activity was found to be less than the standard antifungal drug fluconazole, but the study showed that the plant extract may be used as a potential source of antifungal drugs for pharmaceutical and therapeutic purposes. Further study with compound isolation, minimum inhibitory concentration (MIC), and molecular characterization studies is suggested to isolate the specific antifungal components responsible for its activity.

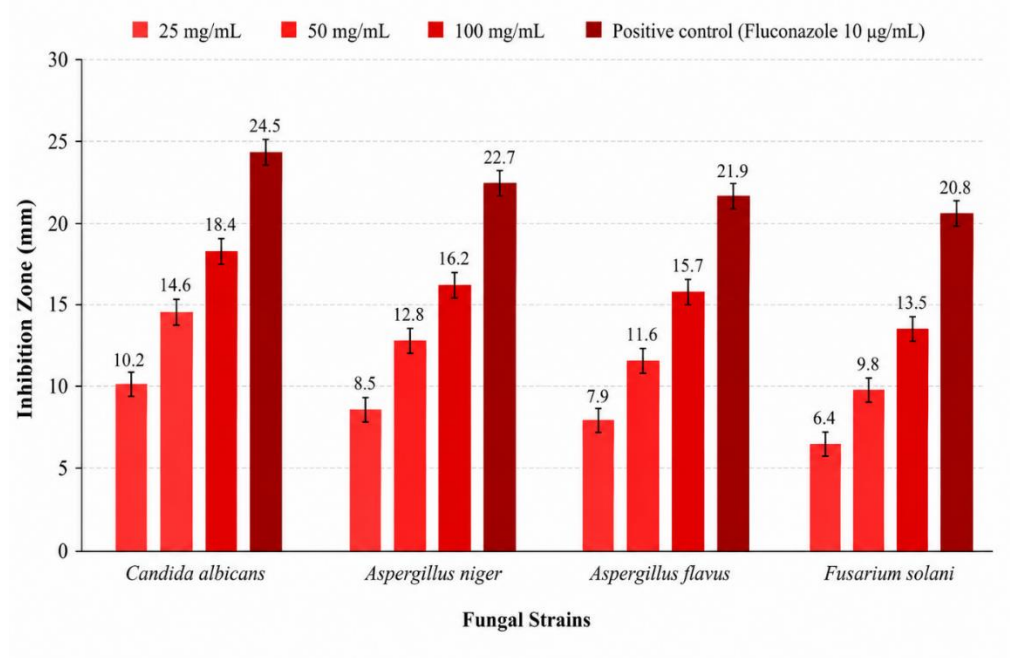


Figure 3. Antifungal Activity of *N. nucifera* Methanolic Extract Against Selected Fungal Strains

2.3.6. Antibacterial Activity

Figure 4 represents the antibacterial activity of the methanolic extract of *N. nucifera* against selected pathogenic bacterial strains with the agar well diffusion method. The diameter of the inhibition zone around each well was used to assess the antibacterial activity. The extract showed the best antibacterial activity against *S. aureus* with a zone of inhibition of 18.0 ± 1.2 mm, followed by *B. subtilis* with 16.0 ± 1.1 mm. On the other hand, *E. coli* exhibited the lowest Antibacterial activity with an inhibition zone of 12.0 ± 1.0 mm (Figure 4).

The results revealed that the methanolic extract of *N. nucifera* possessed better antibacterial activity against the Gram-positive bacteria than the Gram-negative bacteria (Techaoei et al., 2020). This variation could be due to different structures of the bacterial cell walls, as Gram-negative bacteria have an outer lipopolysaccharide membrane, which hinders the penetration of phytochemical compounds. The Gram-positive bacteria have a more simplified structure in the cell wall and are

therefore more sensitive towards antimicrobial agents present in plants (Asimi et al., 2019). The antibacterial activity of the extract may be linked to the presence of phytochemicals like flavonoids, phenolic compounds, alkaloids, tannins, and terpenoids, which are bioactive compounds. They are effective in inhibiting the growth of bacteria by disrupting bacterial cell membranes, inhibiting essential enzymes, and interfering with microbial metabolism (Noumedem et al., 2013). The low values of standard deviation observed in the graph show the good reproducibility and reliability of the experimental results. In general, the results showed that the methanolic extract of *N. nucifera* has antibacterial activity and is a natural source of antimicrobial compounds that can be used in pharmaceuticals and therapeutics.

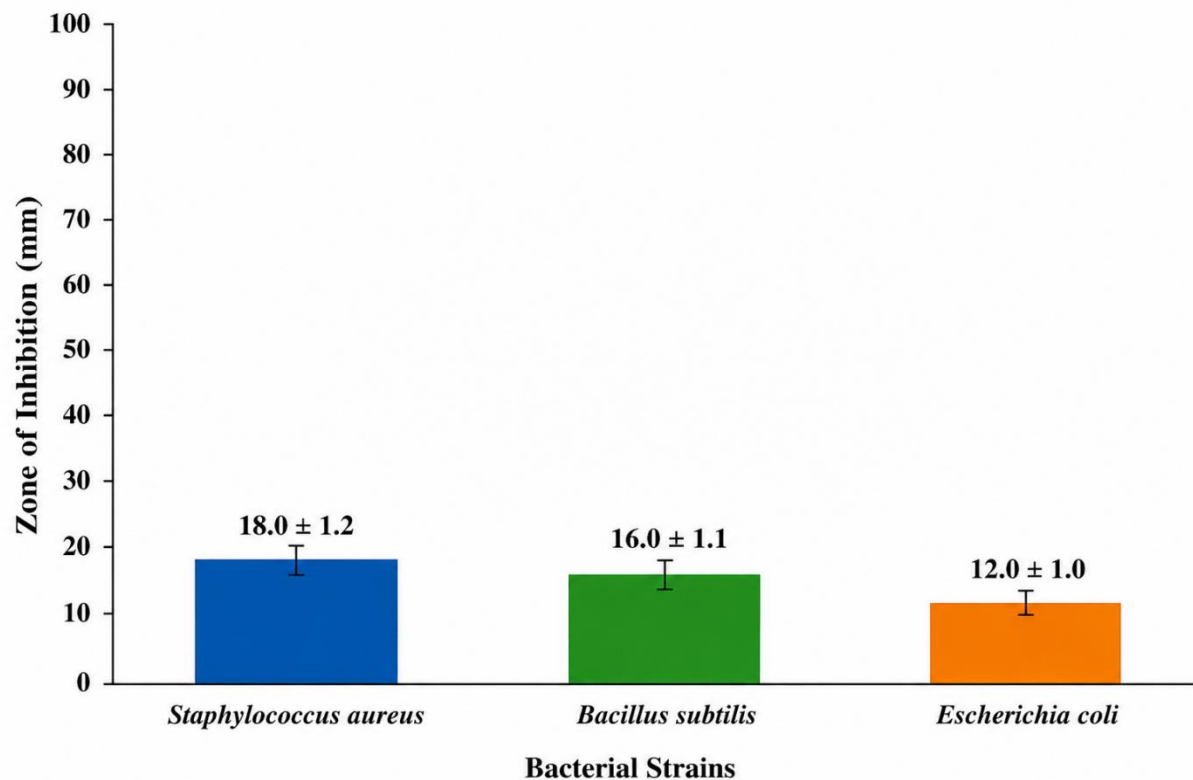


Figure 4. Antibacterial Activity of *N. nucifera* Methanolic Extract Assessed by Agar Well Diffusion Method

4. Conclusion

The present study showed that the post-harvest drying practices have a significant impact on the phytochemical profile, nutritional profile, and biological activities in *N. nucifera*. The qualitative phytochemical screening revealed the presence of some important bioactive compounds, which were found to be the most diverse in methanolic extracts. The nutrient retention, however, was significantly influenced by the drying procedure as shown by proximate analysis. The methanolic extract also showed good concentration-dependent activity in scavenging DPPH radical and had a significant activity on protein denaturation, which makes it a potent natural antioxidant and anti-inflammatory agent. Furthermore, the antiparasitic activity against *Artemia salina* nauplii was pronounced, and antimicrobial activity against different pathogenic bacterial and fungal strains was effective, especially against Gram-positive and *Candida albicans*. This biological activity is probably related to the synergistic activity of the phytochemicals identified. In conclusion, the results suggest that *N. nucifera* has the potential to be a valuable natural source of compounds with nutritional and pharmacological properties. Future research is recommended to isolate, characterize, and evaluate the mechanisms of action of these compounds and to further explore their in vivo effects to support their therapeutic and nutraceutical potential in *N. nucifera*.

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Consent to Participate: Not applicable.

Consent to Publish: Not applicable.

Clinical trial number: Not applicable.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement: Data is available on request from the corresponding author.

Author contributions

Husna Shams, Shah Fahad, Nasreen Ashraf, and Madiha Iqbal: Experiments, Formal Analysis, Writing, Conceptualization, Data curation. Muhammad Sabir Hussain and Asad Ullah: Writing – review & editing, Investigation, Data curation, Conceptualization, Formal analysis. Asif Kamal: Conceptualization, Designed experiments, Data curation, Resources, Supervision, Review & editing, Funding acquisition, Software, and Writing.

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