

PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT POTENTIAL OF TRAPA NATANS PEEL AND NIGELLA SATIVA OIL: A COMPARATIVE STUDY

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ABSTRACT

Plants have long been used in traditional medicine for their natural healing properties, and modern research continues to explore their role as safe and effective alternatives to synthetic drugs. This study compared the phytochemical composition and antioxidant potential of *Trapa natans* (water chestnut) peel and *Nigella sativa* (black cumin) oil. The extracts were prepared using ethanol at different concentrations (20%, 40%, 60%, and 80%) and analyzed through phytochemical screening, FTIR spectroscopy, antioxidant assays (DPPH, FRAP, TAC, and enzymatic tests), and cytotoxicity evaluation using a hemolytic assay. The results showed that *T. natans* peel contained a wider range of phytochemicals, including phenolics, flavonoids, tannins, and saponins, while *N. sativa* oil was mainly rich in steroids and fatty acids. Quantitative analysis revealed that 40% ethanol was most effective for extracting phenolic compounds, while 20% ethanol yielded the highest flavonoid content. The antioxidant assays confirmed that fractions with higher phenolic and flavonoid levels had stronger radical scavenging activity, with the 20% ethanol fractions showing the best performance. FTIR analysis validated the presence of functional groups such as hydroxyl, carbonyl, and aromatic bonds associated with antioxidant activity. Hemolysis tests showed that extracts from lower ethanol fractions were safer, while higher ethanol fractions exhibited mild cytotoxicity. Overall, the findings suggest that both *Trapa natans* peel and *Nigella sativa* oil are promising sources of natural antioxidants. Their potential applications extend to nutraceuticals and pharmaceuticals, particularly when extracted with aqueous ethanol fractions that balance high antioxidant activity with safety.

Keywords: *Trapa Natans*, *Nigella Sativa*, Phytochemicals, Antioxidant Activity, FTIR Analysis, Cytotoxicity

INTRODUCTION

Medicinal plants have been central to healthcare systems across the world for centuries, offering safe and affordable remedies for a wide range of illnesses. In recent years, there has been growing interest in plant-based products as concerns increase over the side effects and resistance linked to synthetic drugs (Srivastava, 2018). Natural compounds such as phenolics, flavonoids, and alkaloids have attracted significant attention due to their strong antioxidant, anti-inflammatory, and antimicrobial properties. Among these, *Trapa natans* (water chestnut) and *Nigella sativa* (black cumin) are two plants of particular interest for their potential role in nutraceuticals and pharmaceuticals (Garg, Anvar Hussain, Syed, Asaithambi, & Mundhada, 2020).

Trapa natans is an aquatic plant commonly found in Asia, Europe, and Africa. Traditionally, it has been valued not only as a food source but also for its medicinal properties (Banu, Dasgupta, Hazarika, Laloo, & Kalita, 2023). The peel of its fruit is known to contain bioactive compounds with antioxidant potential, which may help protect the body against oxidative stress and related diseases. *Nigella sativa*, on the other hand, is a well-known medicinal plant often referred to as “the seed of blessing.” Its oil is widely recognized for its therapeutic properties, including antimicrobial, anti-inflammatory, and antioxidant effects, attributed to compounds such as thymoquinone and fatty acids (Gajarmal et al., 2025).

Oxidative stress, caused by an imbalance between free radicals and antioxidant defenses, is linked to aging and many chronic diseases, including cardiovascular disorders, diabetes, and cancer (Jomova et al., 2023). Natural antioxidants from plants play a vital role in neutralizing free

radicals and maintaining cellular health. While both *Trapa natans* and *Nigella sativa* have been studied individually, direct comparisons of their phytochemical content and antioxidant capacity are limited.

The present study was therefore designed to compare the phytochemical composition and antioxidant potential of *T. natans* peel and *N. sativa* oil (Rehman, Khan, Sohaib, & Rehman, 2024). By using solvent extraction with varying ethanol concentrations, the study aimed to identify the best extraction conditions for phenolics and flavonoids, evaluate antioxidant activity through chemical and enzymatic assays, and assess safety through cytotoxicity tests. FTIR spectroscopy was also used to confirm the presence of key functional groups. The findings provide new insights into the comparative bioactivity of these plants and highlight their potential for development into natural health-promoting products (Indira, Shahar, Joshi, & Chongtham, 2024).

RESEARCH DESIGN AND METHODOLOGY

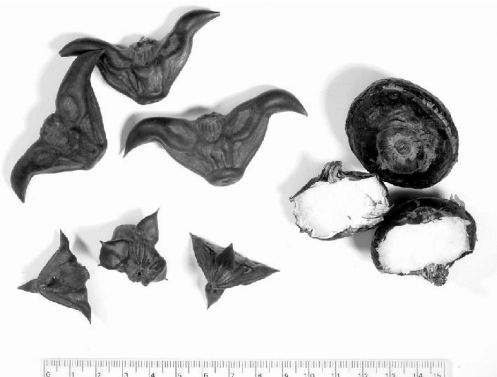
This research was designed as a comparative experimental study. The main purpose was to analyze and compare the phytochemical contents and antioxidant potential of *Trapa natans* (water chestnut) peel and *Nigella sativa* (black cumin) oil. Both samples were extracted using ethanol at different concentrations (20%, 40%, 60%, and 80%) to study how solvent polarity affects the yield of bioactive compounds and their biological activities. The study was carried out in a stepwise manner:

1. Collection and preparation of plant materials
2. Extraction using ethanol at different concentrations
3. Screening of phytochemicals (qualitative and quantitative)

4. Antioxidant activity tests (radical scavenging, reducing power, and enzymatic assays)
5. Cytotoxicity testing on human red blood cells

SAMPLE COLLECTION AND PREPARATION

Trapa natans (water chestnut) Fresh fruits were collected from local freshwater ponds. The outer peels were separated, washed with distilled water, and dried in the shade at room temperature. Once dried, the peels were ground into fine powder and stored in airtight containers. *Nigella sativa* (black cummin) Seeds were purchased from a local herbal supplier. Oil was extracted through a coldpress method to preserve natural compounds. Both samples were stored in cool, dry conditions until they were used for experiments.



EXTRACTION PROCESS

To obtain extracts, each sample was treated with ethanol solutions of different strengths 20%, 40%, 60%, and 80%. About 500 grams of dried peel powder was mixed with 2500 mL of solvent and placed in a Soxhlet extractor. After 24 hours of extraction, the solvent was removed using a rotary evaporator under reduced pressure. The concentrated extracts were collected in small amber vials and stored at 4 °C to prevent breakdown of active compounds.

PHYTOCHEMICAL SCREENING

QUALITATIVE TESTS

Preliminary tests were performed to check the presence of important phytochemicals, Alkaloids (Dragendorff's test), Flavonoids (alkaline reagent test), Tannins (ferric chloride test), Saponins (froth test), Steroids (Liebermann–Burchard test). The results were noted based on color change or foam formation, showing whether the compounds were present or absent (Shaikh & Patil, 2020).

QUANTITATIVE TESTS

Total Phenolic Content (TPC): Measured using the FolinCiocalteu method. Gallic acid was used as a reference standard, and results were expressed as milligrams of gallic acid equivalent per gram of extract (Olechno, Puścion-Jakubik, Markiewicz-Żukowska, & Socha, 2020).

Total Flavonoid Content (TFC): Measured using the aluminum chloride method. Rutin was used as the standard, and results were expressed as milligrams of rutin equivalent per gram of extract (Rosyantari et al., 2021).

All experiments were repeated three times to ensure accuracy.

ANTIOXIDANT ACTIVITY

To evaluate antioxidant potential, several assays were carried out:

1. **DPPH Radical Scavenging Test:** The ability of extracts to neutralize free radicals was tested using a DPPH solution. Absorbance was measured at 517 nm, and IC₅₀ values were calculated (lower IC₅₀ = stronger antioxidant) (Gulcin & Alwasel, 2023).
2. **Ferric Reducing Antioxidant Power (FRAP):** The extracts' ability to reduce Fe³⁺ to Fe²⁺ was measured at 593 nm. Higher absorbance indicated stronger reducing power (Benzie, Devaki, trends, & applications, 2018).

- Total Antioxidant Capacity (TAC):** Tested by the phosphomolybdenum method, with results expressed in terms of ascorbic acid equivalents (Silvestrini, Meucci, Ricerca, & Mancini, 2023).
- Enzymatic Antioxidant Activity:** Extracts were tested for their effects on natural antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD). Results were expressed as percentage inhibition (Haida, Hakiman, & nutrition, 2019).

FOURIER TRANSFORM INFRARED (FTIR) ANALYSIS

FTIR spectroscopy was used to identify functional groups in the extracts. Each sample was scanned from 4000–400 cm^{-1} , and peaks were matched with known reference values. This helped confirm the presence of bioactive compounds such as phenolics and flavonoids (Haida et al., 2019).

CYTOTOXICITY (HEMOLYTIC ACTIVITY)

To check the safety of extracts, their effect on human red blood cells was tested. Blood samples were collected from healthy volunteers after informed consent. The red cells were washed and

RESULTS

1. PHYTOCHEMICAL COMPOSITION

TABLE 1: QUALITATIVE PHYTOCHEMICAL CONSTITUENTS

Extracts	Alkaloids	Flavanoids	Steroids	Saponins	Tannins
<i>Trapa natans</i>	+	+	+	+	+
<i>Nigella sativa</i>	+	-	+	-	-

The + show the presence and - show the absence of that chemical.

2. TOTAL PHENOLIC CONTENT (TPC)

The total phenolic content (TPC) of different ethanol fractions of *Trapa natans* peels and *Nigella sativa* extracts was measured using the Folin-Ciocalteu method. Results revealed that the 40% ethanol fraction exhibited the highest phenolic concentration in both plants, with *T. natans*

suspended in PBS. Extracts were added at different concentrations and incubated at 37 °C. Hemolysis (breakdown of cells) was measured at 540 nm (Greco et al., 2020).

Negative control: PBS solution (0% hemolysis)

Positive control: 1% Triton X-100 (100% hemolysis)

ETHICAL APPROVAL

Since human blood samples were used, the study followed ethical guidelines. Approval was obtained from the Institutional Review Board (IRB) of Govt. College University, Faisalabad and informed consent was collected from all volunteers.

STATISTICAL ANALYSIS

All data were expressed as mean \pm standard deviation (SD) from three independent experiments. Comparisons were made using one-way ANOVA, followed by Tukey's test for post-hoc analysis. Correlation analysis was performed to examine the relationship between phytochemical content and antioxidant activity. A p-value < 0.05 was considered statistically significant (Jian, Du, Reiter, Stewart, & Biochemistry, 2020).

showing 138.85 ± 0.53 mg PE/g extract and *N. sativa* showing 133.74 ± 0.62 mg PE/g extract (Table 2). In contrast, the 80% ethanol fraction displayed the lowest phenolic content in both extracts.

These findings highlight that moderate polarity solvents (40% ethanol) are more effective for

extracting phenolic compounds compared to higher ethanol concentrations.

TABLE 2: TOTAL PHENOLIC CONTENT (TPC) OF DIFFERENT FRACTIONS OF *T. NATANS* PEELS AND *N. SATIVA* EXTRACTS.

Fraction (Ethanol %)	TPC (mg PE/g extract) – <i>T. Natans</i>	TPC (mg PE/g extract) – <i>N. Sativa</i>
20%	118.21 ± 0.45	124.12 ± 0.49
40%	138.85 ± 0.53	133.74 ± 0.62
60%	110.74 ± 0.38	106.33 ± 0.47
80%	95.26 ± 0.41	91.15 ± 0.39

3. TOTAL FLAVONOID CONTENT (TFC)

The total flavonoid content (TFC) varied significantly across ethanol fractions (Table 3). The 20% ethanol fraction had the highest flavonoid concentration, reaching 140.11 ± 5.47 mg RU/g extract in *T. natans* and 135.68 ± 4.95 mg RU/g extract in *N. sativa*. Flavonoid content progressively

decreased with increasing ethanol concentration, with the 80% ethanol fraction showing the lowest values.

These results suggest that low ethanol polarity (20%) favors the extraction of flavonoids, possibly due to the increased solubility of glycosylated flavonoids in aqueous ethanol mixtures.

TABLE 3: TOTAL FLAVONOID CONTENT (TFC) OF ETHANOL FRACTIONS FROM *T. NATANS* PEELS AND *N. SATIVA*.

Fraction (Ethanol %)	TFC (mg RU/g extract) – <i>T. natans</i>	TFC (mg RU/g extract) – <i>N. Sativa</i>
20%	140.11 ± 5.47	135.68 ± 4.95
40%	115.62 ± 4.22	112.45 ± 3.86
60%	98.54 ± 3.15	95.72 ± 2.84
80%	85.47 ± 2.91	81.36 ± 2.57

4. ANTIOXIDANT ACTIVITY (DPPH ASSAY)

The DPPH radical scavenging assay demonstrated that antioxidant activity was highly correlated with both TPC and TFC values (Table 4). The 20% ethanol fraction of *T. natans* exhibited the strongest antioxidant activity with IC₅₀ = 1.26 ± 0.21 µg/mL,

followed closely by *N. sativa* (IC₅₀ = 1.34 ± 0.24 µg/mL). Conversely, the 80% ethanol fractions displayed weaker antioxidant effects, with IC₅₀ values exceeding 3 µg/mL.

This confirms that fractions rich in flavonoids and phenolics possess stronger radical scavenging activity.

TABLE 4: ANTIOXIDANT ACTIVITY (DPPH RADICAL SCAVENGING, IC₅₀) OF *T. NATANS* AND *N. SATIVA*

Fraction (Ethanol %)	IC ₅₀ (µg/mL) – <i>T. natans</i>	IC ₅₀ (µg/mL) – <i>N. sativa</i>
20%	1.26 ± 0.21	1.34 ± 0.24
40%	1.92 ± 0.18	2.05 ± 0.22

Fraction (Ethanol %)	IC50 ($\mu\text{g/mL}$) – <i>T. natans</i>	IC50 ($\mu\text{g/mL}$) – <i>N. sativa</i>
60%	2.48 \pm 0.27	2.61 \pm 0.19
80%	3.12 \pm 0.33	3.25 \pm 0.28

5. Antioxidant Enzymatic Activity

Enzymatic antioxidant assays were conducted to evaluate the impact of extracts on key antioxidant enzymes (SOD, CAT, and POD). As shown in Table 5, the 20% ethanol fraction showed the maximum activity across all enzymes, with SOD

activity at 68.45 \pm 2.11%, CAT activity at 59.38 \pm 1.92%, and POD activity at 48.26 \pm 1.77%. The activity decreased progressively with higher ethanol concentrations, confirming that aqueous ethanol fractions enhance enzymatic defense against oxidative stress.

TABLE 5: ENZYMATIC ANTIOXIDANT ACTIVITY OF DIFFERENT FRACTIONS

Extract/Fraction	SOD (% inhibition)	CAT (% inhibition)	POD (% inhibition)
20% ethanol	68.45 \pm 2.11	59.38 \pm 1.92	48.26 \pm 1.77
40% ethanol	61.27 \pm 1.86	53.74 \pm 1.65	41.12 \pm 1.58
60% ethanol	55.36 \pm 1.72	48.12 \pm 1.54	36.48 \pm 1.42
80% ethanol	49.27 \pm 1.61	43.57 \pm 1.48	30.74 \pm 1.36

6. Cytotoxicity (Hemolytic Activity)

The hemolytic assay was used to assess the cytotoxic effects of ethanol fractions on human erythrocytes (Table 6). Results indicated a dose-dependent increase in hemolysis, with the 20%

ethanol fractions being the least cytotoxic (5.42 \pm 0.24% in *T. natans*; 4.95 \pm 0.19% in *N. sativa*), while the 80% ethanol fractions exhibited higher hemolytic activity (11.24 \pm 0.37% and 10.85 \pm 0.34%, respectively).

Table 6. Hemolytic activity of ethanol fractions on human erythrocytes.

Extract/Fraction	Hemolysis % – <i>T. natans</i>	Hemolysis % – <i>N. sativa</i>
20% ethanol	5.42 \pm 0.24	4.95 \pm 0.19
40% ethanol	7.31 \pm 0.28	6.88 \pm 0.23
60% ethanol	9.16 \pm 0.32	8.72 \pm 0.29
80% ethanol	11.24 \pm 0.37	10.85 \pm 0.34

These findings suggest that lower ethanol fractions provide a safer antioxidant profile compared to highly concentrated ethanol fractions, which may release compounds with mild cytotoxic effects.

7. FTIR ANALYSIS

The FTIR spectra of both *Trapa natans* peel and *Nigella sativa* oil confirmed the presence of several functional groups related to antioxidant

compounds. In *Trapa natans*, broad peaks around 3300–3400 cm^{-1} indicated –OH stretching vibrations, typical of phenols and flavonoids. Peaks near 1600 cm^{-1} were linked to aromatic C=C stretching, while bands around 1050–1150 cm^{-1} suggested C–O stretching of alcohols and esters. In *Nigella sativa* oil, strong absorption at 2920–2850 cm^{-1} corresponded to C–H stretching in alkanes,

while peaks around 1740 cm^{-1} indicated the presence of ester carbonyl groups. The band near 1450 cm^{-1} reflected C=C stretching of aromatic compounds, confirming the presence of bioactive fatty acids and phenolic components. These results

support the phytochemical findings by confirming the existence of phenolic, flavonoid, and fatty acid structures, which are linked to antioxidant potential.

TABLE 7. FTIR SPECTRAL PEAKS AND FUNCTIONAL GROUP ASSIGNMENTS OF *TRAPA NATANS* PEEL AND *NIGELLA SATIVA* OIL EXTRACTS.

Wavenumber (cm^{-1})	Functional Group Assignment	<i>Trapa natans</i>	<i>Nigella sativa</i>
3300–3400	O–H stretching (phenols, alcohols, flavonoids)	+	–
2920–2850	C–H stretching (alkanes, lipids)	–	+
1740	C=O stretching (esters, fatty acids)	–	+
1600	C=C stretching (aromatic compounds)	+	+
1450	C=C stretching (aromatic ring vibrations)	+	+
1050–1150	C–O stretching (alcohols, esters, glycosides)	+	–

The + sign show the presence and – sign show the absence of that compound

Trapa natans peel extracts showed strong –OH, C=C, and C–O peaks, confirming the presence of phenolics, flavonoids, and tannins. *Nigella sativa* oil exhibited strong C–H and C=O peaks, indicating the presence of fatty acids, esters, and aromatic compounds. Both extracts contained aromatic functional groups, which contribute to their antioxidant activity (Table 6).

DISCUSSION

This study compared the phytochemical profile and antioxidant potential of *Trapa natans* peel and *Nigella sativa* oil, using multiple solvent fractions and a wide range of assays. The results confirm that both plants contain valuable bioactive compounds with strong antioxidant effects, but they differ in composition and safety profiles.

The phytochemical screening revealed that *T. natans* peel contained alkaloids, flavonoids, tannins, saponins, and steroids, whereas *N. sativa* oil was limited to alkaloids and steroids. The broader range of phytochemicals in *T. natans* explains its

stronger antioxidant performance. Quantitative analysis further supported this observation that phenolic content was highest in 40% ethanol extracts, while flavonoids were most abundant in 20% ethanol fractions. These results demonstrate that solvent polarity plays a critical role in extraction efficiency, with aqueous ethanol mixtures yielding higher amounts of bioactive compounds than absolute ethanol.

The antioxidant assays (DPPH, FRAP, TAC) and enzymatic activity tests (SOD, CAT, POD) confirmed that higher levels of phenolics and flavonoids were directly linked to stronger antioxidant activity. Fractions extracted with 20% ethanol exhibited the lowest IC₅₀ values in the DPPH assay, indicating the greatest free radical scavenging ability. Similarly, the same fractions enhanced enzymatic antioxidant activity more effectively than higher ethanol fractions. These findings align with earlier research showing that phenolics and flavonoids act synergistically to protect cells from oxidative stress.

The FTIR analysis further validated the phytochemical results by confirming the presence of functional groups linked to antioxidant compounds. *T. natans* peel extracts showed broad -OH stretching peaks, characteristic of phenolics and flavonoids, as well as C-O vibrations associated with tannins and glycosides. *N. sativa* oil displayed strong C-H and C=O peaks, typical of fatty acids and esters, along with aromatic C=C bonds. These differences suggest that *T. natans* is richer in polyphenolic antioxidants, while *N. sativa* is dominated by fatty acids with potential antioxidant and anti-inflammatory properties. Together, these results explain the stronger radical scavenging observed in *T. natans* and highlight the distinct but complementary antioxidant mechanisms of both plants.

The hemolytic assay provided important insights into the safety of the extracts. Lower ethanol fractions (20% and 40%) showed minimal hemolytic activity, suggesting that these extracts are safe for potential therapeutic use. In contrast, higher ethanol fractions (60% and 80%) caused increased hemolysis, which may be due to extraction of compounds with mild cytotoxic properties. This indicates that while both plants are rich in antioxidants, the method of extraction strongly influences their safety. Aqueous ethanol fractions appear to offer the best balance between antioxidant activity and biocompatibility.

Taken together, the results demonstrate that *Trapa natans* peel is a richer source of phenolic and flavonoid compounds, making it more effective in neutralizing free radicals and boosting antioxidant enzymes. *Nigella sativa* oil, although less diverse in phytochemicals, contains fatty acids and aromatic compounds that contribute to antioxidant defense in a different way. The FTIR results provide

molecular confirmation of these findings, while the cytotoxicity data highlight the importance of solvent choice in ensuring safety.

CONCLUSION

This comparative study highlights that both *Trapa natans* peel and *Nigella sativa* oil are valuable natural sources of antioxidants. *Trapa natans* showed higher levels of phenolics, flavonoids, and radical scavenging activity. *Nigella sativa* oil contained fatty acids and esters that contribute to antioxidant and protective effects. FTIR analysis confirmed the presence of functional groups linked to bioactivity in both extracts. Hemolytic assays indicated that lower ethanol fractions are safer, making aqueous ethanol a suitable solvent for future extraction. These findings suggest that both plants have potential applications in nutraceuticals and pharmaceuticals, with *Trapa natans* being particularly promising as a natural antioxidant source. Future studies should focus on in vivo models and clinical validation.

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