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Phytochemical profiling and functional potential of *Trapa natans* (Heikak Yetli): A GC–MS evaluation of bioactive phenolics

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Abstract

Trapa natans, commonly known as Heikak Yetli in Manipuri is an economically and nutritionally important aquatic macrophyte, which is utilized as a folk medicine for the treatment of various ailments. Despite its extensive usage as food and medicine, the phytochemical composition of volatile and semi-volatile constituents of this plant remains poorly characterized. In present study, we report the phytochemical profiling of petroleum ether extract of *T. natans* using Gas Chromatography-Mass Spectrometry (GC–MS). The extract showed 9 distinguishing peaks within a narrow range of retention time (35.725–38.801 min), contributing 2.50% of total chromatographic area with the majority of the area accounting for solvent front, silylation reagents and matrix. The major identified constituents were phenolic and bioactive derivatives such as Hexestrol, 2TMS derivative, 4-Methyl-2,4-bis(p-hydroxyphenyl) pent-1-ene, 2TMS derivative and 4-Tert-octylphenol, TMS derivative. In addition, several siloxane artifacts such as decamethyltetrasiloxane were also observed which necessitates exercise of caution while interpreting GC–MS data of plant extracts. The presence of phenolic derivatives in the extract of *T. natans* further supports its reported antioxidant, anti-inflammatory and antimicrobial properties. The results highlight the ethnopharmacological value of this plant and pave way for future studies to identify and characterize individual phytochemical constituents for functional food and pharmaceutical applications.

Keywords: *Trapa natans*, GC–MS profiling, phenolic derivatives, siloxane artifacts, phytochemicals

Introduction

Medicinal plants still continue to play a significant role in traditional health-care systems as well as served as a major modern drug discovery platform. *Trapa natans* L. belonging to Lythraceae family (Fig. 1) is one such aquatic edible plant, which is consumed as food and used as an indigenous medicine for the treatment of diverse ailments in many parts of the globe (Banu *et al.*, 2023; Kanase *et al.*, 2024) ^[2, 6]. Given its extensive usage as food and medicine, there is a need to understand the phytochemical composition of this plant deeply, which could be useful in nutraceutical as well as pharmaceutical applications. Existing phytochemical studies on leaves of *T. natans* have revealed the presence of alkaloids, tannins, phenolic compounds, flavonoids, steroids, saponins, carbohydrates and glycosides (Chandana *et al.*, 2021; Mendhekar & Rachh, 2023) ^[3, 10]. The fruits and alternative plant parts have shown to contain considerable amounts of gallic acid, caffeic acid, chlorogenic acid, ferulic acid, ellagic acid, quercetin, naringenin and other related polyphenolic constituents (Banu *et al.*, 2023; Kanase *et al.*, 2024) ^[2, 6]. These polyphenolic constituents are known to possess antioxidant, anti-inflammatory and other biological activities (Asha *et al.*, 2024; Huang *et al.*, 2015) ^[1, 5]. In addition to polyphenols, *T. natans* has also been reported to be a rich source of carbohydrates, proteins, fiber, vitamins and minerals (Banu *et al.*, 2023; Kanase *et al.*, 2024) ^[2, 6]. The presence of minerals such as calcium, magnesium and potassium further adds to its nutritional value while its phytochemical constituents make it a potential candidate for functional food development (Kanase *et al.*, 2024; Rehman *et al.*, 2024) ^[6, 11]. Recent studies have also shown that the phytochemical composition of different varieties of this plant as well as the extracts prepared from different plant parts vary significantly with respect to polyphenolic and flavonoid contents, which is directly linked to

their antioxidant activity (Asha *et al.*, 2024; Kiruba *et al.*, 2023) ^[1, 7]. Therefore, it is important to conduct a phytochemical profiling study to identify the bioactive constituents of this plant using appropriate analytical methodologies.

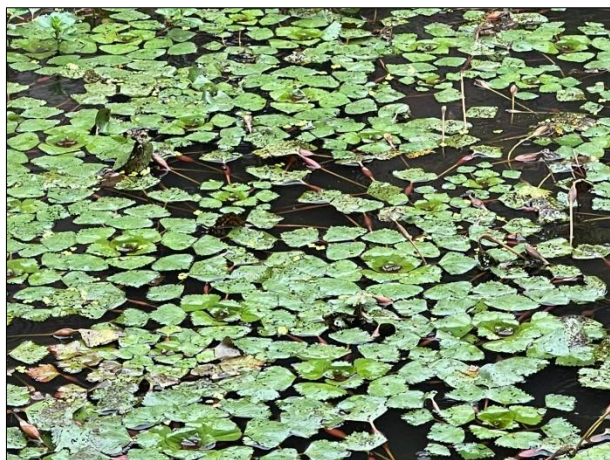


Fig 1: *Trapa natans* in its habitat

Methods

Plant Material Collection and Preparation

Fresh samples of *T. natans* were collected from wetlands area of Manipur during early September 2025 to late February 2026. The collected samples were shade dried and ground to obtain a coarse powder for extraction (Kiruba *et al.*, 2023) ^[7]. The powder was then subjected to petroleum ether extraction at Manipur International University laboratory. Petroleum ether was used as a solvent since it is capable of dissolving a wide range of organic compounds.

GC–MS Analysis

The extracts obtained from *T. natans* were analyzed using GC–MS (PerkinElmer Clarus680 GC/600C MS) to identify phytochemical constituents present in the sample. Peaks were identified by comparing the obtained data with NIST (National Institute of Standards and Technology) Mass Spectral Library followed by summarization of results in terms of retention time, peak area, area percentage,

molecular weight and molecular formula (Mendhekar & Rachh, 2023) ^[10]. Since some of the peaks might have been formed due to derivatisation process, the results were interpreted alongside phytochemical screening results (Mendhekar & Rachh, 2023) ^[10].

Results

GC–MS Profile

GC–MS analysis of the extract obtained from *T. natans* revealed prominent 9 peaks (Table no 1. & Fig No. 2) ranging from a retention time of 35.725–38.801 min. The peak with the highest area was Hexestrol, 2TMS derivative (11,619.8), followed by Tetrasiloxane, 1,1,3,3,5,5,7,7-octamethyl- (11,064.8) and Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- (10,552.8). Other peaks with significant area included 4-Methyl-2,4-bis(p-hydroxyphenyl) pent-1-ene, 2TMS derivative (9,467.1), Tetrasiloxane, decamethyl- (9,235.6) and 4-Tert-octylphenol, TMS derivative (9,105.5). The chromatographic area for any peak can be used to estimate the total area. For example, using Decamethyltetrasiloxane (Peak 1, area 9,235.6, 0.278%):

$$\text{Total area} = 9,235.6 / 0.00278 \approx 3,322,158 \text{ units}$$

Another way to estimate it would be to use the area and percentage values of Hexestrol di-TMS (Peak 9):

$$\text{Total area} = 11,619.8 / 0.00350 \approx 3,319,942 \text{ units}$$

Both calculations give approximately 3.32×10^6 area units. This means that the 9 peaks shown in the GC–MS chromatogram account for only 2.500% of the total area (0.278 + 0.285 + 0.230 + 0.318 + 0.220 + 0.274 + 0.212 + 0.333 + 0.350). The majority of the area (97.5%) belongs to other peaks that were not identified or were considered as solvent front, silylation reagent or matrix background. The identified peaks were mainly siloxane and phenolic derivatives. Overall, the GC–MS analysis showed that the extract of *T. natans* contained a number of phytochemical constituents, which may contribute to the reported biological activity of this plant.

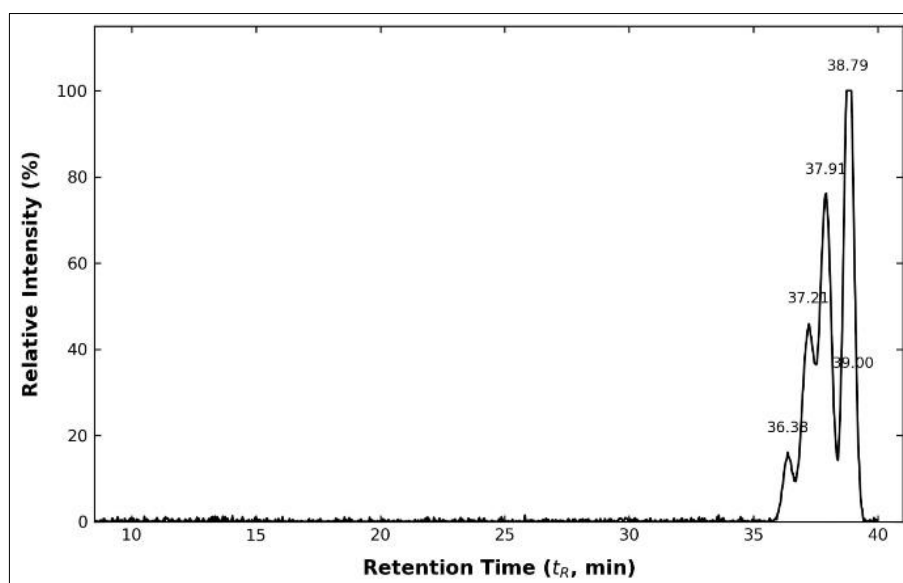


Fig 2: GC-MS chromatogram

Table 1: Presents the nine highest peaks observed in the analysis

Sl. No.	Name of compound	R.T.	Area	Area%	M.W. (g/mol)	Formula	Structure
1	Tetrasiloxane, decamethyl-	35.725	9,235.6	0.278	310.68	C ₁₀ H ₃₀ Si ₄	
2	4-Methyl-2,4-bis(p-hydroxyphenyl) pent-1-ene, 2TMS derivative	36.050	9,467.1	0.285	412.7	C ₂₄ H ₃₆ O ₂ Si ₂	
3	4-(4-Hydroxyphenyl)-4-methyl-2-pentanone, TMS derivative	36.250	7,637.2	0.230	264.43	C ₁₅ H ₂₄ O ₂ Si	
4	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	36.445	10,552.8	0.318	578.2	C ₁₆ H ₅₀ O ₇ Si ₈	
5	Cyclotrisiloxane, hexamethyl-	36.985	7,306.6	0.220	222.46	C ₆ H ₁₈ O ₃ Si ₃	
6	4-Tert-octylphenol, TMS derivative	37.230	9,105.5	0.274	278.5	C ₁₇ H ₃₀ OSi	
7	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	37.051	7,050.2	0.212	578.2	C ₁₆ H ₅₀ O ₇ Si ₈	
8	Tetrasiloxane, 1,1,3,3,5,5,7,7-octamethyl-	38.125	11,064.8	0.333	282.3	C ₈ H ₂₄ O ₃ Si ₄	
9	Hexestrol, 2TMS derivative	38.801	11,619.8	0.350	414.7	C ₂₄ H ₃₈ O ₂ Si ₂	

Discussion

The phytochemical constituents identified in the extract of *T. natans* using GC–MS analysis were mainly phenolic and siloxane derivatives. The presence of phenolic derivatives such as 4-Methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene and 4-Tert-octylphenol suggests that despite being a non-polar solvent, petroleum ether was able to dissolve some of the polar phytochemical constituents of this plant. Although previous studies have shown that highly hydrophilic polyphenolic compounds tend to be found in the polar extracts (Kiruba *et al.*, 2023) [7], our findings demonstrate that the non-polar fraction of *T. natans* also contains certain bioactive phytochemical constituents, which can be easily derivatized and identified using gas chromatography. Other phenolic derivatives identified in this study include 4-(4-Hydroxyphenyl)-4-methyl-2-pentanone, TMS derivative and Hexestrol, 2TMS derivative. These compounds have been reported to possess antioxidant, anti-inflammatory and antimicrobial activities, which further support the potential of *T. natans* as a functional food and a source of bioactive compounds (Banu *et al.*, 2023; Kanase *et al.*, 2024; Huang *et al.*, 2015) [2, 6, 5]. The observed phytochemical profile

correlates well with previous phytochemical screening and pharmacological studies on this plant, which have also revealed presence of phenolic compounds, flavonoids, tannins and other bioactive constituents (Kiruba *et al.*, 2023; Malviya *et al.*, 2010) [7, 9].

In addition to phenolic derivatives, the phytochemical constituents identified in the current study also include siloxane derivatives such as Tetrasiloxane, decamethyl-, Cyclotrisiloxane, hexamethyl- and Octasiloxane, hexadecamethyl-. However, it is important to note that some of these peaks might have been formed due to derivatisation process or might be contaminants originating from GC column or other sources (Mendhekar & Rachh, 2023) [10]. Unfortunately, such an issue is not uncommon in phytochemical analysis since siloxane dimers and trimers (e.g., hexamethylcyclotrisiloxane) as well as silylated alkylphenols (e.g., TMS-4-tert-octylphenol) are often reported in the literature as plant metabolites with diverse biological activity ranging from anti-cancer to anti-microbial properties (Zhang *et al.*, 2018) [12]. Therefore, while interpreting the results of the current study, it is important to bear in mind that some of the identified

phytochemical constituents might not be endogenous to the plant under investigation. The observed phytochemical profile also suggests that the majority of the identified constituents were semi-volatile since they were eluted within a relatively narrow range of retention times (35.725–38.801 min). This could indicate that the extract of *T. natans* contained a number of derivatised compounds, which are more volatile than native phytochemical constituents. Indeed, derivatisation is often used in GC–MS analysis to improve the volatility of various compounds, including phenolic derivatives (Mendhekar & Rachh, 2023) [10]. As a result, the observed GC–MS profile might also reflect the presence of various phytochemical constituents, which have been derivatised during sample preparation.

Overall, the results of the current study are consistent with previous reports on phytochemical and pharmacological properties of *T. natans*. Indeed, several studies have shown that this plant contains phenolic compounds, flavonoids, tannins and other bioactive constituents, which contribute to its antioxidant, anti-inflammatory, antimicrobial and other properties (Banu *et al.*, 2023; Asha *et al.*, 2024) [2, 1]. In addition, the methanolic extracts of this plant have been shown to possess higher antioxidant and antimicrobial activities compared to non-polar extracts, suggesting that the majority of bioactive constituents are likely to be found in the polar fraction (Kiruba *et al.*, 2023) [7]. The results of anti-inflammatory and wound healing studies also support the reported pharmacological properties of this plant (Firoz *et al.*, 2025; Majee *et al.*, 2023; Huang *et al.*, 2015) [4, 8, 5].

Conclusion

T. natans is an economically and nutritionally important aquatic plant, which possesses diverse phytochemical profile and significant biological activity. The current study identified several phytochemical constituents of this plant using GC–MS analysis, which revealed presence of phenolic and siloxane derivatives. The results obtained in this study, alongside previous phytochemical screening and pharmacological investigations, highlight the potential of this plant as a source of bioactive compounds with nutraceutical and pharmaceutical applications.

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