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Phytochemical Profiling and Pharmacological Insights of *Haplanthodes tentaculatus* (L.) R. B. Majumdar Using GC-MS Analysis

PARTH B TRIVEDI[1]* and MADHAVI R BADOLE[1]

[1]Department of Chemistry, Ramnarain Ruia Autonomous College, Matunga, Mumbai-400019, India.
*Corresponding author E-mail: partht6598@gmail.com

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ABSTRACT

Haplanthodes tentaculatus (L.) R. B. Majumdar, an endemic plant of the Acanthaceae family found exclusively in India, has long been utilized in traditional medicine for its healing acceleration and stamina-boosting properties. However, there is limited knowledge about its phytochemical composition and its potential medicinal significance. To address this gap, GC-MS analysis was conducted to unveil the phytochemical profile of *H. tentaculatus*. Moslosooflavone, Squalene, D-Allose, and Phytol were identified as prominent phytoconstituents. These compounds possess known biological activities and are likely responsible for the plant's medicinal properties. This work illustrates the therapeutic capabilities of *H. tentaculatus* and provides a basis for future investigation into its pharmacological uses. Harnessing the rich phytochemical repertoire of *H. tentaculatus* may lead to the development of novel drugs and therapeutic interventions. Future study should prioritise on elucidating the mechanisms of action and clinical efficacy of these phytoconstituents to facilitate their integration into evidence-based healthcare practices.

Keywords: *Haplanthodes tentaculatus*, GC-MS analysis, Moslosooflavone, Therapeutic potential, Pharmacological applications.

INTRODUCTION

Plants have played a significant role in medicine since ancient times.[1] Ayurveda, which has been practiced in India since its inception, remains the most widely recognized and trusted form of traditional medicine.[2,3] One of the key advantages of using Ayurvedic plants is their minimal side effects, leading to the rapid development of plant-based medicines.[4] *Haplanthodes tentaculatus*, or *H. tentaculatus*, is a flowering plant in the Acanthaceae family, Lamiales order, and Magnoliopsida class. It features white flowers and is also recognized as *Haplanthodes tentaculata*. Regionally, it's referred to as "vhadlem kalem kiraytem."Despite an exhaustive literature review, comprehensive documentation of *H. tentaculatus* remains incomplete.[5,6] Therefore, the aim of this study was to utilize the analytical process of GC-MS (Gas Chromatography-Mass Spectrometry) to identify

and isolate biologically active phytocomponents from *H. tentaculatus*.[7] Preliminary research has already been conducted to explore its phytochemical and physicochemical properties. However, more extensive phytochemical investigations were necessary to assess the plant's quality for potential therapeutic use. While chromatographic fingerprinting is commonly employed as a quality control technique, it does not provide a comprehensive phytochemical profile of the plant.[8–10] Consequently, the utilization of GC-MS analysis for different extracts of *H. tentaculatus* was deemed advantageous.[11] This method not only facilitates detailed phytochemical profiling but also aids in the identification of specific phytoconstituents. Motivated by the desire to explore the pharmacological efficacy of plants, we embarked on this research endeavour to uncover the potential applications of *H. tentaculatus*. By investigating its pharmacogenetic quality parameters and conducting in-depth phytochemical studies, we aimed to discover the therapeutic potential of this herbal species.

MATERIALS AND METHODS

Collection of plant material

The plant was collected from Gorai Road Bhayandar, Maharashtra, India. The plant material in the form of an herbarium was authenticated and identified by Blatter Herbarium in St. Xavier's College, Mumbai, India. Specimen matches with the herbarium specimen number 21599 of *H. Santapau*.

Plant Processing and Extraction

The aerial portion of the plant was collected, rinsed with purified water and shade dried for a few days until it dried completely. It was pulverized in a grinder with small pieces. It is an important step for identifying the plant phytochemical profile. Different methods of extraction were used to get maximum extract. Maceration and Sonication Extraction. The solvents methanol, acetone, and n-hexane were used for extraction in order to determine the phytochemical components.

5 gram of pulverized plant material was added to 100 mL of methanol, as was done with acetone and n-hexane. Individually cold maceration of the extract was carried out for 3 days and then used for GC-MS analysis. 5 g of plant material were added to 100 mL of solvent. The extraction process lasted for 30 min, which included 5 min of sonication using an Ultrasonic Bath Sonicator (GSL-200H), with a 1-min hold. The extract obtained will be used for GC-MS examination in further studies.

Gas chromatography-mass spectrometry

All 6 samples were individually analysed on a Shimadzu GCMS-QP2020 NX gas chromatograph-mass spectrometer. System and setup using a fitted fused silica Rtx-5 capillary column (30m X 0.25mm, with 1 µm film thickness). The oven temperature was set to hold at 60°C for 5 min, 124°C for 3 min, 234°C for 3 min and 300°C for 3 min, respectively. Helium was used as the carrier gas with a flow rate of 1mL/minute. The temperature for injection is set at 250°C, pressure 57.4 kPa, split ratio 50, purge flow 3 mL/min, total flow 54 mL/min, and ion source temperature 200°C. The complete analysis time was 41 minutes. The comparison of their mass spectra using the NIST library was used to determine the substance identity.

Phytochemical screening tests

The chemical test was analysed with methanol, acetone and n-hexane extract for qualitative determination of phytochemical test as described by literature.[12–15]

Physicochemical analysis

To analysis the purity and detect adulteration in plants using shade-dried plant material for analysis. The test was performed as mentioned in the literature.[16]

RESULTS

The study utilized GC-MS analysis to profile the phytochemical constituents of *Haplanthodes tentaculatus*, an endemic plant from the Acanthaceae family, to explore its pharmacological potential.

Physicochemical Analysis

The physicochemical properties of the aerial parts of *H. tentaculatus* were quantitatively assessed. The analysis revealed a moisture content of 9.04%, indicating moderate hydration, which is important for the extraction process. The plant material exhibited mineral content and purity, as shown by the respective results of 9.9% for total ash and 8.3% for acid insoluble ash. The different solvent extractive values highlight the plant's diverse solubility profile, essential for effective phytochemical extraction. These physicochemical parameters are in Table 1.

Table 1: Physicochemical Parameters of *Haplanthodes tentaculatus* Aerial Parts: Detailed quantitative assessment of moisture, ash content, and extractive values highlighting the plant's physicochemical properties

Parameters	%(w/w)
Loss on drying	9.04
Total ash value	9.9
Acid insoluble value	8.3
Alcohol soluble extractive value	0.56
Water soluble extractive value	6.24
Alcohol: Aqueous extractive value	5.98

Qualitative Phytochemical Tests

Qualitative screening of the extracts using methanol, acetone, and n-hexane did not detect alkaloids, carbohydrates, glycosides, or cardiac glycosides, indicating a specific phytochemical profile. However, the existence of flavonoids and phenols in methanol and acetone extracts, as well as triterpenoids and reducing sugars in all extracts suggests a rich bioactive compound content conducive to therapeutic uses. These outcomes are summarized in Table 2.

Table 2: Evaluation of Phytochemical Profiles in Various Extracts of *Haplanthodes tentaculatus*: Overview of the existence or nonexistence of essential phytochemical categories, including alkaloids, flavonoids, and terpenoids, in methanol, acetone, and n-hexane extracts

Test	Procedure	Methanol	Acetone	n-hexane	Reference
Alkaloids	Add 1 mL of filtrate along with little amount of Dragendroff's reagent	not present	not present	not present	12
Carbohydrates	Add 1 mL of filtrate with little drops of alcoholic α-naphthol in test tube and add 1 mL of concentrated sulphuric acid	not present	not present	not present	13
Glycosides	Combine 1 mL of sulphuric acid in 1 mL of sulphuric acid in plant extract boil for few minutes, neutralise with 10% NaOH, adding fehling solution A & B	not present	not present	not present	15
Cardiac Glycosides	Add 1 mL of solution, 1.5 mL of glacial acetic acid and 1 drop of 5% $FeCl_3$, added few drops of conc. Sulphuric acid	not present	not present	not present	15
Flavonoids	Combine 1 mL of extract with a little amount of 10% lead acetate solution	present	present	not present	12
Phenols	Add 1 mL of extract along with few drops of 5% ferric chloride solution	present	present	not present	15
Terpenoids	Add 2 mL of chloroform with 5 mL of extract, evaporate and add 3 mL of conc. sulphuric acid	not present	absent not present	not present	14
Triterpenoids	Add 1 mL of extract treat with 1 mL of chloroform next filter. Filtrate with conc. Sulfuric acid shake and allow to stand	present	present	present	15
Reducing sugar	Add 1 mL of filtrate along with little drops of Beneddict's reagent and boil for few minutes	present	present	not present	13

GC-MS Phytochemical Profiling

The GC-MS analysis provided a comprehensive phytochemical profile of *H. tentaculatus*. The analysis of methanol extracts revealed a variety of components, including squalene and flavonoids, known for their antioxidant and anti-inflammatory properties. The acetone and n-hexane extracts predominantly contained diisobutyl cellosolve and hydrocarbons such as n-hexane, which indicate potential for varied therapeutic applications. The detailed phytochemical composition, including area percentages, peak heights, compound names, and retention times, are presented in Tables 3, 4, 5, 6, 7, and 8. Fig. 1, 2, 3, 4, 5, and 6 display the chromatograms of all six plant extracts analysed by GC-MS.

Table 3: Phytoconstituents Identified in Macerated Methanol Extract of Haplanthodes tentaculatus

Peak	R. Time	Area%	Name
1	1.448	29.39	1-Chlorofluoroethane
2	1.547	2.74	Diisobutyl cellosolve
3	1.702	1.39	Acetic acid
4	14.317	3.00	Naphthalene
5	21.034	3.24	Benzenepropanoic acid
6	22.285	2.94	D-Allose
7	24.132	3.25	-
8	28.104	8.55	Pentadecanoic acid
9	29.548	6.04	2-Hexadecen-1-ol , 3,7,11,15-tetramethyl-, acetate,
10	29.817	5.97	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester
11	34.638	5.25	-
12	36.447	17.38	Flavone, 5-hydroxy-7,8-dimethoxy-
13	37.211	10.86	Squalene
	100.00		

Table 4: Phytoconstituents Identified in Macerated Acetone Extract of *Haplanthodes tentaculatus*

Peak	R. Time	Area%	Name
1	1.527	97.96	Diisobutyl cellosolve
2	3.297	0.08	3-Penten-2-one, 4-methyl-
3	4.072	2.02	2-Pentanone, 4-hydroxy-4-methyl-
4	38.725	0.06	
	100.00		

Table 5: Phytoconstituents Identified in Macerated n-Hexane Extract of *Haplanthodes tentaculatus*

Peak	R. Time	Area%	Name
1	1.543	0.34	Acetone
2	1.678	5.15	Pentane, 2-methyl-
3	1.718	5.73	Pentane, 3-methyl-
4	1.764	83.48	n-Hexane
5	1.865	2.15	Acetyl valeryl
6	1.886	2.31	Cyclopentane, methyl-
7	2.054	0.90	Cyclohexane
8	37.195	0.05	
	100.00		

Further analysis using sonication extraction method mirrored the results of the maceration method, confirming the consistency and reliability of the phytochemical data obtained

Table 6: Phytoconstituents Identified in Sonicated Methanol Extract of *Haplanthodes tentaculatus*

Peak	R. Time	Area%	Name
1	1.447	48.54	1-Chloro-1-fluoroethane
2	1.512	18.67	Ethyl alcohol
3	1.546	7.22	Diisobutyl cellosolve
4	14.307	1.65	Naphthalene
5	21.030	1.92	Benzenepropanoic acid
6	24.131	1.52	-
7	26.923	3.97	Neophytadiene
8	28.096	6.00	Pentadecanoic acid
9	34.641	2.15	-
10	36.446	4.51	Flavone, 5-hydroxy-7,8-dimethoxy-
11	37.214	3.75	Squalene
12	39.312	0.10	-

Table 7: Phytoconstituents Identified in Sonicated Acetone Extract of *Haplanthodes tentaculatus*

Peak	R. Time	Area%	Name
1	1.527	97.54	Diisobutyl cellosolve
2	3.304	0.10	2-Pentanone, 4-hydroxy-4-methyl-
3	4.082	2.37	3-Penten-2-one, 4-methyl-
4	39.463	0.02	-

Table 8: Phytoconstituents Identified in Sonicated n-Hexane Extract of *Haplanthodes tentaculatus*

Peak	R. Time	Area%	Name
1	1.544	0.13	Diisobutyl cellosolve
2	1.680	3.29	Pentane, 2-methyl-
3	1.720	4.93	Pentane, 3-methyl-
4	1.766	87.75	n-Hexane
5	1.866	2.19	Pentane, 2,2-dimethyl-
6	1.888	0.77	Cyclopentane
7	2.056	0.73	Cyclohexane
8	39.527	0.21	Hexatriacontane

DISCUSSION

The GC-MS analysis of *Haplanthodes tentaculatus* revealed significant insights into its phytochemical composition, underlining its potential for therapeutic applications. The physicochemical parameters, as shown in Table 1, including the total ash value, acid insoluble ash value, and moisture content suggest that the plant material has retained its integrity with minimal degradation, which is crucial for the extraction of bioactive compounds.

The qualitative phytochemical tests detailed in Table 2 demonstrated the absence of alkaloids, carbohydrates, glycosides, and cardiac glycosides across all extracts, pointing towards a unique phytochemical profile tailored towards specific biological activities. This specificity may enhance the plant's value in developing targeted therapeutic agents with minimal side effects.

The phytochemical profiles derived from different extraction methods, as illustrated in Tables 3 to 8 and depicted in Fig. 1 to 6, provide: Chromatogram of macerated and sonication Haplanthodes tentaculatus in Methanol, acetone, and n-hexane, detailing the peak representations of specific compounds. Figure created by the author using data from GC-MS analysis conducted on April 2023. Notably, compounds such as flavone, squalene, and pentadecanoic acid, identified in multiple extracts, are associated with antioxidant and anti-inflammatory properties, supporting the traditional uses of *H. tentaculatus* for healing and stamina-boosting. Moslosooflavone, a principal component discovered, has substantial antioxidant and anti-inflammatory characteristics, essential for facilitating wound healing by alleviating oxidative stress and diminishing inflammation. These pathways are probable factors in the plant's qualities that expedite healing, as historically used.[17] Moreover, its function in mitigating cellular oxidative damage may contribute to increased stamina by improving overall cellular health and energy metabolism.[18]

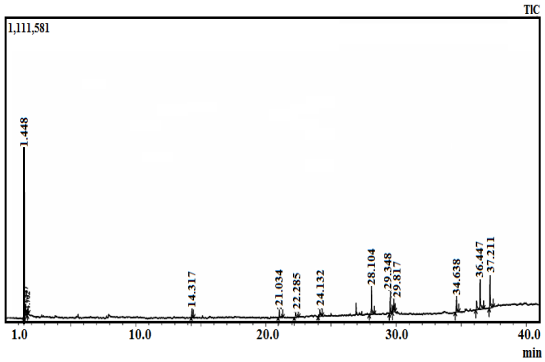

Fig. 1. GC-MS Bands of Macerated *Haplanthodes tentaculatus* in Methanol Extract

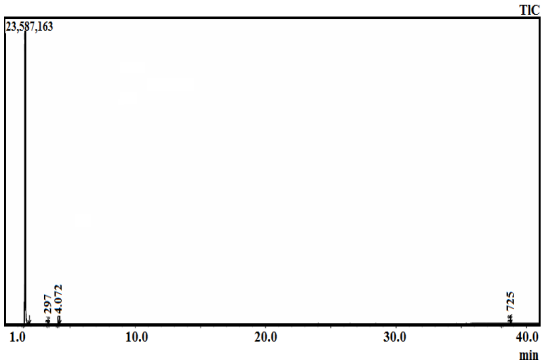

Fig. 2. GC-MS Bands of Macerated *Haplanthodes tentaculatus* in Acetone Extract

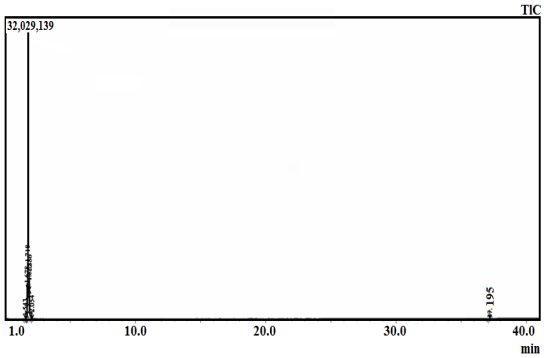

Fig. 3. GC-MS Bands of Macerated *Haplanthodes tentaculatus* in n-Hexane Extract

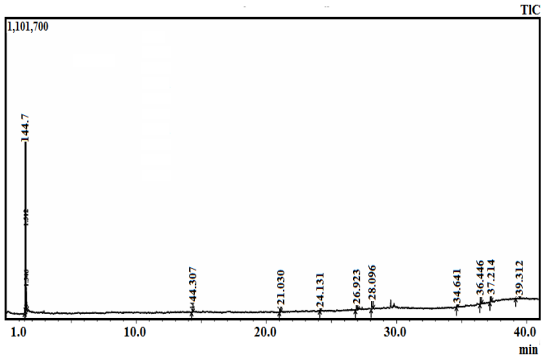

Fig. 4. GC-MS Bands of Sonicated *Haplanthodes tentaculatus* in Methanol Extract

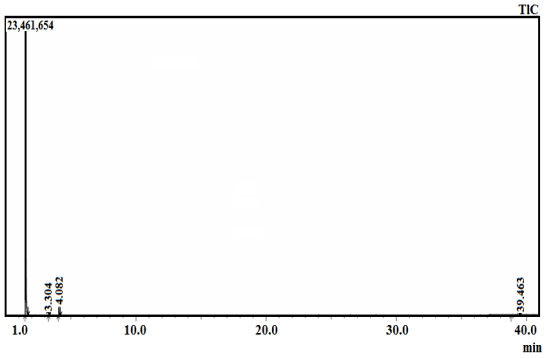

Fig. 5. GC-MS Bands of Sonicated *Haplanthodes tentaculatus* in Acetone Extract

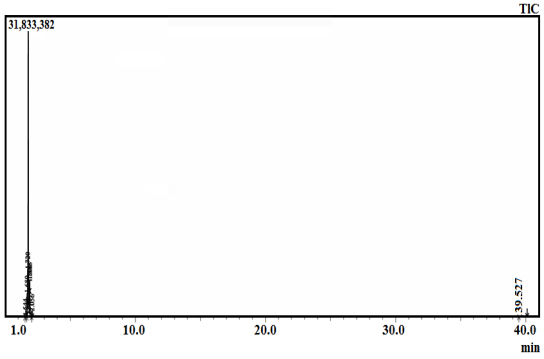

Fig. 6. GC-MS Bands of Sonicated *Haplanthodes tentaculatus* in n-Hexane Extract

Squalene, a significant component in *H. tentaculatus*, is acknowledged for its tissue healing properties attributed to its anti-inflammatory and antioxidant actions. This corresponds with the conventional use of the herb for promoting healing. Furthermore, squalene's involvement in lipid metabolism may enhance stamina by aiding in energy balance and bolstering the immune system during recuperation.[19] D-Allose, while less recognised, has shown antioxidant properties, rendering it advantageous for cellular protection throughout the healing process. Its capacity to influence immunological responses further substantiates its role in the healing acceleration typically ascribed to *H. tentaculatus*. These effects may contribute to increased stamina by bolstering overall immunological resilience after physical exertion.[20] Pentadecanoic acid, found in many extracts, has antibacterial and anti-inflammatory properties that may assist in infection prevention during healing and facilitate tissue repair. Its function in mitigating inflammation corresponds with the historical use of *H. tentaculatus* for wound repair.[21] The statistical analysis confirms the significant variance in the phytochemical compositions between different extracts, highlighting the critical role of solvent choice in maximizing the yield of specific bioactive compounds. The presence of compounds like D-Allose, Pentadecanoic acid, and Squalene, as detailed in Table 9, and their known biological activities underscore the pharmacological relevance of this plant. These compounds have been documented to exhibit antioxidant, antibacterial, and anti-inflammatory properties, which could potentially be harnessed for pharmaceutical development.

Table 9: Biologically Active Compounds Identified in Haplanthodes tentaculatus with Molecular Details and Reported Activities

Sr. No	Compound name/Synonyms	Mol. Weight	Mol. Formula	Activity	Reference
1	D-Allose	180.16	$C_6H_{12}O_6$	Antioxidant	20
2	Pentadecanoic acid	242.40	$C_{15}H_{30}O_2$	Antibacterial, Antifungal	21
3	5-hydroxy-7,8-dimethoxyflavone/ Moslosooflavone/7-O-Methylwogonin	298.29	$C_{17}H_{14}O_5$	Antiviral, Antioxidant, Antitumor, Anti-inflammatory, Anticancer	18
4	Squalene	410.7	$C_{30}H_{50}$	Antioxidant, Anti-inflammatory	19

Moreover, the findings from the GC-MS study and the consistent identification of bioactive compounds in both macerated and sonicated extracts emphasize the reproducibility and efficiency of the extraction methods employed. This robustness in the phytochemical extraction process ensures that the plant's potential for medicinal use is maximized, paving the way for further *In-vivo* studies and clinical trials.

CONCLUSION

This study highlights the significant phytochemical potential of *Haplanthodes tentaculatus*, as evidenced by GC-MS analysis identifying key compounds like Moslosooflavone and Squalene. These findings validate the traditional uses of the plant and underscore its potential for developing novel therapeutic agents. Future research should focus on *In-vivo* studies to explore the clinical efficacy of these compounds, aiming to integrate this plant into evidence-based medicinal practices.

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Conflict of interest

The authors affirm that they do not own any conflicting interests to disclose.

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