International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 11, Issue 11-2024

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2024.11.11.007

Exploring the Phytochemical Diversity of *Haplanthodes tentaculatus* (L.) R. B. Majumdar Through GC-MS Analysis

Parth B. Trivedi*, Madhavi R. Badole, Amrin Mulani
Department of Chemistry, Ramnarain Ruia Autonomous College, Matunga, Mumbai, India
*E-mail correspondent: *partht6598@gmail.com*

Abstract

Haplanthodes tentaculatus (L.) R. B. Majumdar, a member of the Acanthaceae family, is a native plant of southern India with potential medicinal significance. Despite its importance, there is limited research on its chemical composition. In this study, we employed gas chromatography-mass spectrometry (GC-MS) to analyze the phytochemical profile of *H. tentaculatus* methanol extract. We identified 11 compounds, including Quinic acid and Flavone, 5-hydroxy-7,8-dimethoxy-, with notable bioactivities such as antioxidant and potential anti-cancer properties. Additionally, three unknown compounds were detected, suggesting avenues for further exploration. Our findings contribute to understanding the therapeutic potential of *H. tentaculatus* and highlight the importance of studying endemic plants for natural product discovery.

Keywords: Acanthaceae, Bioactive compounds, Gas chromatography-mass spectrometry (GC-MS), Haplanthodes tentaculatus, Medicinal plants, Phytochemical analysis

Introduction

H. tentaculatus, a member of the Acanthaceae family, is indigenous to the southern regions of India, thriving in tropical and subtropical climates like many of its botanical relatives (Awasarkar et al., 2014; Datar & Lakshminarasimhan, 2013; Ibrahim Khan et al., 2017; Turner, 2021). Within the genus *Haplanthodes*, four prevalent species are notably found in Southern India: *Haplanthodes neilgherryensis*, *Haplanthodes plumosa*, *Haplanthodes tentaculata*, and Haplanthodes verticillate (Irwin & Narasimhan, 2011). Neighboring countries host analogous species, such as *Haplanthus hygrophiloides* in Myanmar and *Haplanthuslaxiflorus* distributed across Bangladesh, Bhutan, Cambodia, China, Malesia, Myanmar, Thailand, and Vietnam (Gnanasekaran et al., 2016).

The Acanthaceae family, to which *H. tentaculatus* belongs, exhibits a diverse array of bioactivities attributed to its various plant species. These include antimicrobial, antibacterial, antioxidant, anti-inflammatory, and anti-uric acid properties, as well as anti-cancer and cytotoxic activities. Moreover, specific studies have revealed additional benefits such as antidiabetic and antidiarrheal activities(Mehta et al., 2021; Meurer-Grimes et al., 1996; Sagnia et al., 2014).

Despite its potential medicinal significance, *H. tentaculatus* remains relatively underexplored, with scant literature available on its chemical composition and therapeutic properties. Recognizing the importance of comprehensive research in unlocking the full potential of this species, we embarked on a study aimed at elucidating its phytochemical profile.

To this end, we employed gas chromatography-mass spectrometry (GC-MS), a powerful analytical technique renowned for its ability to provide highly specific and sensitive identification of compounds present in complex mixtures. Methanol was chosen as the solvent for extraction due to its efficiency in extracting a wide range of phytochemicals, including polar compounds, flavonoids, alkaloids, and glucosides(Tiwari, Prashant & Kumar, Bimlesh & Kaur, M. & Kaur, G. & Kaur, 2011). Additionally, methanol offers broad-spectrum extraction capabilities and is well-suited for subsequent analysis.

In this study, we utilized a simple yet effective extraction method, maceration, to obtain crude extracts from *H. tentaculatus*. These extracts were then subjected to GC-MS analysis to identify both volatile and semi-volatile compounds present in the plant material. By documenting the phytoconstituents of *H. tentaculatus*, we contribute to the broader understanding of its chemical composition and potential therapeutic applications(Xu, 2019).

Furthermore, our findings lay the groundwork for future investigations, including the isolation and characterization of novel compounds identified through GC-MS analysis. Such endeavours hold promise for uncovering new bioactive molecules with pharmacological relevance, thereby enriching the field of natural product research and potentially advancing the development of novel therapeutics.

In summary, this study addresses the knowledge gap surrounding *H. tentaculatus* by employing modern analytical techniques to explore its chemical constituents. Our research underscores the importance of preserving and studying endemic plant species like *H. tentaculatus* for their potential medicinal benefits and contributes to the ongoing efforts in natural product discovery and drug development.

Materials and Methods

H. tentaculatus Extract Preparation:

H. tentaculatus specimens were gathered from Bhayandar, Thane, India, in their natural habitat. The entire aerial parts were collected, excluding the roots. Initially, the plant material was meticulously cleaned with fresh water and left to air dry for a period of 15 days. Subsequently, the dried plant material was macerated using a mortar and pestle in 500 ml of 99% pure methanol, with 100 g of dried plant material per extraction. The resulting mixture was subjected to filtration using a vacuum filtration technique to obtain the crude extract, which was then earmarked for further analysis.

GC-MS Analysis:

The obtained extract underwent Gas Chromatography-Mass Spectrometry (GC-MS) analysis using a Shimadzu GCMS-QP2020 NX gas chromatograph-mass spectrometer equipped with an Rtx-5 fused silica capillary column. The analytical run was programmed for 40 minutes with specific parameters: an oven temperature of 60 degrees Celsius, an injection temperature of 250 degrees Celsius, and a column flow rate of 1 mL/minute. The split ratio was set at 50. The temperature program was initiated at 60 degrees Celsius and held for 5 minutes, followed by a ramp to 124 degrees Celsius at a rate of 6 degrees per minute with a 3-minute hold, then a further ramp to 234 degrees Celsius at a rate of 11 degrees per minute with another 3-minute hold, and finally a ramp to 300 degrees Celsius at the same rate with a 3-minute hold. The ion source temperature was maintained at 200 degrees Celsius, and the acquisition mode was set to ACQ scan with a scan speed of 1666. Mass spectral data were acquired over a range from m/z 35 to m/z 500.

Identification of compounds present in the GC-MS chromatograms was facilitated using the NIST library, allowing for the identification of various phytochemical constituents present in the *H. tentaculatus* extract.

Results and Discussion

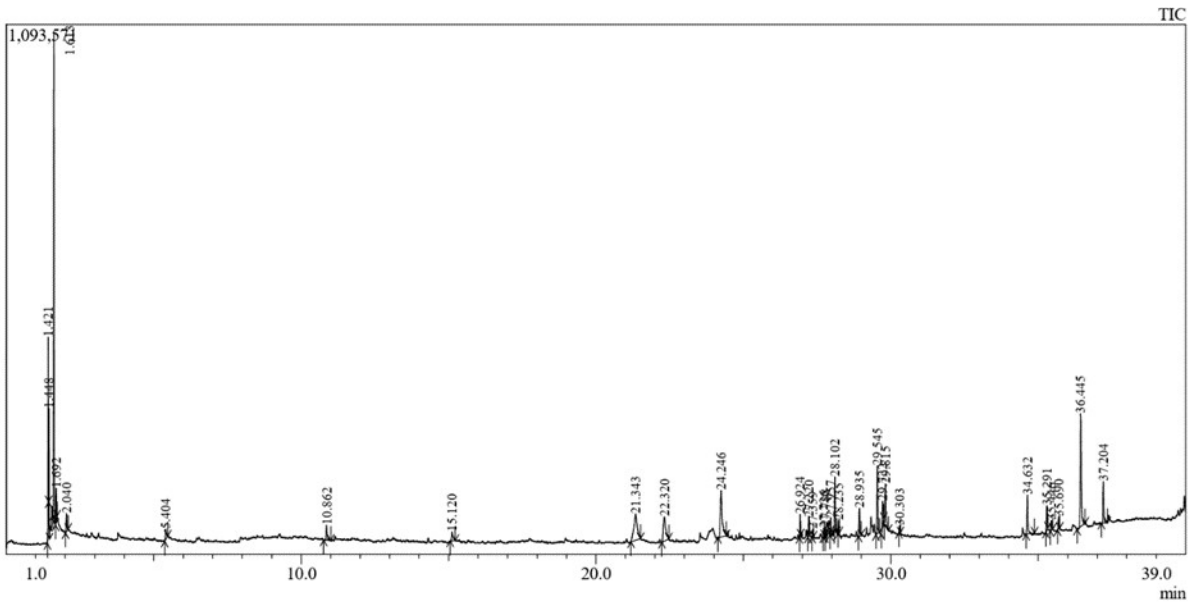
The methanol extract of *H. tentaculatus* was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis, revealing the presence of 11 compounds with notable activity (Table 1). Among these compounds, Quinic acid exhibited a significant area percentage of 7.68%, while Flavone, 5-hydroxy-7,8-dimethoxy- demonstrated an area percentage of 9.34%. The results of the flavonoid test corroborated the presence of flavonoids, providing further validation of the GC-MS findings.

Table 1. Compounds Detected in Methanol Extract of *H. tentaculatus*

Retention time (RT)	Compound	Area %	Mol. formula	Mol. weight	Bio Activity	Reference
21.343	-	7.68	-	-	-	-
24.246	Quinic acid	7.63	$C_7H_{12}O_6$	192	antioxidant, antidiabetic, anticancer activity, antimicrobial	(Benali et al., 2022)
26.924	Neophytadiene	2.07	$C_{20}H_{38}$	278	anti-inflammatory, antioxidant	(Bhardwaj et al., 2020)
27.220	6,10,14,18,22-Tetracosapentaen-2-ol, 3-bromo-2,6,10,15,19,23-hexamethyl-	1.00	$C_{30}H_{51}BrO$	506	-	
27.726	Squalene	3.39	$C_{30}H_{50}$	410	antioxidant, anticancer	(Kim & Karadeniz, 2012)
28.102	Pentadecanoic acid	3.08	$C_{16}H_{32}O_2$	256	antibacterial, antifungal activity	(Chowdhury et al., 2016)
29.545	2-Hexadecen-1-ol	4.76	$C_{22}H_{42}O_2$	338	antioxidant, antimicrobial	(Hidayathulla et al., 2018)
29.772	9,10-Anthracenedione	1.94	$C_{15}H_{10}O_2$	222	antitumor, antimicrobial, antiviral, antioxidant, antiprotozoal, antidiabetic	(Stasevich et al., 2020)

29.815	9,12,15-Octadecatrienoic acid	3.31	$C_{18}H_{30}O_2$	278	analgesic, antidiarrheal, anti-plasmodial, anti-ulcer	(AC et al., 2018)
34.632	-	3.00	-	-	-	-
35.291	-	1.76	-	-	-	-
35.446	Dihydrooroxylin A	1.68	$C_{16}H_{14}O_5$	286	antitubercular	(Jagetia, 2021)
36.445	Moslosooflavone	9.34	$C_{17}H_{14}O_5$	298	anticancer	(Singh et al., 2022)

Figure 1. Chromatogram of Methanol Extract of *H. tentaculatus*



The most prominent activity observed in the extract was antioxidant activity, consistent with previous studies highlighting the antioxidant potential of *H. tentaculatus*. Additionally, the presence of Flavone, 5-hydroxy-7,8-dimethoxy-, with its known anti-cancer properties, suggests the potential therapeutic relevance of this compound in cancer treatment.

Furthermore, three unknown compounds were identified in the extract, exhibiting significant area percentages of 7.68%, 3.00%, and 1.76%, respectively. These compounds, not detected by the NIST library, present intriguing avenues for further exploration and characterization.

Figure 1 provides the Chromatogram of the *H. tentaculatus* extract, highlighting the peaks corresponding to the identified compounds mentioned in Table 1.

Conclusion

In summary, our study sheds light on the phytochemical composition of *H. tentaculatus*, revealing 11 compounds with notable activities, including antioxidant and potential anti-cancer properties. Compounds like Quinic acid and Flavone, 5-hydroxy-7,8-dimethoxy-, demonstrate promising bioactivity, emphasizing the therapeutic potential of this plant species. Our findings advocate for further exploration of *H. tentaculatus* and other endemic plants to uncover novel bioactive molecules. The identification of unknown compounds presents opportunities for future research, promising advancements in natural product discovery. Overall, this study contributes valuable insights into *H. tentaculatus*, laying the groundwork for future pharmacological investigations and potential therapeutic applications.

Acknowledgments

The author acknowledges Ismail Yusuf College, Dr. Dattatraya Bhangare for instrumental support, and Dr. V.R. Naik for plant identification and guidance.

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DOI:10.22192/ijarbs.2024.11.11.007

How to cite this article:
Parth B. Trivedi, Madhavi R. Badole, Amrin Mulani. (2024). Exploring the Phytochemical Diversity of *Haplanthodes tentaculatus* (L.) R. B. Majumdar Through GC-MS Analysis. Int. J. Adv. Res. Biol. Sci. 11(11): 73-78.
DOI: http://dx.doi.org/10.22192/ijarbs.2024.11.11.007