Phytochemical Profiling of *Hydnocarpus pentandrus* (Buch.-Ham.) Oken Leaf Extract for Identification of Bioactive Compounds of Pharmacological Potential using GC-MS

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Abstract

Hydnocarpus pentandrus (Buch.-Ham.) Oken, a member of the family Achariaceae, is a medicinally important plant traditionally used to treat leprosy and skin disorders. In the present study, the leaf extract of *Hydnocarpus pentandrus* was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis to characterise its volatile and semi-volatile phytoconstituents. Analysis was carried out using a gas chromatograph coupled with a triple-quadrupole mass spectrometer operated in electron ionisation mode and compounds were identified by comparison of mass spectra with the National Institute of Standards and Technology (NIST) library. The chromatographic profile revealed the presence of twenty-three phytoconstituents with retention times ranging from 6.458 to 48.788 minutes. The extract was predominantly composed of cyclopentenyl fatty acids, which are characteristic constituents of the Achariaceae family, namely Chaulmoogric acid (42.12%) and Hydnocarpic acid (29.5%), alongside a wide array of flavonoids, including Mangostin (67.5%), Scopoletin (27.01%), Catechin (10.04%), Rutin (13.1%), Luteolin (13.8%), Apigenin (11.4%), Vitexin (16.7%), Quercetin (15.12%) and Chrysoeriol (12.10%). Additional bioactive constituents, including Hydnocarpin, Methoxyhydnocarpin, Hydnowightin, Eugenol, Santonin, Stigmasterol, Diosgenin and Lupeol, were also detected. The co-occurrence of these fatty acids, flavonolignans and phytosterols indicates a chemically rich extract with promising antidiabetic, antimicrobial, anti-inflammatory, antioxidant and antileprotic potential, consistent with the traditional therapeutic use of the leaf extract.

Keywords: *Hydnocarpus pentandrus*, Hydnocarpin, Chaulmoogric acid, Hydnocarpic acid, Flavonoids, Phytochemical profiling, NIST library.

I Introduction

Plants remain a rich reservoir of structurally diverse secondary metabolites that continue to serve as valuable leads for the development of novel therapeutic agents [1]. *Hydnocarpus pentandrus* is a well-recognised medicinal plant, historically valued for its chaulmoogra oil, obtained from its seeds, which was widely used as a principal treatment for leprosy before the advent of modern therapies. In addition to the seeds, the plant's leaves have traditionally been used to manage skin infections, wound healing and inflammatory disorders. However, the phytochemical composition and pharmacological potential of the leaves remain comparatively underexplored [2].

Secondary metabolites, commonly referred to as phytochemicals, are naturally occurring compounds with diverse biological activities that may contribute to disease prevention and therapeutic effects [3]. These metabolites encompass a wide range of bioactive constituents, including volatile oils, steroids, alkaloids, flavonoids and other phenolic compounds with antioxidant, antidiabetic and other properties [4]. The screening of plant-derived bioactive compounds and evaluation of their biological activities have contributed significantly to the discovery of novel therapeutic agents for various diseases. Medicinal plants are widely accessible, relatively inexpensive and often considered safe and effective, with fewer adverse effects than many synthetic drugs. Moreover, modern analytical techniques for identifying and quantifying bioactive constituents in plant materials play an important role in the standardisation and quality control of herbal formulations [5].

Gas chromatography-mass spectrometry (GC-MS) is one of the most widely employed hyphenated analytical techniques for the separation, identification and semi-quantitative estimation of volatile and semi-volatile constituents present in complex plant extracts [6]. The technique combines the high resolving power of capillary gas chromatography with the structural specificity of mass spectrometric detection, enabling the rapid identification and dereplication of known phytoconstituents through spectral matching against reference libraries such as the National Institute of Standards and Technology (NIST) database. GC-MS is particularly valuable for profiling volatile and semi-volatile compounds, including essential oils, fatty acids, lipids and alkaloids, in medicinal plants and herbal preparations [7].

The present investigation was undertaken to characterise the phytochemical profile of the leaf extract of *Hydnocarpus pentandrus* using GC-MS, to identify its major bioactive constituents and correlate the detected compounds with their reported pharmacological activities and traditional medicinal uses. This analysis provides valuable insights into the chemical composition of the leaf extract and supports further evaluation of its therapeutic potential.

II Materials and Methods

II.I Collection of Plant Material

Fresh leaves of *Hydnocarpus pentandrus* were collected from Dapoli, Chandra Nagar, Maharashtra, India (17°44’36.3’’N 73°09’39.1’’E) and authenticated at the Blatter Herbarium, St. Xavier’s College, Mumbai (Accession No. R3060). The leaves were shade-dried and extracted using a suitable solvent system. The resulting extract was concentrated to dryness under reduced pressure prior to GC-MS analysis.

II.II Sample Preparation

The leaf powder was extracted by Soxhlet extraction using a hydroalcoholic solvent (40:60), resulting in a dark green solvent. The extract was collected and the solvent was removed through evaporation. The concentrated extract was used for further investigation. The dried extract was reconstituted in a suitable solvent, filtered through a membrane filter to remove particulate matter and an aliquot of 1 µL of the resulting solution was injected into the GC-MS system for analysis.

II.III Instrumentation and Chromatographic Conditions

GC-MS analysis was performed using a Trace 1300 gas chromatograph coupled with a TSQ 8000 triple-quadrupole mass spectrometer and equipped with a fused-silica capillary column 30 m × 0.25 mm in length, 0.25 μm film thickness. High-purity helium (99.999%) was used as the carrier gas at a constant flow rate of 1.4 mL/min. The injection volume was 1 μL, with a split ratio of 80:1. The injector and ion source temperatures were maintained at 250 °C and 220 °C, respectively. The mass spectrometer was operated in electron ionisation (EI) mode at an ionisation energy of 70 eV and mass spectra were recorded over an m/z range of 50-650. The oven temperature was initially maintained at 70 °C for 1 min and subsequently increased to 260 °C at a rate of 10 °C/min, followed by an isothermal hold for 10 min. The total analytical run time was 35 min. The compounds present in the extract were identified by comparing their mass spectra with those available in the Wiley and NIST spectral libraries, along with reference data reported by Adams and comparison of their retention indices.

II. IV GC-MS Profiling

The individual peaks obtained in the total ion chromatogram (TIC) were identified on the basis of their retention time and characteristic fragmentation pattern by comparison with the mass spectral database of the National Institute of Standards and Technology (NIST) library. The name, molecular formula, molecular weight and percentage peak area of each identified compound were recorded.

III Results

GC-MS analysis of the leaf extract of *Hydnocarpus pentandrus* resulted in the identification of twenty-three phytochemical constituents, with retention times ranging from 6.458 to 48.788 min. The compounds were identified based on their retention times, mass spectral fragmentation patterns and matches with the NIST spectral library. The identified constituents, along with their retention time, molecular formula, molecular weights and percentage peak areas, are presented in Table No. I, Photoplate No.1and Figure No.1.

Table No. I: GC-MS Identified Phytochemical Constituents in the Leaf Extract of *Hydnocarpus pentandrus*

Sr. No.	Retention Time (min)	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area (%)
1	6.458	Mangostin	$C_{24}H_{26}O_6$	410.172	67.5
2	9.195	Scopoletin	$C_{16}H_{18}O_9$	354.095	27.01
3	13.378	Catechin	$C_{15}H_{14}O_6$	290.079	10.04
4	15.642	Cinnamaldehyde	C_9H_8O	132.057	6.83
5	19.378	Hydnocarpin	$C_{22}H_{18}O_6$	378.37	22.4
6	22.340	Eugenol	$C_{10}H_{12}O_2$	164.083	19.7
7	23.617	Rutin	$C_{27}H_{30}O_{16}$	610.153	13.1
8	26.171	Santonin	$C_{15}H_{18}O_3$	246.125	20.07
9	27.391	Luteolin	$C_{15}H_{10}O_6$	286.047	13.8

Sr. No.	Retention Time (min)	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area (%)
10	31.140	Apigenin	$C_{15}H_{10}O_5$	270.052	11.4
11	33.004	Vitexin	$C_{21}H_{20}O_{10}$	432.105	16.7
12	34.218	Quercetin	$C_{15}H_{10}O_7$	302.042	15.12
13	37.718	Citral	$C_{10}H_{16}O$	152.120	7.12
14	38.667	Chrysoeriol	$C_{15}H_{10}O_6$	300.063	12.10
15	39.399	Chaulmoogric acid	$C_{18}H_{32}O_2$	280.240	42.12
16	39.715	Methoxyhydnocarpin	$C_{26}H_{22}O_{10}$	494.121	14.23
17	40.781	Hydnowightin	$C_{35}H_{30}O_{12}$	642.173	21.2
18	41.863	Gorlic acid	$C_{18}H_{30}O_2$	278.224	12.13
19	44.793	Probucol	$C_{31}H_{48}O_2S_2$	516.309	19.4
20	45.159	Hydnocarpic acid	$C_{16}H_{28}O_2$	252.208	29.5
21	48.405	Stigmasterol	$C_{29}H_{48}O$	412.370	21.3
22	48.422	Diosgenin	$C_{27}H_{42}O_3$	414.313	23.12
23	48.788	Lupeol	$C_{30}H_{50}O$	426.386	19.23

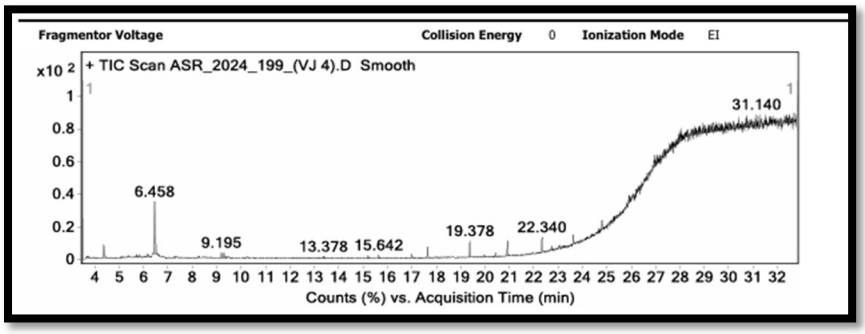

Figure No.1: GC-MS Chromatogram of *Hydnocarpus pentandrus* Leaf Extract

The qualitative analysis was performed using GC-MS (Gas Chromatography–Mass Spectrometry). The analysis was carried out using an injection volume of 0.5 µL. Mass Hunter GC/MS software was used for data acquisition, with Tandem Quadrupole MS software (version 7.6.0.0) used for data processing. The chromatogram and corresponding data are presented in Figure No.1, Photoplate No. 1 and Table No.1, which reveal the presence of twenty-three bioactive compounds with varying retention times and peak area percentages, indicating the complex chemical composition of the extract. The chromatogram showed a predominance of fatty acids and flavonoids, with Chaulmoogric acid (RT 19.399 min, 42.12%) and Hydnocarpic acid (RT 25.159 min, 29.5%), indicating the presence of major constituents. Significant amounts of flavonoids such as Mangostin (67.5%), Scopoletin (27.01%), Catechin (10.04%), Rutin (13.1%), Luteolin (13.8%), Apigenin (11.4%), Vitexin (16.7%) and Quercetin (15.12%) were also detected. Other important phytocompounds identified include Hydnocarpin (22.4%), Methoxyhydnocarpin (14.23%), Hydnowightin (21.2%), Eugenol (19.7%), Santonin (20.07%),

Stigmasterol (21.3%), Diosgenin (23.12%) and Lupeol (19.23%). The presence of these diverse secondary metabolites suggests the extract possesses significant pharmacological potential, as presented.

The phytochemical profile comprises diverse classes, each contributing to a wide range of biological activities. These compounds exhibit significant pharmacological properties, including anti-inflammatory, anti-diabetic, antioxidant, antimicrobial, hepatoprotective and neuroprotective effects. Collectively, the diverse range of bioactive constituents presented in Table No.2 underscores their significant therapeutic potential in the management of metabolic disorders, infectious conditions, chronic diseases and hormonal imbalances such as polycystic ovary syndrome (PCOS).

Table No. II: List of Compounds Summarised as Per Chemical Class Identified in the Extract

Class	Respective Compounds	Count
Xanthones	Mangostin	1
Coumarins	Scopoletin	1
Flavonoid	Catechin, Rutin, Luteolin, Apigenin, Vitexin, Quercetin, Chrysoeriol	7
Phenolic	Cinnamaldehyde, Eugenol, Gorlic acid	3
Cyclopentyl fatty acids	Chaulmoogric acid	1
Flavonolignins	Hydnocarpin, Methoxyhydnocarpin, Hydnowightin	3
Steroids	Probucol, Stigmasterol, Diosgenin	3
Terpenoids	Lupeol	1
Cardiac glycosides	Santonin	1

Table No. III: Biological Activities of Bioactive Compounds Identified in the Extract

Sr. No.	Name of the Compound	Class of Bioactive Compounds	Medicinal Use
1	Mangostin	Xanthones	Alleviates rheumatoid arthritis
2	Scopoletin	Coumarins	Antiinflammatory
3	Catechin	Flavonoid	Antibacterial, antimutagenic and anorexic effects
4	Rutin	Flavonoid	Anti-diabetic and antifungal effects
5	Luteolin	Flavonoid	Enhances muscle activity
6	Apigenin	Flavonoid	Neuroprotective effects
7	Vitexin	Flavonoid	Antiviral and antioxidant
8	Quercetin	Flavonoid	Hepatoprotective
9	Chrysoeriol	Flavonoid	Anti-diabetic, antioxidant and antifungal
10	Cinnamaldehyde	Phenolic	Anticancer, antiviral and antibacterial
11	Eugenol	Phenolic	Metabolic disorders
12	Gorlic acid	Phenolic	Antiaging, antibacterial and antioxidant
13	Chaulmoogric acid	Chaulmoogric acid	Cardioprotective
14	Hydnocarpin	Flavonolignins	Anti-diabetic, antioxidant and antibacterial
15	Methoxyhydnocarpin	Flavonolignins	Anti-diabetic, antioxidant and antiinflammatory
16	Hydnowightin	Flavonolignins	Metabolic disorders
17	Probucol	Steroids	Antiproliferative, antibacterial and apoptotic
18	Diosgenin	Steroids	Antinociceptive
19	Stigmasterol	Steroids	Antimicrobial, antioxidant and antibacterial
20	Lupeol	Terpenoids	Antioxidant
21	Santonin	Cardiac glycosides	Used in treating PCOS

IV Discussion

The predominance of chaulmoogric and hydnocarpic acid in the leaf extract is consistent with the taxonomic identity of *Hydnocarpus pentandrus*, as these cyclopentenyl fatty acids are recognized as chemotaxonomic markers of the genus [10,11]. They are also the principal constituents historically associated with the antileprotic, antidiabetic and antimicrobial properties of chaulmoogra oil. The presence of these compounds in the leaves suggests that the leaves may also contribute to the plant's traditional therapeutic applications, particularly in managing diabetic, skin and infectious disorders [12,13].

The flavonoids and flavonolignans identified in the extract, including mangostin, rutin, quercetin, luteolin, apigenin, vitexin, chrysoeriol, hydnocarpin, methoxyhydnocarpin and hydnowightin, have been widely reported for their antioxidant, antidiabetic, anti-inflammatory, hepatoprotective and antimicrobial properties [14,15]. Among these, hydnocarpin has attracted particular interest due to its reported antidiabetic potentiating effects and suggests a possible synergistic interaction with the fatty acid fraction. The identification of stigmasterol, diosgenin, and lupeol further indicates the potential contribution of phytosterols and triterpenoids to the extract's anti-inflammatory, antidiabetic and anticancer activities [16,17]. In addition, phenylpropanoids such as eugenol and cinnamaldehyde, along with the monoterpenoid citral, are well recognised for their antimicrobial and antioxidant activities and may further contribute to the overall pharmacological potential of the extract [18,19].

Collectively, the co-occurrence of fatty acids, flavonoids, flavonolignans, phenylpropanoids and phytosterols within a single extract reflects its complex phytochemical composition and provides a plausible chemical basis for the diverse traditional therapeutic applications attributed to *Hydnocarpus pentandrus* [20]. Further bioassay-guided fractionation and quantitative validation of the individual constituents are warranted to establish their specific contributions to the observed pharmacological activities [21].

V Conclusion

GC-MS analysis of the leaf extract of *Hydnocarpus pentandrus* revealed a chemically diverse phytochemical profile comprising twenty-three identified constituents. The profile was dominated by the characteristic cyclopentenyl fatty acids, chaulmoogric acid and hydnocarpic acid, along with a diverse range of flavonoids, flavonolignans, phenylpropanoids and phytosterols. These findings provide chemical support for the traditional and pharmacological significance of the plant and establish a foundation for the further isolation, quantitative characterization and bioactivity evaluation of its individual phytoconstituents.

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Conflicts of Interest

The authors have declared that no conflicts of interest exist.

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