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Identification of bioactive constituents in *Lepidagathis sabui* stem extracts using GC-MS and FTIR spectroscopy

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Abstract
Plants have historically been recognized as rich reservoirs of medicinal compounds. *Lepidagathis sabui*, a recently identified endemic species within the Acanthaceae family, was located in the Konkan region of Maharashtra, India. The objective of the current study was to analyze the phytochemical composition and secondary metabolites present in the stem of *L. sabui*, utilizing both Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier Transform Infrared Spectroscopy (FTIR). Stem extracts were obtained using solvent extraction methods with methanol, dichloromethane, and chloroform. Among these, the methanol extract demonstrated the highest concentrations of phenolic compounds and flavonoids. A preliminary screening of this extract indicated the presence of a wide array of bioactive constituents, including alkaloids, flavonoids, phenols, tannins, terpenoids, saponins, glycosides, resins, coumarins, phytosterols, and carbohydrates. GC-MS analysis successfully identified 53 phytochemical compounds, with the predominant constituents being hexadecanoic acid methyl ester, n-hexadecanoic acid, linoleic acid ethyl ester, and 9-octadecenoic acid methyl ester. FTIR analysis further corroborated the presence of various functional groups, including amines, alcohols, phenols, alkanes, aldehydes, and conjugated acids. The results indicate that *L. sabui* may possess noteworthy pharmacological potential, particularly in antimicrobial, anticancer, anti-inflammatory, and antidiabetic contexts. It is recommended that further pharmacological and toxicological studies be conducted to thoroughly investigate its therapeutic capabilities.

Keywords: Bioactive metabolites, FTIR, GC-MS, *Lepidagathis sabui*, phytochemical analysis

Introduction
Plants are valuable sources of secondary metabolites, which are compounds not directly involved in growth but crucial for plant survival and defense. These metabolites are classified into three main groups: terpenoids, alkaloids, and phenolics (Shoker, 2020) [29]. Secondary metabolites possess significant therapeutic potential and are widely used in pharmaceutical applications (Sreenivasulu, 2015; Vaishnav and Demain, 2011) [30, 33]. They exhibit various pharmacological properties, including antimalarial, antidiabetic, hepatoprotective, antiulcer, anti-inflammatory, and antimicrobial activities (Gupta *et al.,* 2018) [9]. Phenolic compounds act as antioxidants and offer protection against various diseases. Alkaloids have a long history of medicinal use and continue to be utilized in modern medicine (Rahman *et al.,* 2021b) [25]. Terpenoids, present in most natural diets, also have important medicinal applications (Cox-Georgian *et al*., 2019) [4]. The study of plant secondary metabolites is crucial for drug discovery and development, as they provide lead compounds for treating a wide range of diseases (Velu *et al.,* 2018) [33].
The identification and characterization of secondary metabolites from medicinal plants require advanced analytical techniques. Fourier Transform Infrared Spectroscopy (FTIR) is widely used to identify functional groups in phytochemicals, playing a crucial role in the characterization of herbal medicines (Wali *et al*., 2022) [35]. Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS) are essential for separating and identifying bioactive compounds (Gabhale & Tapkir, 2020) [8]. These hyphenated techniques combine chromatographic separation with spectroscopic identification, offering advantages in analyzing complex natural extracts. Liquid Chromatography-Nuclear Magnetic Resonance (LC-NMR) is another powerful tool for structural elucidation. These analytical methods are crucial for metabolite profiling, dereplication, and drug discovery from natural sources.

The integration of these techniques with bioassay profiling enhances the identification and characterization of potential therapeutic compounds from medicinal plants (Brusotti *et al.,* 2013) [1].

The genus *Lepidagathis* (Acanthaceae) is known for its diverse medicinal properties and phytochemical constituents. *Lepidagathis sabui*, a newly described species of Acanthaceae, is illustrated and documented from the Konkan region of Maharashtra, India (Chandore *et al.,* 2020a) [2]. This species is closely related to *L. prostrata* but is distinguished by its linear, spinulose, glabrous leaves, axillary spikes, recurved bracts, and outer sepals that have 5-7 nerves. To aid in identification, colored photographs and illustrations of the new species, along with a key to the Indian species featuring spinescent bracts, a five-part calyx, and a two-seeded capsule, are provided (Chandore *et al.,* 2020b) [2]. The Acanthaceae family, including *Lepidagathis*, is rich in secondary metabolites like alkaloids, terpenoids, and flavonoids, which contribute to its medicinal value (Ponnusamy & Balakrishnan, 2023) [22]. GC-MS analysis revealed the presence of various phytocomponents, including cyclopentaneundecanoic acid and n-hexadecanoic acid, which may contribute to its antioxidant properties (Palakkal *et al*., 2017) [19]. These findings highlight the potential of *Lepidagathis* species as sources of natural antioxidants and therapeutic agents.

Recent studies have explored the phytochemical profiles and secondary metabolites of various plant species using advanced analytical techniques. The studies examined phytochemical constituents in various plant extracts using FTIR and GC-MS techniques. FTIR analysis consistently revealed functional groups like OH, C=C, C-H, and C-O (Karki *et al.,* 2020) [12]. GC-MS analysis identified numerous bioactive compounds, with the number of compounds detected ranging from 15 to 30 across studies (Saravanakumar *et al.,* 2016) [27]. Common phytochemicals found included alkaloids, flavonoids, tannins, and saponins (Pakkirisamy *et al.,* 2017) [18]. These techniques proved effective in characterizing phytochemical constituents, supporting the potential use of these plants in pharmaceutical and medicinal applications (Endris *et al*., 2024) [6]. These studies demonstrate the importance of phytochemical profiling in understanding the medicinal properties of endemic plant species and highlight the potential for developing novel therapeutic interventions based on their rich phytochemical repertoires.

Materials and Methods

Collection of Plant Material

Mature and healthy specimens of *Lepidagathis sabui* were collected from the native habitat situated on the lateritic plateaus of the Konkan region, specifically near Rajapur, Maharashtra, India. A herbarium voucher specimen was prepared and photographed prior to submission for taxonomic verification. Identification and authentication of the collected plant material were conducted at the Botanical Survey of India (BSI), Western Regional Circle (WRC), Pune.

Preparation of Plant Extracts

The stem segments of the harvested *Lepidagathis sabui* shoots were separated and shade-dried for a duration of 14 days. Following the drying process, the plant material was reduced to small fragments and ground into a fine powder. The powdered material was then sieved using a muslin cloth to ensure a uniform particle size. For the extraction process, a total of 10 g of the homogenized coarse powder was subjected to extraction with 100 mL of each of the following organic solvents: methanol, dichloromethane, and chloroform. Soxhlet extraction was performed for each solvent over five cycles, utilizing optimal temperatures of 64°C for methanol, 39°C for dichloromethane, and 61°C for chloroform. Each extraction cycle lasted between 5 to 6 hours. Subsequently, the resultant extracts were filtered through sterilized Whatman No. 1 filter paper. The filtrates were concentrated using a rotary evaporator under vacuum at 45-50°C, yielding semi-solid crude extracts, which were subsequently stored at 4°C for further phytochemical analyses.

Quantitative Analysis of Phytochemicals

Quantitative phytochemical analysis was conducted in accordance with the methodology established by Madhu *et al.* (2016) [13]. The extracts prepared with the aforementioned solvents (Methanol, Dichloromethane, and Chloroform) were utilized for comprehensive quantitative evaluations.

Phytochemical Screening

The methanolic extract underwent extensive screening for a variety of phytochemical constituents including tannins, terpenoids, flavonoids, alkaloids, phenols, phytosterols, quinones, and saponins. The presence of these bioactive compounds was confirmed through triplicate qualitative tests, adhering to the standard protocols delineated by Edeoga *et al*. (2005) [5].

GC-MS Analysis

The methanolic extract of the *Lepidagathis sabui* stem was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis using a Shimadzu TQ-8030 instrument. The analysis was performed under optimized conditions at the Central Instrument Facility (CIF), Savitribai Phule Pune University. The column oven temperature was calibrated to 50.0°C, while the injection temperature was maintained at 250.0°C in a splitless mode. A sampling time of 1.00 minutes was employed, with the flow control set to linear velocity at a pressure of 53.5 kPa. The total flow rate was sustained at 14.0 mL/min, with a column flow of 1.00 mL/min and a linear velocity of 36.3 cm/sec. Additional parameters included a purge flow of 3.0 mL/min and a split ratio of 10.0. Functions for high-pressure injection and carrier gas saver were deactivated.

FTIR Spectroscopic Analysis

Fourier Transform Infrared Spectroscopy (FTIR) served as an essential analytical technique for identifying functional groups and characterizing chemical bonds within bioactive compounds. FTIR analysis was conducted following the protocol established by Moumita *et al*. (2017) [15]. Dried powders of the methanolic extract of *Lepidagathis sabui* were subjected to spectroscopic examination. IR spectra were recorded at room temperature using a PerkinElmer Spectrum Two Fourier Transform Infrared Spectrometer located at the Department of Chemistry, Shri Anand College, Pathardi, Maharashtra. Each analysis was replicated three times to ensure the reliability and accuracy of the results.

Results and Discussion

Quantitative Phytochemical Analysis

The concentrations of secondary metabolites in methanol, dichloromethane, and chloroform stem extracts of *Lepidagathis sabui* are presented in Table 1. Among the three solvents, methanol extract exhibited the highest flavonoid

content (4.952 mg/g), while dichloromethane extract showed moderate flavonoid levels (2.262 mg/g). The chloroform extract contained the least concentration of flavonoids (0.416 mg/g) but had a relatively higher phenolic content (0.434 mg/g) than its flavonoid or alkaloid contents. Alkaloids were present in minimal quantities across all extracts.

Table 1: Quantitative estimation of phenols, flavonoids, and alkaloids in *Lepidagathis sabui* stem extracts.

Solvent	Phenols (mg/g ± SE)	Flavonoids (mg/g ± SE)	Alkaloids (mg/g ± SE)
Methanol	1.357±0.048	4.952±0.013	0.197±0.025
Dichloromethane	0.809±0.080	2.262±0.010	0.259±0.010
Chloroform	0.434±0.022	0.416±0.062	0.249±0.012

Phenolic and flavonoid compounds are widely recognized for their antioxidant properties, serving as free radical scavengers and contributing to the therapeutic potential of plant-based formulations (Jones, 1994; Madhu *et al*., 2016) [10, 13]. Our findings align with previous studies reporting significant levels of secondary metabolites in various plant species (Parekh & Chanda, 2007; Narayani, 2012) [21, 17]. Notably, phenol, traditionally used as a urinary antiseptic and in dermatological treatments (e.g., hyperpigmentation), further highlights the pharmacological relevance of these compounds (Forbes, 1953) [7].

Preliminary Phytochemical Screening: Qualitative phytochemical screening revealed the presence of multiple bioactive constituents in the methanol stem extract (Table 2). The extract tested positive for alkaloids, flavonoids, phenols, tannins, terpenoids, saponins, glycosides, resins, coumarins, phytosterols, and carbohydrates. Phlobatannins were absent. These findings confirm the biochemical diversity of the extract, which may underlie its pharmacological potential. Preliminary phytochemical analysis is essential in identifying the bioactive compounds responsible for the therapeutic properties of medicinal plants (Shaikh & Patil, 2020) [28].

Table 2: Preliminary phytochemical screening of *Lepidagathis sabui* stem extract

Sr. No.	Phytochemical Component	Test(s) Used	Result
1	Alkaloids	Hager's test, Wagner's test	+
2	Flavonoids	Ammonia test, H_2SO_4 test, Alkaline reagent test	+
3	Phenols	Iodine test, Lead acetate test, $K_2Cr_2O_7$ test	+
4	Tannins	Mitchell's test, 10% NaOH test	+
5	Terpenoids	Salkowski's test, Liebermann-Burchard test	+
6	Saponins	Foam test	+
7	Glycosides	Bromine water test, Biljet test	+
8	Phlobatannins	HCl test	-
9	Resins	Turbidity test	+
10	Coumarins	Alcoholic NaOH test	+
11	Phytosterols	Salkowski's test	+
12	Carbohydrates	Sulfur test	+

Note: '+' indicates the presence, and '-' indicates the absence, of the phytochemical compound in the methanol extract.

GC-MS Analysis

GC-MS profiling of the methanolic extract identified 53 phytochemical constituents (Figure 1; Table 3). Noteworthy compounds included: n-Hexadecanoic acid (10.16%), Linoleic acid ethyl ester (6.67%), Hexadecanoic acid, methyl ester (5.11%), and 9-Octadecenoic acid, methyl ester (E) (5.82%). The extract also contained sugars (e.g., sucrose, glycerin), aromatic aldehydes (e.g., 5-hydroxymethylfurfural), and a variety of fatty acids and esters. These molecules are often associated with antioxidant, anti-inflammatory, and antimicrobial properties and are widely utilized in pharmaceutical and cosmetic formulations (Mohammad *et al.,* 2021; Rajina *et al.*, 2017) [14, 26].

These results underscore the diverse phytochemical profile of *Lepidagathis sabui*, indicating its potential for developing bioactive formulations.

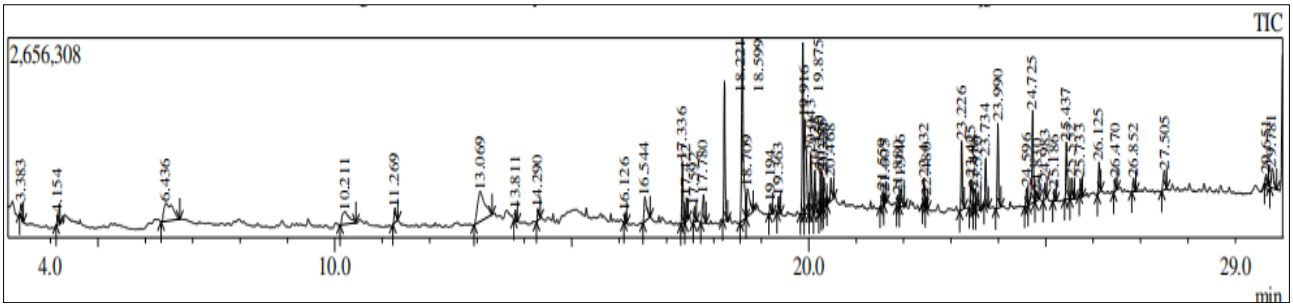

Fig 1: Chromatogram of *Lepidagathis sabui* Stem

Table 3: Phytochemical compounds identified in the methanolic extract of *Lepidagathis sabui* stem using GC-MS.

Peak	R. Time (min)	Area	Area (%)	Height	Height (%)	Compound Name
1	3.383	422200	0.68	192097	0.77	Tetramethyl silicate
2	4.154	260823	0.42	143047	0.58	3,3-Dimethoxy-2-butanone
3	6.436	3067872	4.94	240665	0.97	Glycerin
4	10.211	1694778	2.73	172958	0.70	5-Hydroxymethylfurfural
5	11.269	535423	0.86	198090	0.80	4-Hydroxy-3-methylacetophenone
6	13.069	4219092	6.80	399540	1.61	Sucrose
7	13.811	246172	0.40	144949	0.58	Dodecanoic acid, methyl ester
8	14.290	351825	0.57	156678	0.63	Dodecanoic acid
9	16.126	224483	0.36	138656	0.56	Methyl tetradecanoate
10	16.544	1298320	2.09	331539	1.34	Unknown
11	17.336	1329086	2.14	828229	3.34	Neophytadiene
12	17.422	546301	0.88	313979	1.27	2-Pentadecanone, 6,10,14-trimethyl-
13	17.592	328256	0.53	198523	0.80	Neophytadiene
14	17.780	979328	1.58	377127	1.52	Neophytadiene
15	18.221	3172908	5.11	1850345	7.46	Hexadecanoic acid, methyl ester
16	18.599	6308448	10.16	2431362	9.80	n-Hexadecanoic acid
17	18.709	1851645	2.98	371171	1.50	Benzothiazole, 2-(2-hydroxyethylthio)-
18	19.194	286693	0.46	136437	0.55	Heptadecanoic acid, methyl ester
19	19.363	347764	0.56	221501	0.89	Hexadecanoic acid, trimethylsilyl ester
20	19.875	4140492	6.67	2279070	9.19	Linoleic acid ethyl ester
21	19.916	3615293	5.82	1258799	5.07	9-Octadecenoic acid, methyl ester, (E)-
22	20.043	1827109	2.94	782374	3.15	Phytol
23	20.128	995681	1.60	563634	2.27	Methyl stearate
24	20.250	1052719	1.70	530680	2.14	9,12-Octadecadienoic acid (Z,Z)-
25	20.285	1067824	1.72	493195	1.99	9-Octadecenoic acid, (E)-
26	20.320	1067137	1.72	397335	1.60	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
27	20.468	888054	1.43	300721	1.21	Octadecanoic acid
28	21.559	507353	0.82	171848	0.69	Octacosanol
29	21.605	225821	0.36	132375	0.53	Dotriacontane
30	21.880	270500	0.44	144790	0.58	Methyl 18-methylnonadecanoate
31	21.946	615352	0.99	231365	0.93	Tricyclo[20.8.0.0(7,16)]triacontane
32	22.432	555227	0.89	353038	1.42	Dotriacontane
33	22.480	211231	0.34	135393	0.55	Cyclodecasiloxane, eicosamethyl-
34	23.226	1608366	2.59	921919	3.72	Dotriacontane
35	23.425	987377	1.59	383598	1.55	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)-
36	23.488	677244	1.09	262960	1.06	Docosanoic acid, methyl ester
37	23.570	666788	1.07	237204	0.96	Cyclodecasiloxane, eicosamethyl-
38	23.734	1079936	1.74	625134	2.52	Phthalic acid, di(2-propylpentyl) ester
39	23.990	2009562	3.24	1137335	4.59	Dotriacontane
40	24.596	468696	0.76	238715	0.96	Tetracosamethyl-cyclododecasiloxane
41	24.725	2813386	4.53	1231140	4.96	Dotriacontane
42	24.830	408581	0.66	84591	0.34	Unknown
43	24.983	431710	0.70	220192	0.89	Eicosanoic acid, methyl ester
44	25.186	316736	0.51	158962	0.64	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester
45	25.437	1584273	2.55	764784	3.08	Dotriacontane
46	25.552	620170	1.00	277613	1.12	Tetracosamethyl-cyclododecasiloxane
47	25.733	350831	0.57	224367	0.90	Squalene
48	26.125	711469	1.15	413708	1.67	Tetrapentacontane
49	26.470	329789	0.53	169449	0.68	Tetracosamethyl-cyclododecasiloxane
50	26.852	365964	0.59	177367	0.72	Tetrapentacontane
51	27.505	779521	1.26	256698	1.03	Unknown
52	29.651	358156	0.58	134074	0.54	Ergost-5-en-3-ol, (3β)-
53	29.781	992572	1.60	263967	1.06	Ergostanol
	Total	62072337	100.00	24805287	100.00	

FTIR Spectroscopic Analysis

FTIR spectra (Figure 2, Table 4) of the methanol stem extract revealed characteristic peaks corresponding to various functional groups. Notable absorption bands were observed in the ranges: 1000-650 cm^{-1}: C-O bending (ethers, carboxylic acids); 1054-1050 cm^{-1}: C-O stretching (alcohols); 1550-1350 cm^{-1}: N-O/C-N (nitro compounds and amines); 1680-1615 cm^{-1}: C=C stretching (aromatic compounds); 2915-2929 cm^{-1}: C-H stretching; 3200-3600 cm^{-1}: O-H stretching (alcohols and phenols). These observations confirm the presence of compounds such as esters, alcohols, alkanes, phenols, and aromatic hydrocarbons. Functional groups like alkenes and phenolics are known for their roles in antimicrobial and antioxidant activities (Chen, 2012; Srinivas *et al*., 2006) [3, 31].

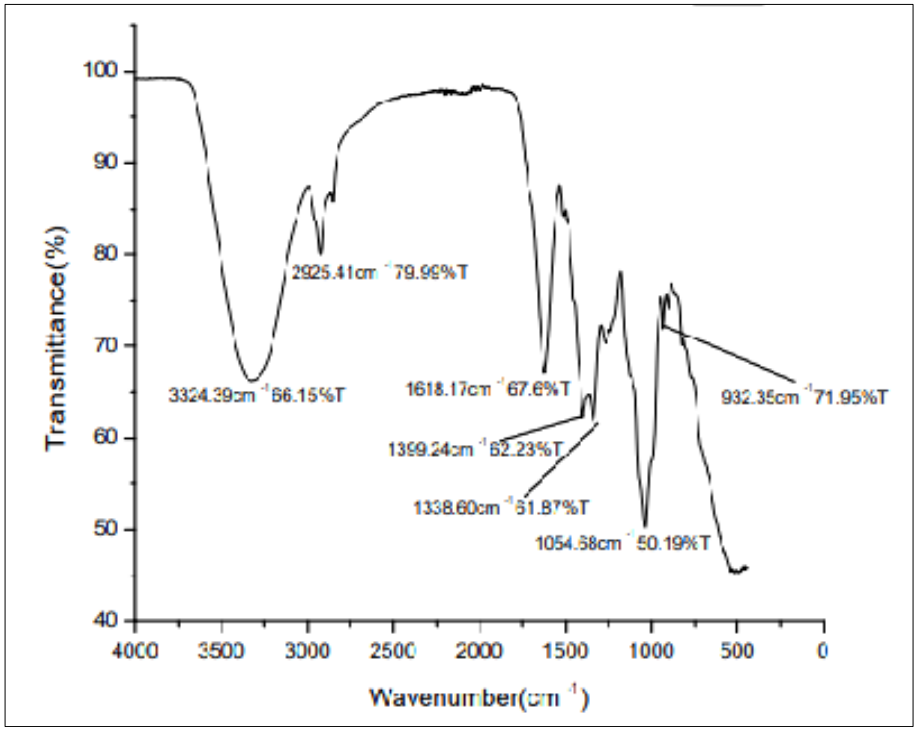

Fig 2: FTIR values of methanolic extract of stem of *Lepidagathis sabui*

Table 4: FTIR peak values and corresponding functional groups identified in the methanolic stem extract of *Lepidagathis sabui*.

Sr. No.	Frequency Peak Value (cm^{-1})	Frequency Range (cm^{-1})	Functional Group Vibration	Functional Group / Class	Representative Compounds
1	932.35	1000-650	C-O bending	Ethers, Carboxylic acids	Carbohydrates, Esters
2	1054.68	1054-1050	C-O stretching	Primary alcohols	Alcohols
3	1399.24	1550-1350	N-O, C-N stretching	Nitro compounds (R-NO_2), Amines	Nitro compounds
4	1618.17	1680-1615	C=C stretching	Aromatic compounds, Alkenes	Aromatic hydrocarbons
5	2925.41	2915-2929	-CH, -CH_2, -CH_3 stretching	Alkanes, C-H bonds	Carbohydrates, Sugars
6	3324.39	3200-3600	O-H stretching (hydrogen bonding)	Alcohols, Phenols	Aromatic phenols

Conclusion

The present study demonstrates the presence of various phytochemical compounds in *Lepidagathis sabui,* which may have potential medicinal applications. The compounds detected in this plant may contribute to its antioxidant and antimicrobial activities. Overall, the results indicate that *Lepidagathis sabui* contains a significant amount of phytochemicals, particularly phenols and flavonoids, which could enhance its medicinal properties. The presence of diverse bioactive compounds, especially hexadecanoic acid methyl ester and phytol, suggests that *Lepidagathis sabui* could serve as a promising source of useful drugs. Further exploration of its extract may reveal valuable pharmacological properties and therapeutic applications.

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