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Analyzing the Chemical Composition of Methanolic Extracts from *Anisomeles indica* Stem: A Comprehensive GC-MS and FT-IR Investigation

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Abstract

The current investigation deals with the study of secondary bioactive compounds found in the stems of *Anisomeles indica* (Lamiaceae). The methanolic extracts of stem were analyzed using Gas Chromatography and Mass Spectrometry (GC-MS), while fine powder was utilized for Fourier Transform Infrared Spectroscopy (FT-IR). The GC-MS analysis of the sample revealed presence of 22 compounds. The prevailing compounds were desogestrel (100%), gamma-sitosterol (63.36%), 3-Hydroxypropyl palmitate, TMS derivative (52.21%), stigmasterol (33.71%), and benzene 1,4-dichloro (19.71), 3,5-dithiahexanol 5,5-dioxide (9.71%), Silane dimethoxydimethyl- (7.27%). The analysis indicated the presence of bioactive compounds viz. fatty acids, polyphenol, coumarins, terpenoids, saponin which shown a wide range of biological activities such as anti-tumor, anti-cancerous, anti-inflammatory, anti-microbial, anti-fungal, and anti-diabetic properties. FT-IR was carried out to determine the peak values associated with functional groups including, alkanes, alcohol, alkyl halide, carboxylic acids, hydroxy compounds, lipids, proteins and phenol. The finding of the study indicated that the methanolic extracts of *A. indica* contain phytochemicals with potential applications in the field of medicine and pharmaceutical.

***Key words*:** *Anisomeles indica,* Lamiaceae, GC-MS, FTIR, Methanolic

Recent years have seen an increase in the use of natural compounds derived from plants for medicinal purpose. Traditional medicine has been recognized by the World Health Organization as playing essential role in primary health care in many poor countries. Plant species are widely employed for the alleviation of diverse ailments including analgesia, psoriasis, skin diseases, uterine infections, diaphoresis and rheumatism stimulation [1-2]. However, the efficacy and safety of herbal therapy are considered [3]. All herbal resources must be subjected to precise scientific testing to ensure their safety. Spectroscopy has emerged as prominent tools in the field of biomedical application and has made substantial advancement in clinical assessments. The utilization of GC-MS and FT-IR techniques has proven to be of significant importance in the field of medication [4]. The GC-MS is commonly used for the separation of volatile substance and finds application in forensic science, food industry, environmental monitoring, fragrance analysis beverages and identification of pesticides [5-6]. FT-IR is widely employed analytical tool utilised for detection of functional groups present in chemical constituents. In addition to its application in quality control, and manufacturing process as FT-IR has found extensive use in the realm of herbal medicines detection [7].

Anisomeles indica R. Br is a medicinal plant commonly known as 'Gopali" belonging to Lamiaceae family. The genus described by three species globally and one species is endemic to peninsular India [8]. *Anisomeles indica* is perennial shrub that grows upright, has a perfume similar to camphor and may be found in Malaysia, South Asia and Northern Australia [9]. The plants consist of bioactive compounds such as polyphenolic, saponins, terpenoids, glycosides, xanthones, coumarins, alkaloids, steroid, and quinines. The phytochemicals present in the plant possesses properties like antioxidants, anti-anaphylactic, anti-cancerous, anti-bacterial, anti-fungal, and analgesic effects. These properties are highly esteemed in the field of conventional medicine [10-11]. The essential oil obtained from *Anisomeles indica* exhibits strong insecticidal properties making it an effective repellant against mosquito [12]. During present investigations the methanolic extracts of *Anisomeles indica* stem were analyzed for identification of phytoconstituents and bioactive components using GC-MS and FT-IR.

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MATERIALS AND METHODS

Collection of plant material and preparation of methanolic extracts

For present study *Anisomeles indica* was collected from Jeur, Kolhapur (Maharashtra), India (74.099139 ºE and 16. 836601 ºN). The plant samples were identified and archived in the herbarium of "The New College" Kolhapur (Maharashtra) India. The collected stems were shade-dried and crushed into fine powder. The powder was subjected to extraction using methanolic solvent. The extracts were filtered, sonicated for 30 min and kept shaking overnight. The extracts were filtered through muslin cloth and centrifuged at 5000 rpm for 10 min. The filtrates were stored at 4 ºC for GC-MS analysis. The fine powder of stem was used for FTIR analysis.

The Elite One-Fused HP 5ms capillary column (30m, 0.25 mm, 0.25 µm) was used for the GC-MS analysis on an Agilent GC 7890 fitted with an MS 5977 B MSD. A 1 mL of extract was injected with constant flow rate of 20 mL/min (split ratio, 20:1) with nitrogen as carrier gas at 200 °C. Oven temperature was held at 50 ºC for 1 min, raised to 250 °C for 1 min and finally to 280 ºC for 9 min. MS data with a mass range m/z of 40-900 amu were acquired at 70 eV. MS analysis was performed using a single quadrupole mass spectrometer detector operating at 150 °C. The scan rate was 1.7/min. The software used was EI source spectral library of NIST 2017. The mass spectrum of the unknown element was compared to that of known component in NIST 2017 database and the percentage of each component was calculated by dividing the average peak area by the total area. The compound names, molecular masses, and structures were determined.

RESULTS AND DISCUSSION

GC-MS analysis

GC-MS analysis of methanolic extracts of *Anisomeles indica* stems showed twenty-two compounds (Table 1, Fig 1). The table also shows the retention time (RT), peak area, percentage, and chemical nature of the compounds. The major phytochemical compounds were dimethyl ether (4.38%), boronic acid ethyl (1.52%), butane 1-methoxy-3-methyl (0.73%), trichloromethane (2.72), silane dimethoxydimethyl (7.27%), 3,5-dithiahexanol 5,5-dioxide (9.71%), 2,4-dimethyl-1-heptene (1.3%), benzene, 1,4-dichloro (19.71%), 1-Hexene, 2,5,5-trimethyl- (0.67%), Octane, 2,2-dimethyl (0.98%), 2,6,8-Trimethyl-4-nonyl acetate (0.73%), oxalic acid, 6-ethyloct-3-yl ethyl ester (1.43%), hexadecanoic acid, methyl ester (2.79%), 7-Tetradecyne (1.74%), Phytol (1.31%), 3-Hydroxypropyl palmitate, TMS derivative (52.21%), 1H-Indene, 1-hexadecyl-2,3-dihydro (15.83%), desogestrel (100%), campesterol (6.58%), stigmasterol (33.71%), and gamma.-sitosterol (63.36%).

Table 1 The compounds identified through GC MS analysis in methanolic extracts of *Anisomeles indica* stem

S. No.	Name of compound	RT time	Area percent	Formula	Molecular weight
1	Dimethyl ether	1.335	4.38	C_2H_6O	46.07
2	Boronic acid, ethyl	1.392	1.52	$C_2H_{11}BO_2$	72.88
3	Butane, 1-methoxy-3-methyl	1.44	0.73	$C_6H_{14}O$	102.78
4	Trichloromethane	1.67	2.72	$C_2H_3Cl_3$	119.38
5	Silane, dimethoxydimethyl	1.851	7.27	$C_1H_{12}O_2Si$	120.22
6	3,5-Dithiahexanol 5,5-dioxide	1.995	9.71	$C_4H_{10}O_3S_2$	130.22
7	2,4-Dimethyl-1-heptene	4.022	1.3	C_9H_{18}	126.23
8	Benzene, 1,4-dichloro	8.65	19.71	$C_6H_4Cl_2$	147.002
9	Cyclopentane, 1,1,3,4-tetramethyl-, cis	9.979	0.44	C_9H_{18}	126.23
10	1-Hexene, 2,5,5-trimethyl	10.094	0.67	C_9H_{18}	126.24
11	Octane, 2,2-dimethyl	13.431	0.98	$C_{10}H_{22}$	142.28
12	2,6,8-Trimethyl-4-nonyl acetate	16.309	0.73	$C_{12}H_{26}O$	186.33
13	Oxalic acid, 6-ethyloct-3-yl ethyl ester	16.339	1.43	$C_{14}H_{26}O_4$	258.35
14	Hexadecanoic acid, methyl ester	30.614	2.79	$C_{17}H_{34}O_2$	270.45
15	7-Tetradecyne	33.942	1.74	$C_{14}H_{26}$	196.37
16	Phytol	34.171	1.31	$C_{20}H_{40}O$	296.53
17	3-Hydroxypropyl palmitate, TMS derivative	40.798	52.21	$C_{19}H_{38}O_3$	314.50
18	1H-Indene, 1-hexadecyl-2,3-dihydro	44.049	15.83	$C_{25}H_{42}$	342.60
19	Desogestrel	48.686	100	$C_{22}H_{30}O$	310.47
20	Campesterol	52.616	6.58	$C_{28}H_{48}O$	400.68
21	Stigmasterol	53.257	33.71	$C_{29}H_{48}O$	412.69
22	Gamma-Sitosterol	54.605	63.36	$C_{29}H_{50}O$	414.70

During the present study desogestrel (100%), was reported in higher concentration in the stem. The steroid is commonly used for hormone contraceptives and antihyperglycemic action [13-14]. It has been used in anticancer activity against colon and liver [15]. The most common phytosterols are campesterol, gamma.-sitosterol and stigmasterol. They help lower cholesterol level in blood which is good for human health. They have hypoglycemic, cancer prevention and anti-inflammatory properties [16]. Phytol is a diterpenes alcohol member [17] observed that phytol has been used in antioxidant, antinociceptive, immune modulators, antiinflammatory, and antimicrobial activity. Phytol is also used in cosmetics, shampoo, bathroom soap and home cleaning products [18-19]. During present study *A. indica* exhibited 1.31% phytol. Hexadecanoic acid has been shown to reduce inflammation, cancer, and glucose level while n-hexadecanoic acid was found to have anti-inflammatory, anticancer, antioxidant antidiabetic activities [20-21]. Arora and Kumar [22] reported that 1H-Indene, 1-hexadecyl-2, 3-dihydro have strong anticancer effect. The amount of anticancer biomolecule

hexadecanoic acid was 2.79% and 1H-Indene 1-hexadecyl-2, 3-dihydro was 15.83 % in *Anisomeles indica* stem (Table 1). Some important molecule reported through GC-MS analysis for their bioactivity in (Table 2).

Table 2 Bioactive compounds identified in the methanolic extracts of *A. indica* stem

S. No.	Name of compound	Activity	Reference
1	Boronic acid, ethyl	Anticancer, Antibacterial	Silva *et al.* [23]
2	3,5-Dithiahexanol 5,5-dioxide	Anticancer	Hameed *et al.* [24]
3	Octane, 2,2-dimethyl-	Anticancer, Antibacterial	Saravanakumar *et al.* [25]
4	Hexadecanoic acid, methyl ester	Antibacterial, Antifungal, Antioxidant, Antitumor	Beschi *et al.* [26] Saljooghianpour and Javaran [27]
5	Phytol	Anti-inflammatory, Antioxidant, Antimicrobial,	Rahman *et al.* [28] Olivia *et al.* [29]
6	1H-Indene, 1-hexadecyl-2,3-dihydro	Anticholinesterase	Koca *et al.* [30]
7	Campesterol	Anticancer, Anti-inflammatory	Choi *et al.* [31] Miras-Moreno *et al.* [16]
8	Stigmasterol	Anti-inflammatory, Anticancer, Antioxidant, Antimicrobial	Bakrim *et al.* [32] Kaur *et al.* [33]
9	Gamma-Sitosterol	Anticancer,	Balamurugan *et al.* [34]

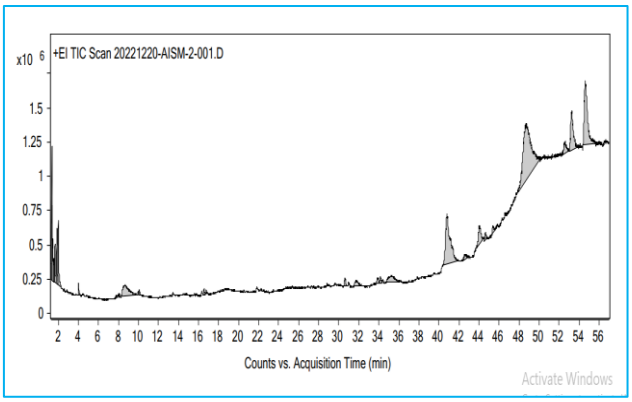

Fig 1 Gas Chromatogram of *A. indica* stem (methanolic extracts)

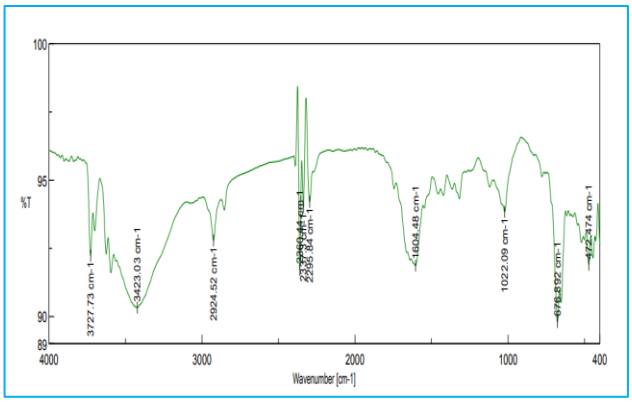

Fig 2 FTIR spectrum of stem powder of *A. indica*

Table 3 FTIR peak value and functional groups in *Anisomeles indica* stem

S. No	Wavelength (cm^{-1})	Functional group	Compound class	Characteristic absorption	Intensity
1	3727.73	O-H stretching	Alcohol	3000-4000	Medium
2	3423.03	O-H stretching	Alcohol	3500-3200	Strong
3	2924.52	C-H stretching	Alkanes	3000-2850	Medium
4	2360.44	O-H stretching	Carboxylic acid	2300-2400	Weak
5	2337.3				
6	1604.48	N-H stretching	Primary amine	1650-1580	Medium
7	1022.09	C-O stretching	Alcohols/Ester	1320-1000	Strong
8	676.892	C-Cl stretching	Alkyl halide	550-850	Medium

FT-IR analysis of *Anisomeles indica* revealing the presence of various compounds as evidence by the bands observed at specific wavelength 3727.73, 3423.03, 2924.52, 2360.44, 2337.3, 1604.48, 1022.09, 676.89, cm^{-1} (Table 3, Fig 2). The O-H stretching in alcohol exhibits strong intensity appearing as a broad band at 3423.03 cm^{-1}. The wavelength of 2924.52 cm^{-1} is commonly associated with the C-H stretching vibration within the alkenes functional group. The wavelength observed at 1604.48 cm^{-1} corresponds to a primary amine. An N-H bending displayed a moderate intensity. The vibrational mode of the small band at 1022.09 cm^{-1} corresponds to the stretching of the C-O bond in the presence of an alcohol functional group. The band observed at 676.82 cm^{-1} can be assigned to the stretching vibration of C-Cl in alkyl halide showed medium intensity. FT-IR analysis was employed to determine the functional groups of active compounds by examining the peak values within the infrared radiations spectrum [35]. Packialakshmi and Periyakkal [36] conducted a screening of the functional groups present in ether, amines, nitro compounds, alkenes, alkynes, and monosubstituted compounds found in *A. malabarica*. These functional groups were found to be associated with diverse medicinal properties. The FT-IR study of *A. indica* revealed the presence of alkyl halides, fatty acids, alcohols, unsaturated hydrocarbons, alkanes, carboxylic compounds, amines and anhydride acid [37]. The present study showed presence of amines, alcohol, alkanes, carboxylic acid and alkyl halide in *Anisomeles indica* stem collected from Western Ghats.

CONCLUSION

The results of current study showed presence of the aforementioned compounds in the methanolic extracts of *Anisomeles indica* stem which have potential application in the field of pharmaceutical research. The utilization of GC-MS analysis served as an initial approach in recognizing the

characteristic of the active component present in the medicinal plants. From this study it can be inferred that *Anisomeles indica* possesses a diverse range of compounds making it a plant of significant pharmaceutical relevance and nutritional worth. However further investigation in determination of efficacy and safety of *Anisomeles indica* to cure various aliment are needed.

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