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Full Length Research Article

GCMS and FTIR Analysis of Phytocompounds in Methanolic Extract of Flower and Seed from *Anisomeles malabarica* (L.) R.Br.

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Abstract

The current study deals with the GCMS and FTIR analysis of phytocompounds in methanolic extract of flower and seed from *Anisomeles malabarica* (L.) R.Br. Plants are used as medicines in various cultures and serve as a source of many potent drugs due to the presence of certain bioactive compounds for pharmaceutical industry. The methanolic extract of plant *Anisomeles malabarica* was prepared by using Soxhlet apparatus. GCMS and FTIR analysis were done to this plant flower and seed extract to find out the bioactive phytocompounds. Using GC-MS were identifying the important volatile constituents and identified compounds were retention time, molecular formula, molecular weight, peak area (%) and activities related to medicinal uses. The GCMS results showed 15 peaks in flower and 10 peaks in seed. The highest area peak (52.25) was presented at ANDROSTAN-17-ONE, 3-ETHYL-3-HYDROXY-, (5. ALPHA)- and lowest peak (0.57) were recorded at SILIKONFETT in flower extract. The maximum area peak (70.37) were recorded at 9,12-Octadecadienoic acid (Z,Z)- and minimum peak (0.21) were noted at Glycidyl oleate in seed extract. FTIR results of this flower and seed extract showed 13 peaks indicate the presence of the bioactive compounds. The strongest peak is presented at the range of 1020.21 cm^{-1} in flower extract and 1019.04 cm^{-1} in seed extract. It was concluded that, an important traditional medicinal plant with an important source of phytocompounds is *Anisomeles malabarica.*

***Key words*:** *Anisomeles malabarica,* Flower, Seed, Methanol, GCMS, FTIR

Plants are a rich source of medicinal substances that have several uses in the pharmaceutical industry. In India, ethnobotanical knowledge has persisted for generations, and people have used plants as a source of medicine, especially for basic healthcare. There are around 45,000 plant species in the nation, and many have been studied for their potential as medicines [1]. Due to their synthesis from organisms, their possible biological effects, and natural stereo-chemistry allowing for their attachment to protein pockets, phytochemicals hold greater promise than synthetic compounds [2]. Secondary metabolites known as bioactive compounds include monoterpenes, flavonoids, sesquiterpenes, alkaloids, saponins, phenols, and glycosides. These are mostly employed in the food, pharmaceutical, chemical, cosmetic, and agricultural industries [3]. Neuropsychiatric disorders like depression, anxiety, and insomnia have an important effect on human behaviour and mood in recent years. More than 9% of people world experience these disorders, which have an effect on their daily lives [4]. Among every illness, depression is the condition with the greatest range. The majority of people suffer significant suffering as a result of depression, and it was projected that in the future years, the amount of cases will significantly increase [5]. There are bioactive components found in many plant species that are used as drugs or pharmacological agents [6]. The infusion of the leaf is used as a traditional medicine to treat fever, epilepsy, and children's teething-related fever. External application of the leaf extract and essential oil is used to treat rheumatoid arthritis [7]. One of the most important non-destructive analytical methods for determining the functional groups of chemical constituents is Fourier transform infrared (FT-IR) spectroscopy, which is frequently used for quality control in the food, beverage, and pharmaceutical industries [8]. Since ancient times, humans have relied heavily on plants as a source of medicine, particularly for folk cures and other broad traditional therapies [9]. One of the quick and accurate methods for identifying volatile chemicals from plant extract is gas chromatography combined with mass spectrometry. The GC-MS method was selected for this particular study to determine the qualitative and quantitative data for the complicated product [10]. The plant is a source for many bioactive compounds found in its bulbs, and it is used as traditional medicine in different countries [11]. The

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simultaneous analysis of a variety of phytochemicals or plant metabolites found in an herbal sample is known as metabolomics [12]. Due to the availability of certain bioactive compounds, plants are employed as medicine in many different cultures and are a source of many powerful pharmaceuticals [13]. In the present study, GCMS and FTIR analysis of phytocompounds in methanolic extract of flower and seed from *Anisomeles malabarica*.

MATERIALS AND METHODS

Sample collection

The *Anisomeles malabarica* plant flowers and seeds were collected from A. Veeriya Vandayar Memorial Sri Pushpam College (Autonomous), Poondi, Thanjavur, Tamil Nadu, India.

Sample preparation

Shade dried flower and seed were ground well by using mixer grinder to get fine powder. For later use, this powder was kept in an airtight container. The thimble of the Soxhlet extractor was filled with 25 grams of this powder, and the distillation flask was loaded using methanol. After soxhlation was finished, the flower and seed extract were eventually collected from the flask. This methanol extract was taken to the GCMS and FTIR analysis [14].

GCMS analysis

Gas chromatography and mass spectrometry (GC-MS) technology, (TSQ QUANTUM XLS) was used to identify the compounds after thin layer chromatography indicated the presence of active chemicals. Gas Chromatography-Mass Spectrometry is the name of the apparatus, and Thermo Scientific is the manufacturer. XCALIBUR (ver-2.2) is the software required for analytical studies. The column sizes are 30mX0.25mmX0.25um for TG-5MS. The interface temperature (°C) and injector temperature were both 280°C.

FTIR analysis

By absorbing light in the infrared spectrum, a Fourier Transform Infrared Spectro Photometer (BRUKER, ALFA MODAL, ABS /Transmittance) with a wavelength range of 4000-400 cm^{-1} and a resolution of 4 cm^{-1} was employed to identify the functional groups of compounds present in the methanolic extract. The plant parts flower and seed methanolic extracts was directly provided into the FTIR spectrometer. In order to generate the spectrum of functional groups, the FTIR measures the bond vibration.

RESULTS AND DISCUSSION

For the purpose of analyzing and categorize the volatile and non-volatile phytochemicals present in the three distinct extracts, such as chloroform, ethyl acetate, and methanol, gas chromatography in conjunction with mass spectroscopy was used. According to the percentage of peak area, the methanolic extract included more than 24, with 8 Phyto-constituents being classified as major and the remaining 16 as minor. Hexadecane (5.39), Phytol (5.33), Heptadecane (4.36%), Nonadecane (4.07), Tridecyl acrylate (31.90), Tetratetracontane (10.90), Phytol, acetate (10.34), Hexadecane (5.39), Phytol (5.33), Heptadecane (4.36%), and Ethyl Palmitate (3.95%). The presence of 7 major and 12 minor phytoconstituents, including Tridecyl acrylate (24.42), 1-Dodecanol (11.41), Phytol, acetate (13.21), n-Decylpropanoate (7.47), Perhydrofarnesyl acetone (6.14), and (E)-phytol (5.03%), was also detected in an extract of ethyl acetate [15]. In the present study, the chromatograms of the samples from flower and seed extracts of *Anisomeles malabarica*, are represented by (Fig 1-2), while the identified compounds and their retention time, molecular formula, molecular weight, peak area (%), and activities related to medicinal uses are shown in (Tables 1-2).

Table 1 Determination of phytochemical compounds from *Anisomeles malabarica* flower by GC-MS method

S. No.	RT	Name of the compound	Molecular formula	Molecular weight	Peak area (%)
1.	5.440	1,2-Cyclopentanedione$$ 1,2-Cyclopentanedione	$C_5H_6O_2$	98	1.2
2.	20.015	E-14-Hexadecenal	$C_{16}H_{30}O$	238	1.57
3.	25.990	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	1.59
4.	28.665	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294	1.96
5.	28.770	6-Octadecenoic acid, methyl ester, (Z)-	$C_{19}H_{36}O_2$	296	1.56
6.	29.180	Methyl stearate	$C_{19}H_{38}O_2$	298	0.71
7.	29.845	Cyclohexane, 1-Ethenyl-1-Methyl-2,4-Bis(1-Methylethenyl)-, [1S-(1.Alpha.,2.Beta.,4.Beta.)]- $$ 2,4-Diisopropen	$C_{15}H_{24}$	204	2.72
8.	32.445	(R)-6-Methoxy-2,8-dimethyl-2-((4R,8R)-4,8,12-trimethyltridecyl)chroman	$C_{28}H_{48}O_2$	416	3.17
9.	37.270	Cholest-20-ene-3,6-diol, (3.beta.,5.alpha.,6.alpha.)-	$C_{27}H_{46}O_2$	402	0.66
10.	38.350	Campesterol	$C_{28}H_{48}O$	400	8.1
11.	38.585	5,8-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	0.83
12.	39.025	Stigmasta-5,23-Dien-3-ol, (3.Beta.)- $$ Stigmasta-5,23-Dien-3.Beta.-ol	$C_{29}H_{48}O$	412	13.67
13.	39.180	Silikonfett	-	9999	0.52
14.	39.615	Lanosterol	$C_{30}H_{50}O$	426	9.51
15.	39.770	Androstan-17-one, 3-ethyl-3-hydroxy-, (5.alpha.)-	$C_{21}H_{34}O_2$	318	52.25

From the results, the 15 compounds were identified in flower extracts of *Anisomeles malabarica* respectively. The flower extracts was detected as a major compound by ANDROSTAN-17-ONE, 3-ETHYL-3-HYDROXY-, (5.ALPHA.) - (52.25%) followed by STIGMASTA-5,23-DIEN-3-OL, (3.BETA.)- (13.67%), LANOSTA-8,24-DIEN-3-OL, (3.BETA.) (9.51%)- and Campesterol (8.1%). The lowest compounds are presented at SILIKONFETT (0.52%), followed by CHOLESTANE-3,5,6,7-TETROL, (3.BETA., 5.ALPHA., 6.ALPHA., 7.BETA.)- (0.66%), Methyl stearate (0.71%) and 5,8-Octadecadienoic acid, methyl ester (0.83%). The highest retention time are noted at 39.768 for the compounds is ANDROSTAN-17-ONE, 3-ETHYL-3-HYDROXY-, (5.ALPHA.)- and lowest was 5.439 in 1,2-CYCLOPENTANEDIONE were recorded (Fig 1, Table 1). The present a GCMS analysis result of *Anisomeles malabarica* seed

extracts were 10 compounds possess many biological properties. The seed extracts was identified as a maximum presented compounds were 9,12-Octadecadienoic acid (Z,Z)- (70.37%), 9,12-Octadecadienoic Acid (Z,Z)-, 2,3-Dihydroxypropyl Ester (9.02%) and Octadecanoic acid (8.6%). The minimum presented compounds were Glycidyl oleate (0.21%), Methyl stearate (0.27%) and 9, 12-Octadecadienoyl chloride, (Z,Z)- (0.56%). The highest retention time are noted at 36.85 in the compound is 9, 12-Octadecadienoic Acid (Z,Z)-, 2,3-Dihydroxypropyl Ester and lowest was 25.992 in Hexadecanoic acid, methyl ester were analyzed (Fig 2, Table 2). Further compared with the flower and seed extract, the *Anisomeles malabarica* seed extracts are presented at maximum area peaks.

Table 2 Determination of phytochemical compounds from *Anisomeles malabarica* seed by GC-MS method

S. No.	RT	Name of the compound	Molecular formula	Molecular weight	Peak area (%)
1.	25.990	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.5
2.	26.720	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	4.95
3.	28.665	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294	4.43
4.	28.770	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	1.08
5.	29.175	Methyl stearate	$C_{19}H_{38}O_2$	298	0.27
6.	29.530	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	70.37
7.	29.865	Octadecanoic acid	$C_{18}H_{36}O_2$	284	8.6
8.	34.015	9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}ClO$	298	0.56
9.	34.090	Glycidyloleate	$C_{21}H_{38}O_3$	338	0.21
10.	36.850	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{38}O_4$	354	9.02

Table 3 FT-IR analysis of flower extract from *Anisomeles malabarica*

S. No.	Range (cm^{-1})	Functional group assignment	Intensity
1.	3397.58	Miscellaneous chromophoric groups, Alcohols and Phenols, O ─ H Stretching vibrations, Intermolecularly hydrogen bonded (change on dilution), polymeric association	Strong
2.	2947.82	Hydrocarbon chromophore, C ─ H Stretching, Alkane	Medium
3.	2864.64	Hydrocarbon chromophore, C ─ H Stretching, Alkane	Weak
4.	2839.55	Carbonyl chromophore, Aldehydes, C ─ H Stretching vibrations; two bands	Weak to medium
5.	2523.15	Carbonyl chromophore, Carboxylic acids, Hydroxyl stretching (bonded), several bands	Weak
6.	2074.40	Miscellaneous chromophoric groups, Unsaturated nitrogen compounds, C ≡ N Stretching vibrations, isocyanides	Weak
7.	1650.08	Miscellaneous chromophoric groups, Unsaturated nitrogen compounds, O ─ NO, Nitrites	Weak
8.	1453.63	Hydrocarbon chromophore, C─H Bending, Alkane, C─H, Alkane, ─CH_2─, Alkane, ─CH_3	Weak
9.	1412.16	Hydrocarbon chromophore, C─H bending, Alkene, monosubstituted (vinyl)	Weak
10.	1111.21	Miscellaneous chromophoric groups, Halogen compounds, C ─ X Stretching vibrations, C ─ F	Weak
11.	1052.49	Miscellaneous chromophoric groups, Sulfur compounds, S = O Stretching vibrations: Sulfoxides, Sulfonic acids	Weak to medium
12.	1019.04	Miscellaneous chromophoric groups, Halogen compounds, C ─ X Stretching vibrations, C─F	Medium to strong
13.	689.62	Miscellaneous chromophoricgroupsk, Hylogen compounds C ─ X Stretching vibrations, C─Cl	Strong to medium

The functional group of active components on the peak values in the infrared radiation area were identified using the FTIR spectrum. The FTIR result peaks in the area of infrared radiation. It was found that numerous functional groups from different molecules were found. It has been shown that FTIR spectroscopy is an effective and sensitive method for determining the composition of biomolecules. Alkynes compounds are denoted by the popular band, which was found at 2375.44 and 2139.33 cm^{-1} in the case of the leaf [6]. Halogen compounds at 1118.27, 116.89, and 530.11 cm^{-1} were used to observe the leaf. The amines group was thought to be responsible for the strong band seen in the *Anisomeles malabarica* sample's absorption spectra at 3976.22, 3905.30, 3762.56, and 3420.95cm^{-1}. Nitro compounds are represented by the band at 1333.69, 1597.01, and 1259.82. The peak at 1114.87 and 1049.72cm^{-1} exhibit C-H stretching, which is indicative of ether existence. Phenol is detected at a wavelength of 1402.4 cm^{-1} in the crude extract of *Anisomeles malabarica*. In the current study, each compound's molecular fingerprint is represented by a unique set of chemical bonds, and FTIR spectroscopy's measurement of substances' atomic level background vibration and frequency are specific. FTIR spectral bands of the flower and seed extract using *Anisomeles malabarica* [6] (Fig 3). The peaks in transmittance were observed at 3397.58, 2947.82, 2864.64, 2839.55, 2523.15, 2074.40, 1650.08, 1453.63, 1412.16, 1111.21, 1052.49, 1019.04, 689.62 using flower extract and (Fig 4) comparable spectral bands were noted at 3387.23, 2946.83, 2865.08, 2838.32, 2525.06, 2033.78, 1652.25, 1453.92, 1411.93, 1111.68, 1051.38, 1020.21, 687.99. The strongest peak is noted at flower extract was 3397.58 and 1019.04 for the compounds of (Miscellaneous chromophoric groups, Alcohols and Phenols, O ─ H Stretching vibrations, Intermolecularly hydrogen bonded (change on dilution), polymeric association) and (Miscellaneous chromophoric groups, Halogen compounds, C ─ X Stretching vibrations, C─F). The weakest peak is observed at 2523.15 and 2074.40 in (Carbonyl chromophore, Carboxylic acids, Hydroxyl stretching (bonded), several bands) and (Miscellaneous chromophoric groups, Unsaturated nitrogen compounds, C ≡ N Stretching vibrations, isocyanides). The medium presented compounds are analyzed at 2947.82 in Hydrocarbon chromophore, C ─ H Stretching, Alkane were

noted in *A. malabarica* flower extract (Table 3). The strong peak is analyzed by seed extract of *Anisomeles malabarica* were 3387.23, 2946.83, 1020.21 in the compound name was (Miscellaneous chromophoric groups, Amines, N ─ H Stretching vibrations, Secondary, free; one band, Imines (N ─ H); one band), (Hydrocarbon chromophore, C ─ H Stretching, Alkane), (Miscellaneous chromophoric groups, Sulfur compounds, S-O Stretching vibrations: sulfonic acids). The weak peak is observed at 2525.06 and 2033.78 in the (Carbonyl chromophore, Carboxylic acids, Hydroxyl stretching (bonded), several bands) and (Hydrocarbon chromophore, C ─ C Multiple bands stretching, Alkyne monosubstituted). The medium peak is noted at 1051.38 in (Miscellaneous chromophoric groups, Sulfur compounds, C-S Stretching vibrations) (Table 4).

Table 4 FT-IR analysis of seed extract from *Anisomeles malabarica*

S. No.	Range (cm^{-1})	Functional group assignment	Intensity
1.	3387.23	Miscellaneous chromophoric groups, Amines, N ─ H Stretching vibrations, Secondary, free; one band, Imines (= N ─ H); one band	Strong
2.	2946.83	Hydrocarbon chromophore, C ─ H Stretching, Alkane	Strong
3.	2865.08	Hydrocarbon chromophore, C ─ H Stretching, Alkane	Strong to weak
4.	2838.32	Carbonyl chromophore, Aldehydes, C ─ H Stretching vibrations; two bands	Weak to medium
5.	2525.06	Carbonyl chromophore, Carboxylic acids, Hydroxyl stretching (bonded), several bands	Weak
6.	2033.78	Hydrocarbon chromophore, C ─ C Multiple band stretching, Alkyne monosubstituted	Weak
7.	1652.25	Carbonyl chromophore, Amides, Carbonyl stretching vibrations, Secondary solid and concentrated solution, Tertiary solid and all solutions	Medium
8.	1453.92	Hydrocarbon chromophore, C ─ H Bending, Alkane, C─H, Alkane, ─CH_2─, Alkane, ─CH_3	Medium
9.	1411.93	Hydrocarbon chromophore, C ─ H Bending, Alkene, disubstituted, *gem*	Medium
10.	1111.68	Miscellaneous chromophoric groups, Halogen compounds, C─X Stretching vibrations, C ─ F	Weak
11.	1051.38	Miscellaneous chromophoric groups, Sulfur compounds, C=S Stretching vibrations	Medium
12.	1020.21	Miscellaneous chromophoric groups, Sulfur compounds, S=O Stretching vibrations: sulfonic acids	Strong
13.	687.99	Miscellaneous chromophoric groups, Halogen compounds, C─X Stretching vibrations, C─Cl	Strong to medium

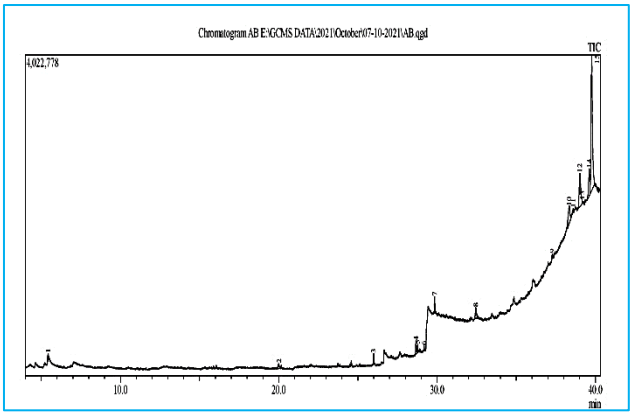

Fig 1 Determination of phytochemical compounds from *Anisomeles malabarica* flower by GC-MS method

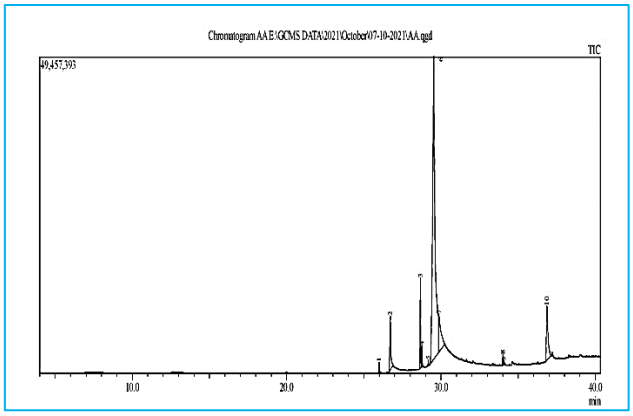

Fig 2 Determination of phytochemical compounds from *Anisomeles malabarica* seed by GC-MS method

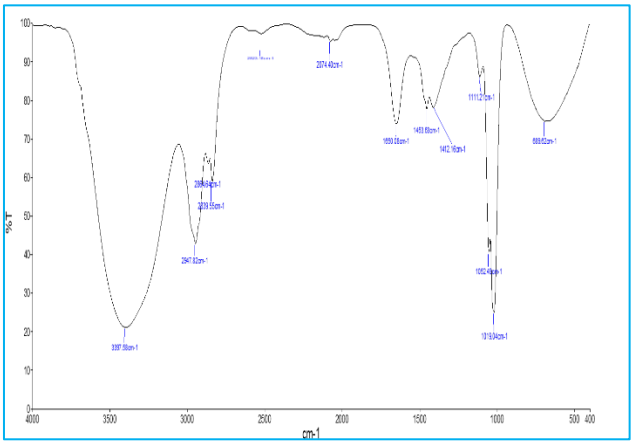

Fig 3 FT-IR analysis of flower extract from *Anisomeles malabarica*

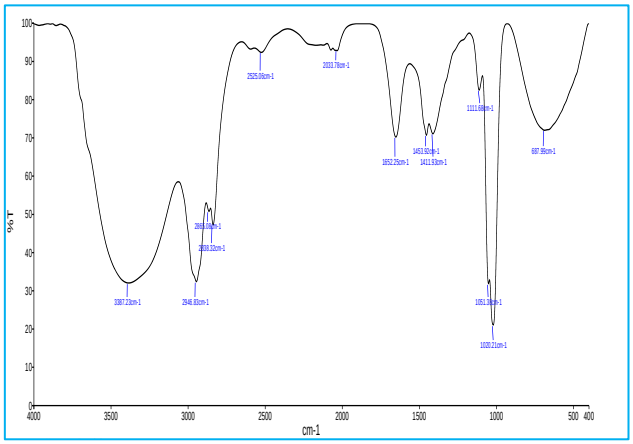

Fig 4 FT-IR analysis of seed extract from *Anisomeles malabarica*

CONCLUSION

The present research study, plant derived secondary metabolites possessed GCMS and FTIR analysis of *Anisomeles malabarica* flower and seed methanolic extract. Gas chromatography mass spectrometry (GC-MS) sample preparation is performed on smaller and more volatile samples including environmental pollutants, industrial byproducts, food contaminants, pesticides, and metabolites of illicit and designer drugs. Fourier Transform Infrared Spectrophotometer (FTIR) is the most powerful tool for identifying the types of chemical bonds and functional groups present in compounds. GC-MS plays an essential role in the phytochemical analysis and chemotaxonomic studies of medicinal plants containing biologically active components. It is also used to detect and measure contaminants from spoilage or adulteration which may be harmful. The presence of several compound specifically alcohol and formaldehyde were responsible for the preservative activity of the plant parts leaves and seed extract. This support the traditional used of the plant as preservative agent.

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