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# PHYTOCHEMICAL EXPLORATION OF *ANISOMELES MALABARICA* R. BR. LEAVES BY SOLVENT EXTRACTION AND GC-MS

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**Keywords:**

*Anisomeles malabarica*, Labiatae, Solvent extraction, phytochemical analysis, GC-MS analysis

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**ABSTRACT:** Medicinal plants belong to the Labiatae family, commonly known as the mint family. This family comprises the *Anisomeles malabarica*, which contain biologically important phytochemicals. Focus on solvent extraction of selected medicinal plant parts and its phytochemical analysis gives an insight for herbal drug discovery. A heat continuous percolation experiment was conducted for 24 hours using three different solvents, namely ethanol, methanol, and chloroform using the Soxhlet apparatus. The phytochemical examination of *Anisomeles malabarica* leaf extracts by the three solvents revealed that the extracts are abundant in alkaloids, saponins, tannins, flavonoids, and glycosides, all of which have been linked to the pharmacological effects of the plant. In the current study, derivatization procedures were employed to analyze both polar and non-polar compound present in the solvent extracts. Based on the GC-MS analysis, large amounts of carboxylic acids, which contain both saturated and unsaturated fatty acids, hydrocarbons, including alkanes and alkenes are responsible for the oily quality of all three extracts. According to analysis, the majority of polar molecules, such as alcohols, phenol, mannose, glucose, fructose, arabinose, myo-inositol, D-glucitol, D-arabitol, D-allose, and glucitol, could be extracted by ethanol. The analysis confirmed that the appropriate solvents must be used in order to extract biologically relevant components. A significant source of information is provided by the results of this study regarding the chemical nature of the phytochemicals isolated and analysed by GC-MS in *Anisomeles malabarica* leaf extract.

**INTRODUCTION:** The threat for life style diseases made human to focus on traditional way of living. Besides the prevalence of medicinal plants in India, it is also known for its traditional medical practices. In India, traditional medicinal practices are used by more than 1.5 million practitioners. Approximately 7800 manufacturing units in India produce natural health products, which consumes more than 2000 tons of medicinal plants every year [1].

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Herbs are offered as dietary supplements or traditional ethnic medicines in more than 1500 forms [2]. Identifying and examining crude drugs is necessary since herbal formulations contain a wide variety of chemical characteristics. Non-standard extraction methods can cause phytochemicals in plants to degrade, and variations can result in inconsistent results [3].

Extraction and screening of bioactive substances from medical plants have been standardized [4]. Recently, scientists have focused on testing and analyzing how different plants and their constituents to treat diseases. Though various techniques are available, Soxhlet's apparatus is generally used to extract phytochemicals from the medicinal plants. Solvent like ethanol, n-hexane,

methanol, chloroform are used for extraction of important pharmacological ingredients. The labiatae family, also known as the mint family, is one of the most important families of medicinal plants. Families of Labiatae are commonly used in folk medicine. Some of them are also used as ornamental or cookery plants, such as mints, thyme, tulsi, spearmint, and coleus [5]. There are more than 20 species of Lamiaceae in *Anisomeles* genus, which are native to tropical Asia and Australia (Kew.org). They are annual or perennial semi-bushy aromatic plants that grow in rocky, arid, or sunny locations. This genus is known for its aromatic and medicinal properties [6]. Three species occur in India yet, *Anisomeles indica*, *Anisomeles malabarica* and *Anisomeles heyneana* [7]. Of these, *A. indica* and *A. malabarica* were investigated for their pharmacological and pharmacological effects [8, 9]. *A. malabarica* (AM, Lamiacea) is a perennial herb, 2 meters in height, which is aromatic and densely pubescent (a short, dense, soft downy hairs on the surface) **Fig. 1.** *A. malabarica*, commonly known as Malabar catmint, is an herbaceous plant native to India, Bangladesh, Sri Lanka, Andaman & Nicobar Islands, Thailand, Malaysia, Indonesia, New Guinea, Bismarck Archipelago, Mauritius, Reunion and Northern Australia [10, 11]. *Anisomeles malabarica* extracts have shown medicinal significance in the treatment of gastrointestinal disorders includes diarrhea, dyspepsia, colic, flatulence, intestinal worms and neurological disorder includes hysteria, amentia, anorexia, epilepsy. Extracts from these plants are also used for intermittent fevers, fevers linked to teething in children, halitosis, gout, and swellings [12, 13]. Several medicinal properties of ethanol extract of *A. malabarica* have been discovered during early research, including anti-allergic effects, anaphylactic properties, anti-bacterial, anticancer, anti-carcinogenic, anti-inflammatory, antiepileptic potential, antifertility, anti-pyretic activity andantispasmodic [14]. In specific, the leaves of *A. malabarica* have been shown to have a variety of therapeutic effects, including antidiabetic, anticancer, antiviral and antiepileptic properties [15, 16, 17, 18, 19]. Therefore, the present study focused on extracting and identifying the phytoconstituents found in different solvent extractions of *A. malabarica* leaves responsible for its medicinal properties and the determination of different bioactive compounds using GC-MS.

## MATERIALS AND METHODS:

**Plant Collection and Identification:** Fresh leaves of *Anisomeles malabarica* free from disease were collected from Tiruvannamalai, Tamil Nadu, India **Fig. 1A**. The leaves were identified and authenticated (Coll. No. 121202) by Centre for Conservation of Medical Resources, The University of TransDisciplinary Health Science and Technology (TDU), Karnataka, India. Chemicals were procured from Sigma Aldrich.

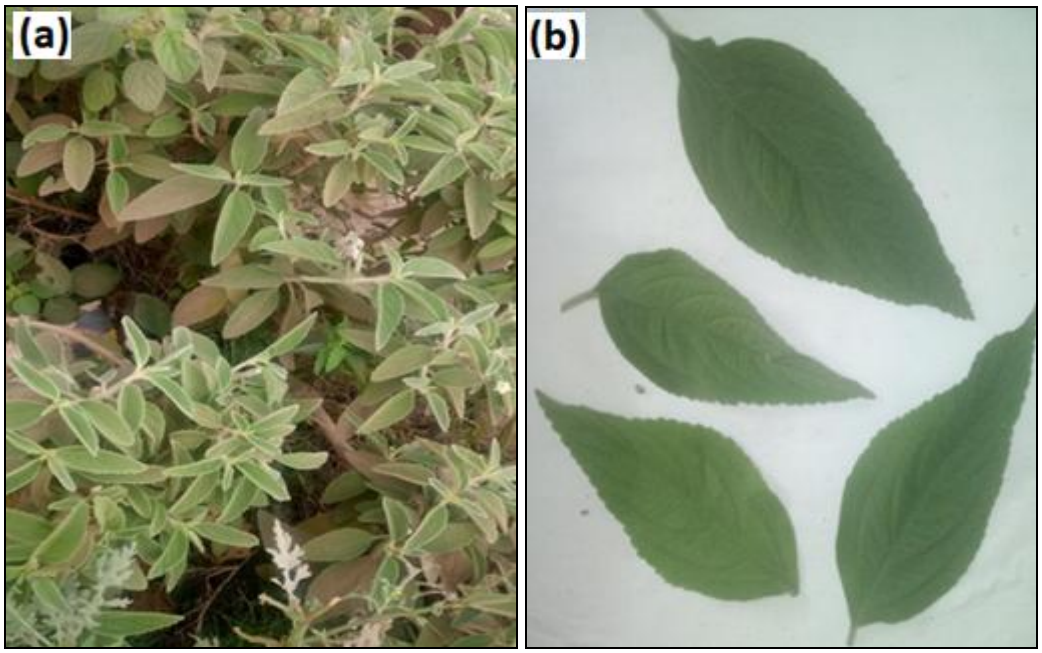


**FIG. 1: *ANISOMELES MALABARICA* (A) PLANT; (B) LEAVES**

**Preparation of Solvent Extract:** Plant materials, leaves **Fig. 1B**, were washed and dried at room temperature (25–27$^{o}$C) and grinded into a coarse powder. 15kg of the dried powder was subjected for solvent extraction was done by hot continuous percolation method in Soxhlet apparatus for 24 hrs using three solvents namely, ethanol, methanol, chloroform [20]. For further phytochemical analysis and GC-MS analysis, all three crude solvent extracts were stored at 4$^{o}$C.

**Phytochemical Analysis:** Phytochemical analysis of solvent extracts was carried out by the standard methods provided [21] for the presence and absence of metabolites such as alkaloids, glycosides, flavonoid, tannins, saponins.

**Detection of Alkaloids:** To 3mL of extracts, 1mL of dilute Hydrochloric acid was dissolved individually and filtered.

**Wagner's Test:** To 1mL of extracts, 2 drops of wagner's reagent (Iodine in Potassium Iodide) was added. Formation of a yellow coloured precipitate indicates the presence of alkaloids.

**Detection of Flavonoids:** To 3mL of extracts, 1mL of sodium hydroxide was added. Formation of yellow color indicates the presence of flavonoids.

**Detection of Tannins:** To 2 drops 5% ferric chloride, 1mL of extract was added. Formation of green precipitate indicates the presence of tannins.

**Detection of Saponins:**
**Froth Test:** Extracts were diluted with distilled water to 20mL and this was shaken in a graduated cylinder for 15minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

**Detection of Glycosides:** To 1mL extract 5mL distilled water was added and filtered. The filtrates were used to test for the presence of carbohydrates.

**Benedict's test:** To 2mL filtrates, 2 to 3 drops of Benedict's reagent was added and kept in boiling water bath for 15 minutes. Formation of red precipitate indicates the presence of reducing sugars.

**GC-MS Analysis:** At the GCMS Central Facility, Indian Institute of Sciences (IISc), Bangalore, GC-MS analyses were performed on each solvent extract.

**Sample Derivatization Procedure for GCMS:** Two steps were followed to detivatize high polar compounds present in the extracts. Firstly, to 5mg crude extract, 40µL of MAHC (Methoxyamine hydrochloride) in pyridine (20mg: 1mL ratio) solution was added and kept for incubation at 65$^{o}$ for 1 hour. Then, 80µL of BSTFA solution (990µL of N, O-Bis (trimethylsilyl) trifluroacetamide and 10µL of chlorotrimethylsilane) was added to the incubated solution and again incubated at 65$^{o}$ for 1 hour. The above derivatized samples were diluted accordingly with hexane and injected 1µL into the GCMS instrument with split mode.

GC–MS was performed on an Agilent Triple Quadrupole MS with an inert mass selective detector (MSD-5975C detector, Agilent Technologies, USA) coupled directly to an Agilent 7890. A gas chromatograph which was equipped with splitless injector, a quick swap assembly, an Agilent 7693A Automatic Liquid Sampler and a DB-5MS fused silica capillary column (Crossbondsilarylene 1, 4-bis (dimethylsiloxy) phenylene dimethyl polysiloxane), 30 m 0.25 mm i.d., film thickness 0.25 µm, Agilent Technologies, USA). The DB-5MS column was operated using an injector temperature of 260$^{o}$C.

Approximately 1µL of each sample diluted in hexane was injected using the split injection mode; the split flow ratio was 10:1. The helium carrier gas was flowed at 1 ml/min. The GC–TIC profiles and mass spectra were obtained using the ChemStation data analysis software, AMDIS (Agilent). All mass spectra were acquired in the EI mode (scan range of *m/z* 45–600 and ionization energy of 70 eV). The temperatures of the electronic-impact ion source and the MS quadrupole were 230°C and 150°C, respectively [22].

**Identification of Phytocomponents:** Interpretation on mass-spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library.

The name, molecular weight, and structure of the components of the test materials were ascertained.

## RESULTS AND DISCUSSION

**Phytochemical Analysis:** In the phytochemical analysis, significant bioactive compounds were identified in the plants **Table 1**. These agents are alkaloids, saponins, tannins, flavonoids, and Glycosides. These bioactive substances have been reported to expert multiple biological effects such as anticataract, antibacterial, antifungal, antiviral, anticancer, antiplatelet, antimalarial, antituberculosis, antineuralgic, antineuropathic, antialcoholic, antihangover, antinauseant, antidepressant, anticonvulsant, antidiabetic, antialopecic, anticirrhotic, cholesterolytic, antihyperlipidemic, antiketotic, lipotropic, antimeniere's, antiear-wax, anti-hypertensive, sweetener, analgesic, sweetener, laxative, emollient, potential prebiotic, fungicide, pesticide. This investigation explores different primary and secondary metabolites of *A. malabarica* leaves by using different solvent extracts **Table 1.**

**TABLE 1: QUALITATIVE ANALYSIS OF PHYTOCHEMICAL EXTRACT OF *ANISOMELES MALABARICA* (L) R. BR**

| Sl. no. | Metabolites | Ethanol extract | Chloroform extract | Methanol extract |
|---|---|---|---|---|
| 1 | Alkaloids | + | + | + |
| 2 | Flavonoids | + | + | + |
| 3 | Tannins | + | + | + |
| 4 | Saponins | + | + | + |
| 5 | Glycosides | + | + | + |

+ indicates presence of particular metabolites. - indicates absences of particular metabolites.

**GC-MS Analysis:** GC-MS chromatogram analysis of the ethanol, methanol and chloroform extract of *A. malabarica* leaves showed twenty-nine, thirty and forty-three phytochemical constituents respectively **Fig. 2, 3, 4**.

The phytocompounds in leaves extract were identified and characterized based on a mass spectrum comparison with the NIST library and their retention time (RT), molecular weight (MW) and molecular formula are presented in **Table 2**. Phytochemical analysis shows fourteen compounds are having pharmacological activity. *A. malabarica* leaves contain a wide variety of phytochemicals which contribute to its medicinal properties **Table 3**.

GC-MS analysis was used to identify different bioactive compounds, responsible for reported biological functions using different solvents extraction.

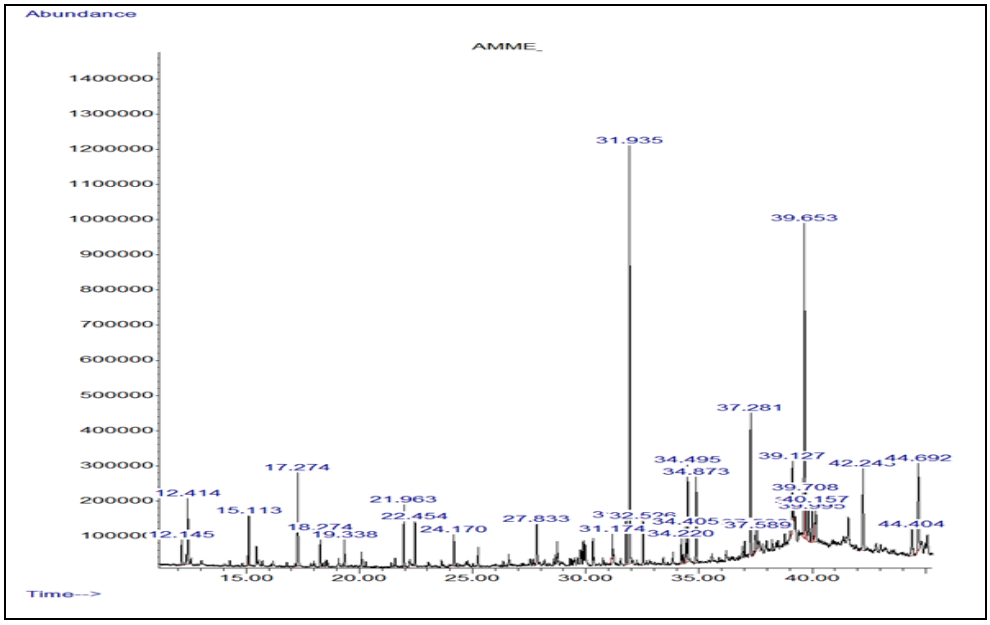


**FIG. 2: GCMS CHROMATOGRAM OF CRUDE METHANOL EXTRACT OF *ANISOMELES MALABARICA* LEAVES**

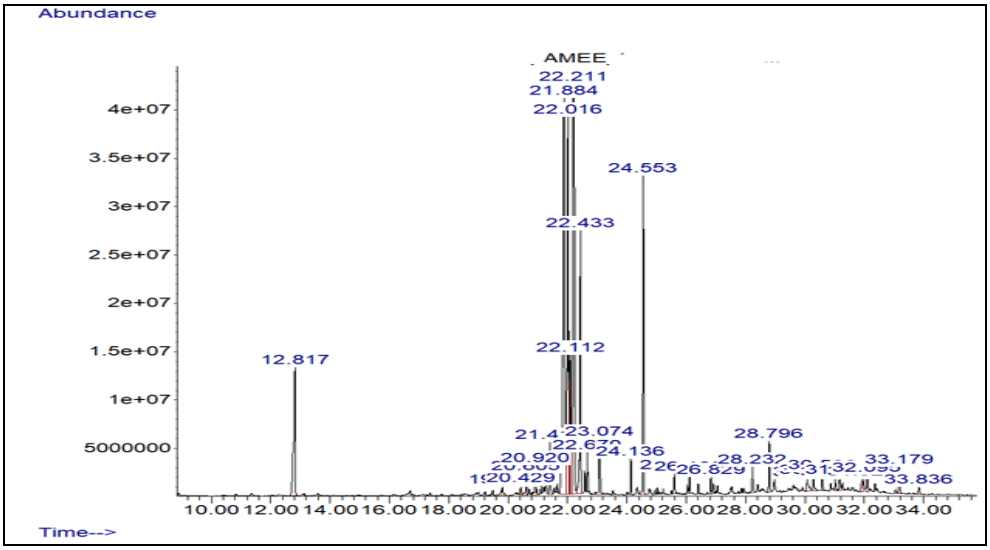


**FIG. 3: GCMS CHROMATOGRAM OF CRUDE ETHANOL EXTRACT OF *ANISOMELES MALABARICA* LEAVES**

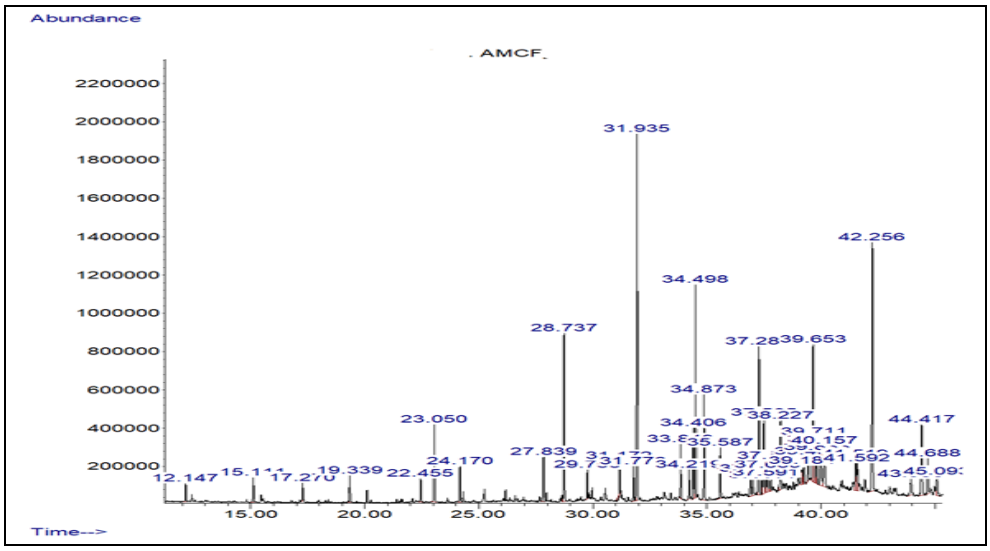


**FIG. 4: GCMS CHROMATOGRAM OF CRUDE CHLOROFORM EXTRACT OF *ANISOMELES MALABARICA* LEAVES GCMS CHROMATOGRAM SHOWS CRUDE EXTRACT CONTAINS**

**TABLE 2: GC-MS ANALYSES FOR METHANOL, ETHANOL, CHLOROFORM EXTRACTS OF *ANISOMELES MALABARICA* LEAVES**

| Nature of chemical compound | Retention time | Area % | Molecular Formula | Molecular weight | Compound Name | Extract |
|---|---|---|---|---|---|---|
| Alcohols/ Phenols | 15.114 | 2.03 | $C_{10}H_{26}O_2Si_2$ | 234.4 | Silane, [(1-methyl-1,3-propanediyl) bis(oxy)] bis[trimethyl- | Methanol |
| | 17.274 | 4.24 | $C_9H_{27}O_4PSi_3$ | 314.5 | Silanol, trimethyl-, phosphate | |
| | 22.452 | 1.82 | $C_{14}H_{22}O$ | 206.32 | Phenol, 2,4-bis(1,1-dimethylethyl) | |
| | 32.525 | 1.93 | $C_{24}H_{60}O_6Si_6$ | 613.2 | Myo-Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)- | |
| | 37.282 | 6.21 | $C_{10}H_{26}O_2S_2Si_2$ | 298.6 | Bis(2-trimethylsiloxyethylthio)disulfide | |
| | 40.158 | 2.13 | $C_{10}H_{26}O_2S_2Si_2$ | 298.6 | Bis(2-trimethylsiloxyethylthio)disulfide | |
| | 12.817 | 5.754 | $C_{12}H_{32}O_3Si_3$ | 308.64 | Trimethylsilyl ether of glycerol | Ethanol |
| | 19.81 | 0.552 | $C_5H_{12}O_5$ | 152.15 | d-(+)-Arabitol | |
| | 20.429 | 0.283 | $C_6H_{14}O_6$ | 182.17 | D-Glucitol | |
| | 22.211 | 19.251 | $C_{22}H_{55}NO_6Si_5$ | 570.1 | D-Allose, pentakis(trimethylsilyl) ether, methyloxime (syn) | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 22.67 | 0.985 | $C_{24}H_{62}NO_6Si_6$ | 615.3 | Trimethylsilyl ether of glucitol | |
| | 23.074 | 1.818 | $C_{24}H_{62}NO_6Si_6$ | 615.3 | Trimethylsilyl ether of glucitol | |
| | 24.553 | 8.566 | $C_{24}H_{60}NO_6Si_6$ | 613.2 | Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-, muco- | |
| | 25.605 | 0.603 | $C_{23}H_{48}NOSi$ | 368.7 | Silane, [(3,7,11,15-tetramethyl-2-hexadecenyl)oxy]trimethyl- | |
| | 26.829 | 0.435 | $C_{18}H_{26}O_2Si_2$ | 330.6 | Silane, [[1,1'-biphenyl]-4,4'-diylbis(oxy)]bis[trimethyl- | |
| | 28.232 | 0.881 | $C_{10}H_{26}O_2S_2Si_2$ | 298.6 | Bis(2-trimethylsiloxyethylthio)disulfide | |
| | 17.27 | 0.48 | $C_9H_{27}O_4PSi_3$ | 314.54 | Silanol, trimethyl-, phosphate | Chloroform |
| | 22.456 | 0.8 | $C_{17}H_{30}OSi$ | 278.5 | Phenol, 2,4-bis(1,1-dimethylethyl) | |
| | 36.916 | 1.09 | $C_{18}H_{34}OSi$ | 294.547 | 3-(1,5-Dimethyl-hexa-1,4-dienyl)-2,2-dimethyl-4-trimethylsilylcyclopentanol | |
| | 37.282 | 5.16 | $C_{28}H_{54}O_2Si_2$ | 478.9 | Docosa-8,14-diyn-cis-1,22-diol, bis(trimethylsilyl) ether | |
| | 40.158 | 2.23 | $C_{10}H_{26}O_2S_2Si_2$ | 298.6 | Bis(2-trimethylsiloxyethylthio)disulfide | |
| | 41.591 | 1.25 | $C_{13}H_{22}$ | 222.399 | Ethanol, 1-(methylenecyclopropyl)-1-(methylene-1-trimethylsilylcyclo propyl)- | |
| | 44.416 | 4.17 | $C_{15}H_{22}O_2$ | 234.33 | Methanol, tris(methylenecyclopropy l)-2(3H)- | |
| | 45.093 | 1.20 | $C_{13}H_{22}OSi$ | 222.399 | Ethanol, 1-(methylenecyclopropyl)--(methylene-1-trimethylsilylcyclo propyl)- | |
| Aldehydes | 22.112 | 4.34 | $C_{22}H_{55}NO_6Si_5$ | 570.1 | d-Mannose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, o-methyloxyme, (1E)- | Ethanol |
| | 22.433 | 7.663 | $C_{21}H_{52}NO_6Si_5$ | 541.1 | d-Glucose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, o-methyloxyme, (1Z)- | |
| | 30.077 | 0.722 | $C_{21}H_{52}O_6Si_5$ | 541.1 | Glucopyranose, pentakis-O-trimethylsilyl- | |
| | 32.095 | 0.526 | $C_{17}H_{42}O_5Si_4$ | 438.854 | Arabinopyranose, tetrakis-O-(trimethylsilyl)-, .alpha.-D- | |
| | 33.836 | 0.276 | $C_{18}H_{44}O_5Si_4$ | 452.9 | Mannose, 6-deoxy-2,3,4,5-tetrakis-O-(trimethylsilyl)-, L-Syn: l-Fucose, tetra-TMS-ether | |
| Alkanes | 24.172 | 1.30 | $C_{20}H_{33}F_7O_2$ | 438.5 | 4-Heptafluorobutyryloxyhexadecane | Methanol |
| | 37.503 | 1.46 | $C_{16}H_{30}O_3Si_2$ | 326.5 | 3,6-Dioxa-2,7-disilaoctane, 2,2,7,7-tetramethyl-3-[(2-methylphenoxy) methyl]- | |
| | 15.11 | 0.81 | $C_{17}H_{40}O_5Si_2$ | 380.7 | 3,7,11,15,18-Pentaoxa-2,19-disilaeicosane, 2,2,19,19-tetramethyl- | Chloroform |
| | 19.34 | 0.79 | $C_6H_{14}OSi$ | 130.26 | Silane, trimethyl[(1-methylnonyl)oxy]- | |
| | 31.777 | 1.08 | $C_{21}H_{46}Si$ | 326.7 | Silane, trimethyloctadecyl- | |
| | 33.847 | 1.67 | $C_{23}H_{48}OSi$ | 368.7 | Silane, [(3,7,11,15-tetramethyl-2-hexadecenyl)oxy]trimethyl- | |
| | 37.668 | 1.31 | $C_8H_{20}OSi$ | 160.33 | Silane, (butoxymethyl)trimethyl- | |
| | 39.183 | 0.63 | $C_{14}H_{28}OSi_2$ | 268.54 | Silane, trimethyl[[1-[(trimethylsilyl)ethynyl]cyclohexyl] oxy]-Or Geraniol, trimethylsilyl ether | |
| | 41.520 | 1.39 | $C_8H_{22}OSSi_2$ | 222.496 | 3-Oxa-6-thia-2,7-disilaoctane, 2,2,7,7-tetramethyl- | |
| | 43.952 | 1.03 | $C_{20}H_{42}$ | 282.5 | Eicosane | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Alkenes | 31.175 | 0.72 | $C_{20}H_{40}$ | 280.5 | 5-Eicosene, (E)- | Methanol |
| | 34.221 | 1.14 | $C_{19}H_{38}$ | 266.5 | 1-Nonadecene | |
| | 39.128 | 2.67 | $C_{15}H_{32}Si_3$ | 296.6 | 1,4-Cyclohexadiene, 1,3,6-tris(trimethylsilyl)- | |
| | 39.710 | 2.02 | $C_{16}H_{32}O_2Si_2$ | 312.5 | Cyclopentene, 3,3-dimethyl-4-methylene-1,2-bis(trimethylsilyloxymethyl)- | |
| | 24.172 | 1.13 | $C_{20}H_{40}$ | 280.5 | 9-Eicosene, (E)- | Chloroform |
| | 27.839 | 1.64 | $C_{19}H_{38}$ | 266.5 | 1-Nonadecene | |
| | 31.171 | 0.7 | $C_{19}H_{38}$ | 266.5 | 1-Nonadecene | |
| | 37.022 | 0.86 | $C_{20}H_{40}$ | 280.5 | 3-Eicosene, (E)- | |
| | 39.124 | 0.96 | $C_{16}H_{32}O_2Si_2$ | 312.59 | Cyclopentene, 3,3-dimethyl-4-methylene-1,2-bis(trimethylsilyloxymethyl)- | |
| | 42.256 | 11.33 | $C_4H_5Br$ | 132.99 | 1,3-Butadiene, 1-bromo- | |
| Amides/Amines | 30.286 | 0.551 | $C_{16}H_{16}BrN_3O_2$ | 362.22 | [4-Bromo-2-(hydrazono-phenyl-methyl)-phenyl]-carbamic acid, ethyl ester | Ethanol |
| | 33.179 | 0.878 | $C_{18}H_{20}N_2O_4$ | 328.4 | 3-Pyrrolidinecarboxamide, 1-(4-ethoxyphenyl)-N-(2-furanylmethyl)-5-oxo- Syn: 1-(4-Ethoxyphenyl)-N-(furan-2-ylmethyl)-5-oxopyrrolidine-3-carboxamide | |
| Arenes | 31.170 | 0.525 | $C_{15}H_{24}$ | 204.35 | 1-(3-Methylbutyl)-2,3,4,6-tetramethylbenzene | Ethanol |
| | 44.687 | 1.89 | $C_{10}H_{10}O_2S$ | 194.25 | Benzene, [(methylenecyclopropyl)sulfonyl]- Or 1,2,4-Triazine | Chloroform |
| Azole | 44.691 | 6.20 | $C_4H_4N_6$ | 136.1 | 1H-1,2,3,4-Tetrazole, 5-(1H-pyrazol-1-yl)- | Methanol |
| | 31.928 | 0.559 | $C_{12}H_{13}N_3S$ | 231.32 | 5(2-Dimethylamino-1-phenyl)-vinyl-1,2,4-thiadiazol Syn: (Z)-N,N-Dimethyl-2-phenyl-2-(1,2,4-thiadiazol-5-yl)ethenamine | Ethanol |
| Carboxylic acid | 18.273 | 1.35 | $C_{10}H_{22}O_4Si_2$ | 262.4 | Butanedioic acid, bis(trimethylsilyl) ester | Methanol |
| | 19.34 | 0.99 | $C_{12}H_{26}O_2Si$ | 230.4 | Nonanoic acid, trimethylsilyl ester | |
| | 21.964 | 2.28 | $C_{13}H_{30}O_5Si_3$ | 350.6 | Butanedioic acid, [(trimethylsilyl)oxy]-, bis(trimethylsilyl) ester | |
| | 27.835 | 2.50 | $C_{20}H_{39}ClO_2$ | 347 | 3-Chloropropionic acid, heptadecyl ester | |
| | 31.773 | 1.77 | $C_{13}H_{28}O_3Si_2$ | 288.5 | 2-Ethyl-3-ketovalerate, bis(trimethylsilyl) | |
| | 31.935 | 16.92 | $C_{19}H_{40}O_2Si$ | 328.6 | Hexadecanoic acid, trimethylsilyl ester | |
| | 34.406 | 1.53 | $C_{21}H_{40}O_2Si$ | 352.6 | 9,12-Octadecadienoic acid (Z,Z)-, trimethylsilyl ester | |
| | 34.496 | 4.18 | $C_{21}H_{38}O_2Si$ | 350.6 | alpha.-Linolenic acid, trimethylsilyl ester | |
| | 34.874 | 3.66 | $C_{21}H_{44}O_2Si$ | 356.6 | Octadecanoic acid, trimethylsilyl ester | |
| | 37.589 | 0.65 | $C_{23}H_{48}O_2Si$ | 384.7 | Eicosanoic acid, trimethylsilylester | |
| | 39.651 | 15.04 | $C_{23}H_{32}O_3$ | 356.5 | 5,16-Pregnadiene, 20-acetoxy-3-oxo | |
| | 39.840 | 2.29 | $C_{11}H_{22}O_4S_3Si_2$ | 370.7 | ((((Carboxymethyl)thio)carbothioyl)thio) acetic acid ditms | |
| | 20.605 | 0.659 | $C_{13}H_{18}O_{10}$ | 334.28 | D-Glycero-L-manno-Heptonic acid, | Ethanol |
| | 24.136 | 0.89 | $C_{19}H_{40}NO_2Si$ | 328.6 | Hexadecanoic acid, trimethylsilyl | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | ester | |
| | 26.109 | 0.471 | $C_{21}H_{38}NO_2Si$ | 350.6 | alpha.-Linolenic acid, trimethylsilyl ester | |
| | 28.796 | 1.374 | $C_{13}H_{20}O_4Si$ | 268.38 | 3,5-Dimethoxyphenylacetic acid, trimethylsilyl ester | |
| | 30.588 | 0.496 | $C_{17}H_{32}O_4Si_3$ | 384.7 | Benzeneacetic acid, 2,5-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester | |
| | 31.018 | 0.336 | $C_6H_{10}O_7$ | 194.14 | Per-O-(trimethylsilyl)-.alpha.,.beta.-L-idopyranuronic acid | |
| | 12.147 | 0.59 | $C_{12}H_{30}O_4Si_3$ | 322.62 | Propanoic acid, 2-[(trimethylsilyl )oxy]-, trimethylsilyl ester | Chloroform |
| | 28.736 | 5.26 | $C_{17}H_{36}O_2Si$ | 300.6 | Tetradecanoic acid, trimethylsilyl ester | |
| | 31.935 | 11.69 | $C_{19}H_{40}O_2Si$ | 328.6 | Hexadecanoic acid, trimethylsilyl ester | |
| | 34.217 | 0.94 | $C_{19}H_{36}Cl_2O_2$ | 367.4 | Dichloroacetic acid, heptadecyl ester | |
| | 34.406 | 2.24 | $C_{21}H_{40}O_2Si$ | 352.6 | 9,12-Octadecadienoic acid (Z,Z)-, trimethylsilyl ester | |
| | 34.496 | 6.94 | $C_{21}H_{38}O_2Si$ | 350.6 | Alpha.-Linolenic acid, trimethylsilyl ester | |
| | 34.874 | 3.44 | $C_{21}H_{44}O_2Si$ | 356.7 | Octadecanoic acid, trimethylsilyl ester | |
| | 35.586 | 1.79 | $C_{13}H_{22}O_2Si$ | 238.4 | Myrtenoic acid, trimethylsilylester | |
| | 37.506 | 3.56 | $C_{23}H_{36}O_2Si$ | 372.6 | 2,4,6,8-Nonatetraenoic acid, 3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-, trimethylsilyl ester, (all-E)- | |
| | 37.589 | 0.47 | $C_{23}H_{48}O_2Si$ | 384.7 | Eicosanoic acid, trimethylsilyl ester | |
| | 37.802 | 1.13 | $C_{13}H_{28}O_4Si_2$ | 304.53 | Heptanedioic acid, bis(trimethylsilyl) ester | |
| | 39.434 | 0.83 | $C_{25}H_{54}O_4Si_2$ | 474.9 | Hexadecanoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester | |
| | 39.651 | 5.37 | $C_{23}H_{32}O_3$ | 356.5 | 5,16-Pregnadiene, 20-acetoxy-3-oxo | |
| | 39.710 | 1.66 | $C_{12}H_{16}O_4Si$ | 252.34 | 3,4-Methylenedioxyphenylacetic acid, trimethylsilyl ester | |
| | 39.832 | 1.46 | $C_{15}H_{26}O_4Si_2$ | 326.53 | 3,4-Methylenedioxyphenylacetic acid, trimethylsilyl ester | |
| | 39.985 | 2.52 | $C_{14}H_{24}O_3Si_2$ | 296.51 | Acetic acid, [o-(trimethylsiloxy)phenyl]-, trimethylsilyl ester | |
| Ester | 39.997 | 2.04 | $C_9H_{14}O_5$ | 202.2 | Cyclopentane-R1,(trans)-2-dicarboxylic acid, 3,3-dimethyl-4-methylene-(cis)-5-trimethylsilyl-, dimethyl ester | Methanol |
| | 42.244 | 4.84 | $C_{25}H_{54}O_4Si_2$ | 474.9 | 2-[(Trimethylsilyl)oxy]tetradecanoic acid, bis(trimethylsilyl) ester | |
| Ether/ Epoxides | 12.147 | 1.00 | $C_9H_{22}O_2Si_2$ | 218.4 | Silane, trimethyl[1-methyl-2-oxo-2-trimethylsilyl)ethoxy]-, (R)- | Methanol |
| | 12.414 | 2.84 | C7H18OSi | 146.3 | Silane, trimethyl(2-methylpropoxy) | |
| | 37.412 | 0.54 | $C_{28}H_{54}O_2Si_2$ | 478.9 | Docosa-8,14-diyn-cis-1,22-diol, bis(trimethylsilyl) ether | Chloroform |
| Ketones | 44.404 | 2.21 | $C_8H_9N_5O_2$ | 207.1 | Furazano[3,4-b]pyrazin-5(4H)-one, 6-(1-pyrrolidinyl)- | Methanol |
| | 20.920 | 0.776 | $C_{19}H_{46}O_6Si_4$ | 482.9 | Fructofuranoside | Ethanol |
| | 21. | 1.462 | $C_{22}H_{55}NO_6Si_5$ | 570.1 | D-Fructose, 1,3,4,5,6-pentakis-O- | |

| | | | | | |
|---|---|---|---|---|---|
| 411 | | | | (trimethylsilyl)-, O-methyloxime | |
| 21.882 | | $C_{22}H_{55}NO_6Si_5$ | 570.1 | D-Fructose, 1,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxime | |
| 21.884 | 22.103 | | 570.1 | D-Fructose, 1,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxime | |
| 22.016 | 16.267 | $C_{22}H_{55}NO_6Si_5$ | 570.1 | D-Fructose, 1,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxime | |
| 23.05 | 2.46 | $C_{16}H_{12}N_2O_2$ | 264.28 | 7-Methyldiftalone | Chloroform |
| 29.755 | 0.82 | $C_{17}H_{24}O_3$ | 276.4 | 7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione | |

**TABLE 3: BIOACTIVITY OF PHYTOCOMPONENTS IDENTIFIED IN THE LEAVES EXTRACT OF *ANISOMELE SMALABARICA* BY GC-MS**

| Sl. no. | Name | Biological activity | Reference |
|---|---|---|---|
| 1 | Trimethylsilyl ether of glycerol | Anticataract, Antiear-wax, Antiketotic, AntiMeniere's, Antineuralgic, Arrhythmigenic, Emollient, Hyperglycemic. | [23] |
| 2 | D-Glucitol, | Laxative | [24] |
| 3 | D-Fructose, 1,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxime | Antialcoholic, Antidiabetic, Antihangover, Antiketotic, Antinauseant, Laxative, Neoplastic, Sweetener. | [25, 26] |
| 4 | Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-, muco- | Antialopecic, Anticirrhotic, Antidiabetic, Antineuropathic, Cholesterolytic, Lipotropic, Sweetener. | [23,25] |
| 6 | [4-Bromo-2-(hydrazono-phenyl-methyl)-phenyl]-carbamic acid, ethyl ester | Analgesic, Antibacterial, Antifungal, Antiviral, Anti-hypertensive, Antidepressant, Anticancer, Antiplatelet, Antimalarial and Anticonvulsant | [27, 2829,30] |
| 7 | Benzeneacetic acid, 2,5-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester | Fungicide, Pesticide | [23] |
| 8 | Per-O-(trimethylsilyl)-.alpha.,.beta.-L-idopyranuronic acid Syn: alpha-L-Idopyranuronic acid | Antiviral acitivty | [31] |
| 9 | Silane, [(3,7,11,15-tetramethyl-2-hexadecenyl)oxy]trimethyl- | Antimycobacterial Activity | [32] |
| 10 | 5(2-Dimethylamino-1-phenyl)-vinyl-1,2,4-thiadiazol | Antifungal and Antibacterial Agents | [33] |
| 11 | Arabinopyranose, tetrakis-O-(trimethylsilyl)-, .alpha.-D- | Potential prebiotic | [34] |
| 12 | 3-Pyrrolidinecarboxamide, 1-(4-ethoxyphenyl)-N-(2-furanylmethyl)-5-oxo- Syn: 1-(4-Ethoxyphenyl)-N-(furan-2-ylmethyl)-5-oxopyrrolidine-3-carboxamide | Antituberculosis agent | [35] |
| 13 | 5,16-Pregnadiene, 20-acetoxy-3-oxo | Antihyperlipidemic agent | [36] |

**CONCLUSION:** Phytochemicals derived from pharmaceutically important plants can be used to design drugs for many dreadful diseases. Extracts of *Anisomeles malabarica* leaves are rich in alkaloids, saponins, tannins, flavonoids, and glycosides that appear to possess anti-diabetic, anticancer, antiviral and anti-epileptic properties [15, 16, 17, 18, 19]. In order to extract phytochemicals using suitable solvents of different polarities, several standard protocols have been followed. Medicinal and aromatic properties of *Anisomeles members* are attributed to their high essential oil concentrations [13]. As part of the analysis of essential oils, Gas Chromatography- mass spectrometry (GC-MS) make excellent tools because of their ability to separate, identify and quantify semi-volatile and volatile analytes. According to their relative polarity, three solvents were selected in this study: methanol (0.762), ethanol (0.654) and chloroform (0.259). A GC-MS chromatogram analysis of the ethanol, methanol, and chloroform extracts of *A. malabarica* leaves revealed 29 phytochemicals, 30 phytochemicals and 41 phytochemicals, respectively.

A few of these phytochemicals must possess polyfunctional groups, which makes them polar and reduce their volatility. By using trimethylsilyl (TMS), these phytocomponents can be derivatized to decrease their polarity and improve their retention time. A silylation agent that substitutes protons in functional groups groups (-OH, -COOH, $-NH_2$, -NH, -SH, -OP (=O) $(OH)_2$) to form trimethylsilyl (TMS) derivatives [23].

It has found that all three extracts contain carboxylic acids (includes saturated and unsaturated fatty acids), hydrocarbon (includes alkanes, alkenes), alcohol, and phenol compounds in abundant. Moreover, the physical nature of the extract also reflects these oily rich components. In addition, ethanol can extract majority of the polar compounds, including mannose, glucose, fructose, arabinose, myo-inositol, D-glucitol, D-arabitol, D-allose, glucitol, *etc* **Table 2**.

Derivatization techniques were used in the current work to analyze both polar and non-polar compounds found in the solvent extracts. However, these experiments showed that in order to extract biologically significant components, proper solvents must be chosen. The study suggests that *Anisomeles malabarica* leaves contain a variety of bioactive chemicals with therapeutic characteristics **Table 3**. Further research will be needed to isolate and define pharmacologically significant phytocomponents in the crude leaf extract of *Anisomeles malabarica.*

**Footnote:**

**Ethics Statement:** This article does not contain any studies with human participants or animals performed by any of the authors.

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## REFERENCES:

1. Gupta P and Shukla A: The scope and market of alternative medicine and India's potential to become a global distributor. Int J Adv Res Ideas Innov Technol 2019; 5(2): 1146-50.
2. Shi Y, Zhang C and Li X: Traditional medicine in India. J Tradit Chin Med 2021; 8: 51-55.
3. Balamurugan V, Fatima S, Velurajan S. A guide to phytochemical analysis. Int J Adv Res 2019; 5(1): 236-45.
4. Fu Y, Luo J, Qin J and Yang M: Screening techniques for the identification of bioactive compounds in natural products. Journal of Pharmaceutical and Biomedical Analysis 2019; 168(10): 189-200.
5. Naghibi F, Mosaddegh M, Motamed SM and Ghorbani A: Labiatae family in folk medicine in Iran: from ethnobotany to pharmacology. Iranian Journal of Pharmaceutical Research 2022; 4(2): 63-79.
6. Kirtikar KR and Basu BD: Indian Medicinal Plants. International Book Distributors Book Sellers and Publishers, Deheradun 1999; (3).
7. Sastri BN: The Wealth of India. A Dictionary of Indian Raw Materials and Industrial Products. Raw Materials. The Wealth of India. A Dictionary of Indian Raw Materials and Industrial Products. Raw Materials 1950.
8. Dharmasiri M, Thabrew M and Ratnasooriya W: Anti-inflammatory effects of *Anisomeles indica*. Phytomed 2000; 7: 97.
9. Ushir YV, Chidrawar VR, Patel KN, Tatiya AU and Surana SJ: Pharmacognostic studies on the leaves of *Anisomeles indica* Linn. (Labiatae) 2009; 44-48.
10. *Anisomeles malabarica* (L.) R.Br. ex Sims. [online] India Biodiversity Portal, Available at: https://indiabiodiversity.org/biodiv/species/show/228742 [Accessed date Aug 15, 2018].
11. Vattakaven T, George RM, Balasubramanian D, Réjou-Méchain M, Muthusankar G, Ramesh BR and Prabhakar R: India Biodiversity Portal: An integrated, interactive and participatory biodiversity informatics platform. Biodiversity Data Journal 2016; (4).
12. Yogesh U and Krishnakant P: Chemical composition and antibacterial activity of essential oil from *Anisomeles* species grown in India. Pharmacognosy Journal 2011; 2(18): 55-9.
13. Annapoorani S: A review on *Anisomeles malabarica* and their usage. International Journal of Life Sciences (Amravati) 2019; 7(1): 140-2.
14. Ramaraj R and Unpaprom Y: Medicinally Potential Plant of *Anisomeles malabarica* (L.) R. Br. Journal of Agr. Research & Extension 2013; 30(3): 29-39.
15. Choudhary N, Bijjem KR and Kalia AN: Antiepileptic potential of flavonoids fraction from the leaves of *Anisomeles malabarica*. Journal of Ethnopharmacology 2011; 135(2): 238-42.
16. Preethy CP, Alshatwi AA, Gunasekaran M and Akbarsha MA: Analysis of the cytotoxic potential of Anisomelic Acid Isolated from *Anisomeles malabarica*. Scientia Pharmaceutica 2013; 81(2): 559-66.
17. Preethy CP, Padmapriya R, Periasamy VS, Riyasdeen A, Srinag S, Krishnamurthy H, Alshatwi AA and Akbarsha MA: Antiproliferative property of n-hexane and chloroform extracts of *Anisomeles malabarica* (L). R. Br. in HPV16-positive human cervical cancer cells. Journal of Pharmacology and Pharmacotherapeutics 2012; 3(1): 26-34.
18. Kotha P, Badri KR, Nagalapuram R, Allagadda R and Chippada AR: Anti-diabetic potential of the leaves of *Anisomeles malabarica* in streptozotocin induced diabetic rats. Cellular Physiology and Biochemistry 2017; 43(4): 1689-702.
19. Kotha P, Marella S, Allagadda R, Badri KR and Chippada AR: Evaluation of biochemical mechanisms of anti-

diabetic functions of *Anisomeles malabarica*. Biomedicine & Pharmacotherapy 2019; 112: 108598.
20. López-Bascón MA and De Castro ML: Soxhlet extraction. In Liquid-phase extraction 2020; (1) 327-354. Elsevier.
21. Shaikh JR and Patil M: Qualitative tests for preliminary phytochemical screening: An overview. International Journal of Chemical Studies 2020; 8(2): 603-8.
22. Wang M, Avula B, Wang YH, Zhao J, Avonto C, Parcher JF, Raman V, Zweigenbaum JA, Wylie PL and Khan IA: An integrated approach utilising chemometrics and GC/MS for classification of chamomile flowers, essential oils and commercial products. Food Chemistry 2014; 152: 391-8.
23. Duke JA: Handbook of phytochemical constituent grass, herbs and other economic plants. CRC Press 1992.
24. Ibrahim OO: Sugars alcohols: Chemical structures, manufacturing, properties and applications. EC Nutrition 2016; 4(2): 817-24.
25. Puri B and Hall A: Phytochemical dictionary: a handbook of bioactive compounds from plants. CRC press; 1998.
26. Duke JA: Handbook of medicinal herbs. CRC Press 2002.
27. Giles DP, Kerry JC, Kozlik A, Palmer BH, Shutler SW and Willis RJ: Substituted benzophenone hydrazones, process for their preparation, pesticidal compositions containing them and method of combating pests. European Patent 1981; 26: 040.
28. Böger M, Dürr D, Gsell L, Hall RG, Karrer F, Kristiansen O, Maienfisch P, Pascual A and Rindlisbacher A: Synthesis and structure–activity relationships of benzophenone hydrazone derivatives with insecticidal activity. Pest Management Science: formerly Pesticide Science 2001; 57(2):191-202.
29. Khalid MK, Fazal R, Ajmal K, Sajjad A, Muhammad T, Syed M S, Momin K, Najeebullah, Shaikh, Shahnaz P and Muhammad IC: Synthesis of benzophenone hydrazone analogs and their DPPH radical scavenging and urease inhibitory activities. J Chem Soc Pak 2015; 37(03): 479.
30. Hussain I and Ali A: Exploring the pharmacological activities of hydrazone derivatives: a review. J Phytochem Biochem 2017; 1(1): 1-1.
31. Hallak LK, Collins PL, Knudson W and Peeples ME: Iduronic acid-containing glycosaminoglycans on target cells are required for efficient respiratory syncytial virus infection. Virology 2000; 271(2): 264-75.
32. Rajab MS, Cantrell CL, Franzblau SG and Fischer NH: Antimycobacterial activity of (E)-phytol and derivatives: a preliminary structure-activity study. Planta Medica 1998; 64(01): 2-4.
33. Camoutsis C, Geronikaki A, Ciric A, Soković M, Zoumpoulakis P and Zervou M: Sulfonamide-1, 2, 4-thiadiazole derivatives as antifungal and antibacterial agents: synthesis, biological evaluation, lipophilicity and conformational studies. Chemical and Pharmaceutical Bulletin 2010; 58(2): 160-7.
34. Degnan BA and Macfarlane GT: Transport and metabolism of glucose and arabinose in *Bifidobacterium breve*. Archives of Microbiology 1993; 160(2): 144-51.
35. He X, Alian A, Stroud R and Ortiz de Montellano PR: Pyrrolidine carboxamides as a novel class of inhibitors of enoyl acyl carrier protein reductase from Mycobacterium tuberculosis. J of Med Chemistry 2006; 49(21): 6308-23.
36. Ramakrishna R, Kumar D, Bhateria M, Gaikwad AN and Bhatta RS: 16-Dehydropregnenolone lowers serum cholesterol by up-regulation of CYP7A1 in hyperlipidemic male hamsters. The Journal of Steroid Biochemistry and Molecular Biology 2017; 168: 110-7.
37. Rohloff J: Analysis of phenolic and cyclic compounds in plants using derivatization techniques in combination with GC-MS-based metabolite profiling. Molecules 2015; 20(2): 3431-62.

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