

GC-MS Analysis of Bioactive Components of *Cynoglossum zeylanicum* (Vahl Ex Hornem) Thunb. Ex. Lehm. (Boraginaceae).

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Abstract

Cynoglossum zeylanicum belongs to the family Boraginaceae. It is commonly known as “Jathakkai”. The present investigation was carried out to determine the possible bioactive components of whole plant of *Cynoglossum zeylanicum* using GC-MS analysis. Twenty compounds were identified. The prevailing compounds in the ethanol extract of whole plant of *Cynoglossum zeylanicum* were 9,12-Octadecadienoic acid(Z-Z)- (44.18%), n-Hexadecanoic acid (15.46%), Borazine,2,4,6-trimethyl (9.36%), Oleic acid (4.76%), 9,12-Octadecadienoyl chloride,(Z-Z)- (4.00%), Isosorbide (3.72%), Ethanamine, N-ethyl-N-nitro (3.24%), 2-Furancarboxaldehyde, 5-(hydroxyl methyl)- (2.83%) and Phytol (2.60%).

Key Words

Jathakkai, GC-MS, Bioactive compounds, Phytol.

Introduction

Phytochemicals are biologically active constituents of plants. These biologically active compounds contain some medicinal properties to cure several diseases. Herbal medicine represents one of the most important fields of traditional medicine all over the world. To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants, which have folklore reputation in a more intensified way¹. The past decade has been seen considerable change in opinion regarding ethnopharmacological therapeutic applications. The presence of various life sustaining constituents in plants has urged scientists to examine these plants with a view to determine potential medicinal properties². *Cynoglossum zeylanicum* belongs to Boraginaceae family. It is commonly known as “Jathakkai” Decoction prepared from the whole plant is used to arrest vomiting by Badaga community in Nilgiri Biosphere Reserve, Tamil Nadu³. Taking into consideration of the medicinal importance of *Cynoglossum zeylanicum*, the ethanol extract of whole plant of *Cynoglossum zeylanicum*, were analyzed for the first time using GC-MS. Persual of literature reveals that

information on the chemical analysis of *Cynoglossum zeylanicum* is totally lacking. This work will help to identify the compounds of therapeutic value.

Materials and methods

Collection of plant sample

Whole plant of *Cynoglossum zeylanicum* was collected from Kothagiri, Nilgiri Biosphere Reserve, Western Ghats, Tamil Nadu. With help of local flora, voucher specimen were identified and preserved in the Ethnopharmacology unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin, Tamil Nadu for further references.

Plant sample extraction

The whole plants were cleaned, shaded dried and pulverized to powder in a mechanical grinder. Required quantity of powder was weighed and transferred to Stoppard flask, and treated with ethanol until the powder is fully immersed. The flask was shaken every hour for the first 6 hours and then it was kept aside and again shaken after 24 hours. This process was repeated for 3 days and then the extract was filtered. The extract was collected and evaporated to dryness by using a vacuum distillation unit. The final residue thus obtained was then subjected to GC-MS analysis.

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GC-MS Analysis

GC-MS analysis of these extracts were performed using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a Elite-I, fused silica capillary column (30mmX0.25mm 1D X 1 µMdf, composed of 100% Di methyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml/min and an injection volume of 2µl was employed (split ratio of 10:1); Injector temperature 250°C; Ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbomass.

Identification of Compounds

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 component was compared with the spectrum of the known components stored in the NIST library. The Name, Molecular weight and structure of the components of the test materials were ascertained

Result and Discussion

The compounds present in the ethanol extract of whole plant of *Cynoglossum zeylanicum* were identified by GC-MS analysis (Fig 1). The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the ethanol extract of whole plant of *Cynoglossum zeylanicum* are presented in Table 1. Twenty compounds were detected in ethanol extract of *Cynoglossum zeylanicum* whole plant. The results revealed that 9,12-Octadecadienoic acid(Z-Z)- (44.18%), n-Hexadecanoic acid (15.46%), Borazine,2,4,6-trimethyl (9.36%), Oleic acid (4.76%), 9,12-Octadecadienoyl chloride,(Z-Z)- (4.00%), Isosorbide (3.72%), Ethanamine, N-ethyl-N-nitro (3.24%), 2-Furancarboxaldehyde, 5-

(hydroxyl methyl)- (2.83%) and Phytol (2.60%).were found as the major compounds in the ethanol extract of *Cynoglossum zeylanicum* whole plant. Figure 2, 3, 4 and 5 shows all spectrum and structure of 9, 12-Octadecadienoic acid (Z-Z)-, n-Hexadecanoic acid, Borazine, 2, 4, 6-trimethyl, Oleic acid. Table 2 listed the major phytochemicals and its biological activities obtained through GC-MS study of *Cynoglossum zeylanicum*. In the present study, 20 compounds have been identified from ethanol extract of the whole plant of *Cynoglossum zeylanicum* by Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Among the identified phytochemicals, tetradecanoic acid and n-Hexadecanoic acid have the property of antioxidant activity. 9, 12- Octadecadienoic acid (Z-Z) has the property of anti-inflammatory and antiarthritic as reported by earlier workers^{4, 5}. Squalene has the property of antioxidant⁶. Recently qualene possesses chemopreventive activity against colon carcinogenesis⁷. Phytol is detected in *Cynoglossum zeylanicum* whole plant which was also found to be effective at different stages of the arthritis. It was found to give food as well as preventive and therapeutic results against arthritis. The results show that, reactive oxygen species- promising novel class of pharmaceutical for the treatment of rheumatic arthritis and possibly other chronic inflammatory diseases⁸. Thus, this type of GC-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will be helpful for further detailed study. Further investigation into the pharmacological importance of *Cynoglossum zeylanicum* and their diversity and detailed Phytochemistry may add new knowledge to the information in the traditional medicinal systems.

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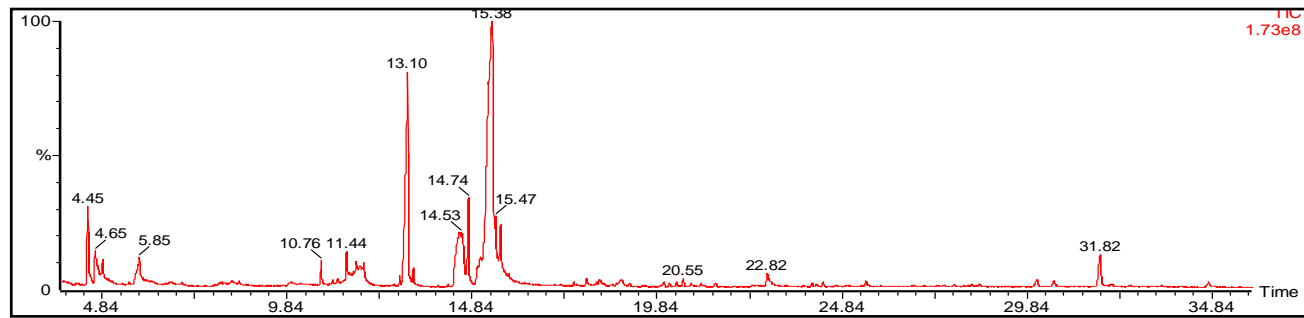


Fig. 1: GC-MS chromatogram of the ethanol extract of the whole plant of *Cynoglossum zeylanicum*.

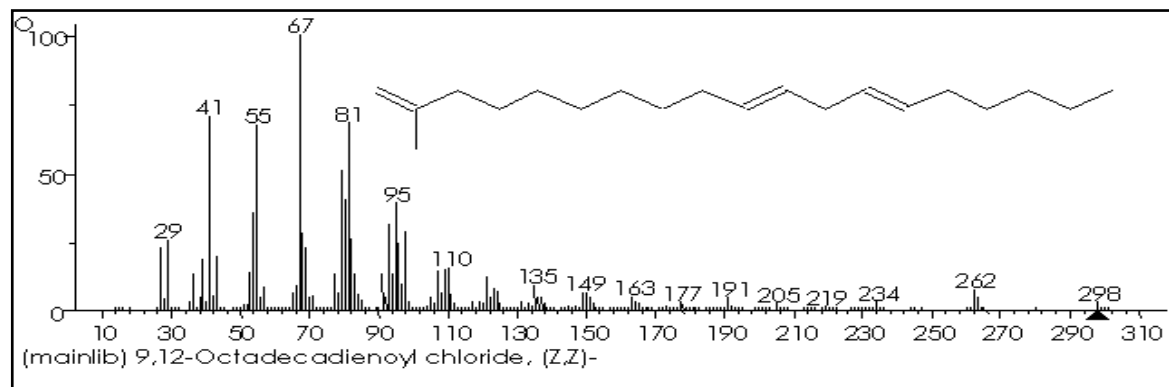


Fig. 2: Mass spectrum of 9, 12-Octadecadienoyl chloride, (Z, Z)-

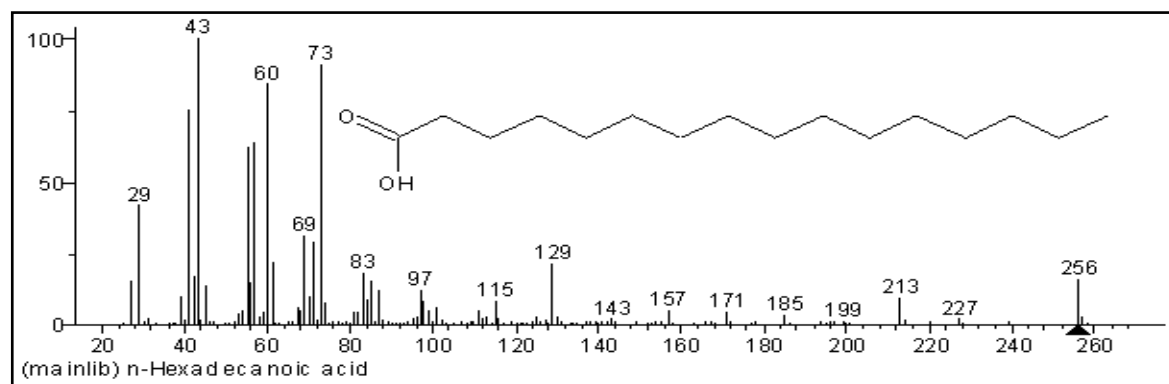


Fig. 3: Mass spectrum of n-Hexadecanoic acid.

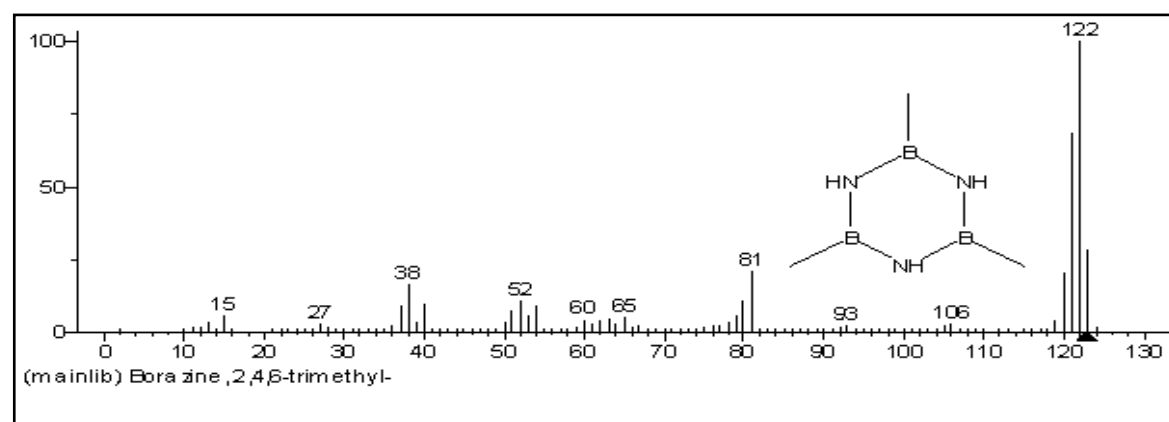


Fig. 4: Mass spectrum of Borazine, 2, 4, 6-trimethyl.

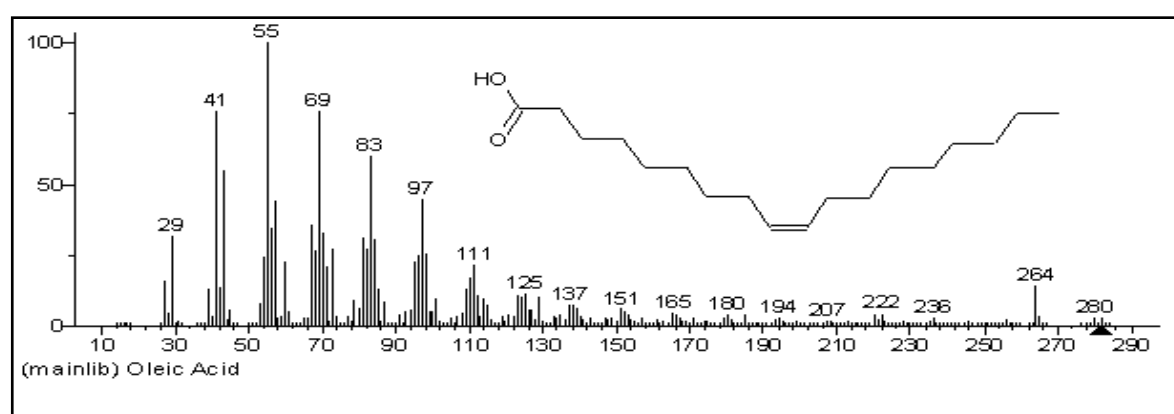


Fig. 5: Mass spectrum of Oleic acid.

Table 1: Components detected in the whole plant of ethanol extract of *Cynoglossum zeylanicum*.

No.	RT	Name of the compound	Molecular formula	MW	Peak Area %
1	4.45	Isosorbide	C ₆ H ₁₀ O ₄	146	3.72
2	4.65	2-Furancarboxaldehyde, 5-(hydroxymethyl)	C ₆ H ₆ O ₃	126	2.83
3	4.85	Conhydrin	C ₈ H ₁₇ NO	143	1.52
4	5.85	Ethanamine, N-ethyl-N-nitro-	C ₄ H ₁₀ N ₂ O ₂	118	3.24
5	10.76	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228	0.88
6	11.44	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	1.46
7	13.10	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	15.46
8	13.25	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284	0.48
9	14.53	Borazine, 2,4,6-trimethyl-	C ₃ H ₁₂ B ₃ N ₃	123	9.39
10	14.74	Phytol	C ₂₀ H ₄₀ O	296	2.60
11	15.38	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	280	44.18
12	15.47	9,12-Octadecadienoyl chloride, (Z,Z)-	C ₁₈ H ₃₁ ClO	298	4.00
13	15.61	Oleic Acid	C ₁₈ H ₃₄ O ₂	282	4.76
14	20.55	1,2-Benzenedicarboxylic acid, diisooctyl ester	C ₂₄ H ₃₈ O ₄	390	0.32
15	22.82	1-Docosene	C ₂₂ H ₄₄	308	1.35
16	24.33	Squalene	C ₃₀ H ₅₀	410	0.21
17	25.48	Heptadecane, 2,6,10,14-tetramethyl-	C ₂₁ H ₄₄	296	0.27
18	30.11	Stigmastan-6,22-dien, 3,5-dedihydro-	C ₂₉ H ₄₆	394	0.56
19	30.57	Stigmasterol	C ₂₉ H ₄₈ O	412	0.39
20	31.82	á-Sitosterol	C ₂₉ H ₅₀ O	414	2.39

Table 2: Activity of phytocomponents identified in the whole plant of ethanol extract of *Cynoglossum zeylanicum*.

No.	Name of the compound	Molecular formula	Compound Name	**Activity
1	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	C ₆ H ₆ O ₃	Aldehyde	Antimicrobial Preservative
2	Conhydrin	C ₈ H ₁₇ NO	Alkaloid	Antimicrobial Antiinflammatory
3	Ethanamine, N-ethyl-N-nitro-	C ₄ H ₁₀ N ₂ O ₂	Nitrogen compound	Antimicrobial
4	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	Myristic acid	Antioxidant, Cancer preventive, Nematicide, Lubricant Hypocholesterolemic
5	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	Terpene alcohol	Antimicrobial Antiinflammatory

6	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	Palmitic acid	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic, 5-Alpha reductase inhibitor
7	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	Fatty acid ester	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic, 5-Alpha reductase inhibitor
8	Borazine, 2,4,6-trimethyl-	C ₃ H ₁₂ B ₃ N ₃	Boron compound	Antimicrobial
9	Phytol	C ₂₀ H ₄₀ O	Diterpene	Anticancer Antioxidant Antiinflammatory Diuretic
10	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	Linoleic acid	Antiinflammatory, Hypocholesterolemic Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistaminic Antieczemic, Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge
11	Oleic Acid	C ₁₈ H ₃₄ O ₂	Mono unsaturated fatty acid	Antiinflammatory, Antiandrogenic Cancer preventive, Dermatitogenic Hypocholesterolemic, 5-Alpha reductase inhibitor, Anemiagenic Insectifuge, Flavor
12	1,2-Benzenedicarboxylic acid, diisooctyl ester	C ₂₄ H ₃₈ O ₄	Plasticizer compound	Antimicrobial Antifouling
13	Squalene	C ₃₀ H ₅₀	Triterpene	Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor, Pesticide
14	Stigmastan-6,22-dien, 3,5-dedihydro-	C ₂₉ H ₄₆	Steroid	Antimicrobial Antioxidant Antiinflammatory Antiarthritic, Antiasthma, Diuretic
15	Stigmasterol	C ₂₉ H ₄₈ O	Steroid	Antimicrobial Antioxidant Antiinflammatory Antiarthritic, Antiasthma, Diuretic
16	á-Sitosterol	C ₂₉ H ₅₀ O	Steroid	Antimicrobial Antioxidant Antiinflammatory Antiarthritic, Antiasthma, Diuretic

**Source: Dr. Duke's: Phytochemical and Ethnobotanical Databases
