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Research

Gas Chromatography-Mass Spectrometry Analysis Of Hydroalcoholic Extract of *Heliotropium indicum* Linn Leaves

A. Krishnaveni[1*], M. Bairavi[2], S. Prithivirajan[2], K. Umamageshwari[2], T. Venkata Rathina Kumar[3]

[1]Assistant Professor, [2]II year M.Pharm, [3]Head of the Department
Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai -20. Affiliated to the Tamilnadu Dr.M.G.R. Medical University, Chennai-32

*Author for Correspondence: Dr. A. Krishnaveni, M.Pharm, Ph.D,
Email: akrishnaveni72@rediffmail.com

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Abstract

Heliotropium indicum Linn belongs to Boraginaceae, is an annual herb commonly known as Indian Heliotrope. *H.indicum* is native to Asia, Tamil Nadu, India and is widely used in traditional medicine. In Kancheepuram district of Tamilnadu, India, tribal communities used this plant to treat skin infections, poisonous animal bites,stomach related problems and nervous diseases. Phytochemical report on *H.indicum* revealed the presence of alkaloids, triterpenes, sterols, amines and volatile oils. The plant is reported to possess antibacterial, antitumor, wound healing, anti-inflammatory, anti-nociceptive and diuretic activity. The fresh leaves of *Heliotropium indicum* were authenticated, collected, shade dried coarsely powdered and extracted with hydroalcohol. The extract was concentrated and stored in airtight container for further use. The aim of this study was to characterize the bioactive molecules in *H.indicum* leaves extract using Gas chromatography – mass spectrometry (GC-MS). GC-MS chromatogram showed bioactive molecules which may attribute to pharmacological properties.

Keywords: Gas chromatography –mass spectrometry (GC-MS), *Heliotropium indicum.*

INTRODUCTION

Heliotropium indicum Linn belongs to Boraginaceae, is an annual herb commonly known as Indian Heliotrope [1]. *H.indicum* is native to Asia and India, tribal communities of Kancheepuram district, Tamilnadu, used the plant preparations to treat skin infections, poisonous animal bites, stomach related problems and nervous diseases [2]. Malasar tribal communities of Tamilnadu used leaves sap preparations to protect dandruff [3]. In Cachhar district of Assam, India folklore claim revealsroot sap is used to treat eye diseases, fresh leaf paste is

applied externally to cure wounds [4]. In Southern parts of India leaf extract is used externally to prevent rheumatoid arthritis [5]. In African countries tribal people use *H.indicum* to treat inflammation and tumors [6].In Jamaica traditional system of medicine, infusion of the whole plant is used to treat fever Stomach infections and to cure cold and cough [7]. In West Indies traditional system of medicine decoction of the flower and buds is taken orally by the women as an emmenagogue and as abortifacient [8]. The phytochemical review showed the presence of alkaloid, tannins, triterpenes, sterols, amines and volatile oils etc [9]. Pharmacological survey reported its activities such as antimicrobial, anti-inflammatory, antitumor, antitubercular, to treat cataract, analgesic and wound healing [10]. It is immense and essential to identify the bioactive phytoconstituents present in the *Heliotropium indicum.* The present research is to investigate the Gas Chromatography –Mass Spectrometry of hydroalcoholic extract of *H.indicum* leaves.

Sample collection and identification

Plant collected from the domestic garden Mavadukurichi village, Thanjavur district, Tamil Nadu in the month of April 2024. It was identified and authenticated by DR. Stephen, Professor, Department of Botany The American College, Madurai-625002. The herbarium of this specimen was kept in the department for further reference.

Preparation of hydroalcoholic extract of *Heliotropium indicum* (HAEHI)

The collected leaves were washed with water; shade dried and powdered, extracted with hydroalcohol (70%) by maceration technique until the complete extraction of material and filtered. The extract was concentrated under reduced pressure to obtain a residue.

Gas chromatography- mass spectroscopy analysis

Gas chromatography – Mass spectrometry (GC-MS) (Shimadzu QP 2020) is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample. It is a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification of biochemical components of medicinal plants. GC-MS analysis was carried out to identify some of the potent volatile and semi-volatile constitutes present in the hydroalcoholic extract of *Heliotropium indicum* (L.).

Column

Column is fused silica, packed with SH-Rxi-5 Sil MS (30 m x 0.25 mm ID x 250 µm df) and the components were separated using helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 280º C.

Condition

1 µL of hydroalcoholic extract sample injected into the instrument, oven temperature was as follows: 50º C (3 min) followed by 180º C at the rate of 15º C min-1.

Mass detector

The mass detector conditions were: transfer line temperature 290º C; ion source temperature 230º C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 50 to 600 Da. The spectrums of the components were compared with database of spectrum of known components stored in the GC-MS NIST (2017) library.

RESULTS AND DISCUSSION

Gas chromatography – Mass spectrometry (GC-MS) identified the presence of twenty five bioactive constituents are shown in the Table 1 and its biological activity are shown in Table 2 and the chromatogram was displayed in Fig 1.

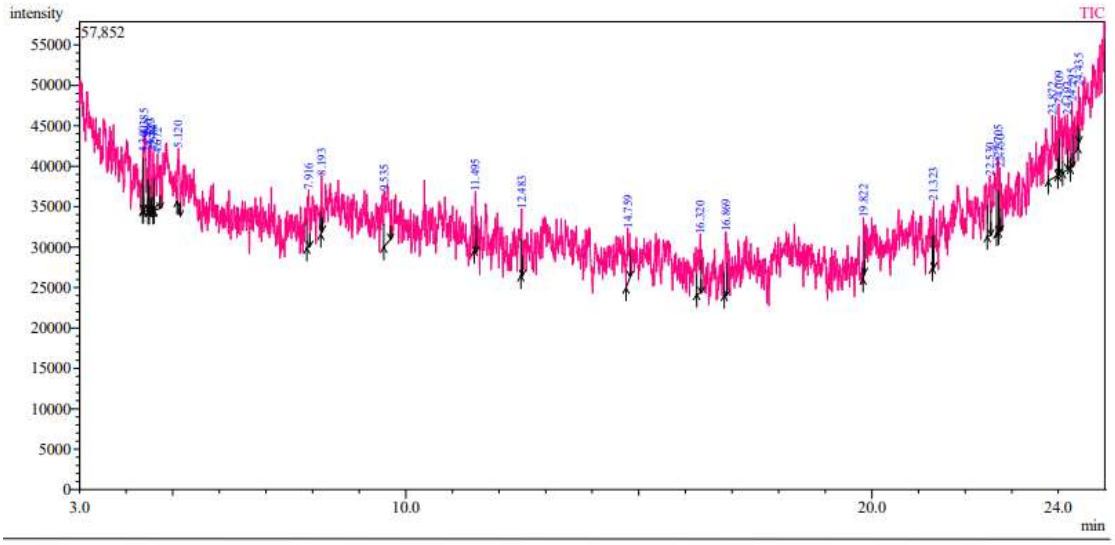

Fig 1: Gas Chromatography - Mass Spectrometry of HAEHI

Table 1: Identification of bioactive compounds in HAEHI by GC-MS

S.No	R. Time	Area	Area%	Molecular formula	Molecular weight	Name
1	4.360	7848	1.66	$C_{20}H_{13}N_5O_2$	355	2,7-Diphenyl-1,6- dioxopyridazino [4,5:2',3']pyrrolo[4',5'-d]pyridazine
2	4.385	37743	7.99	$C_7H_{16}O_3$	148	1,3,3-Trimethoxybutane
3	4.480	8940	1.89	$C_{14}H_{28}O_2$	228	Heptanoicacid,heptylester
4	4.523	15317	3.24	$C_{13}H_{10}N_6O_2S_2$	346	4-[3-(3,4-Dimethoxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl]-1,2,3-thiadiazole
5	4.584	7846	1.66	$C_8H_{10}O_4$	170	1,3-Cyclohexadiene-1-carboxylic acid, 5-hydroxy-6-methoxy-, trans
6	4.672	33075	7.00	$C_{16}H_{27}NO_4Si_2$	353	Anisuricacid,bis(O_trimethylsilyl)-
7	5.120	17802	3.77	$C_9H_9N_3S_2$	223	4-Allyl-5-thiophen-3-yl-2,4-dihydro-[1,2,4]triazole-3-thione
8	7.916	19476	4.12	$C_{17}H_{18}N_4O_2S$	342	2-acetylpyridine4-Carbethoxyphenyl]-3-thiosemicarbazone
9	8.193	8337	1.76	$C_{18}H_{38}S_2$	318	Di-n-nonyldisulphide
10	9.535	43552	9.22	$C_{12}H_{15}NO_3$	221	1-Hexen-3-ol,5-nitro-1-phenyl-,(R*,R*)-
11	11.495	9425	1.99	$C_{15}H_{26}O$	222	(3R,5aR,9S,9aS) Tetramethyloctahydro -2,2,5a,9- -2H-3, 9a- methanobenzo[b]oxepine
12	12.483	11480	2.43	$C_9H_{16}O_9S$	300	Methyl3-O-mesyl-5-O-methoxycarbonyl-d-xylofuranoside
13	14.759	22089	4.67	$C_{12}H_{13}N_3O_3$	247	2-Isobutyl-5-(2-nitro-phenyl)-[1,3,4]oxadiazole
14	16.320	25897	5.48	$C_9H_8N_4O_4S$	268	Benzaldehyde,3,4-methylenedioxy-2-nitro-, thiosemicarbazone
15	16.869	17610	3.73	$C_{13}H_{24}OSi_2$	252	1-Pentamethyldisilyloxy-2-phenylethane
16	19.822	9881	2.09	$C_{20}H_{40}O_3Si$	356	2-Trimethylsiloxy-6-hexadecenoicacid,methylester
17	21.323	13552	2.87	$C_7H_8N_6O_3$	224	1H-1,2,3-Triazole-4-carboxylicacid,1-(4-amino-3-furazanyl)-5-ethyl
18	22.530	21001	4.44	$C_{14}H_{22}O$	206	2,5,9-Trimethylcycloundeca-4,8-dienone

19	22.705	20145	4.26	$C_{13}H_{22}OSi$	222	2-tert-Butylphenol,trimethylsilyether
20	22.750	14353	3.04	$C_{32}H_{46}$	430	1,1'-Biphenyl,4-(4-pentylcyclohexyl)-4'-(4-propylcycl
21	23.872	43772	9.26	$C_{10}H_{13}NO_3Si$	223	Benzaldehyde,2-nitro-4-trimethylsilyl-
22	24.009	10079	2.13	$C_{12}H_{22}Si_2$	222	1,2-Bis(trimethylsilyl)benzene
23	24.192	33674	7.13	$C_{19}H_{36}F_3NO$	351	1-Aminoheptadecane, N-trifluoroacetyl-
24	24.295	13444	2.85	$C_{14}H_{32}O_8Si_3$	412	Ethyl 4,4,6,6,8,8-hexamethyl-11-oxo-3,5,7,9,12-pentaoxa-4,6,8-trisilatetradecan-1-oate
25	24.435	6201	1.31	$C_{15}H_{29}NO_3Si_2$	327	Ginkgotoxin, bis(trimethylsilyl) ether

Among the identified bioactive molecules 2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyridazine proved Renal disease treatment, Heart failure treatment, Antiepileptic, Alzheimer's disease treatment. 1,3,3-Trimethoxybutane proved as Antioxidant, Anti-inflammatory, Antibacterial. Heptanoic acid, heptyl esters serve as artificial flavors and fragrances in the food and cosmetics industries.4-[3-(3,4-Dimethoxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl]-1,2,thiadiazole proved as Anti-inflammatory, anticancer, antibacterial, antidepressant, antifungal, antiviral, antiparasitic, anticonvulsant, anti-HIV and herbicidal. 1,3-Cyclohexadiene-1carboxylic acid, 5-hydroxy-6-methoxy-trans proved as Antibacterial effect. Anisuric acid, bis(O-trimethyl silyl) proved anti-urolithic, analgesic and free radical scavenging agents.4-Allyl-5-thiophe14n-3-yl-2,4-dihydro-[1,2,4]triazole-3-thione proved Anti-inflammatory, CNS stimulants, sedatives, antianxiety, antimicrobial and antimigraine agents (rizatriptan) and antimycotic activity 2-acetylpyridine4-[4-Carbethoxyphenyl]-3-thiosemicarbazone proved Antifungal, Anti-inflammatory, Anticancer, Antiviral. Dinonyl di sulphide proved as Neuroprotective, Neurotoxic, Nociceptive, Diuretic.1-Hexen-3-ol,5-nitro-1-phenyl-,(R*,R*)- proved as Oligosaccharideprovider,oligospermy,oliguria.3R,5aR,9S,9aS)-2,2,5a,9-Tetramethyloctahydro-2H-3,9a-methanobenzo[b]oxepine proved Repellant, Arrhythmia, Arthritis, scorpion bite Antidiabetic, Mast cell stabilizers. Methyl3-O-mesyl-5-O-methoxycarbonyl-d-xylofuranoside proved as Catechol O-methyl transferase inhibitor, Methyl guanidine inhibitor.2-Isobutyl-5-(2-nitro-phenyl)-[1,3,4]oxadiazole used as antimicrobial agent. 2Trimethylsiloxyhexadecenoicacid, methylester, Proved Antioxidant, Antiarthritic, Hepatoprotective, Anti-inflammatory, Anticancer, Nematicide, Antiulcer**.** 1H-1,2,3-Triazole-4-carboxylicacid,1-(4-amino-3-furazanyl)-5-ethyl used as antimicrobial agent. 2,5,9-Trimethylcycloundeca-4,8-dienoneproved Antiproliferavtive, Antioxidant, Antibacterial. 2-tertButylphenol, trimethylsilyether Used as a chemical intermediate for synthetic resins, plasticizers, surface-active agents, perfumes, antioxidants, and agrochemicals. Benzaldehyde,2-nitro-4-trimethylsilyl proved as antifungal, Ginkgotoxin, bis(trimethylsilyl) ether proved to improve memory, brain. 1,2-Bis(trimethylsilyl)benzene proved Antibacterial Antimicrobial, Antioxidant. 1-Aminoheptadecane, N-trifluoroacetyl-proved as Anti-inflammatory, Ginkgotoxin, bis(trimethylsilyl) ether proved Anticancer, Anti-inflammatory, Diuretic, Antimicrobial, Hepatoprotective, Anti- arthritic, Antiasthma, Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Anti-androgenic, Flavor, Hemolytic, 5-Alpha reductase inhibitor.[11-31]

Table 2: Biological activity of phytocompounds identified in HAEHI by GC-MS

S.No	Name Of The Bioactive Compound	Derivatives	Biological Activity
1	2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyridazine	Pyrazine	Renal disease treatment, Heart failure treatment, Antiepileptic, Alzheimer's disease treatment [11]
2	1,3,3-Trimethoxybutane	Acetal	Antioxidant, Anti-inflammatory Antibacterial[12]
3	Heptanoicacid, heptyl ester	Ester	Artificial flavors and fragrances in the food and cosmetics industries. It also serves as an intermediate in the production of various chemical compounds in the pharmaceutical industry.[13]
4	4-[3-(3,4-Dimethoxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl]-1,2,3-thiadiazole	Thiadiazole	Anti-inflammatory, Anticancer, Antibacterial, Antidepressant, Antifungal, Antiviral, Antiparasitic,

			Anticonvulsant, Anti-HIV and Herbicidal etc [14]
5	1,3-Cyclohexadiene-1carboxylic acid, 5-hydroxy-6-methoxy-trans	carboxylic acids	Antibacterial effect [15]
6	Anisuricacid,bis(O-trimethylsilyl)	Silyl groups	Antiurolithic, Analgesic and free radical scavenging agents [16]
7	4-Allyl-5-thiophe14n-3-yl-2,4-dihydro-[1,2,4]triazole-3-thione	Thiophene	Anti-inflammatory, CNS stimulants, Sedatives, Antianxiety, Antimicrobial and Antimigraine agents (rizatriptan) and Antimycotic activity.[17]
8	2-acetylpyridine4-[4-Carbethoxyphenyl]-3-thiosemicarbazone	Pyridine	Antifungal, Anti-inflammatory, Anticancer, Antiviral [18]
9	Di-n-nonyldisulphide	Sulfur	Neuroprotective ,Neurotoxic, Nociceptive Diuretic[19]
10	1-Hexen-3-ol,5-nitro-1-phenyl-,(R*,R*)-	Nitro	Oligosaccharide provider, Oligospermy, Oliguria [20]
11	(3R,5aR,9S,9aS)-2,2,5a,9-Tetramethyloctahydro-2H-3, 9a-methanobenzo[b]oxepine	Oxepine	Repellant, Arrhythmia, Arthritis, scorpion bite, Antidiabetic, Mast cell stabilizers [21]
12	Methyl3-O-mesyl-5-O-methoxycarbonyl-d-xylofuranoside	Furan	Catechol O methyl transferase inhibitor, Methyl guanidine inhibitor [22]
13	2-Isobutyl-5-(2-nitro-phenyl)-[1,3,4]oxadiazole	Oxadiazole	Antimicrobial activity [23]
14	Benzaldehyde,3,4-methylenedioxy-2-nitro-,thiosemicarbazone	-	-
15	1-Pentamethyldisilyloxy-2-phenylethane	-	-
16	2-Trimethylsiloxy-6-hexadecenoicacid,methylester	Silylated fatty acid ester	Antioxidant, Anti arthritic, Hepatoprotective, Anti-inflammatory, Anticancer, Nematicide, Antiulcer [24]
17	1H-1,2,3-Triazole-4-carboxylicac id,1-(4-amino-3-furazanyl)-5-ethyl	Triazole	Antimicrobial agent [25]
18	2,5,9-Trimethylcycloundeca-4,8-dienone	Carbonyl compound	Antiproliferavtive, Antioxidant, Antibacterial [26]
19	2-tert- Butylphenol, trimethylsilyether	Phenol	Used as a chemical intermediate for synthetic resins, plasticizers, surface-active agents, perfumes, antioxidants, agrochemicals. [27]
20	1,1'-Biphenyl,4-(4-pentylcyclohexyl)-4'-(4-propylcycl	Cyclohexyl ring	-
21	Benzaldehyde,2-nitro-4-trimethylsilyl-	Benzaldehyde	Antifungal [28]
22	1,2-Bis(trimethylsilyl)benzene	Phenol	Antibacterial Antimicrobial, Antioxidant [29]
23	1-Aminoheptadecane, N-trifluoroacetyl-	Fluorinated amide	Anti-inflammatory [30]
24	Ethyl 4,4,6,6,8,8-hexamethyl-11-oxo-3,5,7,9,12-pentaoxa-4,6,8-trisilatetradecan-1-oate	-	-
25	Ginkgotoxin, bis(trimethylsilyl) ether	Phenol	Anticancer, Anti-inflammatory, Diuretic, Antimicrobial, Hepatoprotective, Anti- arthritic, Antiasthma, Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Anti-androgenic, Flavor, Hemolytic, 5-Alpha reductase inhibitor [31]

Benzaldehyde,3,4-methylenedioxy-2-nitro-,thiosemicarbazone,1-Pentamethyldisilyloxphenylethane,1,1'-Biphenyl,4-(4-pentylcyclohexyl)-4'-(4-propylcycl,Ethyl4,4,6,6,8,8-hexamethyl-11-3,5,7,9,12--pentaoxa-4,6,8-trisilatetradecan-1-oate did not show any biological activity.

CONCLUSION

The current investigation concluded that the hydroalcoholic extract of *Heliotropium indicum* leaves reported the presence of twenty-one bioactive compounds with potential pharmacological activities. It may serve as potential source of therapeutic drugs due to the presence of important phytochemical bioactive compounds which contribute the activities namely antimicrobial, anti-inflammatory, antioxidant, antiarthritic and other activities. The results indicate the pharmacological potential and provide a basis for further research.

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