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Research

Gas Chromatography-Mass Spectrometry analysis of Hydroalcoholic extract of *Hibiscus vitifolius* Linn Leaves.

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Abstract

Hibiscus vitifolius also known as tropical rose mallow and grape leaved mallow, belongs to the family of Malveceae. It is a found in India and in United States of America. *Hibiscus vitifolius* is traditionally used for the treatment of jaundice, diabetics, inflammation, urease activity in India. The literature look over revealed the presence of alkaloids, flavones, gossypin, carotenoids, atropine, promethazine, aldose, galactose, bioflavonoids, morphine, pentahydroxy glycosyl flavones. The plant exhibited antibacterial, antimicrobial, antioxidant, antihepatotoxic, anticancer, diuretic, anti-inflammatory and antiepilepsy effect. The fresh leaves of *Hibiscus vitifolius* Linn were authenticated, collected, shade dried and coarsely powdered, was extracted with hydroalcohol. The extract was concentrated and stored in air tight container for further use. The aim of the present research study was to carry out for the identification of bioactive moleculess from hydroalcoholic extract of *Hibiscus vitifolius* Linn leaves by Gas chromatography-Mass spectrometry (GC-MS). GC-MS chromatogram showed twentyfive bioactive molecules which may attribute to different pharmacological properties.

Keywords: Gas chromatography- Mass spectrometry, *Hibiscus vitifolius* (L).

INTRODUCTION

Hibiscus vitifolius Linn is medicinal herb, belongs to Malvaceae family. *Hibiscus vitifolius* also known as tropical rose mallow, Grape leaved mallow [1]. *Hibiscus vitifolius* is a common plant of India and United state of America. *Hibiscus vitifolius* is widely distributed in tropical Asia, Africa, Australia and it is also naturalized in West Indian Islands, Cuba, Hispaniola, Jamaica Central America [2]. *Hibiscus vitifolius* is traditionally used for the treatment of jaundice, diabetics, inflammation, urease activity in India [3]. Ethnomedicinal claim of *Hibiscus vitifolius* leaf powder is used to treat inflammation in Chittur Taluk in Palakkad District, Kerala. Triupur district,

Tamilnadu, leaf juice is used to treat cuts and wounds, Sivagangai district and Tirunelveli district, Tamilnadu, used leaf preparations to control diarrhoea [4-7]. The plant having alkaloids, flavones, gossypin, carotenoids, atropine, promethazine, aldose, galactose, bioflavonoids, morphine, pentahydroxy glycosyl flavones [8]. The pharmacological survey reported antibacterial, antioxidant, anti-inflammatory, hepatoprotective activity, antiepilepsy, anti-cancer activity and anti-microbial. [9-15]. It is immense and essential to identify the bioactive phytoconstituents present in the *Hibiscus vitifolius*. The present research is to investigate the Gas-chromatography-Mass spectrometry of hydroalcoholic extract of *H.vitifolius* Leaves.

Authentication and collection

Leaves were collected from a street side of Rayagiri village, Sivagiri Taluk, Tenkasi District, Tamil Nadu in the month of Mar 2024. The fresh leaves were identified and authenticated by a botanist 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 *Hibiscus vitifolius* linn (HAEHV)

Collected leaves were washed, shade dried and coarsely powdered (50 gm), passed through sieve no: 40, was extracted with hydroalcohol by maceration technique for 72 hours. The extracts were collected, concenrtated to dryness and stored in air tight container. The hydroalcoholic extract was analysed by GC-MS.

Gas chromatography- Mass spectrometry 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 *Hibiscus vitifolius* Linn

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 280^0 C.

Condition

1 µL of hydroalcoholic extract sample injected into the instrument, oven temperature was as follows: 50^0 C (3 min) followed by 180^0 C at the rate of 15^0 C min-1.

Mass detector

The mass detector conditions were: transfer line temperature 290^0 C; ion source temperature 230^0 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 twentyfive bioactive constituents are shown in the table 1 and its biological activitiy are shown in Tbale 2 and the chromatogram was displayed in Fig 1.

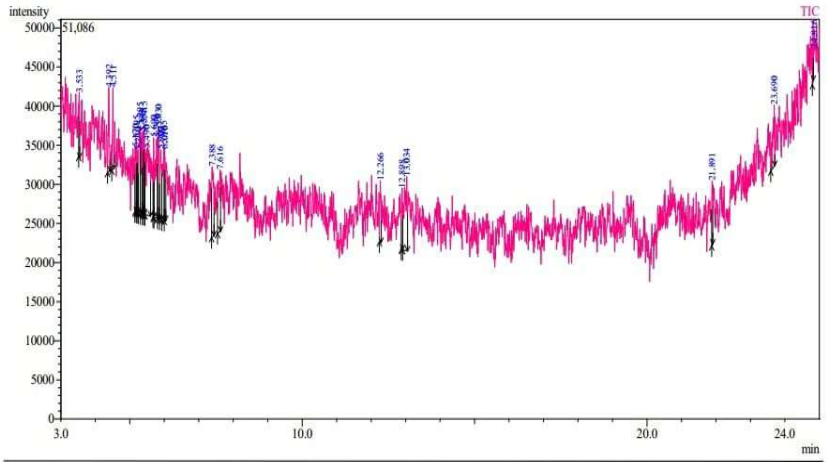

Fig 1: Gas chromatography- Mass spectrometry of HAEHV

Table 1: List of bioactive compounds present in HAEHV leaves by GC-MS

Peak#	Retention Time	Area %	Mol. Weight	Mol. formula	Name of the Bioactive compound
1	3.533	1.47	145	C_6H_5ClFN	Benzenamine, 3-chloro-4-fluoro-
2	4.392	3.59	218	$C_9H_{18}O_4Si$	Methyl 2-(trimethylsilyl)ethyl malonate
3	4.511	2.89	144	$C_8H_{16}O_2$	2-Isopropyloxan-4-ol
4	5.170	2.69	154	$C_6H_9F_3O$	2,2-Difluorobut-3-en-1-yl 2-fluoroethyl ether
5	5.195	1.92	130	$C_7H_{11}Cl$	2-Heptyne, 7-chloro-
6	5.230	2.19	222	$C_{11}H_{10}O_5$	4,6-Diformyl-2,5-dimethoxytropone
7	5.295	5.20	224	$C_{10}H_{12}N_2O_4$	Benzene, 1,2,3,5-tetramethyl-4,6-dinitro-
8	5.349	4.91	236	$C_9H_{19}BCl_2Si$	2-Pentene, 3-(chloroethylboryl)-2-(chlorodimethylsilyl)-, (E)-
9	5.385	2.99	175	$C_{10}H_9NO_2$	3H-Indol-3-one, 1-acetyl-1,2-dihydro-
10	5.415	3.01	162	$C_{11}H_{14}O$	2(1H)-Naphthalenone, 4a,5,8,8a-tetrahydro-4a-methyl-, trans
11	5.490	11.83	422	$C_{16}H_{15}IN_4O_2$	N-(4-Iodophenyl)-3-(isonicotinoylhydrazono)butyramide
12	5.690	2.11	202	$C_6H_6N_2O_4S$	Benzenesulfonamide, 4-nitro-
13	5.790	6.96	152	$C_{10}H_{16}O$	Bicyclo[3.1.1]heptane-2-carboxaldehyde, 6,6-dimethyl
14	5.830	4.96	154	$C_8H_{10}O_3$	3,8,11-Trioxatetracyclo [4.4.1.0(2,4).0(7,9)] undecane, (1.alpha.,2.alpha.,4.alpha., 6.alpha.,7.beta.,9.beta.)-
15	5.900	4.16	414	$C_{28}H_{34}N_2O$	[5,9-Dimethyl-1-(3-phenyl-oxiran-2-yl)-deca-4,8-dienylidene]-(2-phenyl-aziridin-1-yl)-amine
16	5.985	5.14	240	$C_8H_{16}O_8$	l-Gala-l-ido-octose
17	6.010	3.15	252	$C_{10}H_{12}N_4O_4$	2-Butanone, (2,4-dinitrophenyl)hydrazone
18	7.988	4.85	280	$C_{12}H_{16}N_4O_2S$	4-Morpholinethiocarboxylic acid 2-[1-[2-pyridyl]-2-hydroxyethylidene]hydrazide
19	7.616	5.32	224	$C_8H_{16}O_5S$	Ethyl-1-thio-.beta.-d-glucopyranoside
20	12.266	2.85	292	$C_{16}H_{20}O_5$	2,4-Dioxabicyclo[3.3.0]octan-6-ol, 7-(2-carboxy-1-propyl)-3-phenyl
21	12.898	2.46	163	$C_9H_9NO_2$	Benzene, 1-cyclopropyl-2-nitro-
22	13.034	8.31	306	$C_{10}H_{12}F_6N_2O_2$	1,2-Diaminocyclohexane, N,N'-bis(trifluoroacetyl)- (stereoisomer 2)

23	21.891	1.92	220	$C_{11}H_{16}N_4O$	1H-[1,2,4]Triazole-3-carboxylic acid (2-cyclohex-1-enyl-ethyl)-amide
24	23.690	3.81	223	$C_{10}H_{13}NO_3Si$	Benzaldehyde, 2-nitro-4-trimethylsilyl-
25	24.816	1.28	207	$C_{13}H_{21}NO$	N-Methyl-1-adamantaneacetamide

Among the identified bioactive molecules Benzenamine, 3-chloro-4-fluoro- used as intermediate in synthesis of antibiotic, 2-Heptyne, 7-chloro- used as preparation of grignard reagent, Benzene , 1,2,3,5-tetramethyl-4,6-dinitro- used to be manufacturing of dye , 3H-Indol-3-one, 1-acetyl-1,2-dihydro- is used to applied schmidt rearrangement, 2(1H)-Naphthalenone, 4a,5,8,8a-tetrahydro-4a-methyl-, trans and 4-Morpholinethiocarboxylic acid 2-[1-[2-pyridyl]-2-hydroxyethylidene]hydrazide showed as anti-inflammatory properties, N-(4-Iodophenyl)-3-(isonicotinoylhydrazono)butyramide showed as anticonvulsant activity, Benzenesulfonamide, 4-nitro- reported as acetylcholine inhibitor, Bicyclo[3.1.1]heptane-2-carboxaldehyde, 6,6-dimethyl showed as alzhemer's disease, 3,8,11Trioxatetracyclo[4.4.1.0(2,4).0(7,9)]undecane, (1.alpha.,2.alpha.,4.alpha.,6.alpha., 7.beta.,9.beta.)-and 2,4-Dioxabicyclo[3.3.0]octan-6-ol, 7-(2-carboxy-1-propyl)-3-phenyl reported as antiallergy, [5,9-Dimethyl-1-(3-phenyl-oxiran-2-yl)-deca-4,8-dienylidene]-(2-phenyl-aziridin-1-yl)-amine and 1,2-Diaminocyclohexane, N,N'-bis(trifluoroacetyl)- (stereoisomer 2) showed as antimicrobial activity and also 1,2-Diaminocyclohexane, N,N'-bis(trifluoroacetyl)- (stereoisomer 2) reported as antitumor activity , 2-Butanone, (2,4-dinitrophenyl)hydrazone and N-Methyl-1-adamantaneacetamide showed antioxidant properties, l-Gala-l-ido-octose showed helps in improvement memory, Ethyl-1-thio-.beta.-d-glucopyranoside reported as anti-bacterial properties, Benzene, 1-cyclopropyl-2-nitro- used as degradation of polystyrene , 1H-[1,2,4]Triazole-3-carboxylic acid (2-cyclohex-1-enyl-ethyl)-amide reported as Covid, Benzaldehyde, 2-nitro-4-trimethylsilyl and N-Methyl-1-adamantaneacetamide reported as antifungal and N-Methyl-1-adamantaneacetamide showed as anti-aflatoxigenic, antibacterial.

Table 2: List of phytocompounds identified with biological activity presnt in HAEHV by GC-MS

Peak#	Name of the bioactive compound	Derivative	Biological activity
1	Benzenamine, 3-chloro-4-fluoro-	Aniline	Intermediate in synthesis of antibiotic [17]
2	Methyl 2-(trimethylsilyl)ethyl malonate	Ethyl	-
3	2-Isopropyloxan-4-ol	Isopropyl alcohol	-
4	2,2-Difluorobut-3-en-1-yl 2-fluoroethyl ether	Ether	-
5	2-Heptyne, 7-chloro-	Aliphatic alkyl	preparation of Grignard reagent [18]
6	4,6-Diformyl-2,5-dimethoxytropone	Tropane	-
7	Benzene, 1,2,3,5-tetramethyl-4,6-dinitro-	Benzene	Manufacturing of dye [19]
8	2-Pentene, 3-(chloroethylboryl)-2-(chlorodimethylsilyl)-, (E)-	Pentene	-
9	3H-Indol-3-one, 1-acetyl-1,2-dihydro-	Indole	Role of Schmidt rearrangement [20]
10	2(1H)-Naphthalenone, 4a,5,8,8a-tetrahydro-4a-methyl-, trans	Napthalene	Anti-inflammatory [21]
11	N-(4-Iodophenyl)-3-(isonicotinoylhydrazono)butyramide	Butyramide	Anticonvulsant activity [22]
12	Benzenesulfonamide, 4-nitro-	Benzosulfonamide	Acetylcholine inhibitor [23]
13	Bicyclo[3.1.1]heptane-2-carboxaldehyde, 6,6-dimethyl	Bicycloheptane	Alzhemer's disease [24]
14	3,8,11-Trioxatetracyclo[4.4.1.0(2,4).0(7,9)]undecane, (1.alpha.,2.alpha.,4.alpha.,6.alpha.,7.beta.,9.beta.)-	Tetracycloundecane	Antiallergy [25]
15	[5,9-Dimethyl-1-(3-phenyl-oxiran-2-yl)-deca-4,8-dienylidene]-(2-phenyl-aziridin-1-yl)-amine	Epoxide	Anti microbia[26]
16	l-Gala-l-ido-octose	Octose	Helps in improvement memory [27]
17	2-Butanone, (2,4-dinitrophenyl)hydrazone	Butanone	Antioxidant [28]

18	4-Morpholinethiocarboxylic acid 2-[1-[2-pyridyl]-2-hydroxyethylidene]hydrazide	Morpholine	Anti-inflammatory [29]
19	Ethyl-1-thio-.beta.-d-glucopyranoside	glucopyranoside	Antibacterial [30]
20	2,4-Dioxabicyclo[3.3.0]octan-6-ol, 7-(2-carboxy-1-propyl)-3-phenyl	Dioxabicyclooctane	Allergic asthma [31]
21	Benzene, 1-cyclopropyl-2-nitro-	Benzene	Degradation of polystyrene [32]
22	1,2-Diaminocyclohexane, N,N'-bis(trifluoroacetyl)- (stereoisomer 2)	Cyclohexane	Antitumor activity and antimicrobial activity [33,34]
23	1H-[1,2,4]Triazole-3-carboxylic acid (2-cyclohex-1-enyl-ethyl)-amide	Triazole	Covid [35]
24	Benzaldehyde, 2-nitro-4-trimethylsilyl-	Aromatic aldehyde	Antifungal activity [36]
25	N-Methyl-1-adamantaneacetamide	Adamantaneacetamide	Antifungal, anti-aflatoxigenic, antioxidant, antibacterial [37]

Methyl 2-(trimethylsilyl) ethyl malonate, 2-Isopropyloxan-4-ol, 2,2-Difluorobut-3-en-1-yl 2-fluoroethyl ether, 4,6-Diformyl-2,5-dimethoxytropone, 2-Pentene, 3-(chloroethylboryl)-2-(chlorodimethylsilyl)-, (E)- does not show any biological activity, these compounds were identified for the first time in this plant.

CONCLUSION

The current investigation concluded that the hydroalcoholic extract of *Hibiscus vitifolius* (L) leaves reported the presence of twenty 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 contributre the activities namely antiallergy, anti-inflammatory, anti-tumor, anti -bacterial and other activities.the results indicate the pharmacological potential and provide a basic for further research.

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