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Research

Gas Chromatography-Mass Spectroscopy Study of Hydro-alcoholic Extract of *Carissa carandas* (L.) Leaves.

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Abstract

GC-MS chromatogram of the extract revealed a diverse range of thirty-six compounds, including terpenoids, fatty acids, heterocyclic compounds, steroids, and vitamins. The identified compounds possess various potential applications, including flavoring, antimicrobial activity, anti-inflammatory properties and anticancer effects. Notably, the extract contained caryophyllene derivatives known for their anti-inflammatory, neuroprotective, and antimicrobial properties. These findings suggest that *Carissa carandas* leaves could be a valuable source of natural bioactive compounds with potential medicinal applications. However, further research is needed to isolate and characterize the specific components responsible for these therapeutic effects.

Keywords: Gas Chromatography – Mass Spectroscopy, Carissa carandas (Linn.)

INTRODUCTION

Carissa carandas, a thorny shrub native to India, belongs to Apocynaceae, It has been traditionally employed in Ayurvedic, Unani, and Siddha systems of medicine for centuries [1]. Its fruits, leaves, roots, and bark, have been used to treat a wide range of ailments such as diarrhea, dysentery, fever, and skin disorders [2,3]. Phytochemical studies have revealed the presence of alkaloids, flavonoids, saponins, and cardiac glycosides [4,5]. It exhibited antioxidant, anti-inflammatory, anti-microbial, and anti-diabetic activities [6,7]. It is immense and essential to identify the bioactive phytoconstituents present in the *Carissa carandas (L)*. The present research is to investigate the Gas-chromatography-Mass Spectroscopy of hydroalcoholic extract of *C.carandas* Leaves.

MATERIALS AND METHODS

Authentication and Collection

Fresh leaves of *Carissa carandas* Linn., were collected from domestic garden of Indian Medical

Association convention centre, Madurai Medical College, Madurai dist, Tamilnadu during the month of March - 2024 and was authenticated by Dr. D. Stephen, M.Sc., Ph.D., Professor, Department of Botany, American College, Madurai-02. The herbarium of this specimen was kept in the department forfurther reference.

Preparation of powder

The leaves were collected and shade dried, was powdered using blender. The powder was sieved in a No.60 sieve and kept in a well closed container in a dry place.

Preparation of hydroalcoholic extract of *Carissa carandas* (HAECC)

The powder of *Carissa carandas* were extracted successively with hydroalcoholic extract using maceration process. The extracts were combined, concentrated, evaporated to dryness and the solid mass was noted, stored in an air-tight container for further use. The hydro alcoholic extract of *Carissa carandas* were analysed by GC-MS.

Gas chromatography-Mass Spectroscopy analysis

Gas chromatography – Mass Spectroscopy (GC-MS) (Shimadzu QP 2020) is an analytical method that combines the features of gas-chromatography and mass Spectroscopy 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 [4]. GC-MS analysis was carried out to identify some of the potent volatile and semi-volatile constitutes present in the hydroalcoholic extract of *Carissa carandas (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 2800 C.

Condition

1 µL of hydro alcoholic extract sample injected into the instrument, oven temperature was as follows: 500 C (3 min) followed by 1800 C at the rate of 150 C min-1.

Mass detector

The mass detector conditions were: transfer line temperature 2900 C; ion source temperature 2300 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 spectroscopy (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify bio-active substances present in HAECC [5].

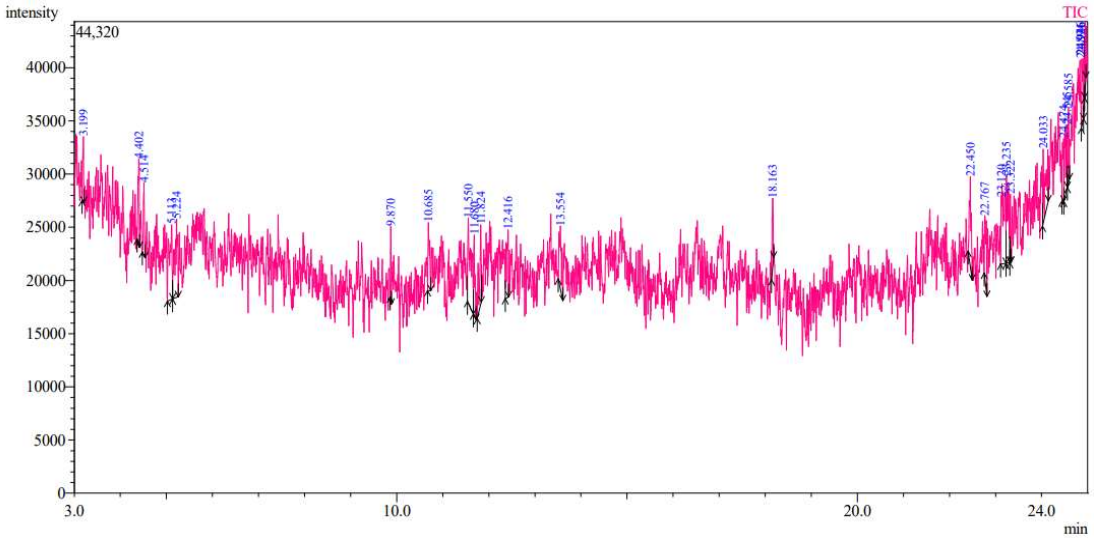

Fig 1: Gas chromatography-Mass Spectroscopy of Hydroalcoholic extract of *Carissa carandas*

Table 1: Phytoconstituents present in the hydroalcoholic extract of *Carissa carandas*

Peak #	R.Time	Area %	Mol. Formula	Mol. weight	Name of the phytoconstiuents
1	3.199	1.89	$C_9H_{16}O$	140	4-Cyclononen-1-ol
2	4.402	3.75	$C_8H_{16}O_3$	160	2-Hydroxyisocaproic acid, methyl ether, methyl ester
3	4.514	2.54	$C_9H_{20}O_2$	160	2,3,4,4-Tetramethyl-pentane-1,3-diol
4	5.113	7.52	$C_{10}H_{14}BNO$	175	1,3,2-Oxazaborolidine, 2,4-dimethyl-5-phenyl-
5	5.224	7.29	$C_7H_6N_2O_4$	182	Benzene, 4-methyl-1,2-dinitro-
6	9.870	1.24	$C_9H_{15}ClO_3$	206	Dimethylmalonic acid, monochloride, isobutyl ester
7	10.685	3.53	$C_{16}H_{22}F_7NO_2$	393	2,3,9-Trimethyl-5-heptafluorobutyryloxy-2-azabicyclo[4.4.0]decane
8	11.550	9.01	$C_9H_8N_2O_5$	224	(4-Carbamoyl-2-nitrophenyl)acetic acid
9	11.680	2.91	$C_7H_7F_3N_2$	176	2,3-Diazabicyclo[3.2.0]hept-2-ene, 1,6,6,-trifluoro-4-spirocyclopropan
10	11.824	4.34	$C_{15}H_{24}$	204	Bicyclo[7.2.0]undec-4-ene, 4,11, 11-trimethyl-8-methylene-,[1R-(1R*
11	12.416	4.12	$C_6H_6O_3S$	158	Benzenesulfonic acid
12	13.554	4.69	$C_{14}H_{12}N_2O_3$	256	3-[2-(1-Methyl-1H-pyrazol-5-yl)-2-oxoethyl]-1,3-dihydro-2-benzofuran
13	18.163	2.62	$C_{17}H_{32}O_2$	268	Z-11-Tetradecen-1-ol propionate
14	22.450	4.92	$C_9H_{14}O_2$	154	3-Nonynoic acid
15	22.767	3.81	$C_{19}H_{30}O_2$	290	8,11-Octadecadiynoic acid, methyl ester
16	23.120	8.74	$C_5H_{12}OS$	120	1-Pentanol, 5-mercapto-
17	23.235	6.23	$C_{12}H_{28}O_2Si_2$	260	2-Hydroxymethylcyclopentanol, (Z)-, 2TMS derivative
18	23.322	1.69	$C_5H_8O_2S$	132	Ethyl propargyl sulfone
19	24.033	5.38	$C_{24}H_{38}O_2Si_2$	414	Hexestrol, 2TMS derivative
20	24.474	2.29	$C_{19}H_{25}NO_2$	363	Glutaric acid, cyclohexylmethyl 3-nitrobenzyl ester
21	24.545	4.45	$C_{10}H_9NO_2S$	207	2-Ethoxycarbonylphenyl isothiocyanate
22	24.585	1.92	$C_{10}H_{16}N_2OSi$	208	N-Methyl nicotinimidate, O-trimethylsilyl
23	24.894	2.90	$C_{14}H_{29}O_4PS$	324	Ethanethioic acid, S-[8-(diethylphosphono)octyl] ester
24	24.926	1.14	$C_{14}H_{22}O_4Si$	282	Ethyl homovanillate, TMS derivative
25	24.946	1.10	$C_{12}H_{19}NO_2$	209	3-(Aminomethyl)adamantane-1-carboxylic acid

Table 2: Bioactive compounds present in the hydroalcoholic extract of *Carissa carandas*

Peak #	Name	Nature of the compound	Applications
1	4-Cyclononen-1-ol	Cycloalkanols	Flavouring Agent [8]
2	2-Hydroxyisocaproic acid, methyl ether, methyl ester	Hydroxycarboxylic acid	Antimicrobial, Antinflammatory [9]
3	2,3,4,4-Tetramethyl-pentane-1,3-diol	Branched alkane diol	Colouring agent [10]
4	1,3,2-Oxazaborolidine, 2,4-dimethyl-5-phenyl-	Substituted oxazaborolidine	Antimicrobial[11]
5	Benzene, 4-methyl-1,2-dinitro-	Nitroaromatic Compound	Plasticizer, Propellant [12]
6	Dimethylmalonic acid, monochloride, isobutyl ester	Carboxylic acid derivative	---
7	2,3,9-Trimethyl-5-heptafluorobutyryloxy-2-azabicyclo[4.4.0]decane	Substituted azabicyclic heterocyclic compound.	Antiinflammatory, Antimicrobial [13]
8	(4-Carbamoyl-2-nitrophenyl)acetic acid	Nitroaromatic Acid	Immunomodulators for cancer [14]
9	2,3-Diazabicyclo[3.2.0]hept-2-ene, 1,6,6,-trifluoro-4-spirocyclopropan	Substituted azabicyclic heterocyclic compound.	Against Leishmaniasis(Antiparasitic) [15]
10	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*	Caryophyllene	Antiinflammatory, Neuroprotective [16] Antimicrobial, Anticancer[17]
11	Benzenesulfonic acid	Sulfonamide derivatives	Antidiabetic [18]
12	3-[2-(1-Methyl-1H-pyrazol-5-yl)-2-oxoethyl]-1,3-dihydro-2-benzofuran	Cyclic ether	Antibacterial [19]
13	Z-11-Tetradecen-1-ol propionate	Fatty acid ester	---
14	3-Nonynoic acid	Carboxylic acid	Anti inflamatory [20]
15	8,11-Octadecadiynoic acid, methyl ester	Acetylenic fatty acid	Anti microbial [21]
16	1-Pentanol, 5-mercapto-	Secondary thiol	Flavoring agent [22]
17	2-Hydroxymethylcyclopentanol, (Z)-, 2TMS derivative	Cyclopentane diol	Building blocks for Synthesis [23]
18	Ethyl propargyl sulfone	Sulfanones	Bio catalyst [24]
19	Hexestrol, 2TMS derivative	Stilbestrol	Anticancer [25]
20	Glutaric acid, cyclohexylmethyl 3-nitrobenzyl ester	Nitrobenzyl ester	Selective muscarinic antagonists [26]
21	2-Ethoxycarbonylphenyl isothiocyanate	Isothiocyanates	Antimicrobial [27]
22	N-Methyl nicotinimidate, O-trimethylsily	Nicotinamide derivatives	Neuroprotective [28], Antimicrobial [29]
23	Ethanethioic acid, S-[8-(diethylphosphono)octyl] ester	Thioacetic acid	Antioxidant [30]
24	Ethyl homovanillate, TMS derivative	Vannilin derivative	Flavoring agent [32]
25	3-(Aminomethyl)adamantane-1-carboxylic acid	Adamantane derivative	Anti-allodynic for Neuropathic pain [33]

Hydroalcoholic extract of *Carissa carandas* contains a diverse array of bioactive compounds with various applications. 4-cyclononen-1-ol, 1-Pentanol, 5-mercapto-, Ethyl homovanillate, and TMS derivative contribute to flavoring agent [8,22,31]. While 2-hydroxyisocaproic acid, 2,3,9-Trimethyl-5-heptafluorobutyryloxy-2-azabicyclo[4.4.0]decane, Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R* exhibit antimicrobial and anti-inflammatory properties [9,13,16,17]. 2,3,4,4-tetramethyl-pentane-1,3-diol is used as coloring agent [10]. 1,3,2-oxazaborolidine,2,4-dimethyl-5-phenyl-, 8,11-Octadecadiynoic acid, methyl ester, 2-Ethoxycarbonylphenyl isothiocyanate and N-Methyl nicotinimidate, O-trimethylsilyl demonstrate antimicrobial activity[11,21,27,29]. Benzene, 4-methyl-1,2-dinitro- are employed as plasticizers and propellants [12]. 2,3,9-trimethyl-5-heptafluorobutyryloxy-2-azabicyclo[4.4.0]decane and N-Methyl nicotinimidate, O-trimethylsilyl exhibit neuroprotective effects [14,28]. (4-carbamoyl-2-nitrophenyl) acetic acid are known for their

immunomodulatory properties against cancer [14]. 2,3-diazabicyclo[3.2.0]hept-2-ene, 1,6,6,-trifluoro-4-spirocyclopropan- display antiparasitic activity against Leishmaniasis [15]. bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-[1R-(1R*)] and Hexestrol, 2TMS derivative exhibit anticancer properties [17,25]. Benzenesulfonic acid demonstrate antidiabetic activity [18]. 3-[2-(1-methyl-1H-pyrazol-3-yl)-2-oxoethyl]-1,3-dihydro-2-benzofuran possess antibacterial properties [19]. 3-nonynoic acid exhibit anti-inflammatory properties [20]. Ethyl propargyl sulfone are employed as bio catalysts [24]. Glutaric acid, cyclohexylmethyl 3-nitrobenzyl ester are selective muscarinic antagonists [26]. Ethanethioic acid, S-[8-(diethylphosphono)octyl ester possess antioxidant properties [30]. 3-(aminomethyl) adamantane-1-carboxylic acid exhibit anti-allodynic properties for neuropathic pain [40].

Dimethylmalonic acid, monochloride, isobutyl ester & Z-11-tetradecen-1-ol propionate have not been reported to possess any biological activity so far.

CONCLUSION

Gas chromatography-Mass Spectroscopy analysis of hydroalcoholic leaf extract of *Carissa carandas* reported the presence of 25 bioactive molecules. Among the identified compounds 23 exhibited biological activity, while 2 compounds showed no reported activity. It may serve as potential source of therapeutic drugs due to the presence of important phytochemical bioactive compounds which contributre the activities namely anti-inflammatory, anti-cancer, anti –microbial, neuroprotective and other activities. Still, further studies are essential to isolate, characterize the active components responsible for therapeutic activity.

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