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Research

Gas Chromatography-Mass Spectroscopy Analysis Of Hydroalcoholic Extract Of *Ziziphus Oenoplia* (L.) Mill Leaves.

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Abstract

Ziziphus oenoplia Mill is medicinal herb, belongs to Rhamnaceae, commonly known as jackal jujube. The plant is used in India and Thailand traditional system of medicine for treatment of uterus inflammation, anthelmintic, spermatorrhoea, healing of cuts and boils. The literature look over revealed the presence of flavanoids, phenols, alkaloids, glycosides, pentacyclic triterpenes, carboxylic acids, aromatic compounds, nitro compounds, and esters. The plant exhibits antibacterial, antimicrobial, wound healing, anthelmintic, antioxidant, antihepatotoxic, antiplasmodial, anticancer, antinociceptive and antidiarrhoeal activity. The fresh leaves of Ziziphus oenoplia 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 Ziziphus oenoplia leaves by Gas chromatography- Mass spectroscopy (GC-MS). GC-MS chromatogram showed twenty bioactive molecules which may attribute to pharmacological properties.

Keywords: Gas chromatography - Mass spectroscopy, Ziziphus oenoplia

INTRODUCTION

Ziziphus oenoplia (L.) Mill is medicinal herb, belongs to Rhamnaceae, commonly known as jackal jujube grown in tropical and subtropical regions of Asia and Australia[1]. The plant is used in Indian and Thailand system of medicine for the treatment of uterus inflammation, as anthelmintic, spermatorrhoea, healing of cuts & boils [2]. The phytochemical review showed the presence of cyclopeptide alkaloids, phenols, flavanoids, pentacyclic triterpenes, fatty acids, aromatic compounds, hydroxycarboxylic acids [3-5]. The pharmacological survey reported antibacterial, antimicrobial, wound healing, anthelmintic, antioxidant, anti-hepatotoxicity, antiulcer, anticancer & antiplasmodial activity [6-8]. The literature survey indicated that this plant have not been

investigated by Gas Chromatography- Mass Spectroscopy method. The present research is to investigate organic active compounds furnished in hydroalcoholic leaf extract of *Ziziphus oenoplia* (L.) Mill.

Authentication and collection

The fresh leaves of *Ziziphus oenoplia* were collected from foot hills of Azhagar kovil, Madurai, Madurai district, Tamil Nadu in the month of Nov 2023. The collected plant materials were authenticated by Dr. Stephen, Professor, Department of Botany, American College Madurai-625002. The herbarium was made and kept in the department for further reference.

Preparation of hydroalcoholic extract of *Ziziphus oenoplia* (HAEZO)

The collected leaves were washed, shade dried and coarsely powdered (80 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 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 [9]. GC-MS analysis was carried out to identify some of the potent volatile and semi-volatile constitutes present in the hydroalcoholic extract of *Ziziphus oenoplia* (L.)Mill.

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) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample [10].

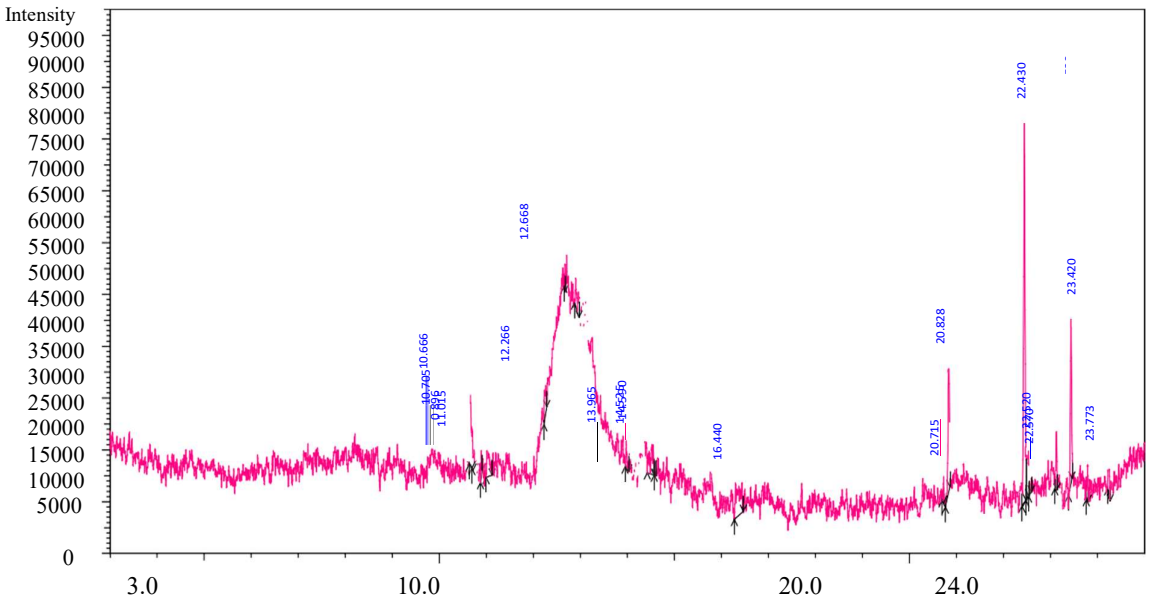

Fig 1: Gas Chromatography - Mass Spectroscopy of HAEZO

GC-MS analysis of *Ziziphus oenoplia* leaves extract revealed the presence of 20 bioactive compounds . The active principles with their retention time (Rt), molecular formula, molecular weight percentage (%) was presented in Table 1 and the chromatogram was depicted in Fig 1.

The biacctuive molecules are found to be Phenol derivative: 2-Methoxy-4-vinylphenol; beta.-D-Galactopyranoside, 4-nitrophenyl - retention time 10.666 min, area of 4.77% ,height of 13.568 and R.T 24.248, area of 1.94%, height of 5115 respectively.

Piperidine derivative: 1-Methyl-4-[nitromethyl]-4-piperidinol, Piperidine, 4,4-dimethoxy- R.T 10.705, area of 1.44%, height of 7165 and R.T. 13.965, area of 1.70% , height of 5095 respectively.

Indolizidine derivative : deacetyl-slafranine with R.T 16.440, area of 8.59% and height of 5763.

Furan derivative: 6-Methoxyhexahydrocyclopenta[b]furan-2-one with R.T. 12.669 min, area of 0.71% and height 4806.

Furazone derivative: 4-Acetoxy-7,8-dihydro(6H)furazano[3,4-c]azepin-1-oxide with R.T 20.715, area of 0.80% and height 4732.

Benzoic acid derivative: Pseudosmilagenin bis[3,5-dinitrobenzoate] with R.T 11.015, area of 4.04% and height 4903.

Aliphatic diterpene derivative: Phytol with R.T 22.430 , area of 30.63 and height 73309.

Ester derivative: 1a-Chloro-2,3-dioxo-6a-phenyloctahydro-1-oxa-2a-aza-cyclopropa[f]inden-6-carboxylic acid, ethyl ester; 2-Hexen-1-ol, acetate, (E)-; Acetic acid, cyclohexyl ester; 12-Methyloctadec-11-enoic acid trimethylsilyl ester; 3,3-Difluoro-3-phenoxy-2-trifluoromethyl-propionic acid methyl ester; Hexadecanoic acid, ethyl ester; Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester; 11-Dodecyn-1-ol acetate; Octadecanoic acid, ethyl ester; 2,2-Dimethyl-6-methylene-1-[3,5-dihydroxy-1-pentenyl]cyclohexan-1-perhydrol; with R.T of 10.896, 12.266, 12.897, 14.525, 14.590, 20.828, 22.570, 23.111, 23.420, 23.773; area % of 0.20, 2.62, 2.87, 5.17, 1.56, 10.25, 1.51, 2.97, 11.67, 1.53 and height of 4837, 5153, 5374, 5664, 6447, 24054, 5008, 11039, 32401, 6779 respectively.

Table 1: Identification of bioactive compounds in hydroalcoholic extract of *Ziziphusoenoplia* leaves by GC-MS

Peak #	Retention Time	Area %	Molecular Weight	Molecular formula	Name of the bioactive compound
1	10.666	4.77	150	$C_9H_{10}O_2$	2-Methoxy-4-vinylphenol
2	10.705	1.44	174	$C_7H_{14}N_2O_3$	1-Methyl-4-[nitromethyl]-4-piperidinol
3	10.896	0.80	349	$C_{17}H_{16}ClNO_5$	1a-Chloro-2,3-dioxo-6a-phenyloctahydro-1-oxa-2a-aza-cyclopropa[f]inden-6-carboxylic acid, ethyl ester
4	11.015	4.04	804	$C_{41}H_{48}N_4O_{13}$	Pseudosmilagenin bis[3,5-dinitrobenzoate]
5	12.266	2.62	142	$C_8H_{14}O_2$	2-Hexen-1-ol, acetate, (E)-
6	12.668	0.71	156	$C_8H_{12}O_3$	6-Methoxyhexahydrocyclopenta[b]furan-2-one
7	12.897	2.87	142	$C_8H_{14}O_2$	Acetic acid, cyclohexyl ester
8	13.965	1.70	145	$C_7H_{15}NO_2$	Piperidine, 4,4-dimethoxy-
9	14.525	5.17	368	$C_{22}H_{44}O_2Si$	12-Methyloctadec-11-enoic acid trimethylsilyl ester
10	14.590	1.56	284	$C_{11}H_9F_5O_3$	3,3-Difluoro-3-phenoxy-2-trifluoromethyl-propionic acid methyl ester
11	16.440	8.59	156	$C_8H_{16}N_2O$	deacetyl-slafranine
12	20.715	0.80	211	$C_8H_9N_3O_4$	4-Acetoxy-7,8-dihydro(6H)furazano[3,4-c]azepin-1-oxide
13	20.828	10.25	284	$C_{18}H_{36}O_2$	Hexadecanoic acid, ethyl ester
14	22.430	30.63	296	$C_{20}H_{40}O$	Phytol
15	22.520	4.44	166	$C_{11}H_{18}O$	1-Oxaspiro[2.2]pentane, 5-isopropylidene-2,2,4,4-tetramethyl-
16	22.570	1.51	374	$C_{25}H_{42}O_2$	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl] methyl]-, methyl ester
17	23.111	2.97	224	$C_{14}H_{24}O_2$	11-Dodecyn-1-ol acetate
18	23.420	11.67	312	$C_{20}H_{40}O_2$	Octadecanoic acid, ethyl ester
19	23.773	1.53	256	$C_{14}H_{24}O_4$	2,2-Dimethyl-6-methylene-1-[3,5-dihydroxy-1-pentenyl]cyclohexan-1-perhydrol
20	24.248	1.94	301	$C_{12}H_{15}NO_8$	beta.-D-Galactopyranoside, 4-nitrophenyl

Among the identified bioactive molecules hexadecanoic acid ethyl ester; 9,12-octadecanoic acid; 2,2-Dimethyl-6-methylene-1-[3,5-dihydroxy-1-pentenyl]cyclohexan-1-perhydrol; Cyclopropanebutanoic acid, 2-[[2-[[2-[(2 pentylcyclopropyl)methyl cyclopropyl] methyl]cyclopropyl]methyl]-, methyl ester 3,3-Difluoro-3-phenoxy-2-trifluoromethyl-propionic acid methyl ester; 12-Methyloctadec-11-enoic acid trimethylsilyl ester and 11-dodecyn-1- ol acetate proved as antioxidant, antimicrobial, flavouring purpose, antiprotozoal, hypocholesterolemic agent and germicidal activity . Phytol proved as antimicrobial, anticancer, antioxidant, anxiolytic, anti-inflammatory & antinociceptive activities.1-Methyl-4-[nitromethyl]-4-piperidinol; Piperidine, 4,4-dimethoxy- proved as antibacterial, antifungal, antidiabetic, anticancer and antipsychotic activity.

2-Methoxy-4-vinylphenol; beta.-D-Galactopyranoside, 4-nitrophenyl proved as flavouring agent and degradation of glycosaminoglycan enzyme. Pseudosmilagenin bis[3,5-dinitrobenzoate] proved to be used in flourimetric analysis of creatinine [11-19].

The biological activities of bioactive molecules are listed in Table 2.

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

S.No	Name of the bioactive compound	Derivative	Biological activity
1	2-Methoxy-4-vinylphenol	Phenol	Antimicrobial [11].
2	1-Methyl-4-[nitromethyl]-4-piperidinol	Piperidine	Antimalarial [12].
3	1a-Chloro-2,3-dioxo-6a-phenyloctahydro-1-oxa-2a-az		-
4	Pseudosmilagenin bis[3,5-dinitrobenzoate]	Benzoic acid	Fluorometric analysis of creatinine
5	2-Hexen-1-ol, acetate, (E)-	Ester	-
6	6-Methoxyhexahydrocyclopenta[b]furan-2-one	Furan	-
7	Acetic acid, cyclohexyl ester	Ester	-
8	Piperidine, 4,4-dimethoxy-	Piperidine	-
9	12-Methyloctadec-11-enoic acid trimethylsilyl ester	Ester	Antioxidant, Antimicrobial, Antiinflammatory [13].
10	3,3-Difluoro-3-phenoxy-2-trifluoromethyl-propionic a	Ester	Antiprotozoal [14].
11	deacetyl-slafranine	Indolizidine	Parasympathomimetic agent – salivation, lacrimation, urination, defecation [15].
12	4-Acetoxy-7,8-dihydro(6H)furazano[3,4-c]azepin-1-o	Furazone	Antibiotic, Antiinflammatory [16].
13	Hexadecanoic acid, ethyl ester	Ester	Antioxidant, Hypocholestrolemic, Nematicide[17].
14	Phytol	Aliphatic diterpene	Cytotoxic, Antioxidant, Antiinflammatory, Antinociceptive, Antimicrobial, Precursor of Vit E and K, Anticancer, Diuretic [18].
15	1-Oxaspiro[2,2]pentane, 5-isopropylidene-2,2,4,4-tetramethyl	Oxaspiro	-
16	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl] methyl ester	Ester	-
17	11-Dodecyn-1-ol acetate	Ester	-
18	Octadecanoic acid, ethyl ester	Ester	-
19	2,2-Dimethyl-6-methylene-1-[3,5-dihydroxy-1-pentenyl cyclohexan-1-perhydrol	Ester	-
20	beta.-D-Galactopyranoside, 4-nitrophenyl	Phenol	Detection of glycosylated enzyme [19].

1a-Chloro-2,3-dioxo-6a-phenyloctahydro-1-oxa-2a-aza-cyclopropa[f]inden-6-carboxylic acid, ethyl ester; 2-Hexen-1-ol, acetate ester; 6-Methoxyhexahydrocyclopenta[b]furan-2-one; Acetic acid, cyclohexyl ester; 1-Oxaspiro[2.2]pentane, 5-isopropylidene-2,2,4,4-tetra, Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclop)] methyl]cyclopropyl]methyl] methyl ester; 11-Dode cyn-1-ol acetate; Octadecanoic acid, ethyl ester; 2,2-Dimethyl-6-methylene-1-[3,5-dihydroxy-1-pentenyl]; does not show any biological activity. These comnpounds were identified for the first time in this plant.

CONCLUSION

The current investigation concluded that the hydroalcohol leaf extract of *Ziziphus oenoplia* resulted the presence of twenty bio- active constituents. These may be organcic compounds responsible for biological activities. From this study it can be concluded that *Ziziphus oenoplia* may serve as a new potential source of therapeutic drugs due to the presence of numerous important phytochemical bioactive compounds. This is the first analytical report of *Ziziphus oenoplia* showing the bioactive compounds which may facilitate to extend the research to isolation, and charctersitaion of those compounds.

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REFERENCES

1. Pullaiah T. Medicinal Plants in Andhra Pradesh. Illustrated Edn, Daya books 406-407, 2002.
2. Suksamrarn S, Suwannapoch N, Aunchai N, Kuno M, Ratananukul P, Haritakun R. Ziziphine N, O, P and Q, new antiplasmodial cyclopeptide alkaloids from *Ziziphus oenoplia* var.brunoniana. *Tetrahedron,* 61: 1175-1180, 2005.
3. Pundir R, Singh G, Pandey AA, Saraf SA. Demand of herbal hepatoprotective formulations in Lucknow-A Survey. *Pharmaceutical Research*, 1: 23-33, 2009.
4. Jadhav SA, Prassanna SM. Evaluation of antiulcer activity of Ziziphus oenoplia (L.) Mill roots in rats. *Asian Journal of Pharmaceutical and Clinical Research,* 1(1): 92-95, 2011.
5. Jadhav SA, Chavan SD. In vitro antioxidant activity of Ziziphus oenoplia (L.) Mill Root extract. *Journal of Pharmaceutical Science*, 4(4): 586-588, 2012.
6. David B. *In-vitro* anti-denaturation and antibacterial activities of *Zizyphus oenopli. Scholars Research Library Der Pharmacia Lettre*, 2010; 2(1): 87-93.
7. Eswari LM, Bharathi VR, Jayshree N. Hypolipidemic Activity on Ethanolic Extract of Leaves of *Ziziphus oenoplia* (L) Mill *International Journal of Pharmaceutical & Biological Archives,* 2013; 4(1): 136-141.
8. Rao Ch V, Rawat AKS, Singh AP, Singh A, Verma N. Hepatoprotective potential of ethanolic extract of Ziziphus oenoplia (L.) Mill roots against antitubercular drugs induced hepatotoxicity in experimental models. *Asian Pacific Journal of Tropical Medicine.s* 2 283- 288, 2012.
9. Ronald Hites A. Gas Chromatography Mass Spectroscopy: Handbook of Instrumental Techniques for Analytical Chemistry, 609-611, 1997.
10. Kell DB, Brown M, Davey HM, Dunn WB, Spasic I, Oliver SG. "Metabolic footprinting and systems biology. *Nature Reviews Microbiology*. 3:557-65, 2005.
11. Barathi Kannan. "Bioactive potential of 2-Methoxy-4-vinylphenol abd Benzofuran from Brassica oleracea L.var.capitate on oxidative and microbiological stability of beef meat. *Foods*, 9(5): 568, 2020.
12. Hardeep Kaur. Hybrid alkaloids an approach toward development of better antimalarial therapeutics. *Studies in Natural Products Chemistry*. 76: 199-245, 2023.
13. Natarajan P.GC-MS analysis of bioactive compounds present in Oeophylla smaragdiba. *Research Journal of Pharmacy and Technology*, 2019; 12(6): 2736-2741.
14. Pubchem.ncbi.nlm.nih.gov accessed on 2 April 2024.
15. Hagler et al. Salivary syndrome in Horses: Identification pf Slaframine in Red clover hay. *Applied and Environmental Microbiology*. 1067-1073, 1981.
16. National Institute of Standards and Technology- 28562-53-0.
17. Vijayabaskar G. Determination of phytocompounds in Withania somnifera and Smilax china using GC-MS technique. *Journal of Pharmacognosy and Phytochemistry*. 7(6):554-557, 2018.
18. Ahmed Darwish El-Gamal, Characterization of bioactive compounds with antioxidant activity and antimicrobial activity from freshwater cyanobacteria. *Egyptian Journal of Chemistry*. 723-735, 2022.
19. Sigma-Aldrich; N1252 accessed on 2 April 2024.