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GC-MS ANALYSIS OF BIOACTIVE COMPOUNDS IN THE LEAF EXTRACTS OF CAPPARIS DIVERSIFOLIA WIGHT & ARN. – AN ENDEMIC TAXON OF SOUTHERN WESTERN GHATS

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ABSTRACT

Capparis diversifolia Wight & Arn. is a shrub belonging to capparaceae, endemic to southern Western Ghats. The stem and leaf of this plant is used in traditional folk medicine to relieve variety of pains, skin diseases and to clean eyes. Gas chromatography and mass spectrometric analysis of methanol extract of leaf revealed the presence of thirty compounds. The major phytoconstituents were 6, 6, 7-Trimethyl-9-oxo-3 oxabicyclo (3.3.1) nonane 2, 4-dinitrophenyl hydrazone (15.36%), 2,6-dimethyl-N-(2methyl-à-phenylbenzyl) aniline (12.87%), 1, 12-octadecandiol (9.46%) and phytol (9.37%). From the study it is obvious that *Capparis diversifolia* leaf extract contains many bioactive compounds and it also gives a detailed insight about the phytochemical profile which could be exploited for the development of plant based drugs.

KEYWORDS: GC-MS analysis, *Capparis diversifolia*, methanolic extract.

INTRODUCTION

Medicinal plants are the nature's gift to make disease free healthy life. It plays a vital role to preserve our health. In India large number of plant species had been screened for their pharmacological potential but still a vast wealth of species are unexplored. Medicinal plants are at interest to the field biotechnology as drug industries depend in part on plant source for the production of pharmaceutical compounds.[1] Capparaceae family comprises various important medicinal properties distributed in tropical and subtropical India, whose medicinal usage has been reported in the traditional systems of medicine such as Ayurvedha, Siddha and Unani. Plants belonging to capparaceae have been used extensively as an adaptogen to

increase the non-specific resistance of antioxidant and immunostimulant effects.[2] *Capparis* is a dominating genus of the family capparaceae. Capparis species are used to treat diseases like asthma, paralysis, rheumatism, diabetes and cancer.[3] *Capparis diversifolia* Wight & Arn. commonly known as Travancore caper is an erect shrub with slightly curved thorns endemic to southern Western Ghats. Leaves dimorphic, linear to oblong; flowers showy, purple, confected in 3-8 flowered in subumbels; fruits smooth and ovoid "Fig:1". *C. diversifolia* leaves and flowers are consumed with milk to cure headache.[4] Irula tribes of Nellithurai Beat used the leaf extraction for fever.[5] No reports are available with respect to the phytochemical and pharmacological properties of the plant. Keeping this in view the present study is carried out to identify the phytochemical compounds present in the leaves of *Capparis diversifolia* with the help of GC-MS technique.

MATERIALS AND METHODS

Collection and identification of plant material

The leaves of *Capparis diversifolia* were collected from areas of Thovalai and Mupundal, Kanyakumari district of Tamilnadu. The plant was identified and authenticated by Botanical Survey of India, Southern Circle, Coimbatore as *Capparis diversifolia* Wight & Arn. (Capparaceae). Voucher specimens (SMCH-3011& SMCH-3012) were preserved in, St. Mary's College Herbarium, Department of Botany, Thoothukudi, Tamil Nadu, India.

Preparation of powder and extract

Fresh leaves were shade dried and pulverized to powder in a mechanical grinder. 25 gm. of leaf powder was packed in soxhlet apparatus and extracted with methanol. The filtrate was evaporated to dryness using a rotary evaporator. The final residue obtained was then subjected to GC-MS analysis. The dried extract was stored at 20^0C in vials for further studies.

Gas chromatography-mass spectrometry analysis[6]

GC- MS analysis of the extracts were carried out using GC-MS Clarus500 Perkin Elmer system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) employing the following conditions: column Elite -1 fused silica capillary column (30 mm×0.25 mm ID × 1µmdf,composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; Helium (99.999%) was used as a carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 µl 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 9 minute, isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 550 Da. Total GC running time was 40 min.

Characterization of compounds

Interpretation on mass spectra of GC-MS was conducted using the database of National Institute of Standard and Technology (NIST). The mass spectra of the unknown compounds were compared with that of the known components stored in the NIST-library and the name, molecular weight and structure of the components were ascertained. The biological activities of phytocomponents were ascertained based on Dr. Duke's Phytochemical and Ethno botanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA.

RESULTS AND DISCUSSION

GC-MS chromatogram of the methanol leaf extract of *Capparis diversifolia* depicted 30 peaks indicates the presence of 30 compounds "Fig.2". The phytocomponents identified in the methanol leaf extract of *Capparis diversifolia* with the retention time ranging from 3.07 to 39.34 minutes were presented in Table 1. 6, 6, 7- Trimethyl-9-oxo-3 oxabicyclo (3.3.1) nonane 2, 4-dinitrophenyl hydrazone (15.36%) "Fig. 3(a)", 2, 6-dimethyl-N-(2 methyl-à-phenylbenzyl) aniline (12.87%) "Fig. 3 (b)", 1, 12-octadecandiol (9.46%) "Fig. 3 (c)" and phytol (9.37%) "Fig. 3 (d)" were the phytocomponents with high peak areas. The identified compounds possess many biological activities. For instance, phytol shows anticancer, antiinflammatory and antimicrobial properties.[7] Phytol is a diterpene with antimicrobial properties, significantly against many bacterial strains.[8] It can be used for the manufacture of synthetic forms of vitamin E.[9] and vitamin K_1.[10] 2,4-dinitrophenyl hydrazone is a ketoacid had diverse biological properties such as antiinflammatory, antitubercular, antimicrobial, antimalarial, antitumor activities.[11,12,13] 1, 12 – octadecanediol is a long chain fatty alcohol possess bactericidal activity against *Staphylococcus aureus*.[14] 2,6-dimethyl-N-(2 methyl-à-phenyl benzyl) aniline is a derivative of aniline which has safer antimicrobial and *in vitro* antioxidant property.[15] Most of the phytocomponents identified from the leaf samples have antimicrobial, antiinflammatory, antioxidant and anticarcinogenic activity (Table 1). Further studies are obligatory to isolate actual phytoconstituents responsible for specific bioactivity and the exact mechanism of action at the molecular level.

Table 1: Phytocomponents identified in the methanol leaf extracts of *Capparis diversifolia* (GC-MS Study)

No.	RT	Name of the compound	Molecular formula	MW	Peak area %	** Activity
1.	3.07	(6E,8Z)-6,8-dodecadienyl acetate	$C_{14}H_{24}O_2$	224	0.80	Antibacterial Antibiotic Anti-inflammatory
2.	6.41	2-(2,2-Diphenyl-2-hydroxy-1-methylethyl)-3-[2-(1naphthalene)propionylamino)quinazolin-4(3H)-one	$C_{36}H_{31}N3O_3$	553	1.28	Anti-inflammatory Anti-diabetic
3.	6.78	3,8-Dihydroxy-4-methoxy-2-methoxycarbonyl-1-methylanthraquino	$C_{18}H_{14}O_7$	342	3.25	Antineurotoxic Antiviral
4.	10.25	(2-Methoxyethoxy)methyl 2,12-Dibromo-7-phenyl-5,6,8,9-tetrahydrobenz[a,j]anthracene-14-carboxylate	$C_{33}H_{28}Br_2O_4$	646	0.81	Anticancer
5.	12.72	Benzyl (S)-3-Hydroxy-2-methylenepentanoate	$C_{13}H_{16}O_3$	220	1.61	Anti-inflammatory
6.	13.07	Cadinene	$C_{15}H_{24}$	204	0.51	No activity reported
7.	13.42	cis-N-Methyl-8-hydroxy-1-azaspiro[5.5]undecane-2-one	$C_{11}H_{19}NO_2$	197	0.47	Antimicrobial Antioxidant
8.	13.97	Carbamate, N-(2-naphthyl)-, 3-pentynyl ester	$C_{16}H_{15}NO_2$	253	0.45	Antioxidant
9.	15.09	Pregna-1,4-diene-3,20-dione, 9chloro-11-hydroxy-16-methyl-17,21-bis(1-oxopropoxy)-, (11á,16á)-	$C_{28}H_{37}ClO_7$	520	0.53	No activity reported
10.	17.64	1,6-Methanofluorene	$C_{14}H_{12}$	180	2.07	Antioxidant
11.	19.88	Nitrazepam	$C_{15}H_{11}N_3O_3$	281	0.70	Antiviral
12	21.72	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	2.61	Anthelmintic
13	22.32	12,24-dimethyl-1,13-dioxacyclotetracosane-2,14-dione	$C_{24}H_{44}O_4$	396	3.75	Ant carcinogenic Antidiabetic Antibacterial
14	22.86	Benzoic acid, 4-formyl-, ethyl ester	$C_{10}H_{10}O_3$	178	3.68	Antioxidant Anti-inflammatory Antimicrobial
15	23.50	(-)-Loliolide	$C_{11}H_{16}O_3$	196	1.41	Antioxidant Antibacterial Antipyretic
16	23.88	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276	2.34	Antimicrobial Anti-inflammatory Antioxidant
17	25.19	Phytol	$C_{20}H_{40}O$	296	9.37	Antimicrobial Anti-inflammatory Anticancer
18	25.95	Pseudosolasodine diacetate	$C_{31}H_{49}NO_4$	499	0.45	Antioxidant Antimicrobial
19	27.91	Aspidospermidin-21-ol, 1-acetyl-	$C_{18}H_{20}N_2O$	280	3.31	Antioxidant

		15,16,17-trimethoxy- acetate (ester) (CAS)				Antimicrobial Anti-inflammatory
20	28.95	Methyl 5-(à-hydroxy-2-chlorobenzyl)-3-(4-methoxyphenyl)-3,4-dihydro-1H-[1,2]-oxazole-5-carboxylate	$C_{19}H_{18}ClNO_5$	375	4.65	Anti-inflammatory Antiprotozoal
21	30.19	6,6,7-Trimethyl-9-oxo-3-oxabicyclo(3.3.1)nonane 2,4-dinitrophenylhydrazone	$C_{17}H_{22}N_4O_5$	362	15.36	Anti-inflammatory Antineurotoxic Antibiotic
22	31.52	(2RS)-1,3,8-trimethyl-4-propyl-5-ethyl-2-(1-hydroxyethyl)-7-methoxycarbonylethyl-6,.gmma.-methylenecarbonyl-porphine	$C_{36}H_{42}N_4O_4$	594	4.28	Antineurotoxic Antiviral
23	33.19	2,6-dimethyl-N-(2-methyl-à-phenylbenzyl)aniline	$C_{22}H_{23}N$	301	12.87	Antimicrobial
24	34.51	Naphthalene, 1,5-dibromo-2,6-bis(bromomethyl)-	$C_{12}H_8Br_4$	468	0.61	Antiviral Anti-inflammatory
25	34.81	cis-trans-cis-2,4,6,8-Tetramethyltetraphenylcyclotetrasiloxane	$C_{28}H_{32}O_4Si_4$	544	1.44	Anti-inflammatory
26	36.22	1,3-Di(3,4-dichlorobenzyl)-2-phenylimidazolidine	$C_{23}H_{20}Cl_4N_2$	464	0.14	Antiallergic Anti-inflammatory
27	36.63	Lycopersen	$C_{40}H_{66}$	546	5.89	Antimicrobial
28	37.85	1,12-octadecandiol	$C_{18}H_{38}O_2$	286	9.46	Antibacterial
29	38.98	3,4-bis[5'-(2''-Naphthyl)-2'-methylthiophen-3'-yl]-2,5-dihydrothiophene	$C_{34}H_{26}S_3$	530	2.51	Antiviral Anti-inflammatory
30	39.34	1,13-Tetradecadiene	$C_{14}H_{26}$	194	3.14	No activity reported

***Dr. Duke's Phytochemical and Ethno botanical Database.*

Fig. 1. ***Capparis diversifolia*** **Wight & Arn.twig with flower and fruit.**

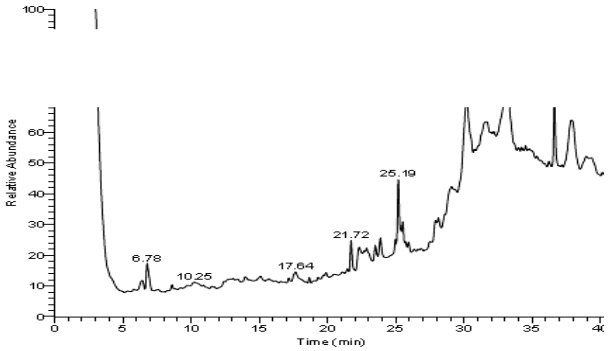
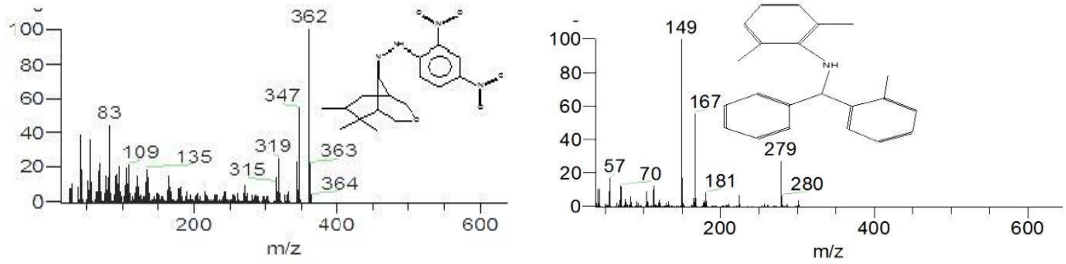
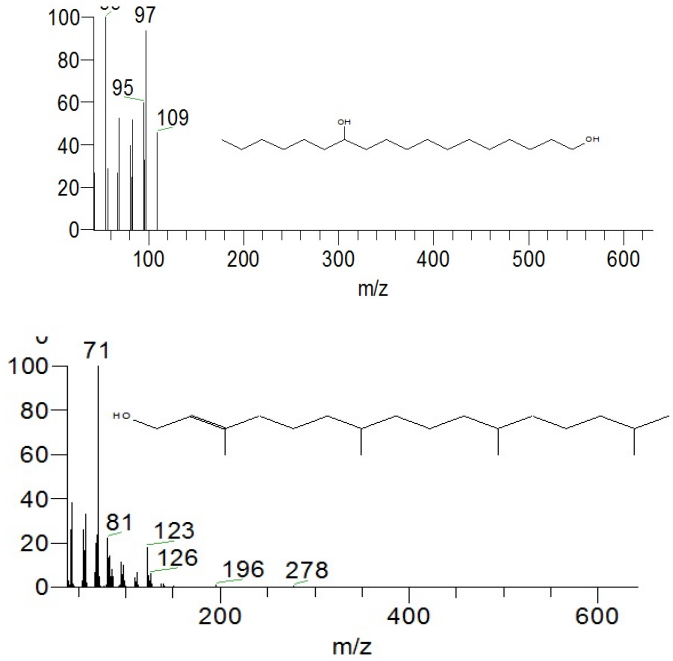


Fig 2: GC-MS Chromatogram of ethanol leaf extract of *Capparis diversifolia*



(a) 6,6,7-Trimethyl-9-oxo-oxaicyclo(3.3.1)nonane2,4,-dinitrophenyl hydrazone

(b) 2, 6-dimethyl-N-(2 methyl-à-phenyl benzyl) aniline



(c) 1, 12-octadecandiol

(d) Phytol

Fig 3: Mass spectra of some of the phytocompounds identified

CONCLUSION

Capparis diversifolia is an unexploited and underutilized member of capparaceae. The phytocomponents identified might serve as source for drug formulations in future.

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