GAS CHROMATOGRAPHY AND MASS SPECTROSCOPHY OF ETHANOLIC EXTRACT OF *GARCINIA GUMMI GUTTA* (L.) *ROXB* FRUIT

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ABSTRACT

The plant Garcinia cambogia belongs to family Guttiferae (Clusiaceae). It is a wild subtropical tropical plant. The plants are shrubs or trees with yellow-greenish juice. It is widely used in Kerala as a culinary spice as well as have many medicinal properties. Mass spectrometry coupled with chromatographic separation such as gas chromatography and mass spectrometry analysis [GC-MS] is normally used for direct analysis of components existing in traditional medicines and medicinal plants. By GC-MS study various compounds are identified with various retention time.

KEYWORDS: Hyphenated techniques, Alzheimer's disease, retention time.

INTRODUCTION

Garcinia is one of the largest genera of the Clusiaceae family comprising of 390 species and it is one in all the foremost medicinally important members. These polygamous trees or shrubs are mainly spread in tropical Asia, Polynesia, and Africa **(Soni et al., 2004)**. Garcinia gummi-gutta (L.) Roxb., commonly known as Garcinia gummi-gutta (a former scientific name), as well as Malabar tamarind,

brindle berry, Garcinia cambogia (**Tinworth et al., 2010).** The plant Garcinia cambogia belongs to family Guttiferae (Clusiaceae). It is a wild subtropical tropical plant. The plants are shrubs or trees with yellow-greenish juice.

Garcinia gummi-gutta is a medium-sized evergreen tree that produces light green fruits. These fruits are juicy and feature distinct grooves. Among its varieties, gummi-gutta stands out due to its large fruit size, pulpy aril, and higher seed count (4-8 seeds per fruit), making it widely cultivated and preferred. The processed pericarp of gummi-gutta is highly prized for its delicate taste and flavour. Variability analysis revealed that nipple length exhibited the highest variability (74.8%), while fruit girth showed the lowest (12.8%). On average, the fruit weighs approximately 161g.

Mass spectrometry coupled with chromatographic separation such as gas chromatography and mass spectrometry analysis [GC-MS] is normally used for direct analysis of components existing in traditional medicines and medicinal plants. (**Vardharaj V et al., 2015**) Gas chromatography and mass spectroscopy is a reliable technique to identify the bioactive constituents present in the plant extract. There is no report for the GC-MS analysis of the phytoconstituent present in the ethanolic extract of *Garcinia gummi-gutta* fruit. So, this is a chosen research study.

Thus, the aim of the study is to identify the bioactive constituent present in the ethanolic extract of the chosen fruit and identify its biological uses for the further research purpose.

MATERIALS AND METHODS

Collection and authentication of fruit

Garcinia gummi-gutta fruit collected from Thiruvanathapuram, Kerala in the month of July 2023. The species for the proposed study was identified and authenticated by DR.Stephen, Professor, and Department of Botany American College Madurai-625002. The fruits are shade dried and was powdered in a mixer. The coarse powder was sieved and was stored in a well closed container.

Preparation of extract of *Garcinia gummi gutta* powder

The powder is macerated with various different solvent for 24 hrs to the extractive yield. From that observation, the polar solvent ethanol gives more yield than other solvents. So, Ethanol is chosen as the solvent for extraction. Maceration technique is chosen for the

extraction. Because there is no damage for the chemical constituent present in the plant. The powder is defatted using pet ether for several days. After that, powder macerated with ethanol for about 3 days. Then filter the extract and evaporate for the final yield.

GC –MS ANALYSIS

EEGG was analysed by GC-MS

Make: Perkin Elmer

GC model: Clarus 680

Mass Spectrometer: Clarus 600 (EI)

Software: Turbo Mass ver 5.4.2

Instrumentation Acquisition Parameters

Oven: Initial temp 60°C for 2 min, ramp 10°C/min to 300°C, hold 6 minutes

Total Run Time: 32 minutes

Injected Temperature: 260°C

Volume:1 μL

Split: 10:1

Flow Rate: 1 mL/minute

Carrier Gas: Helium

Solvent Delay: 1min

Column: Fused silica column, Elite-5MS (30.0m, 0.25mmID, 250μm df)

Transfer line temperature: 210 °C

Ion source temperature: 210 °C

Scan time:0.2 sec, 40 to 600Da

Scan interval:0.1 sec

PROCEDURE (pavia et al., 2014)

The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250μm 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 260°C during the chromatographic run. The 1μL of extract sample injected into the instrument the oven temperature was as follows: 60°C (2 min), followed by 300 °C at the rate of 10 °C min−1, and 300 °C, where it was held for minutes. The mass detector conditions were: transfer line temperature 240 °C, ion source temperature 240 °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 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

RESULT

The results of GC- MS analysis of ethanolic extract of *garcinia gummi gutta L (robs)* was tabulated and its major compounds also tabulated.

GC-MS quantitative report of ethanolic extract of *garcinia gummi gutta L (robs)*

Peak	Retention time	Start time	End Time	m/z	Area	Area %	Height	Height %	A/H
1	6.337	6.26	6.42	TIC	406046	1.21	77450	0.66	5.24
2	12.057	11.99	12.14	TIC	1261494	3.76	326484	2.78	3.86
3	13.277	13.18	13.38	TIC	496436	1.48	72427	0.62	6.85
4	13.536	13.49	13.595	TIC	157702	0.47	49243	0.42	3.2
5	15.212	15.17	15.23	TIC	197907	0.59	90466	0.77	2.19
6	15.255	15.23	15.305	TIC	221529	0.66	97636	0.83	2.27
7	15.5	15.47	15.525	TIC	123630	0.37	54301	0.46	2.28
8	15.558	15.525	15.655	TIC	581298	1.73	209592	1.79	2.77
9	17.878	17.835	17.93	TIC	279256	0.83	122778	1.05	2.27
10	18.357	18.305	18.43	TIC	1716704	5.12	727104	6.2	2.36
11	18.807	18.755	18.86	TIC	315450	0.94	99760	0.85	3.16
12	20.645	20.61	20.69	TIC	119928	0.36	50883	0.43	2.36
13	21.905	21.86	21.97	TIC	246315	0.73	65755	0.56	3.75
14	22.015	21.97	22.06	TIC	411967	1.23	166026	1.41	2.48
15	22.544	22.505	22.58	TIC	132399	0.4	61524	0.52	2.15
16	22.82	22.785	22.87	TIC	105022	0.31	51498	0.44	2.04
17	23.074	23.03	23.11	TIC	126834	0.38	46092	0.39	2.75
18	23.255	23.195	23.405	TIC	1700101	5.07	254383	2.17	6.68
19	23.449	23.405	23.5	TIC	331820	0.99	116074	0.99	2.86
20	23.642	23.605	23.695	TIC	217804	0.65	97215	0.83	2.24
21	24.583	24.54	24.635	TIC	128658	0.38	55954	0.48	2.3
22	25.666	25.625	25.755	TIC	1295946	3.87	490370	4.18	2.64
23	25.941	25.89	25.995	TIC	646060	1.93	234717	2	2.75
24	26.04	25.995	26.08	TIC	216367	0.65	66227	0.56	3.27
25	26.42	26.385	26.45	TIC	79105	0.24	44292	0.38	1.79
26	26.479	26.45	26.53	TIC	111179	0.33	40888	0.35	2.72
27	26.646	26.59	26.815	TIC	1957765	5.84	360782	3.07	5.43
28	28.214	28.16	28.265	TIC	1036148	3.09	439132	3.74	2.36
29	29.062	29.02	29.1	TIC	136717	0.41	65108	0.55	2.1
30	29.705	29.67	29.74	TIC	149742	0.45	46805	0.4	3.2
31	29.786	29.74	29.865	TIC	990949	2.96	215664	1.84	4.59
32	29.895	29.865	29.935	TIC	115354	0.34	45059	0.38	2.56
33	30.273	30.22	30.35	TIC	1772687	5.29	754830	6.43	2.35
34	32.178	32.13	32.255	TIC	2159535	6.44	882457	7.52	2.45

35	33.976	33.92	34.04	TIC	2679597	8	1114278	9.5	2.4
36	34.812	34.765	34.87	TIC	916142	2.73	377807	3.22	2.42
37	35.18	35.155	35.215	TIC	70201	0.21	39114	0.33	1.79
38	35.666	35.61	35.76	TIC	3092684	9.23	1233099	10.51	2.51
39	37.25	37.18	37.325	TIC	3082224	9.2	1180927	10.06	2.61
40	38.435	38.41	38.45	TIC	49720	0.15	35582	0.3	1.4
41	38.46	38.45	38.565	TIC	221183	0.66	39059	0.33	5.66
42	38.742	38.68	38.815	TIC	2808087	8.38	951782	8.11	2.95
43	39.17	39.155	39.175	TIC	48035	0.14	44281	0.38	1.08
44	39.251	39.175	39.265	TIC	330428	0.99	78422	0.67	4.21
45	39.325	39.265	39.35	TIC	271251	0.81	61587	0.52	4.4

GC-MS chromatogram of ethanolic extract of *garcinia gummi gutta L (robs)*
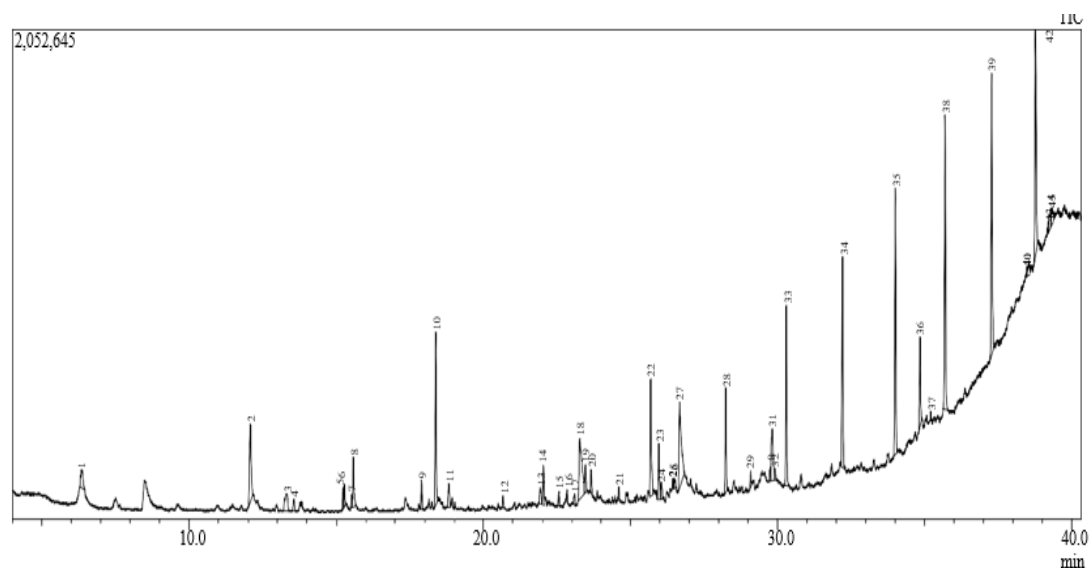
Major compounds present in ethanolic extract of *Garcinia gummi-gutta* fruit

SI. NO	COMPOUND	M.W	MOLECULAR FORMULA	BIOLOGICAL ACTIVITY
1	Cyclopentasiloxane, Decamethyl	370	$C_{10}H_{30}O_5Si_5$	Antimicrobial activity, act as antistatic, emollient, humectant, hair conditioning agent
2	Phosphanoacetic acid	356	$C_{11}H_{29}O_5Psi_3$	Antiviral agent against Herpes simplex virus
3	7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodizepine-2-thione	342	C18h19c1N2osi	Competetive antagonist of GABA agonist at the fatty acid site.
4	Cyclohexane Dodecamethyl	444	$C_{12}H_{36}O_6Si_6$	Antibacterial and cytoxicity activity
5	**Dodecanedioic acid**	458	$C_{24}H_{50}O_4Si_2$	Antibacterial, antioxidant, anti-apoptopic. Antinflammatory, antioxidant and **neuroprotective property through inhibition of IL-1β,IL-6 and** TNF-α.
6	Eicosane	282	C20h42	Anti-fungal, Antitumour, Anti-bacterial,

				larvicidal, Antimicrobial, cytotoxic effects. Volatile biomarkers of parkinsons disease from sebum (Varsha jayakar et.,al 2021)
7	**2,4-Di-tert-butylphenol**	206	C14H22O	Possess antifungal and antioxidant Property. 2015), neuronal protective thus has significant anti-amnesic effect
8	Hexadecane	226	C16H34	Antioxidant and antimicrobial activity
9	Tetradecanoic acid	228	C14H28O2	Antivirulence activity (Borkotoky et.,al 2023)
10	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	276	C17H24O3	Anti-microbial activity (Varsha jayakar et.,al 2020)
11	Tetradecane	198	C14H30	Antimicrobial, Cytotoxicity, Antipyretic, Anthelmentic, Tumour, Bronchitis, Asthma, Tuberculosis, Dyspepsia, Constipation, Anemia, Throat diseases, Elephantiasis, Antidiabatic, Anti-inflammatory, Antidiarrhoeal activity
13	N-Hexadecanoic acid	256	C16H32O2	Antifungal,antioxidant,hypocholestrelomic, nemacitide,anti-androgenic, haemolytic 5-α reductase inhibitor, antimicrobial, antimalarial activity
14	Tetracosane	310	C24H50	Anti-oxidant and antimicrobial activity
15	Octadecanoic acid	284	C18H36O2	Antibacterial action, Decreases cardiovascular and cancer risks, reduces LDL cholesterol levels, reduces blood pressure, improved heart function

DISCUSSION

In Alzheimer's disease, neuroprotective agents are pivotal for preserving neuronal structure and function, thereby slowing the progression of the condition. These agents play key roles in reducing neuroinflammation, exerting antioxidant effects, modulating neurotrophic factors, and inhibiting amyloid plaque accumulation. Here 2,4-Di-tert-butylphenol and Dodecanedioic acid are neuro protective agents. Among these compounds, 2,4-Di-tert-butylphenol demonstrates anti-amnesic activity. This suggests that it may specifically target the cognitive decline associated with Alzheimer's disease, potentially improving memory and cognitive function in affected individuals. The presence of neuroprotective compounds in the ethanolic extract of Garcinia gummi-gutta holds promise for the development of therapeutic interventions for Alzheimer's disease. Further research into the mechanisms of action and potential synergistic effects of these compounds is warranted to fully understand their therapeutic potential in the treatment of neurodegenerative disorders.

CONCLUSION

The ethanolic extract of *Garcinia gummi-gutta* was found to contain a wide range of biologically active phytochemicals with notable antimicrobial, antioxidant, antiviral, and neuroprotective properties. GC-MS analysis highlighted the presence of key compounds such as 2,4-Di-tert-butylphenol and Dodecanedioic acid, both of which exhibit significant neuroprotective potential. These results offer a promising basis for further investigation into the use of *Garcinia gummi-gutta* as a source of plant-derived therapeutic agents, particularly for the prevention and treatment of Alzheimer's disease and other neurodegenerative disorders.

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