GC-MS Study of the *Excoecaria agollocha* Leaf extract from Pitchavaram, Tamil nadu , India

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Abstract: The secondary metabolites in herbal samples could be analyzed by GC-MS technique. The present study is involved using plant extract from mangrove (Excoecaria agallocha -Euphorbiaceous). Four bioactive compounds are identified in the plant sample such as Stigma 4 en 3 one; 9, 12, octadecadienoic acid (z,z) octyl ester; 1,2,3 - Benzenetriol and Ambelline. The compound 1,2,3 - Benzenetriol and Ambelline were found to be useful for the treatment of anti fungal, anti-microbial, anti-inflammatory and anti cancer activities.
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Keywords: metabolite;herbal; GC-MS technique; mangrove; fungal, microbial; inflammatory

Introduction

Excoecaria agollocha is a typical mangrove species that occur along the coastal areas of Pitchavaram , Tamil Nadu ,India . The plant nature is commonly causes damage to the human eye .The plant produces white milky sap. This tree has a religious significance for the local community and people visit to Hindu temple at Chidambaram town and reserve this sacred plant. Various parts of plant are used in the traditional medicinal treatment to ulcers and Leprosy and also as an aphrodisiac for decades. The plant leaf was collected from Pitchavaram mangrove forest (11 2 and 79 44'E) located in Tamil Nadu. The area is situated in the southeast coast of India. The mangrove forest located at 225 Km south of Chennai and 5 Km north east of Chidambaram, Cuddalore district.

Materials and methods

PRINCIPLE AND APPLICATION OF GAS CHROMOTOGRAPHY - MASS DETECTOR (GC-MS) IN PHYTO CHEMICAL ANALYSIS

GC-MC Plays a key role in the analysis of unknown components of plant origin. GC-MS ionizes compound and measures their mass numbers. Ionization method includes EI (Electron Ionization). The EI method produces ions by colliding thermal electrons emitted from a filament with sample gas molecules. This method provides high stability in ionization and obtained mass spectra show good reproducibility.

The EI method provides good result for quantitative analysis as well. Quantitative analysis with GC-MS, in which only ions specific to the compounds are measured, is highly selective method without interfering components.

Gas chromatography Technique involves the separation of volatile components in a test sample using suitable capillary column coated with polar or non –polar or intermediate polar chemicals. Elite-1 column (100% Dimethyl poly siloxane) is a non – polar column used for analysis of phyto-components .Elite -5 column (5% phenyl and 95% methyl poly siloxane) is an intermediate column and also used for the estimation of phytochemicals.

An inert gas such as hydrogen or nitrogen or helium is used as a carrier gas .The compounds of test sample is evaporated in the injection port of the GC equipment and segregated in the column by absorption and adsorption technique with suitable GC programme.

TABLE-1. Phyto components identified in the plant extract by GC MS technique

S. No	RT	Name of the Compound	Molecular Formula	Molecular Weight	Peak area %	Compound Nature
1	74	Stigma 4 en 3 one	$C_{29}H_{48}O$	412	8	Steroid compound
2	99	9,12,octadecadienoic acid (z,z) octyl ester	C18H32O2	280	1.84	Linoleic Acid
3	140	1,2,3 - Benzenetriol	C6H6O3	126.11	2.7	Polyphenolic Compound
4	158	Ambelline	C18H21NO5	331.4	6.5	Alkaloid

TABLE-2. Activity of Components identified in the sample [GC MS study]

No.	RT	Name of the compound	Molecular formula	MW	Peak Area %	Compound Nature	**Activity
1	74	Stigma 4 en 3 one	$C_{29}H_{48}O$	412	8	Steroid compound	Antimicrobial Antioxidant Antiinflammatory Antiarthritic Antiasthma Diuretic
2	99	9,12 octadecadienoic acid (z,z) octyl ester	C18H32O2	280d compound	1.84	Linoleic acid ester	Antiinflammatory, Hypocholesterolemic, Cancer preventive Hepatoprotective Nematicide, Insectifuge Antihistaminic, Antieczemic Antiacne, Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge
3	140	1,2,3 - Benezenetriol	C6H6O3	126.11	2.7	Poly Phenolic compound	Antimicrobial Antioxidant Antiinflammatory Anticancer
4	158	Ambelline	C18H21NO5	331.4	6.5	Alkaloid	Antimicrobial Antiinflammatory Anticancer Antioxidant

Activity Source:
Dr. Duke's Phytochemical and Ethanobotanical Databases
Mass Spectrum and structure of compounds identified:

I. 9,12 octadecadienoic acid (z,z) octyl ester

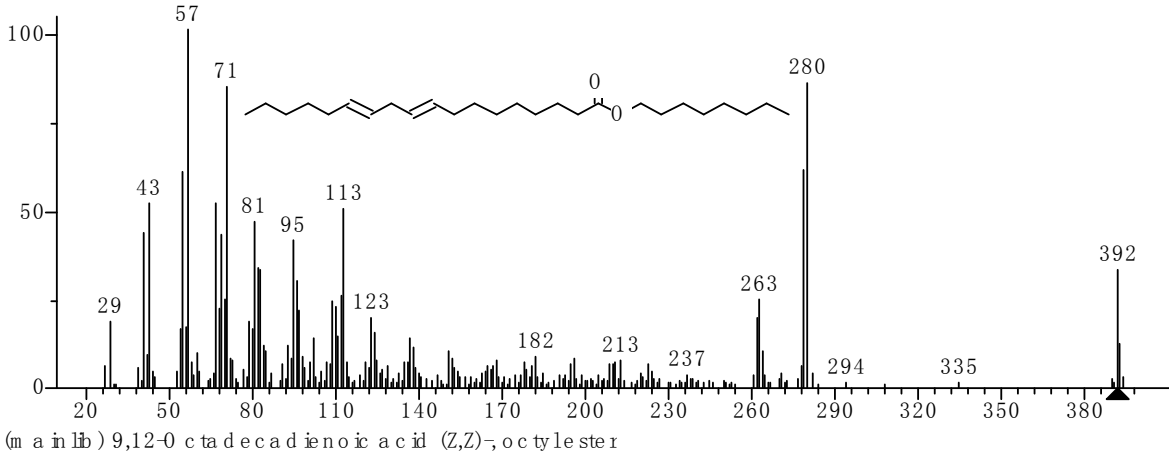

Figure 1. Activity of Component 9,12-Octadecadienoic acid (Z,Z)-, octyl ester

Name: 9,12-Octadecadienoic acid (Z,Z)-, octyl ester
Formula: $C_{26}H_{48}O_2$
MW: 392 CAS#: 64022-34-0 NIST#: 187640 ID#: 21867 DB: mainlib
Other DBs: None
Contributor: Chemical Concepts
10 largest peaks:
57 999 | 280 848 | 71 837 | 279 604 | 55 601 |
67 514 | 43 511 | 113 500 | 81 463 | 41 429 |
Synonyms: 1.Octyl (9E,12E)-9,12-octadecadienoate

2. 1,2,3-Benzenetricarboxylic acid

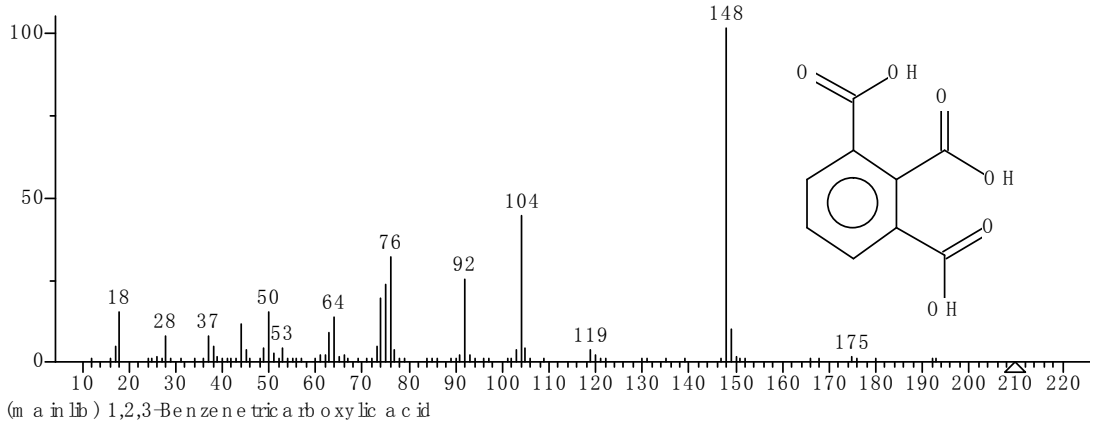

Figure 2. Activity of Component 1,2,3-Benzenetricarboxylic acid

Name: 1,2,3-Benzenetricarboxylic acid
Formula: $C_9H_6O_6$
MW: 210 CAS#: 569-51-7 NIST#: 230908 ID#: 86790 DB: mainlib
Other DBs: None
Contributor: Japan AIST/NIMC Database- Spectrum MS-NW-2162
10 largest peaks:
148 999 | 104 436 | 76 310 | 92 247 | 75 228 |
74 189 | 18 147 | 50 145 | 64 129 | 44 112 |
Synonyms:
1.Benzene-1,2,3-tricarboxylic acid
2.Hemimellitic acid
3.1,2,3-Tricarboxybenzene

3. Ambelline

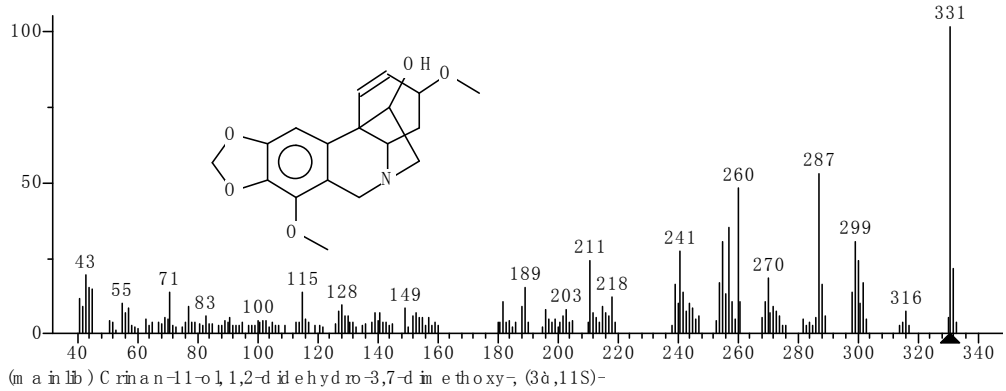

Figure 3. Activity of Component Crinan-11-ol, 1,2-didehydro-3,7-dimethoxy-, (3à,11S)-

Name: Crinan-11-ol, 1,2-didehydro-3,7-dimethoxy-, (3à,11S)-
Formula: $C_{18}H_{21}NO_5$
MW: 331 CAS#: 3660-62-6 NIST#: 29178 ID#: 140026 DB: mainlib
Other DBs: None
10 largest peaks:
331 999 | 287 517 | 260 470 | 257 345 | 255 298 |
299 294 | 241 266 | 211 235 | 300 235 | 332 207 |
Synonyms:
1.Crinan-12-ol, 1,2-didehydro-3à,7-dimethoxy-
2.Ambelline
3.Crinan-18-ol, 1,2-didehydro-3,9-dimethoxy-, (3à,18S)-
4.Amberline
5.3H,6H-5,11b-Ethano[1,3]dioxolo[4,5-j]phenanthridine, crinan-11-ol deriv.
6.Ambellin
7.3,7-Dimethoxy-1,2-didehydrocrinan-11-ol #

4. Stigmast-4-en-3-one

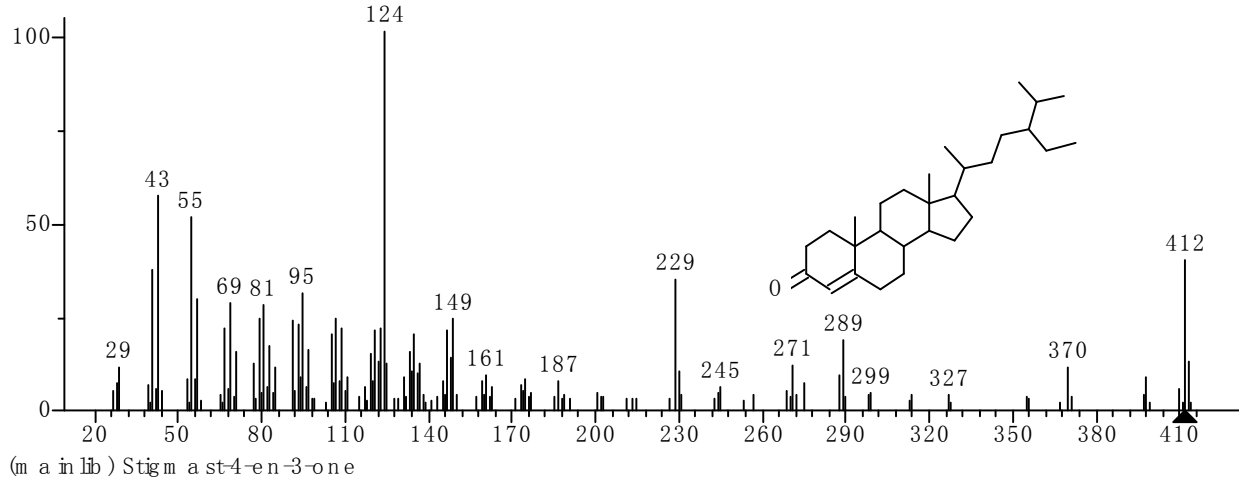

Figure 4. Activity of Component Stigmast-4-en-3-one

Name: Stigmast-4-en-3-one
Formula: $C_{29}H_{48}O$
MW: 412 CAS#: 1058-61-3 NIST#: 17165 ID#: 71556 DB: mainlib
Other DBs: None
Contributor: S.HAYASHI, DEPT. OF CHEM., HIROSHIMA UNIV., HIROSHIMA, JAPAN.
10 largest peaks:
124 999 | 43 567 | 55 506 | 412 394 | 41 366 |
229 344 | 95 308 | 57 292 | 69 283 | 81 275 |
Synonyms:
1.4-Stigmasten-3-one
2.Sitostenone
3..DELTA.4-Sitosterol-3-one

Discussion

The Excoecaria agallocha plant was tested in GC MS and indentified four active compounds .The major compound was ambilline which is an alkaloid compound with 6.5%. This compound contains a very high anti cancer activity, anti bacterial, anti fungal and anti viral activity.

Conclusion

The com pounds 1,2,3 -Benezenetriol and Ambelline are having good antioxidant and anti-inflammatory property with anticancer activity. The other two compounds responsible for development of phytomedicine against microbes are Stigma 4 en 3 one and 9,12 Octadecadienoic acid (z,z) octyl ester According to spectral data, the identified compounds aare active phyto components gainst all pathogens and where significant to the marine plant of mangrove. Further research is needed to Excoecaria agallocha for a comprehensive pharmacological investigation.

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