ISSN 1996-5052

Current Research in Chemistry

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Current Research in Chemistry 7 (1): 1-8, 2015
ISSN 1996-5052 / DOI: 10.3923/crc.2015.1.8

Chemical Compositions of Medicinal Mangrove Species *Acanthus ilicifolius*, *Excoecaria agallocha*, *Rhizophora apiculata* and *Rhizophora mucronata*

K. Satyavani, S. Gurudeeban, V. Manigandan, E. Rajamanickam and T. Ramanathan
Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai, 608502, Tamil Nadu, India

Corresponding Author: S. Gurudeeban, Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai, 608502, Tamil Nadu, India Tel: + 91 97894 45377

ABSTRACT

Chemical composition of medicinal mangrove species *Acanthus ilicifolius*, *Excoecaria agallocha*, *Rhizophora apiculata* and *Rhizophora mucronata* were isolated by simultaneous steam distillation using petroleum ether, diethyl ether and ethanol (75:75:0.5 mL). The extract was subjected to GC-MS analysis. A total of 135 chemical constituents were identified and compared with retention time in the NIST library 2011. The chemical constituents characterized into essential oils, higher alkanes, acid, alcohol and esters. Major peaks were indicated the presence of 8-pentadecane, 1, 2, 5-trimethylphyrrole, Di-(2-ethylhexyl) phthalate, diethyl phthalate, epoxyhexobarbital and cyclooctacosane. Further studies will determine the commercial application of these constituents in pharma and cosmetics industry.

Key words: Alkene, halophytes, Likens-Nickenrson distillation, retention time

INTRODUCTION

India has a rich heritage of plants and utilized 80% of plant materials for the drug development process. Volatile Organic Compounds (VOC) are constituents of essential oils which are commonly used as flavoring agents and fragrances in the food industry (Bicchi, 2004). Moreover, VOC from floral system are attracting pollinators and involved in stress responses Essential oils are complex and contain many different chemical ingredients that are extracted from plants through distillation, a process of purifying liquids by boiling and condensing its vapors. Essential oils serve as an invaluable element of natural healing for mankind. Ancient period oils were used in religious practices and the preservation of the dead for the afterlife. The Greek and Roman cultures used oil of lavender for wounds which is still utilized today. While there is still limited understanding of the mechanisms through which most essential oils act, research continues to indicate their effectiveness as agents for both treatment and preventive measures for several chronic diseases such as; cancer, HIV and even diabetes (Dudareva *et al*., 2003).

Rapid and systematic measurement of specific plant volatiles metabolites is a challenge for phytochemists because of their inherent structural diversity and choice of techniques to be used in the experimental method. Gas Chromatography coupled with Mass Spectrometry (GC-MS) is widely used for the analysis of plant volatiles (Tholl *et al*., 2006). One of the important coastal biodiversity

is mangroves; those are trees, shrub, palms or ground ferns which exist in muddy, wet soil in tropical or subtropical tidal waters. They are variously adapted to cope with the coastal unfavourable environmental conditions for reproduction and growth of high-energy wave action (Jagtap *et al.*, 2002). Traditional medicinal mangrove species *Acanthus ilicifolius* (Family: Acanthaceae), *Excoecaria agallocha* (Family: Euphorbiaceae), *Rhizophora apiculata* and *Rhizophora mucronata* (Family: Rhizophoraceae) are distributed along the Southeast coast of India. Mangroves have two basic factors which attract researchers to validate its chemical constituents. Firstly, mangroves are one of the easiest tropical forest types to generate with unusual morphology, carbohydrates metabolism and the path of photosynthesis from other glycophytes. The second reason is the mangroves are widely used in folklore medicine along the fisherwomen community (Bandaranayake, 2002). Previously, the extracts of these four mangrove species reported to have anti-diabetic, antimicrobial, anticancer, anti-nociceptive, cytotoxic and dipeptidyl peptidase IV inhibitory activities (Gurudeeban, 2013). Apart from that, those species involved in environmental protection from natural disasters like Tsunami. Therefore, the present study aimed to investigate the possible chemical constituents in *A. ilicifolius*, *E. agallocha*, *R. apiculata* and *R. mucronata*.

MATERIALS AND METHODS

Plant material: Leaves of *A. ilicifolius*, *E. agallocha*, *R. apiculata* and *R. mucronata* were collected from Kodiyampalayam coastal village, Nagapattinam district, Tamil Nadu (Southeast coast of India) during the month of January 2012. Authentication done in the herbarium maintained at Centre of Advanced Study in Marine Biology, Annamalai University, India (Voucher No. AUCASMB01/2010, AUCASMB07/2010, AUCASMB10/2010 and AUCASMB11/2010).

Clevenger distillation: Fresh leaves of four mangrove species were taken to the round bottom flask separately and distilled water was added. The separator and condenser were fixed and distilled for 3 h. No volatiles were separated in the unit.

Likens-Nickenrson distillation: Briefly 250 g of fresh leaves of *A. ilicifolius*, *E. agallocha*, *R. apiculata* and *R. mucronata* was blended with distilled water separately and continuously steam distilled with mixture of solvents mixture of petroleum ether, diethyl ether, ethanol (75:75:0.5 mL) extracted in a Likens-Nickerson apparatus for 120 min. The three leaves extract was dried over anhydrous sodium sulphate separately and concentrated using a Kuderna-Danish evaporator and finally, reduced the volume about to 1 mL under a gentle stream of nitrogen (Gurudeeban *et al.*, 2011).

GC-MS analysis: Isolated volatiles were subjected to gas chromatography mass spectrometric analysis (Perkin-Elmer Auto system XL coupled with Turbo Mass Gold MS system) under following conditions; SPB-1 column -30×0.32 mm, 0.25 m film thickness; oven temperature -50° (3 min)-2°/min-250°C (7 min); injection temperature-200°C; detector temperature -250°C, carrier gas-helium, flow rate -1 mL/min. Standard alkanes were analysed under the similar conditions. Constituents were identified by comparison of their mass spectra with those in NBS and

our IDENT data base and confirmed in many compounds, by their relative retention indices. Quantitative data were obtained by electronic integration of the TIC peak areas with the use of the internal standard method. Retention indices of all the constituents were determined by Kovats method (Jennings and Shibambto, 1980). The components of the volatile oil were identified by comparison of the Kovats indices as well as mass spectra presented in NIST library 2011.

RESULTS

The chemical constituents of *A. ilicifolius, E. agallocha, R. apiculata* and *R. mucronata* were determined. The retention indices for all the compounds were determined by Kovats method using alkanes as standards. In results, 20, 52, 30 and 33 compounds were analysed from *A. ilicifolius, E. agallocha, R. apiculata* and *R. mucronata* with peak area ranges from 1.01-11.42, 0.19-12.47, 0.48-12.79 and 0.27-20.45% Table 1-4, respectively. The majority of compound belongs to essential oil, higher alkanes, acid, alcohol and esters. While some of the same compounds were repeated with different peak areas like L-alanine, benzene methanol and Undecane. Mass spectrometer determines the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to the appearance of peaks at different Retention Time (RT). Each mass spectral fingerprint of that compound can be identified from the data library. Among the four species large number of compounds was determined from the *E. agallocha*.

Table 1: Chemical compounds in *Acanthus ilicifolius*

Compounds	Retention time	Peak area (%)
Inositol	20.583	1.01
Alpha-d-mannofuranosidel	20.583	1.01
2-hexadecen-1-ol	23.631	4.96
2-hexadecen-1-ol	23.631	4.96
9-Eicosyne	24.650	1.74
Cyclopentene	24.650	1.74
Palmitic acid	26.915	2.32
1, 2-Benzenedicarboxylic acid	28.703	11.42
Di-(2-ethylhexyl)phthalate	28.703	11.42
9, 12, 15-Octadecatrienoic acid	30.650	2.36
Ethyl linoleolate	30.650	2.36
11, 14, 17-Eicosatrienoic acid	30.650	2.36
Pentadecanoic acid	30.984	2.26
Pentadecane	30.984	2.26
Ethyl linoleolate	34.266	1.26
Hexane	37.102	1.56
Tetracosahexaene	37.102	1.56
8, 11-Eicosadienoic acid	38.638	1.01
Ethyl linoleolate	38.759	1.25
Tetracontane	42.057	2.71
Tetrapentacosan	47.391	2.05

Table 2: Chemical compounds in *Rhizophora apiculata*

Compounds	Retention time	Area (%)
Linalool	22.70	1.83
α-Terpineol acetate	26.68	2.30
3, 7-Dimethyl-2-octen-1-ol	27.14	3.57
Ethylene oxide	27.80	3.53
2-Ethyl-2-butenal	28.52	0.94
Imidazo 1, 4 diazepine	32.54	1.40
Diazene	33.68	0.86
α-Isomethyl ionone	34.14	2.47
4, 6-Bis (dimethyl amino) methyl	34.63	1.99
Acetamide	35.05	0.79
2-Methyl-pentanol-2	35.20	2.18
Allyldimethylphenylsilane	35.55	1.48
α-Ionyl acetate	35.95	3.17
2-Methoxynaphthalene	36.11	3.32
Oxyphenylon	36.22	0.63
2, 5-Diethylphenol	36.77	0.48
N-Oxo pyrimidine	37.82	2.19
O-Acetotoluide	38.43	4.16
Dehydrococcinine	38.68	1.92
Mintlactone	38.98	5.35
Trimethyl (pentafluorophenyl) silane	39.19	2.19
Diethyl phthalate	39.40	12.79
Epoxy purine	40.02	5.05
1, 2 Epoxyhexobarbital	41.01	18.50
o-Kresol-glycidaether	41.12	5.53
Bromobimane	42.31	1.48
1, 3-Cyclohexadiene	43.29	1.64

DISCUSSION

Plants synthesize around 200,000 secondary metabolites includes essential oils those could be extracted from various parts of the plants viz., leaves, stem, fruit etc (Sharma *et al*., 2013). In the present study, fresh leaves of *A. ilicifolius, E. agallocha, R. apiculata* and *R. mucronata* were selected because of its phenolic nature. Bakkali *et al*. (2008) pointed the importance of distillation technique in the volatile isolation, the simultaneous distillation and solvent extraction were followed in the present study. The essential oil and volatiles are the mixture of aldehydes, aliphatic hydrocarbons, alcohols, esters, terpenes and alkanes which might be responsible for the industrial and pharmacological applications (Dorman and Deans, 2000). In accordance with that, cyclohexane (3.83%), octadecane (4.70%), n-hexadecanoic acid (4.86%), 8-pentadecane (9.80%), cyclooctacosane (20.45%); O-Acetotoluide (4.16%), Epoxy purine (5.05%), mintlactone (5.35%), diethyl phthalate (12.79%), epoxyhexobarbital (18.50%); decane (4.96%), ethylene oxide (5.85%), diethylphalate (7.32%), 1, 2, 5-trimethylphyrrole (10.55%), 1, 2-Benzenedicarboxylic acid (11.42%) and hexadecane (12.47%) were identified as major peak in these mangrove species. Commercially, cyclohexane produced is converted into KA oil by catalytic oxidation used as a nonpolar organic solvent, cyclohexane is also for calibration of Differential Scanning Calorimetry (DSC) instruments

Table 3: Chemical compounds in *Rhizophora mucronata*

Compounds	Retention time	Area (%)
Undecanol	11.24	0.47
Acetamide, N-3-pyrrolidinyl	12.11	0.31
Lactamide	12.23	0.37
Thiosemicarbazide	14.57	1.84
Ortetamine	17.95	0.35
Dihydropinene	18.45	0.96
Methylethylbenzene	19.34	0.27
Hexyl pentyl ether	19.93	0.55
1, 2-Benzenediol	20.43	0.62
1-Iodo-2-methylnonane	21.57	0.33
Actamide	22.72	0.27
α-Terpineol	26.70	0.59
Linalyl butyrate	27.81	0.27
Phenylephrine	37.80	1.90
L-Alanine-4-nitroanilide	37.95	0.80
Norephedrine	38.10	0.96
Phenylpropanolamine	38.24	0.82
1, 2-Benzenediol	38.46	1.66
Ethyl isopropylureidoacetate	38.77	2.48
Cyclohexane	38.96	3.83
n-Hexadecanoic acid	39.18	4.86
8-Pentadecanone	39.48	9.80
Acridinedione	39.74	2.95
Phenylephrine	39.91	2.04
p-nitro phenyl methylphosphonate	40.37	3.52
2-Propenamide	40.53	3.56
1-Butanamine, N-methyl	40.72	3.36
Octadecane	41.09	4.70
Cyclooctacosane	41.89	20.45
Piperidine	42.23	2.65
1, 4-Butanediamine	42.77	1.64
2, 5-Cyclohexadien-1-one	43.36	0.89

and heat treating equipment manufacturer for surface combustion (Price, 1995). Palmitic acid is used primarily to produce soaps, cosmetics and release agents. Sodium palmitate is inexpensive and adds texture to process foods and natural additive in organic products. Rats fed a diet of 20% palmitic acid and 80% carbohydrate for extended periods showed alterations in central nervous system, control of insulin secretion and suppression of the body's natural appetite-suppressing signals (Benoit *et al*., 2009). Diethyl phthalate is a suitable solvent for many organic molecules, cosmetics and fragrances also used as plasticizers, detergent bases and aerosol sprays Industries (Ghorpade *et al*., 2002). Ethylene oxide is part of the most important raw materials used in the large-scale chemical production of ethylene glycols, ethylene glycol ethers, ethanolamines and ethoxylates. Ethylene oxide is used as a sterilizing agent, disinfecting agent and fumigant as a mixture with carbon dioxide, nitrogen and applied for gas-phase sterilization of medical equipment and instruments, packaging materials and clothing, surgical and scientific equipment, clothing, furs and valuable documents (Lyon, 2008). Enormous quantity of diterpenoids, triterpenoids, flavonoids and phorbole esters was present in the leaf extracts of *E. agallocha* (Zou *et al*., 2006). The volatiles

Table 4: Chemical compounds in *Excoecaria agallocha*

Compounds	Retention time	Area (%)
Dodecanediol	31.16	2.25
L-Alanine-4-nitroanilide	32.24	1.96
Benzene methanol	33.78	0.38
1, 1-diethoxyundecane	33.98	0.71
Hexadecane, 2-methyl	35.03	0.83
Metaraminol	35.27	0.69
1, 2-Benzenediol	35.53	0.75
Tetradecane	35.90	0.91
Benzyl alcohol	36.10	0.43
Hexadecane	36.64	12.47
Benzenemethanol	36.97	1.17
4-trifluoroacet benzyl alcohol	37.10	0.70
L-Alanine-4-nitroanilide	37.73	1.13
Alanine	28.14	0.28
2, 6-Octadiene-4, 5-diol	28.88	0.72
Undecane	29.00	0.66
Tetradecane	31.17	1.85
2, 4-Dimethylamphetamine	31.67	0.32
Cathine	32.29	0.72
2-pyridone	32.55	0.34
L-Alanine, N-glycyl	32.83	0.31
Cyclopentane	33.05	2.63
Octadecane	34.01	1.22
Hexadecanoic acid	34.29	1.43
Pfenylacetic acid	34.70	0.08
Heptadecane	35.06	1.17
Undecane	35.24	1.56
Pentanoic acid	35.98	1.21
Hydroxybenzenepropanoic acid	36.12	1.61
Diethyl methylphosphonate	36.30	0.68
Acridine	36.39	1.07
Hexadecane	36.70	5.99
Trifluoroacetic acid	37.18	4.50
Triethyl (pentafluorophenyl) silane	37.58	0.49
Ngainone	37.86	3.05
N-1-Adanantyl-p-methylbenzalimine	38.28	0.59
Pentachlorophenol	38.48	1.58
Isohumulone	38.74	2.34
Octadecanoic acid	39.02	2.84
Decane	39.22	4.96
Diethyl phthalate	39.45	7.32
Benzamide	39.90	2.94
Pentanenitrile	40.07	3.31
Diacetate,	40.42	3.09
Clivorine	40.59	2.79
1, 2, 5-Trimethylpyrrole	41.06	10.55

Table 4: Continue

Compounds	Retention time	Area (%)
Disilylamine silanamine	41.53	3.21
Ethylene oxide	41.93	5.87
N-Acetoacetyl-deacetylcolchicine	42.08	1.25
Ketostearic acid	42.36	1.88
N-1-Adanantyl-p-methylbenzalimine	43.16	1.11
O-Methylhydroxylamine	43.42	0.58

such as hexadecanoic acid, pentadecanoic acid and decanal were observed at 39.22, 39.18 and 39.48, retention time in *E. agallocha*, *R. apiculata* and *R. mucronata* than compared to GC-MS analysis of *Exocarpium citri* using solvent extraction method identified hexadecanoic acid, pentadecanoic acid and decanal at 1975, 1857 and 1026 retention indices (Xie *et al*., 2013).

CONCLUSION

We conclude that there are number of constituents in medicinal mangrove species were identified, characterized and reported in the first time. This study also indicates GC-MS was suitable analytical technique to determine chemical components. Further experimental studies will determine the commercial application of these constituents in pharma and cosmetics industry.

ACKNOWLEDGEMENTS

The authors thank to the authorities of Annamalai University and BioMed Research Management Services, Tamil Nadu, India for providing necessary support.

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