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BIOACTIVE PRINCIPLES IN THE ESSENTIAL OIL OF TWO SPECIES OF *MELALEUCA* L.

Oommen P. Saj*¹ and John E. Thoppil²

1. Dept. of Botany and Research Centre, University College, Trivandrum, Kerala-695034
2. Genetics and Plant Breeding Division, Dept. of Botany, University of Calicut, Kerala-673635.

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For Correspondence:

Dr. Oommen P. Saj

Dept. of Botany and
Research Centre, University
College, Trivandrum, Kerala-
695034

E-mail:

sajanmaranad@gmail.com

ABSTRACT

Angiosperms are the sleeping giants of drug development but their economic potential is not exploited sufficiently. Myrtaceae is a family of many members having volatile essential oil, which contain different classes of bioactive principles such as monoterpenoids, sesquiterpenoids and phenolic compounds. *Melaleuca leucadendron* and *M. styphelioides* are two economically important and widely cultivated plants of the family. The former is detected as oil rich taxa while the latter is oil poor. The chemical compositions of their oil content on GLC also show wide variation. The essential oil of *M. leucadendron* was composed of 44.09% monoterpenoids, 40.11% sesquiterpenoids, 3.53% phenolic compounds and 12.27% remain as undetected trace compounds. This belonged to mixed terpenoid chemotype. The essential oil of *M. styphelioides* was consisted of 71.65% sesquiterpenoids, 16.34% monoterpenoids 1.09% phenolic compounds and 10.92% undetected trace chemicals and this belonged to mixed sesquiterpenoid chemotype. Several bioactive principles are found in their essential oil which can be used variously.

INTRODUCTION

The higher plants are the sleeping giant of drug development¹. The economic potential of various products obtained from them are not used satisfactorily. The potential for the usage of higher plants is vast, since for an estimated total of approximately 3 lakh species, only about 500 have been studied extensively for medicinal and biological applications². More formulations are hence developed in plant based pharmaceuticals to produce anticarcinogenic drugs³. One of the earliest reports is the use of extract of *Colchicum autumnale* L. bulb for reducing uncontrolled cell division. The property of preventing carcinogenesis has been reported on many plant extracts^{4,5,6}. Apart from these reports, antiseptic, antifungal, antibacterial and antibiotic activities of essential oils and leaf extracts were reported from different plants^{7,8,9,10,11}.

Two commonly cultivated species of *Melaleuca* L. (Family Myrtaceae) were selected for the present study- *Melaleuca leucadendron* L. and *M. styphelioides* Sm. *Melaleuca leucadendron* is commonly called 'Cajaput Tree or Paper bark tree' and cultivated in gardens as an avenue tree^{12,13}. Cajaput oil is extracted from the leaves is used in various medicines and also used as a mosquito repellent¹⁴. *Melaleuca styphelioides* is commonly called 'Prickly paper bark tree or Black Tea tree'. This tree is a native of Australia, but now widely cultivated in gardens world over. The leaves are pointed and spiny^{15,16,17}.

MATERIALS AND METHODS

The material for the present study, *Melaleuca leucadendron* L. was collected from the Botanical Garden, University of Calicut and *M. styphelioides* Sm. from Govt. Botanical Gardens, Ootty. Identified, authenticated and voucher specimens were deposited in the herbarium, University of Calicut (CU 88010 & 88011 respectively).

The mature leaves of the plants were collected in the morning, cleaned and dried under shade. 50g of each of the flaked and powdered material was hydrodistilled in a Clevenger apparatus¹⁸ at 100°C for 4 hours. The aromatic essential oils were collected into small amber coloured bottles and stored at 4-6°C.

Gas liquid chromatographic analysis were carried out in Perkin Elmer HS - 40 Auto system Gas chromatograph, equipped with FID and connected with a chromatograph data processor PE Nelson 1022. Neat samples of the cooled essential oils were analyzed.

The GLC conditions used were as follows :

Column character: SS (Stainless Steel), SE-30 (Silicon E-30), solid phase; chemical in the column – 100% methyl silicon gum, Mesh size – 100/100, Column measurements: length 6 ft, Internal diameter: 2 mm, Carrier gas: Nitrogen, Inlet pressure: 8 psi, Flow rate: 30 ml/minute.

Temperature programme: from 80⁰C (Initial temperature) to 220⁰C (Final temperature) at a rate of 5⁰C/minute. Injector temperature 200⁰C and detector temperature 300⁰C. The percentage composition of the oil was computed from the GLC peak areas without using correction factor. The identity of the major components was assigned by comparing their GLC retention times with those of the standards, peak enrichment by co-injection with the standards and by comparison with literature data.

RESULTS

Essential oil showed wide dissimilarities in the quality and quantity among the two species. *M. leucadendron* is comparatively oil rich taxa (2.5%) where as *M. styphelioides* is oil poor (0.02%) and both the oil were yellow coloured.

The essential oil obtained from the leaves of *M. leucadendron* on GC analysis showed the presence of several compounds. 25 compounds were detected. β – caryophyllene was found in substantial amount. Other major compounds include 1,8- cineole, α – terpinolene, γ – cadinene, linalool, sabinene, myrcene, α – pinene, methyl iso eugenol, iso caryophyllene, β – cadinene, farnesal, germacrene, β – elemene, β – bourbonene, β – terpinene, globulol and camphene. Seven oil components were also detected in trace amounts. The details are shown in the Table 1 and Fig.1. The leaf essential oil of *M. styphelioides* on GC analysis showed the presence of several volatile compounds of which 17 were detected. The prominent ones include γ –cadinene, α -cadinene, β - farnesene, α - terpinene, α - cadinol, α - farnesene, β - caryophyllene and aromadendrene. Other major oil components include α – terpineol, germacrene, terpinen – 4 – ol, β – elemene, sabinene and eugenyl acetate. Three compounds were detected in traces (Table 2 and Fig.2).

Table 1 Details of the GC analysis of leaf essential oil of *M. leucadendron*

No.	Name of the compound	Class	Percentage yield
1	α – pinene	Monoterpenoid	4.30
2	camphene	”	1.27
3	sabinene	”	4.81
4	myrcene	”	4.77
5	linalool	”	5.86
6	1,8 - cineole	”	9.70
7	limonene	”	0.25
8	α – terpinolene	”	8.96
9	sebenyl acetate	”	0.21
10	β – terpinene	”	1.53
11	citronellyl acetate	”	0.58

12	methyl benzoate	”	0.57
13	terpinyl acetate	”	0.51
14	citriodorol	Sesquiterpenoid	0.58
15	globulol	”	1.31
16	α – terpineol	Monoterpenoid	0.77
17	methyl iso eugenol	Phenolic compound	3.53
18	β – elemene	Sesquiterpenoid	1.54
19	β – bourbonene	”	1.50
20	germacrene	”	1.77
21	β – caryophyllene	”	19.49
22	farnesal	”	1.88
23	β – cadinene	”	2.01
24	iso caryophyllene	”	2.06
25	γ - cadinene	”	7.97

Table 2 Details of the GC analysis of leaf essential oil of *M. styphelioides*

No.	Name of the compound	Class	Percentage yield
1	sabinene	Monoterpenoid	1.20
2	linalool	”	0.95
3	1,8 – cineole	”	0.34
4	estragole	”	0.28
5	α – terpinene	”	8.21
6	terpinen – 4 – ol	”	1.57
7	α – terpineol	”	3.79
8	eugenyl acetate	Phenolic compound	1.09
9	β – elemene	Sesquiterpenoid	1.27
10	aromadendrene	”	4.22
11	germacrene	”	2.89
12	β – caryophyllene	”	4.31
13	α – cadinene	”	16.64
14	γ – cadinene	”	19.63
15	α – farnesene	”	6.64
16	β – farnesene	”	8.52
17	α – cadinol	”	7.53

DISCUSSION

In the leaf essential oil of *M. leucadendron* the sesquiterpenoid, β -caryophyllene (19.49%) was detected in substantial amount. The major monoterpenoids detected were 1,8- cineole (9.7%), α -terpinolene (8.96%), linalool (5.86%), sabinene (4.81%), myrcene (4.77%), α -pinene (4.3%), β -terpinene (1.53%) and camphene (1.27%). The other major sesquiterpenoids found were γ -cadinene (7.97%), iso caryophyllene (2.06%), β -cadinene (2.01%), farnesal (1.88 %), germacrene (1.77%), β -elemene (1.54%), β -bourbonene (1.5%) and globulol (1.31%). Methyl

iso eugenol (3.53%) was the only phenolic compound detected. The essential oil of *M. leucadendron* was composed of 44.09% monoterpenoids, 40.11% sesquiterpenoids, 3.53% phenolic compounds and 12.27% of undetected chemical compounds (Table 1 and Fig. 1).

Earlier works conducted on the leaf essential oil of *M. leucadendron*^{20,21} and related species^{22,23} reveal the presence of many of these compounds. From the present study it can be suggested that the herb oil of *M. leucadendron* may belong to a mixed terpenoid class and the chemotype seems to be "Mixed terpenoid".

17 volatile compounds were detected from the leaf essential oil of *M. styphelioides*. The major monoterpenoid compound include α -terpinene (8.21%) and the sesquiterpenoids were γ -cadinene (19.63%), α -cadinene (16.64%), β -farnesene (8.52%), α -cadinol (7.53%), α -farnesene (6.64%), β -caryophyllene (4.31%) and aromadendrene (4.22%). Nine other constituents were found in traces. Eugenyl acetate (1.09%) was the only phenolic compound detected.

The herb oil of *M. styphelioides* was composed of 16.34% monoterpenoids, 71.65% sesquiterpenoids and 1.09% phenolic compounds. 10.92% of the essential oil remains undetected (Table 2 and Fig. 2). Previous works on the essential oil of *M. styphelioides*^{21,22,24} also reveals the dominance of sesquiterpenoid compounds in this taxa.

Many of the monoterpenoids, sesquiterpenoids and phenolic compounds detected in the essential oils were reported to function as anticancerous, antitumourous^{3,4,5,6,7,8} insecticidal, fungicidal, bactericidal and antiseptic agents^{25,26,27,28}. These principles can be used for the manufacture of herbal pesticides.

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