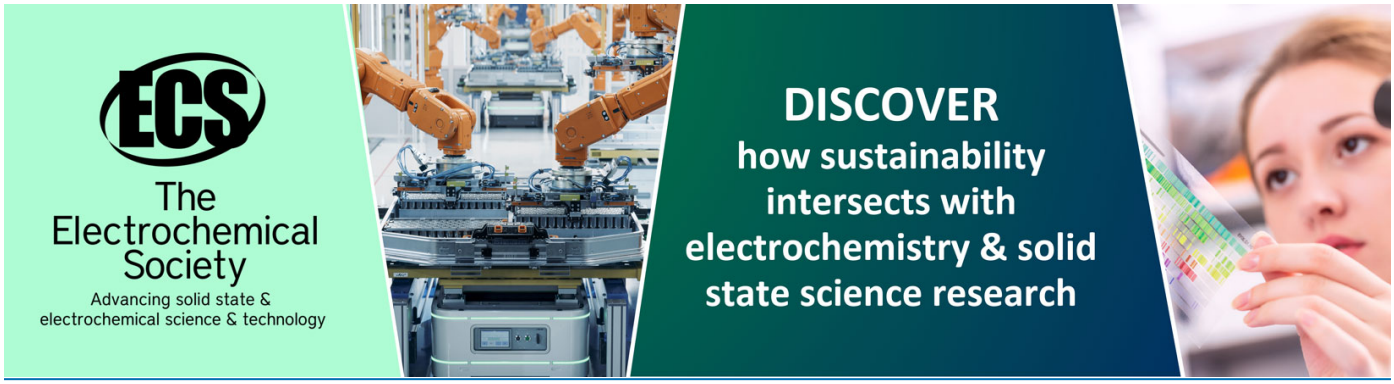
Biochemical Aspect, Antimicrobial and Antioxidant Activities of *Melaleuca* and *Syzygium* Species (Myrtaceae) Grown in Egypt



Biochemical aspect, antimicrobial and antioxidant activities of *Melaleuca* and *Syzygium* species (Myrtaceae) grown in Egypt

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Abstract:
The aim of the present work was to determine antimicrobial activities of the methanolic extracts of four *Melaleuca* species (i.e., *Melaleuca leucandron*, *Melaleuca armillaris*, *Melaleuca linarifolia*, & *Melaleuca ericifolia*) and five *Syzygium* species (i.e., *Syzygium samaragense*, *Syzygium jambos*, *Syzygium gratum*, *Syzygium paniculatum* & *Syzygium malaccense*). Also, to investigate the chemical composition of the most promising extracts. The antimicrobial activity was evaluated via disc agar plate method against four pathogenic microbial strains *viz.*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Aspergillus niger*, the antioxidant activity was evaluated via 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH), while the chemical composition was determined via gas chromatography coupled to a mass spectrometry system (GC/MS). For *Melaleuca* species, *S. aureus* pathogens were inhibited after treatment with their methanolic extracts with range 8.0-20.0 mm of inhibition zones, *E. coli* with range 0.0-21.0 mm of inhibition zones, *C. albicans* with range 9.0-18.0 mm of inhibition zones, and *A. niger* with range 0.0-15.0 mm of inhibition zones. While, for *Syzygium* species, *S. aureus* pathogens were inhibited after treating with their methanolic extracts with range 10.0-20.0 mm of inhibition zones, *E. coli* with range 0.0-14.0 mm of inhibition zones, *C. albicans* with range 0.0-21.0 mm of inhibition zones, and *A. niger* with range 0.0-9.0 mm of inhibition zones. In the DPPH assay, the IC_{50} values ranged from 34.60 to 60.97μg/ml; for *Melaleuca* species. While, for *Syzygium* species the IC_{50} values ranged from 29.81 to 52.95μg/ml relative to 7.35μg/ml of the standard ascorbic acid. GC/MS analysis revealed that the methanolic extract of *Syzygium gratum* consists of 39 compounds representing 99.08%, in which the major compounds are Veridiflorol (7.16%), and 2-methyl, 3-Hexanone (5.74%). While, the methanolic extract of *Melaleuca armillaris* consists of 30 compounds representing 97.66%, in which the major compounds are Veridiflorol (18.36%), and Globulol (12.57%).

Keywords: Myrtaceae; *Melaleuca* sp.; *Syzygium* sp.; Antimicrobial, DPPH, GC/MS.

Introduction:

The resistance of the pathogenic microbial strains against the existence antibiotics is still a major challenge. However, infectious diseases caused by bacterial and fungal infections are regarded as a great health issue. Recently, there is a dramatic increasing in microbial resistance to antimicrobial agents, so it is very important to search for alternative antimicrobial agents from natural source like plants or herbs to overcome this challenge (1-2). Consequently, several plant, fungal and marine extracts were screened for their antimicrobial activities (3-12). Moreover, plants produce a high diversity of secondary metabolites with a prominent function for protection against predators and microbial pathogens due to their biomedical properties against microbes (13).

Reactive oxygen species (ROS) are generated as secondary products during normal oxygen metabolism. The over-production of such species leads to damage of vital cells and tissues in the human body including; DNA, proteins, and lipids. This phenomenon is known by oxidative stress and is associated with the chronic destroyed diseases like cancer, coronary artery disease, hypertension and diabetes (14-20).

Myrtaceae family, involved in the Myrtales Order, has approximately 130 genera and about 3800-5800 species of mainly tropical and subtropical distribution, being concentrated in the Neotropics and Australia (21). The genus *Melaleuca* L. (Myrtaceae) includes about 250 species mainly occurs in Australia. Essential oils are the most prominent chemical constituents in this genus (22), as well as flavonoidal (23-24), phenolic acids (25), and tannins compounds (25-26).

Moreover, the genus *Syzygium* (Myrtaceae), the genus comprises about 1200-1800 species especially flowering plants. Species of this genus widely spread in Africa, and southern east Asia (27-28). Several studies demonstrated the efficacy of *Syzygium* species against different types of bacterial strains (29-30). Numerous classes of secondary metabolites were reported in the different *Syzygium* species among them are flavonoids (31), proanthocyanidins (32), chalcones (33), and phenolic acids (34, 31). In this context, the current study has described the chemical profiles, antimicrobial activities and antioxidant activities of some *Melaleuca* and *Syzygium* species grown in Egypt.

Materials and Methods:

Plants materials

Fresh leaves of four *Melaleuca* (i.e., *Melaleuca leucandron*, *Melaleuca armillaris*, *Melaleuca linarifolia*, and *Melaleuca ericifolia*) and five *Syzygium* (i.e., *Syzygium samaragense*, *Syzygium jambos*, *Syzygium gratum*, *Syzygium paniculatum* and *Syzygium malaccense*) species were collected from different locations including; Zoo Garden, El-Orman Garden and Mazhar Botanical Garden, Giza, Egypt during April, 2019. The plant was taxonomically identified by Dr. Tearse Labib, Department of Flora and Taxonomy, El-Orman Botanical Garden, Giza, Egypt.

Extraction

The dried leaves were grinded and extracted with methanol (50 gm for each plant sample) at room temperature for four days (8×500 ml). The combined extracts were filtered evaporated under vacuum until becoming dry at 40°C.

In vitro antimicrobial evaluation

The antimicrobial activities were evaluated by using disc agar plate assay against four different pathogenic microbial strains, *Staphylococcus aureus*, *Escherichia coli*, *Candida*

albicans and *Aspergillus niger* according to the reported procedures (35-36). Neomycin (100 µg/disc) and Cyclohexamide (100 µg/disc) were used as antibacterial and antifungal standards, respectively

Antiradical activity:

The antiradical action of the tested samples was evaluated according to the reported methodology illustrated by (37), briefly different dilutions of each sample (2 ml) were added to (2 ml) solution of 0.1 mmol/l 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH). An equal amount of methanol and DPPH were acted as a regulator. After 20 min of incubation at 37^{o}C in the dark, the absorbance was registered at 517 nm. The test was accomplished in triplicate. The antiradical action was estimated and the SC_{50} (concentration of analyte needed to sweep fifty percent of the radical) value was calculated. The reduction in the absorbance of DPPH solution reveals an increase of the DPPH radical masking potential. The DPPH radical scavenging activity was estimated according to the following equation:

$$\% \text{ DPPH radical scavenging activity} = [(1- A_{sample} / A_{control})] \times 100$$

Where A_{sample} and $A_{control}$ are the absorbance of the sample and control.

GC/MS analysis

GC/MS investigation of the most active samples was carried out according to the reported procedures (7), using a Thermo Scientific, Trace GC Ultra/ISQ Single Quadrupole MS,TG-5MS fused silica capillary column (30 m, 0.251 mm, 0.1 mm film thickness). For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used, Helium gas was used as the carrier gas at a constant flow rate of 1ml/min. The injector and MS transfer line temperature was set at 280°C. The oven temperature was programmed to an initial temperature of 50°C (hold 2 min) to 150 °C at an increasing rate of 7°C/min. then to 270 at an increasing rate of 5°C/min (hold 2 min) then to 310°C as a final temperature at an increasing rate of 3.5°C/min (hold 10 min). The quantification of all the identified components was investigated using a percent relative peak area. A tentative identification of the compounds was performed based on the comparison of the irrelative retention time and mass spectra with those of the NIST, WILLY library data of the GC/MS system.

Results and Discussion:

In vitro evaluation of the antimicrobial activities of Melaleuca species

The findings related to antimicrobial inhibition zones of the methanolic extracts from leaves of four *Melaleuca* species against four pathogenic microbial strains are shown in **Table 1 and Figure 1**. The inhibition zones for *M. leucandron* were between 10 and 20 mm. While, for *M. armillaris* were between 8 and 18 mm, for *M. linarifolia* were between 8 and 15 mm, and for *M. ericifolia* were 8 and 21 mm. The most susceptible microbial strains to the *M. leucandron* extract were *S. aureus* and *A. niger* with inhibition zones of 20 and 15 mm, respectively. While, the most susceptible microbial strains to the *M. armillaris* extract was *C. albicans* with inhibition zone of 18 mm. Also, the most potent activity was recorded for *M. ericifolia* against *E. coli* with inhibition zone of 21 mm. To date, very little reports in the literature describe the antimicrobial activity of *Melaleuca* species extracts, but the most published papers concerned with the antimicrobial activities of their essential oils.

The antimicrobial activity of crude leaf extract of *M. quinquenervia* was evaluated against five microbial strains. Inhibition zones at concentration 10 mg were 13.4 mm against *S. aureus*, 11.5 mm against *B. cereus*, 14.1 mm against *E. coli*, and there is any activity recorded against *C. albicans* and *S. typhimurium* (38).

The antibacterial activity of the methanolic extracts of the leaves and flowers of *M. cajuputi* were evaluated against eight pathogens, *viz.*, *Staphylococcus aureus, Escherichia coli,*

Bacillus cereus, Staphylococcus epidermidis, Salmonella typhimurium, Klebsiella pneumonia, Streptococcus pneumoniae, and *Pasteurella multocida*. The extracts demonstrated activity against Gram + ve bacterial strains; *B. cereus* 6.33 mm/12.33 mm (leaves/flowers), *S. aureus* 12.33 mm/ 12.33 mm (leaves/flowers), and *S. epidermidis* 13.66 mm/ 17.33 mm (leaves/flowers), on the other hand, there is no any activity recorded against Gram-negative bacterial strains (39). Furthermore, a recent study has revealed that the aqueous extract of *M. alternifolia* grown in Australia showed antimicrobial activity against *P. aeruginosa* with MIC of 0.25 mg/ml (40).

Table 1. The antimicrobial activity of the methanolic extracts of four *Melaleuca* species using four pathogenic microbes

Sample	Clear zone (φmm)			
	S. aureus	***E. coli***	***C. albicans***	***A. niger***
M. leucandron	20	0	10	15
M. armillaris	8	0	18	9
M. linarifolia	10	15	11	8
M. ericifolia	8	21	9	0

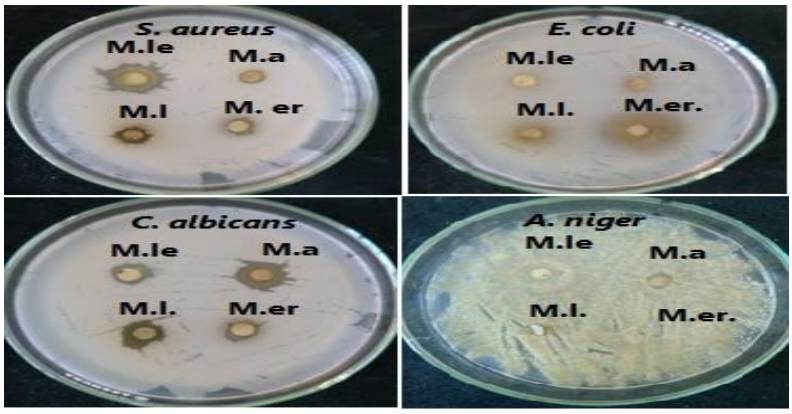

Figure 1. Antimicrobial inhibition zones of the methanolic extracts of four *Melaleuca* species against four pathogenic microbes. M.le: *Melaleuca leucandron*; M.a: *Melaleuca armillaris*; M.l: *Melaleuca linarifolia*; M.er: *Melaleuca ericifolia*.

***In vitro* evaluation of the antimicrobial activities of Syzygium species**

The methanolic extracts from the leaves of five *Syzygium* species were subjected to *in vitro* antimicrobial activity test against four pathogenic microbial strains, i.e., *S. aureus, E. coli, C. albicans,* and *A. niger*. Results presented in **Table 2 and Figure 2** revealed the antimicrobial activity of these extracts. It has been found that *S. jambos* and *S. paniculatum* showed a remarkable activity against all test microbes except the fungus. However, *S. jambos* showed almost the highest antimicrobial activity against *S. aureus* (20 mm), *E. coli* (8 mm), *C. albicans* (21 mm), and *A. niger* (7 mm). Our findings are in agreement with some extent with several previous studies (41-42).

A present study has reported that the inhibition zones of *S. polyanthum* leaves extract against foodborne pathogens were 7.00 mm, 9.33 mm, 9.67 mm, 7.00 mm, 6.67 mm, 9.33 mm, 6.67 mm, 8.33 mm, and 6.67 mm on *E. coli, K. pneumoniae, L. monocytogenes, P. aeruginosa, P.*

mirabilis, *S. aureus*, *S. typhimurium*, *V. cholerae*, and *V. parahaemolyticus*, respectively (42). Moreover, the acetone extract of the bark of *Syzygium cordatum* gave a diameter of zone of inhibition of 22 mm against *Staphylococcus aureus*, 19 mm against *Bacillus subtilis* and 18 mm against each of *Enterococcus fecalis*, *Enterobacter cloacae* and *Proteus mirabilis* (43).

Interestingly, a previous study has revealed that the hydroalcoholic extract of the leaves of *S. cumini* have shown antimicrobial activity against six pathogenic microbial strains viz., *S. mutans*, *S. oralis*, *S. parasanguis*, *S. salivarius*, *S. sp* and *L. casei* with inhibition zones of 15 mm, 15 mm, 19 mm, 13.5 mm, 15.5 mm and 15.5 mm, respectively (44). The antimicrobial activities of the different solvent extracts of *Syzygium alternifolium* leaves were evaluated. The inhibition zones were ranged from 4-8 mm (*Staphylococcus aureus*), 4-7 mm (*Escherichia coli*), 3-15 mm (*Pseudomonas aeruginosa*), 3-9 mm (*Candida albicans*), 5-10 mm (*Pencillium notatum*), and 2-6 mm (*Enterococcus*) (45).

The antimicrobial activity of the hydroalcoholic extract of *Syzygium cumini* leaves was evaluated via agar diffusion method. The inhibition zones were 12.0 mm, 14.7 mm, 9.7 mm, 8.7 mm, 10.0 mm, 9.0 mm, and 8.3 mm against *Candida albicans*, *Candida krusei*, *Enterococcus faecalis*, *Kocuria rhizophila*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Shigella flexneri*, respectively (46).

The ethanol extracts of the fruits and seeds parts of *Syzygium samaragense* were evaluated for their antimicrobial activities against certain clinical isolates, the inhibition zones were 16 mm fruits, 25 mm seeds, 10 mm fruits, 18 mm seeds, 11 mm fruits, 23 mm seeds, and 9 mm fruits, 21 mm seeds, respectively against *S. aureus*, *S. typhi*, *P. aeruginosa* and *E. coli*. While, the aqueous extracts showed low antimicrobial activities with inhibition zones of 7 mm fruits, 11 mm seeds, 0 mm fruits, 9 mm seeds, 0 mm fruits, 10 mm seeds, and 0 mm fruits, 9 mm seeds, respectively against *S. aureus*, *S. typhi*, *P. aeruginosa* and *E. coli* (47).

In accordance with a recent study, the aqueous methanol extract (85% MeOH) of *Syzygium jambos* leaves grown in Egypt showed antimicrobial activity against four microbial strains with inhibition ones of 13.5 mm, 11.0 mm, 13.5 mm, and 11.5 mm, respectively for *Staphylococcus aureus*, *Methicillin-resistant Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* (41).

Table 2. The antimicrobial activity of the methanolic extracts of five *Syzygium* species using four pathogenic microbes

Sample	**Clear zone (φmm)**			
	S. aureus	***E. coli***	***C. albicans***	***A. niger***
S. samaragense	15	14	10	0
S. jambos	20	8	21	7
S. gratum	10	0	0	0
S. paniculatum	12	13	8	9
S. malaccense	13	9	12	0

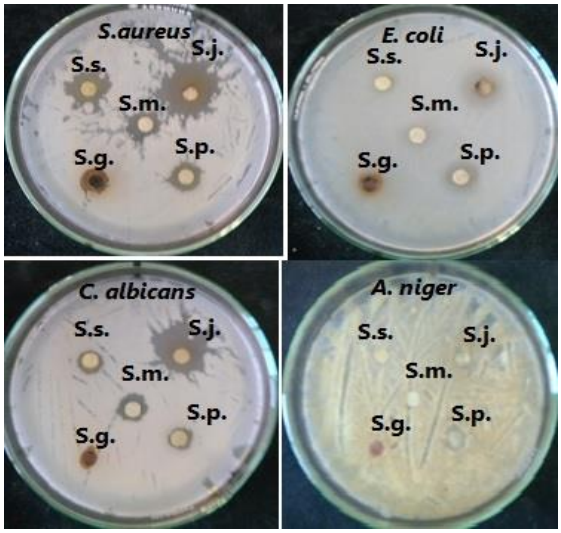

Figure 2. Antimicrobial inhibition zones of the methanolic extracts of five *Syzygium* species against four pathogenic microbes. S.s.: *Syzygium samaragense*; S.j.: *Syzygium jambos*; S.g.: *Syzygium gratum*; S.p.: *Syzygium paniculatum*; S.m.: *Syzygium malaccense*.

DPPH free radical scavenging biochemical activity

The DPPH free radical masking properties of the crude methanolic extracts of four *Melaleuca* and five *Syzygium* species are reported in Table 3. For *Melaleuca* species, the IC_{50} values for the tested extracts ranged from 34.60 to 60.79 µg/ml compared to ascorbic acid with IC_{50} equal to 7.35 µg/ml. The results are in the order: *Melaleuca armillaris* (IC_{50}: 34.60 µg/ml) > *Melaleuca ericifolia* (IC_{50}: 49.92 µg/ml) > *Melaleuca linarifolia* (IC_{50}: 59.54 µg/ml) > *Melaleuca leucandron* (IC_{50}: 60.97 µg/ml). While for *Syzygium* species, the *Syzygium gratum* extract showed the highest antioxidant activity with IC_{50} value of 29.81µg/ml, followed by *Syzygium paniculatum* (IC_{50}: 40.95 µg/ml), *Syzygium samaragense* (IC_{50}: 41.50 µg/ml), *Syzygium jambos* (IC_{50}: 48.13 µg/ml), and *Syzygium malaccense* (IC_{50}: 52.95 µg/ml), respectively, compared to ascorbic acid with IC_{50} equal to 7.35 µg/ml.

In this regard, a recent study dealt with the DPPH antiradical activity of the methanolic extracts of the leaves parts of seven *Syzygium* species from Indonesia has suggested that IC_{50} values were in the order; *S. jambos* (7.90 µg/ml), *S. malaccences* (10.77 µg/ml), *S. samarangense* (13.85 µg/ml), *S. cumini* (16.91µg/ml), *S. aqueum* (20.24 µg/ml), *S. aromaticum* (21.51 µg/ml), and *S. polyanthum* (26.03 µg/ml). The obtained results were matched to some extent with our current findings (48).

IC_{50} values of DPPH free radical scavenging activities of *M. leucadendron* solvents extracts were 5.1, 55.7, 4.8 and 60.0 µg/ml, respectively for methanol, chloroform, butanol and water extracts (49). Also, free radical masking antioxidant activity of the methanolic extract of the leaves part of *Melaleuca leucadendra* from Indonesia was evaluated and IC_{50} value was 22.46 µg/ml (48). The methanolic extract from flowers and leaves parts of *Melaleuca cajuputi* were evaluated for their DPPH free radical scavenging antioxidant activity, the leaves extract showed a higher scavenging activity with IC_{50} value of 10 µg/ml, while the flower extract showed an IC_{50} value of 25 µg/ml (50, 57). Also, the Inhibition percent's of DPPH radical by the aqueous leaves extract of *Syzygium cumini* were 62.23%, 82.6%, and 87.13% at 100, 150 and 200 µg/ml, respectively. While, for the ethanolic extract 6%, 9%, and 25% were 100, 150 and 200 µg/ml, respectively (51). IC_{50} values of DPPH free radical scavenging activities of aqueous and methanolic extracts of *S. cumini* were 24.77, and 9.97 µg/ml, respectively for aqueous and methanolic extracts (52).

Table 3. DPPH free radical scavenging activity of the methanolic extracts of four *Melaleuca* and five *Syzygium* species

Sample	DPPH free radical scavenging activity SC_{50} (μg/ml)[a]
Melaleuca leucandron	60.97 ± 0.59
Melaleuca armillaris	34.60 ± 0.15
Melaleuca linarifolia	59.54 ± 0.38
Melaleuca ericifolia	49.92 ± 0.13
Syzygium samaragense	41.50 ± 0.36
Syzygium jambos	48.13 ± 0.24
Syzygium gratum	29.81± 0.27
Syzygium paniculatum	40.95 ± 0.17
Syzygium malaccense	52.95 ± 0.20
Ascorbic acid	7.35 ± 0.47

[a]SC_{50}: concentration in μg/ml required for scavenging the DPPH radical (100 μg/ml) by 50 %, it was calculated by probit-graphic interpolation for ten concentration levels.

GC/MS analysis of the methanolic extract of *Syzygium gratum*

GC/MS analysis of the methanolic extract of *Syzygium gratum* comprises 39 ingredients. The overall peak areas of the identified components constitutes 99.08 %, the prospects of the chemical skeletons of the identified components are recorded in table (4): The main biochemical compounds are Veridiflorol $C_{15}H_{26}O$ (7.16%), 2-methyl, 3-Hexanone $C_7H_{14}O$ (5.74%), Pentadecanoic acid, 14-methyl-, methyl ester $C_{17}H_{34}O_2$ (4.98%), Nonadecane $C_{19}H_{40}$ (4.77%), 2,6,10,15-tetramethyl, and Heptadecane $C_{21}H_{44}$ (4.12%), collectively represented 26.77 % of the total peak areas **(Figure 3)**. The identification was accomplished using computer search user-generated reference libraries, incorporating mass spectra (53-55). Peaks were examined by single-ion chromatographic reconstruction to confirm their homogeneity. Occasionally, when identical spectra have not been found, only the structural type of the isomer component was proposed on the bases of its mass spectral fragmentation. Reference compounds were co-chromatographed when possible to confirm GC retention times. 3-Piperidinamine, 1-ethyl-, N-[3-[n-aziridyl]propylidene]-3-methylaminopropylamine, Carbamic acid, hydroxy-, ethyl ester, and 3-Oxabicyclo[3.3.0]octan-2-one,7-methylene were detected by GC/MS analysis as major constituents in the methanolic extract of *Syzygium calophyllifolium* (56).

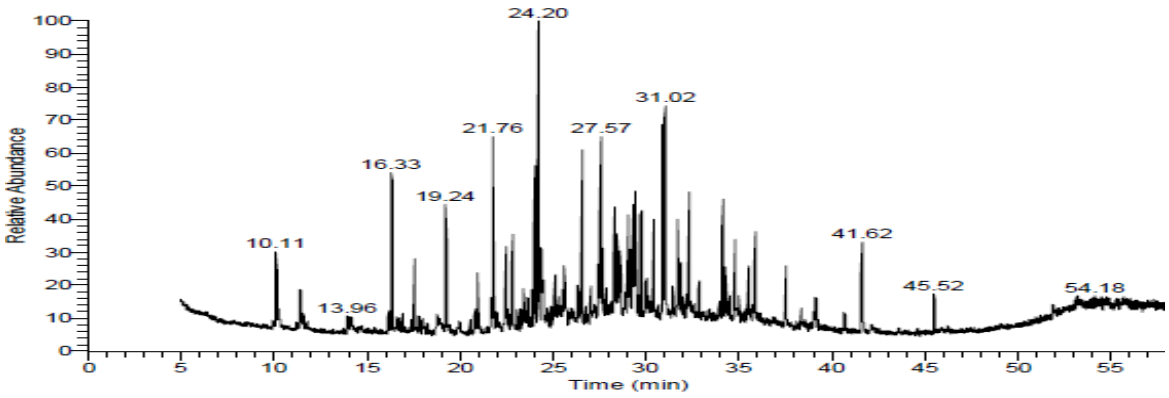

Figure 3. GC/MS profile of the methanolic extract of *Syzygium gratum*.

Table 4. GC/MS investigation of the methanolic extract of *Syzygium gratum*

Peak No.	R_t (min.)	M.W.	M.F.	Area %	Identified compounds
1	10.11	184	$C_{13}H_{28}$	2.02	2, 4, 6, Trimethyl, Decane
2	11.40	160	$C_9H_{20}O_2$	1.14	(2RS,3RS)-2-Butyl-3-methylbutane-1,4-diol
3	16.33	156	$C_{11}H_{24}$	3.30	2,6,6-trimethyl, Octane
4	17.55	212	$C_{15}H_{32}$	1.49	2,6,11-trimethyl, Dodecane
5	19.24	196	$C_{14}H_{28}$	2.65	3-Tetradecene
6	20.95	184	$C_{13}H_{28}$	1.37	2,3,7-trimethyl, Decane
7	21.76	198	$C_{14}H_{30}$	3.84	2,3,5,8-tetramethyl, Decane
8	22.47	206	$C_{14}H_{22}O$	2.17	2,4-bis(1,1-dimethylethyl), Phenol
9	22.82	226	$C_{16}H_{34}$	2.17	Hexadecane
10	24.01	238	$C_{17}H_{34}$	3.93	1-Heptadecene
11	24.20	222	$C_{15}H_{26}O$	7.16	Veridiflorol
12	24.35	192	$C_{13}H_{20}O$	2.14	α-Ionone
13	25.12	220	$C_{15}H_{24}O$	1.19	Aromadendrene oxide-(1)
14	25.61	222	$C_{15}H_{26}O$	1.21	α-Cadinol
15	26.57	296	$C_{21}H_{44}$	4.12	2,6,10,15-tetramethyl, Heptadecane
16	27.49	268	$C_{19}H_{40}$	4.77	Nonadecane
17	27.57	150	$C_{10}H_{14}O$	3.98	1-carboxaldehyde,4-(1-methyletheny), 1-Cyclohexene
18	27.67	234	$C_{15}H_{22}O_2$	1.48	7-(1,3-Dimethylbuta-1,3-dienyl)-1,6,6-trimethyl-3,8-dioxatricyclo[5.1.0.0(2,4)]octane
19	28.31	242	$C_{16}H_{34}O$	2.71	1-Hexadecanol
20	28.44	254	$C_{18}H_{38}$	2.35	2,2,4,9,11,11-hexamethyl, Dodecane
21	28.62	236	$C_{16}H_{28}O$	2.02	7,11-Hexadecadienal
22	29.04	270	$C_{17}H_{34}O_2$	2.03	Isopropyl myristate
23	29.14	306	$C_{20}H_{34}O_2$	1.32	Butyl, 6,9,12-hexadecatrienoate
24	29.33	208	$C_{14}H_{24}O$	2.29	à,2,6,6-tetramethyl,1-Cyclohexene-1-butanal
25	29.42	268	$C_{18}H_{36}O$	3.25	6,10,14-trimethyl-2-Pentadecanone
26	29.74	258	$C_{15}H_{14}O_4$	3.77	4-(3-Hydroxyphenoxy)benzoic acid ethyl ester
27	30.90	114	$C_7H_{14}O$	5.74	2-methyl, 3-Hexanone
28	31.02	270	$C_{17}H_{34}O_2$	4.98	Pentadecanoic acid,14-methyl-, methyl ester
29	31.46	292	$C_{18}H_{28}O_3$	1.15	Benzenepropanoic acid,3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester
30	31.70	282	$C_{20}H_{42}$	1.07	2,6,11,15-tetramethyl, Hexadecane
31	32.32	282	$C_{20}H_{42}$	2.52	Eicosane
32	34.14	394	$C_{28}H_{58}$	2.43	Octacosane
33	34.27	282	$C_{18}H_{34}O_2$	1.25	dihydro-5-tetradecyl, 2(3H)-Furanone
34	34.79	380	$C_{27}H_{56}$	2.06	Heptacosane
35	35.52	408	$C_{29}H_{60}$	1.33	Nonacosane
36	35.87	422	$C_{30}H_{62}$	1.90	Triacontane
37	37.54	310	$C_{22}H_{46}$	1.42	Docosane
38	41.62	390	$C_{24}H_{38}O_4$	2.28	1,2-Benzenedicarboxylicacid, bis(2-ethylhexyl) ester
39	45.51	410	$C_{30}H_{50}$	1.08	Squalene
T%				**99.08 %**	

R_t: Retention time; M.W.: Molecular Weight; M.F.: Molecular Formula

GC/MS investigation of the methanolic extract of *Melaleuca armillaris*

GC/MS analysis of the methanolic extract of *Melaleuca armillaris* comprises 30 ingredients. The overall peak areas of the identified components constitutes 97.66%, the prospects of the chemical skeletons of the identified components are recorded in table (5): The main biochemical compounds are Veridiflorol $C_{15}H_{26}O$ (18.36%), Globulol $C_{15}H_{26}O$ (12.57%), (+) spathulenol $C_{15}H_{24}O$ (7.53%),Cyclopropa[c,d]pentalene-1,3-dione, hexahydro-4-(2-methyl-2-propenyl)-2,2,4-trimethyl $C_{15}H_{20}O_2$ (4.71%), 1H-Indene, 1-ethylideneoctahydro- 7a-methyl $C_{12}H_{20}$ (5.35%), and Docosane $C_{22}H_{46}$ (5.31%), for which represented 53.83% of the total peak areas **(Figure 4)**. The identification was accomplished using computer search user-generated reference libraries, incorporating mass spectra (53-55). GC/MS analysis of the methanolic extract of *Melaleuca cajuputi* grown in Malaysia led to identification of major compounds namely Ethanone, 4H-1-Benzopyran-4-one, 1,4-Naphthalenedione, Alpha.-Tetralone, Caryophyllene Bicyclo[7.2.0]undec-4ene, 1H-Cycloprop[e]azulen-7-ol, 2-Naphthalenemethano, Squalene, and Stigmast-5-en-3-ol (57) .

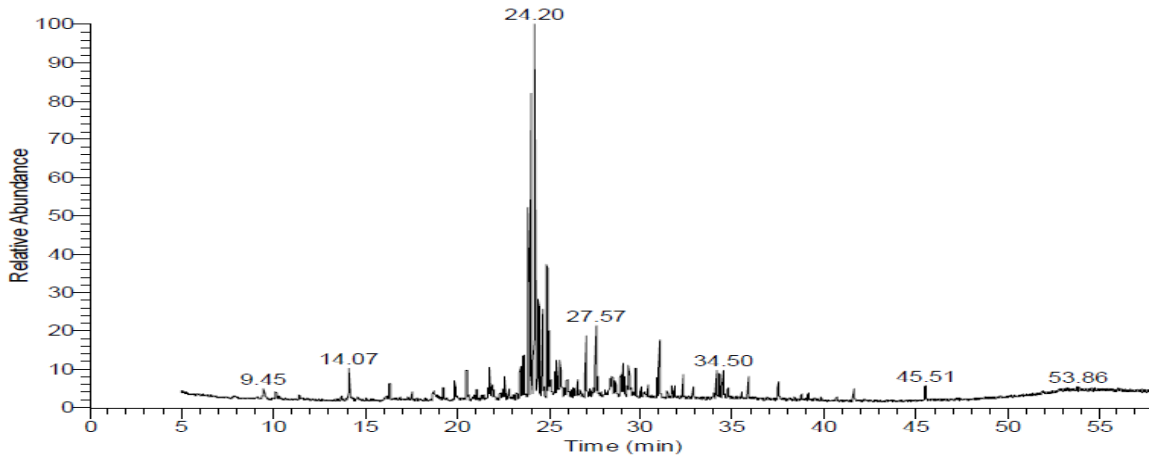

Figure 4. GC/MS profile of the methanolic extract of *Melaleuca armillaris*.

Table 5. GC/MS investigation of the methanolic extract of *Melaleuca armillaris*

Peak No.	R_t (min.)	M.W.	M.F.	Area %	Identified compounds
1	14.07	154	$C_{10}H_{18}O$	1.62	3-Cyclohexene-1-methanol, à,à,4-trimethyl
2	19.85	178	$C_{11}H_{14}O_2$	1.10	2-Butanone,4-(4-methoxyphenyl)-
3	20.52	204	$C_{15}H_{24}$	1.96	trans-Caryophyllene
4	22.85	202	$C_{15}H_{22}$	0.90	trans-calamenene
5	23.45	222	$C_{15}H_{26}O$	3.21	Epiglobulol
6	23.88	220	$C_{15}H_{24}O$	7.53	(+) spathulenol
7	24.02	222	$C_{15}H_{26}O$	12.57	Globulol
8	24.20	222	$C_{15}H_{26}O$	18.36	Veridiflorol
9	24.35	177	C_9H_7NOS	1.03	5-(2'-Thienyl)pyrrole-2-carbaldehyde
10	24.44	232	$C_{15}H_{20}O_2$	4.71	Cyclopropa[c,d]pentalene-1,3-dione,hexahydro-4-(2-methyl-2-propenyl)-2,2,4-trimethyl
11	24.65	222	$C_{14}H_{22}O_2$	3.62	2-Heptanone,6-(3-acetyl-2-methyl-1-cyclopropen-1-yl)-6-methyl

12	24.90	164	$C_{12}H_{20}$	5.35	1H-Indene,1-ethylideneoctahydro-7a-methyl
13	25.01	222	$C_{15}H_{26}O$	2.95	Cubenol
14	25.11	290	$C_{19}H_{30}O_2$	0.69	Methyl 8,10-octadecadiynoate
15	25.32	222	$C_{15}H_{26}O$	2.35	1,4-Methanoazulen-7-ol, decahydro-1,5,5,8a-tetramethyl
16	25.61	222	$C_{15}H_{26}O$	1.36	α-acorenol
17	27.02	220	$C_{15}H_{24}O$	2.79	Isoaromadendrene epoxide
18	27.57	232	$C_{15}H_{20}O_2$	3.33	(-)-oxidoselina-1,3,7(11)- trien-8-one
19	27.67	324	$C_{23}H_{32}O$	0.70	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en1-Carboxaldehyde
20	28.31	254	$C_{16}H_{30}O_2$	0.76	9-Hexadecenoic acid
21	28.43	310	C22H46	5.31	Docosane
22	28.62	254	$C_{15}H_{26}O_3$	1.08	Perhydrocyclopropa[e]azulene-4,5,6-triol,1,1,4,6-tetramethyl
23	29.04	270	$C_{17}H_{34}O_2$	1.56	Isopropyl myristate
24	29.14	292	$C_{19}H_{32}O_2$	2.52	Methyl3-cis,9-cis,12-cis-octadecatrienoate
25	29.42	268	$C_{18}H_{36}O$	1.07	2-Pentadecanone, 6,10,14-trimethyl-
26	29.73	285	$C_{19}H_{14}O$	1.92	6-methyl-7(12h)-benz[a]anthracenone
27	31.03	270	$C_{17}H_{34}O_2$	2.50	Pentadecanoic acid, 14-methyl-, methyl ester
28	34.13	422	C30H62	1.18	Triacontane
29	34.28	314	$C_{19}H_{38}O_3$	1.59	Octadecanoic acid,4-hydroxy-, methyl ester
30	34.50	296	$C_{20}H_{40}O$	1.28	Phytol
T%				**97.66 %**	

R_t: Retention time; M.W.: Molecular Weight; M.F.: Molecular Formula

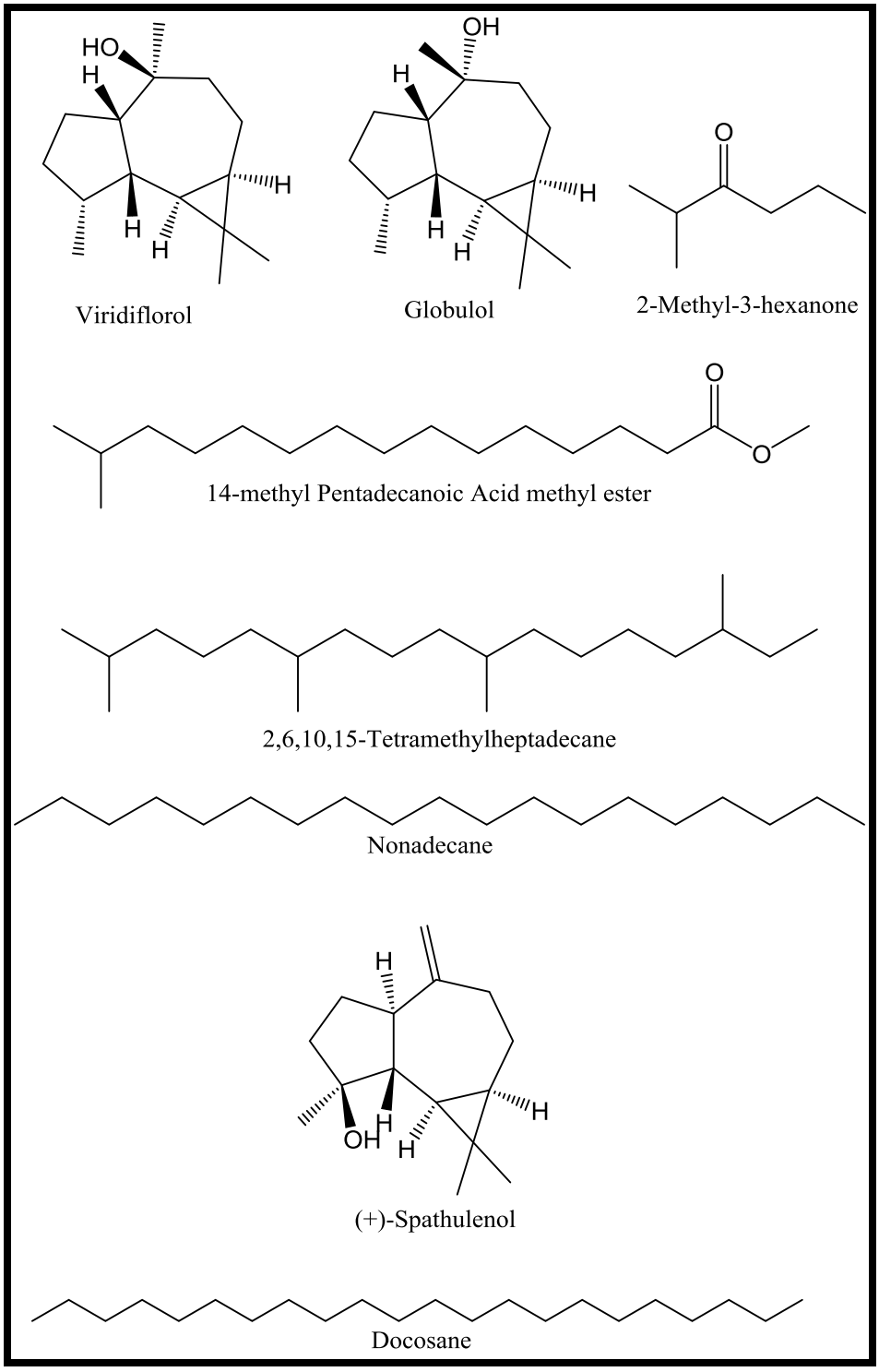

Figure 5. Chemical structures of the major biochemical compounds identified by GC/MS analysis from the methanolic extract of *Syzygium gratum* and *Melaleuca armillaris*.

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