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 leucadron, Melaleuca armillaris, Melaleuca linariifolia, & 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 treatment 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 IC50 values ranged from 34.60 to 60.97 µg/ml; for Melaleuca species. While, for Syzygium species the IC50 values ranged from 34.60 to 60.97 µg/ml; for Melaleuca species. While, for Syzygium species the IC50 values ranged from 29.81 to 52.95 µg/ml relative to 7.35 µg/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 leucadron, Melaleuca armillaris, Melaleuca linearifolia, 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 grind 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°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:

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

Where \(A_{\text{sample}}\) and \(A_{\text{control}}\) 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 \(M. \text{ leucandron}\) were between 10 and 20 mm. While, for \(M. \text{ armillaris}\) were between 8 and 18 mm, for \(M. \text{ linearifolia}\) were between 8 and 15 mm, and for \(M. \text{ ericifolia}\) were 8 and 21 mm. The most susceptible microbial strains to the \(M. \text{ leucandron}\) extract were \(S. \text{ aureus}\) and \(A. \text{ niger}\) with inhibition zones of 20 and 15 mm, respectively. While, the most susceptible microbial strains to the \(M. \text{ armillaris}\) extract was \(C. \text{ albicans}\) with inhibition zone of 18 mm. Also, the most potent activity was recorded for \(M. \text{ ericifolia}\) against \(E. \text{ coli}\) 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 \(M. \text{ quinquenervia}\) was evaluated against five microbial strains. Inhibition zones at concentration 10 mg were 13.4 mm against \(S. \text{ aureus}\), 11.5 mm against \(B. \text{ cereus}\), 14.1 mm against \(E. \text{ coli}\), and there is any activity recorded against \(C. \text{ albicans}\) and \(S. \text{ typhimurium}\) (38).

The antibacterial activity of the methanolic extracts of the leaves and flowers of \(M. \text{ cauputi}\) were evaluated against eight pathogens, viz., \(Staphylococcus \text{ aureus}\), 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, 10 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 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 |
|-----------------|------------------|-----------|---------|-------------|---------|
|                 |                  | φ         |         |             |         |
| S. samaragense  | 15               | 14        | 10      | 0           | 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 IC50 values for the tested extracts ranged from 34.60 to 60.79 µg/ml compared to ascorbic acid with IC50 equal to 7.35 µg/ml. The results are in the order: *Melaleuca armillaris* (IC50: 34.60 µg/ml) > *Melaleuca ericifolia* (IC50: 49.92 µg/ml) > *Melaleuca linarifolia* (IC50: 59.54 µg/ml) > *Melaleuca leucadendron* (IC50: 60.97 µg/ml). While for *Syzygium* species, the *Syzygium gratum* extract showed the highest antioxidant activity with IC50 value of 29.81 µg/ml, followed by *Syzygium paniculatum* (IC50: 40.95 µg/ml), *Syzygium samaragense* (IC50: 41.50 µg/ml), *Syzygium jambos* (IC50: 48.13 µg/ml), and *Syzygium malaccense* (IC50: 52.95 µg/ml), respectively, compared to ascorbic acid with IC50 equal to 7.35 µg/ml.

In this regard, a recent study dealt with the DPPH anti radical activity of the methanolic extracts of the leaves parts of seven *Syzygium* species from Indonesia has suggested that IC50 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* (16.91 µg/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).

IC50 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 IC50 value was 22.46 µg/ml (48). The methanolic extract from flowers and leaves parts of *Melaleuca caipupati* were evaluated for their DPPH free radical scavenging antioxidant activity, the leaves extract showed a higher scavenging activity with IC50 value of 10 µg/ml, while the flower extract showed an IC50 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). IC50 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<sub>50</sub> (µg/ml)<sup>a</sup> |
|-------------------------|-------------------------------------------------|
| Melaleuca leucadron     | 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                                     |

<sup>a</sup>SC<sub>50</sub>: 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<sub>15</sub>H<sub>26</sub>O (7.16%), 2-methyl, 3-Hexanone C<sub>7</sub>H<sub>14</sub>O (5.74%), Pentadecanoic acid, 14-methyl-, methyl ester C<sub>17</sub>H<sub>34</sub>O<sub>2</sub> (4.98%), Nonadecane C<sub>19</sub>H<sub>40</sub> (4.77%), 2,6,10,15-tetramethyl, and Heptadecane C<sub>21</sub>H<sub>44</sub> (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<sub>t</sub> (min.) | M.W. | M.F. | Area % | Identified compounds |
|----------|----------------------|------|------|--------|----------------------|
| 1        | 10.11                | 184  | C<sub>3</sub>H<sub>2</sub>8 | 2.02   | 2,4,6, Trimethyl, Decane |
| 2        | 11.40                | 160  | C<sub>6</sub>H<sub>12</sub>O<sub>2</sub> | 1.14   | (2RS,3RS)-2-Butyl-3-methylbutane-1,4-diol |
| 3        | 16.33                | 156  | C<sub>11</sub>H<sub>22</sub> | 3.30   | 2,6,6-trimethyl, Octane |
| 4        | 17.55                | 212  | C<sub>21</sub>H<sub>42</sub> | 1.49   | 2,6,11-trimethyl, Dodecane |
| 5        | 19.24                | 196  | C<sub>21</sub>H<sub>42</sub> | 2.65   | 3-Tetradecene |
| 6        | 20.95                | 184  | C<sub>21</sub>H<sub>42</sub> | 1.37   | 2,3,7-trimethyl, Decane |
| 7        | 21.76                | 198  | C<sub>35</sub>H<sub>70</sub> | 3.84   | 2,3,5,8-tetramethyl, Decane |
| 8        | 22.47                | 206  | C<sub>18</sub>H<sub>34</sub>O | 2.17   | 2,4-bis(1,1-dimethylethyl), Phenol |
| 9        | 22.82                | 226  | C<sub>17</sub>H<sub>36</sub> | 2.17   | Hexadecane |
| 10       | 24.01                | 238  | C<sub>17</sub>H<sub>36</sub> | 3.93   | 1-Heptadecene |
| 11       | 24.20                | 222  | C<sub>18</sub>H<sub>36</sub> | 7.16   | Veridiflorol |
| 12       | 24.35                | 192  | C<sub>18</sub>H<sub>36</sub> | 2.14   | α-Ionone |
| 13       | 25.12                | 220  | C<sub>18</sub>H<sub>36</sub> | 1.19   | Aromadendrene oxide-(1) |
| 14       | 25.61                | 222  | C<sub>18</sub>H<sub>36</sub> | 1.21   | α-Cadinol |
| 15       | 26.57                | 296  | C<sub>18</sub>H<sub>36</sub> | 4.12   | 2,6,10,15-tetramethyl, Heptadecane |
| 16       | 27.49                | 268  | C<sub>18</sub>H<sub>36</sub> | 4.77   | Nonadecane |
| 17       | 27.57                | 150  | C<sub>18</sub>H<sub>36</sub> | 3.98   | 1-carboxaldehyde, 4-(1-methylethenyl), 1-Cyclohexene |
| 18       | 27.67                | 234  | C<sub>18</sub>H<sub>36</sub> | 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<sub>18</sub>H<sub>36</sub> | 2.71   | 1-Hexadecanol |
| 20       | 28.44                | 254  | C<sub>18</sub>H<sub>36</sub> | 2.35   | 2,2,4,9,11,11-hexamethyl, Dodecane |
| 21       | 28.62                | 236  | C<sub>18</sub>H<sub>36</sub> | 2.02   | 7,11-Hexadecadienal |
| 22       | 29.04                | 270  | C<sub>18</sub>H<sub>36</sub> | 2.03   | Isopropyl myristate |
| 23       | 29.14                | 306  | C<sub>18</sub>H<sub>36</sub> | 1.32   | Butyl, 6,9,12-hexadecatrienoate |
| 24       | 29.33                | 208  | C<sub>18</sub>H<sub>36</sub> | 2.29   | à,2,6,6-tetramethyl-1-Cyclohexene-1-butanal |
| 25       | 29.42                | 268  | C<sub>18</sub>H<sub>36</sub> | 3.25   | 6,10,14-trimethyl-2-Pentadecanone |
| 26       | 29.74                | 258  | C<sub>18</sub>H<sub>36</sub> | 3.77   | 4-(3-Hydroxyphenoxo)benzoic acid ethyl ester |
| 27       | 30.90                | 114  | C<sub>19</sub>H<sub>38</sub> | 5.74   | 2-methyl, 3-Hexanone |
| 28       | 31.02                | 270  | C<sub>19</sub>H<sub>38</sub> | 4.98   | Pentadecanoic acid, 14-methyl-, methyl ester |
| 29       | 31.46                | 292  | C<sub>19</sub>H<sub>38</sub>O<sub>3</sub> | 1.15   | Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester |
| 30       | 31.70                | 282  | C<sub>20</sub>H<sub>42</sub> | 1.07   | 2,6,11,15-tetramethyl, Hexadecane |
| 31       | 32.32                | 282  | C<sub>20</sub>H<sub>42</sub> | 2.52   | Eicosane |
| 32       | 34.14                | 394  | C<sub>20</sub>H<sub>42</sub> | 2.43   | Octacosane |
| 33       | 34.27                | 282  | C<sub>20</sub>H<sub>42</sub> | 1.25   | dihydro-5-tetradecyl, 2(3H)-Furanone |
| 34       | 34.79                | 380  | C<sub>20</sub>H<sub>42</sub> | 2.06   | Heptacosane |
| 35       | 35.52                | 408  | C<sub>20</sub>H<sub>42</sub> | 1.33   | Nonacosane |
| 36       | 35.87                | 422  | C<sub>20</sub>H<sub>42</sub> | 1.90   | Triacantone |
| 37       | 37.54                | 310  | C<sub>20</sub>H<sub>42</sub> | 1.42   | Docosane |
| 38       | 41.62                | 390  | C<sub>21</sub>H<sub>42</sub>O<sub>4</sub> | 2.28   | 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester |
| 39       | 45.51                | 410  | C<sub>30</sub>H<sub>60</sub> | 1.08   | Squalene |

R<sub>t</sub>: 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$_{26}$O (7.53%), Cyclopropa[c,d]pentalene-1,3-dione, hexahydro-4-(2-methyl-2-propenyl)-2,2,4-trimethyl C$_{15}$H$_{26}$O$_2$ (4.71%), 1H-Indene, 1-ethylideneoctahydro- 7a-methyl C$_{12}$H$_{26}$ (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*.](image)

| Peak No. | R$_t$ (min.) | M.W. | M.F. | Area % | Identified compounds |
|---------|--------------|------|------|--------|----------------------|
| 1       | 14.07        | 154  | C$_{10}$H$_{16}$O | 1.62 | 3-Cyclohexene-1-methanol, ã,ã,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$_{24}$ | 0.90 | trans-calamenene |
| 5       | 23.45        | 222  | C$_{15}$H$_{26}$O | 3.21 | Epiglobulol |
| 6       | 23.88        | 220  | C$_{15}$H$_{26}$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$_{4}$H$_{8}$NOS | 1.03 | 5-(2'-Thienyl)pyrrole-2-carbaldehyde |
| 10      | 24.44        | 232  | C$_{13}$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$_{4}$H$_{12}$O$_2$ | 3.62 | 2-Heptanone,6-(3-acetyl-2-methyl-1-cyclopropen-1-y)-6-methyl |
| No. | Rt  | T%  | M.W. | M.F. | Compound Description |
|-----|-----|-----|------|------|----------------------|
| 12  | 24.90 | 164 | C_{13}H_{30} | 5.35 | 1H-Indene,1-ethylideneoctahydro-7a-methyl |
| 13  | 25.01 | 222 | C_{14}H_{20}O | 2.95 | Cubenol |
| 14  | 25.11 | 290 | C_{15}H_{30}O_{2} | 0.69 | Methyl 8,10-octadecadiynoate |
| 15  | 25.32 | 222 | C_{15}H_{30}O | 2.35 | 1,4-Methanoazulen-7-ol, decahydro-1,5,5,8a-tetramethyl |
| 16  | 25.61 | 222 | C_{16}H_{30}O | 1.36 | α-acoreno1 |
| 17  | 27.02 | 220 | C_{16}H_{28}O | 2.79 | Isoaromadendrene epoxide |
| 18  | 27.57 | 232 | C_{16}H_{30}O_{2} | 3.33 | (−)-oxidoselina-1,3,7(11)-trien-8-one |
| 19  | 27.67 | 324 | C_{22}H_{48}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 | C_{22}H_{46} | 5.31 | Docosane |
| 22  | 28.62 | 254 | C_{16}H_{30}O_{3} | 1.08 | Perhydrocyclopropa[e]azulene-4,5,6-triol,1,1,4,6-tetramethyl |
| 23  | 29.04 | 270 | C_{17}H_{30}O_{2} | 1.56 | Isopropyl myristate |
| 24  | 29.14 | 292 | C_{18}H_{32}O_{2} | 2.52 | Methyl3-cis,9-cis,12-cis-octadecatrienoate |
| 25  | 29.42 | 268 | C_{19}H_{30}O | 1.07 | 2-Pentadecanone, 6,10,14-trimethyl- |
| 26  | 29.73 | 285 | C_{19}H_{32}O | 1.92 | 6-methyl-7(12h)-benz[a]anthracenone |
| 27  | 31.03 | 270 | C_{17}H_{30}O_{2} | 2.50 | Pentadecanoic acid, 14-methyl-, methyl ester |
| 28  | 34.13 | 422 | C_{30}H_{62} | 1.18 | Triacontane |
| 29  | 34.28 | 314 | C_{19}H_{30}O_{3} | 1.59 | Octadecanoic acid,4-hydroxy-, methyl ester |
| 30  | 34.50 | 296 | C_{22}H_{48}O | 1.28 | Phytol |

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

T% 97.66 %
Figure 5. Chemical structures of the major biochemical compounds identified by GC/MS analysis from the methanolic extract of *Syzygium gratum* and *Melaleuca armillaris*. 
References:
1. Ghareeb MA, Habib MR, Mossalem HS, and Abdel-Aziz MS. Phytochemical analysis of *Eucalyptus camaldulensis* leaves extracts and testing its antimicrobial and schistosomicidal activities. Bulletin of the National Research Centre. 2018a; 42(16): 1-9.
2. Hamed MM, Ghareeb MA, Shafei AA, Abdel-Aziz MS, and Tolba SS. The *in vitro* evaluation of antioxidant, anticancer and antimicrobial properties of *Araucaria heterophylla* grown in Egypt. PharmacologyOnline. 2019; 1(1): 221-235.
3. Ghareeb MA, Saad AM, Abdel-Aleem AH, Abdel-Aziz MS, Hamed MM, and Hadad AH. Antioxidant, antimicrobial, cytotoxic activities and biosynthesis of silver & gold nanoparticles using *Syzygium jambos* leaves growing in Egypt. *Der Pharma Chemica*. 2016a; 8: 277-286.
4. Ghareeb MA, Ahmed WS, Refahy LA, Abdou AM, Hamed MM, and Abdel-Aziz MS. Isolation and characterization of the bioactive phenolic compounds from *Morus alba* L. growing in Egypt. *PharmacologyOnline*. 2016b; 3: 157-167.
5. Hathout AS, EL-Neekety AA, Abdel Aziz MS, Sabry BA, Hamed AA, Ghareeb MA, and Aly SE. Novel Egyptian bacterial exhibiting antimicrobial and antiaflatoxigenic activity. *Journal of Applied Pharmaceutical Science*. 2016; 6: 001-010.
6. El-Neekety AA, Abdel-Aziz MS, Hathout AS, Hamed AA, Sabry BA, Ghareeb MA, Aly SE, and Abdel-Wahhab MA. Molecular identification of newly isolated non-toxigenic fungal strains having antiaflatoxigenic, antimicrobial and antioxidant activities. *Der Pharma Chemica*. 2016; 8: 121-134.
7. Madkour HMF, Ghareeb MA, Abdel-Aziz MS, Khalaf OM, Saad AM, El-Ziaty AK, and Abdel-Mogib M. Gas chromatography-mass spectrometry analysis, antimicrobial, anticancer and antioxidant activities of *n*-hexane and methylene chloride extracts from *Senna italica*. *Acta Chromatographica*. 2018; 30(4): 243-249.
8. Saad AM, Abdel-Aleem AH, Ghareeb MA, Hamed MM, Abdel-Aziz MS, and Hadad AH. *In vitro* antioxidant, antimicrobial and cytotoxic activities and green biosynthesis of silver & gold nanoparticles using *Callistemon citrinus* leaf extract. *Journal of Applied Pharmaceutical Science*. 2017; 7: 141-149.
9. Abdel-Aziz MS, Ghareeb MA, Saad AM, Refahy LA, and Hamed AA. Chromatographic isolation and structural elucidation of secondary metabolites from the soil-inhabiting fungus *Aspergillus fumigatus* 3T-EGY. *Acta Chromatographica*. 2018; 30(4): 243-249.
10. Khalaf OM, Ghareeb MA, Saad AM, Madkour HMF, El-Ziaty AK, and Abdel-Aziz MS. Phenolic constituents, antimicrobial, antioxidant and anticancer activities of ethyl acetate and *n*-butanol extracts of *Senna italica*. *Acta Chromatographica*. 2019; 31(2): 138-145.
11. Ghareeb MA, Hamed MM, Saad AM, Abdel-Aziz MS, Hamed AA, and Refahy LA. Bioactive secondary metabolites from the locally isolated terrestrial fungus *Penicillium* sp. SAM16-EGY. *Pharmacognosy Research*. 2019; 11: 162-170.
12. Ghareeb MA, Khalaf OM, Abdel-Aziz MS, Saad AM, Madkour HMF, El-Ziaty AK, and Refahy LA. Chemical profiles and bio-Activities of different extracts of *Terfezia* species and their other associated Fungi. Current Bioactive Compounds. 2020; 16(3): 308-319.
13. Bassolé IHN, and Juliani HR. Essential oils in combination and their antimicrobial properties. *Molecules*. 2012; 17: 3989-4006.
14. Hasan N, Al Mamun, Beial H, Rahman A, Ali H, Tasnin N, Ara T, Rabbi A, Asaduzzaman M, and Islam A. A report on antioxidant and antibacterial properties of *Callistemon viminalis* leaf. *International Journal of Pharmaceutical Science and Research*. 2016; 1(7): 36-41.
15. Ghareeb MA, Mohamed T, Saad AM, Refahy LA, Sobeh M, and Wink M. HPLC-DAD-ESI-MS/MS analysis of fruits from *Firmiana simplex* (L) and evaluation of their antioxidant and antigenotoxic properties. *Journal of Pharmacy and Pharmacology*. 2018b; 70: 133-142.
16. Ghareeb MA, Saad AM, Ahmed WS, Refahy LA, and Nasr SM. HPLC-DAD-ESI-MS/MS characterization of bioactive secondary metabolites from Strelitzia nicolai leaf extracts and their antioxidant and anticancer activities in vitro. Pharmacognosy Research. 2018c; 10(4): 368-378.

17. Ghareeb MA, Sobeh M, Rezq S, El-Shazly AM, Mahmoud MF, and Wink M. HPLC-ESI-MS/MS profiling of polyphenolics of a leaf extract from Alpinia zerumbet (Zingiberaceae) and its anti-Inflammatory, anti-nociceptive, and antipyretic activities in vivo. Molecules. 2018d; 23: 3238.

18. Sobeh M, Mahmoud MF, Hasan RA, Abdelfattah MAO, Sabry OM, Ghareeb MA, El-Shazly AM, and Wink M. Tannin-rich extracts from Lannea stuhlmannii and Lannea humilis (Anacardiaceae) exhibit hepatoprotective activities in vivo via enhancement of the anti-apoptotic protein Bcl-2. Scientific Reports. 2018; 8: 9343.

19. Boulonouar B, Gherib A, Bronze MR, and Ghareeb MA. Identification, quantification, and antioxidant activity of hydroalcoholic extract of Artemisia campestris from Algeria. Turkish Journal of Pharmaceutical Sciences. 2019; 16(2): 234-239.

20. Ghareeb MA, Sobeh M, El-Maadawy WH, Mohammed HS, Khalil H, Botros SS, and Wink M. Chemical profiling of polyphenolics in Eucalyptus globulus and evaluation of its hepato-renal protective potential against cyclophosphamide induced toxicity in mice. Antioxidants. 2019b; 8(9): 415.

21. Barbosa LCA, Silva CJ, Teixeira RR, Meira RMSA, and Pinheiro AL. Chemistry and biological activities of essential oils from Melaleuca L. species. Agriculturae Conspicuus Scientifica. 2013; 78(1): 11-23.

22. Silva JC, Barbosa LCA, Demuner AJ, Montanari RM, Pinheiro AL, Iara Dias L, and Andrade NJ. Chemical composition and antibacterial activities from the essential oils of Myrtaceae species planted in Brazil. Quim. Nova. 2010; 33(1): 104-108.

23. Yao L, Jiang Y, Singanusong R, D'arcy B, Datta N, Caffin N, and Raymont K. Flavonoids in Australian Melaleuca, Guioa, Lophostemon, Banksia and Helianthus honeys and their potential for floral authentication. Food Research International. 2004; 37: 166-174.

24. Yoshimura M, Ito H, Miyashita K, Hatano T, Taniguchi S, Amakura Y, and Yoshida T. Flavonol glucuronides and C-glucosidic ellagitannins from Melaleuca squarrosa. Phytochemistry. 2008; 69: 3062-3069.

25. Hussein SAM, Hashim ANM, El-Sharawy RT, Seliem MA, Linscheid M, Lindequist U, and Nawwar MAM. Ericifolin: An eugenol 5-O-galloylglucoside and other phenolics from Melaleuca ericifolia. Phytochemistry. 2007; 68: 1464-1470.

26. Tuiwawa SH, Crave LA, Sam C, and Crisp MD. The genus Syzygium (Myrtaceae) in Vanuatu. Blumea. 2013; 58: 53-67.

27. Chen J, and Craven LA. Syzygium P. Browne ex Gaertner: Flora of China Online. Fruct. Sem. Pl. 1. 2015; 13: 166. 1788.

28. Djipa CD, Delmée M, and Quetin-Leclercq J. Antimicrobial activity of bark extracts of Syzygium jambos (L.) Alston (Myrtaceae). Journal of Ethnopharmacology. 2000; 71: 307-313.

29. Chandrasekaran M, and Venkatesalu V. Antibacterial and antifungal activity of Syzygium jambolanum seeds. ). Journal of Ethnopharmacology. 2004; 91(1):105-108.

30. Ghareeb MA, Hamed MM, Abdel-Aleem AH, Saad AM, Abdel-Aziz MS, and Hadad AH. Extraction, isolation and characterization of bioactive compounds and essential oil from Syzygium jambos. Asian Journal of Pharmaceutical and Clinical Research. 2017; 10: 194-200.

31. Gordon A, Jungfer E, da Silva BA, Maia JG, and Marx F. Phenolic constituents and antioxidant capacity of four underutilized fruits from the Amazon region. Journal of Agricultural and Food Chemistry. 2011; 59(14): 7688-7699.
33. Simirgiotis MJ, Adachi S, To S, Yang H, Reynertson KA, Basile MJ, Gil RR, Weinstein IB, and Kennelly EJ. Cytotoxic chalcones and antioxidants from the fruits of *Syzygium samarangense* (Wax Jambu). Food Chemistry. 2008; 107: 813-819.

34. Kasetti R, Nabi S, Swapna S, and Apparao C. Cinnamic acid as one of the antidiabetic active principle(s) from the seeds of *Syzygium alternifolium*. Food and Chemical Toxicology. 2012; 50: 1425-1431

35. Bauer AW, Kirby WM, Sherris JC, and Turck M. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology. 1966; 45(4): 493-496.

36. Ghareeb MA, Refahy LA, Saad AM, Osman NS, Abdel-Aziz MS, El-Shazly MA, and Mohamed AS. *In vitro* antimicrobial activity of five Egyptian plant species. Journal of Applied Pharmaceutical Science. 2015; 5: 045-049.

37. Shirwaikar A, Rajendran K, and Punitha ISR. *In vitro* antioxidant studies on the benzyl tetra isoquinoline alkaloid Berberine Annie. Biological and Pharmaceutical Bulletin. 2006; 29(9): 1906-1910.

38. Soonthornchareonnon N, Wiwat C, and Chuakul W. Biological activities of medicinal plants from Mangrove and Beach Forests. Mahidol University Journal of Pharmaceutical Science. 2012; 39(1): 9-18.

39. Al-Abd NM, Nor ZM, Mansor M, Azhar F, Hasan MS, and Kassim M. Antioxidant, antibacterial activity, and phytochemical characterization of *Melaleuca cajuputi* extract. BMC Complementary and Alternative Medicine. 2015; 15:385

40. Wigmore SM, Naiker M, and Bean DC. Antimicrobial activity of extracts from native plants of temperate Australia. Pharmacognosy Communications. 2016; 6(2): 80-84.

41. Ghareeb MA, Saad AM, Abdel-Aleem AH, Abdel-Aziz MS, Hamed MM, and Hadad AH. Antioxidant, antimicrobial, cytotoxic activities and biosynthesis of silver & gold nanoparticles using *Syzygium jambos* leaves growing in Egypt. Der Pharma Chemica. 2016; 8(17): 277-286.

42. Ramli S, Radu S, Shaari K, and Rukayadi Y. Antibacterial activity of ethanolic extract of *Syzygium polyanthum L.* (Salam) leaves against foodborne pathogens and application as food sanitizer. BioMed Research International. 2017; 2017: 1-13.

43. Ramadhania ZM, Insanu M, Gunarti NS, Wirasutisna KR, Sukrasno S, and Hartati R. Antioxidant activity from ten species of Myrtaceae. Asian Journal of Pharmaceutical and Clinical Research. II-Indonesian Conference on Clinical Pharmacy. 2017: 5-7.

44. Kumar MS, and Yasmeen N. Antibacterial activity of methanolic extract of *Syzygium alternifolium* leaves. American Journal of Advanced Drug Delivery. 2013; 1(5): 628-634.

45. Surh J, and Yun J. Antioxidant and anti-inflammatory activities of butanol extract of *Melaleuca leucadendron* L. Preventive Nutrition and Food Science. 2012; 17: 22-28.

46. Ghareeb MA, Refahy LA, Saad AM, Osman NS, Abdel-Aziz MS, El-Shazly MA, and Mohamed A.S. *In vitro* antimicrobial activity of five Egyptian plant species. Journal of Applied Pharmaceutical Science. 2015; 5(2): 045-049.
51. Bhati GS, Vaidya X, Sharma P, and Agnihotri A. Evaluation of phytochemicals and free radical scavenging behavior in different parts of *Syzygium cumini*. International Journal of Current Pharmaceutical Research. 2017; 9(5): 180-185.

52. Eshwarappa RSB, Iyer RS, Subbaramaiah SR, Richard SA, and Dhananjaya BL. Antioxidant activity of *Syzygium cumini* leaf gall extracts. Bioimpacts. 2014; 4(2): 101-107.

53. Madkour HMF, Ghareeb MA, Abdel-Aziz MS, Khalaf OM, Saad AM, El-Ziati AK, and Abdel-Mogib M. Gas chromatography-mass spectrometry analysis, antimicrobial, anticancer and antioxidant activities of *n*-hexane and methylene chloride extracts from *Senna italica*. Journal of Applied Pharmaceutical Science. 2017; 7: 023-032.

54. Abdel-Wareth MTA, El-Hagrassi AM, Abdel-Aziz MS, Nasr SM, and Ghareeb MA. Biological activities of endozoic fungi isolated from *Biomphalaria alexandrina* snails maintained in different environmental conditions. International Journal of Environmental Studies. 2019: 76(5): 780-799.

55. Shawky BT, Nagah M, Ghareeb MA, El-Sherbiny GM, Moghannem SAM, and Abdel-Aziz MS. Evaluation of antioxidants, total phenolics and antimicrobial activities of ethyl acetate extracts from Fungi grown on rice straw. Journal of Renewable Materials. 2019; 7(7): 667-682.

56. Sathyanarayanan S, Chandran R, Thankarajan S, Abrahamse H, and Thangaraj P. Phytochemical composition, antioxidant and anti-bacterial activity of *Syzygium calophyllifolium* Walp. fruit. Journal of Food Science and Technology. 2018; 55(1): 341-350.

57. Al-Abd NM, Nor ZM, Mansor M, Azhar F, Hasan MS, and Kassim M. Antioxidant, antibacterial activity, and phytochemical characterization of *Melaleuca cajuputi* extract. BMC Complementary and Alternative Medicine. 2015; 15: 385.