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# Chemical Composition, and Evaluation of Antibacterial, Antibiofilm and Synergistic Effects with Conventional Antibiotics of Essential Oil from *Mallotus repandus*

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**Abstract:** The essential oil (EO) of aerial parts of *Mallotus repandus* (Willd.) Muell. Arg. was extracted by hydrodistillation and characterized by GC/FID and GC/MS. Fifty-one compounds comprising 97.1% of the EO were identified, of which *α*-humulene (18.7%), *β*-selinene (12.8%), aciphyllene (10.7%), (*E*)-caryophyllene (8.4%), *α*-copaene (5.5%), humulene epoxide II (4.9%) and caryophyllene oxide (4.3%) were the major compounds. The EO was evaluated for antibacterial properties using broth microdilution method and crystal-violet static biofilm formation assay. The *M. repandus* EO possessed a bactericidal effect against tested gram-positive bacteria strains (MIC = MBC: 0.05-0.10 mg/mL). Further, the EO showed the ability to inhibit the biofilm formation of *Staphylococcus aureus*. In addition, the potential synergistic effect was assessed by checkerboard method. Combination of the *M. repandus* EO with Streptomycin showed synergistic effects against the tested bacterial strains. This study demonstrates that *M. repandus* EO could be further explored as good alternative for potential pharmaceuticals.

**Keywords:** *Mallotus repandus* (Willd.) Muell. Arg.; essential oil; antibacterial activity; antibiofilm activity; synergistic effects. 

## 1. Plant Source

Fresh aerial parts of *Mallotus repandus* (Willd.) Muell. Arg were collected in August 2019 from Lishui in Zhejiang Province of China, and authenticated by Prof. Hong Zhao. A voucher specimen (number NAS00414674) was deposited in the herbarium of Institute of Botany, Jiangsu Province and Chinese Academy of Sciences.

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## 2. Previous Studies

*M. repandus*, a climbing shrub belonging to the genus *Mallotus* (Euphorbiaceae family), is utilized traditionally in treating fever, tendon inflammation, rheumatic arthritis, ulcer and snakebite [1, 2]. In previous studies, the methanol extracts of *M. repandus* leaves and stems possessed considerable antinociceptive and anti-inflammatory activities [2, 3], and the extracts of *M. repandus* stems and roots showed great superoxide and hydroxyl radical scavenger activities [4]. The plant has also been used to treat a variety of liver disorders [5]. Previous studies have demonstrated that the stem extract of *M. repandus* exhibited remarkable hepatoprotective effect in animal models [6]. However, reviewing the obtainable existing studies, no research on the essential oil of *M. repandus* has been reported.

## 3. Present Study

The current study was carried out to identify the chemical constituents of the essential oil of *M. repandus*, and to investigate the potential antibacterial and antibiofilm activities as well as the synergistic effects with conventional antibiotics of *M. repandus* essential oil. Hydrodistillation of the aerial parts of *M. repandus* resulted in 0.11 ± 0.03% (w/w) yield on a dry mass basis. The GC-FID and GC/MS results are detailed in Table 1. A total of fifty-one constituents were detected from the EO, accounting for 97.1% of the total oil. *α*-Humulene (18.7%), *β*-selinene (12.8%), aciphyllene (10.7%), (*E*)-caryophyllene (8.4%), *α*-copaene (5.5%), humulene epoxide II (4.9%) and caryophyllene oxide (4.3%) were the predominant constituents identified.

**Table 1**. Chemical content of the essential oil of *Mallotus repandus*

| **Compound** [a] | **RI** [b] | **RI** [c] | **RI range** [d] | **%** |
|---|---|---|---|---|
| Benzaldehyde | 955 | 952[e] | 947–982 | 0.2 |
| 3-Ethyl-4-methylpentanol | 1018 | 1023[f] | 1020,1027 | 0.3 |
| Linalool | 1102 | 1095[e] | 1088–1109 | 2.1 |
| Nerol oxide | 1153 | 1154[e] | 1146–1172 | 0.2 |
| Methyl salicylate | 1196 | 1190[e] | 1160–1192 | 1.4 |
| *β*-Cyclocitral | 1222 | 1217[e] | 1205–1225 | 0.1 |
| Isobornyl formate | 1229 | 1235[e] | 1189-1271 | 0.2 |
| Geraniol | 1253 | 1249[e] | 1238–1269 | 0.2 |
| Dihydroedulan | 1291 | 1293[f] | 1293 | 0.1 |
| Epidolichodial | 1309 | 1314[f] | 1314 | 0.1 |
| Hexyl tiglate | 1336 | 1330[e] | 1313-1340 | 0.2 |
| *α*-Cubebene | 1352 | 1348[e] | 1340–1373 | 0.3 |
| *α*-Copaene | 1379 | 1374[e] | 1363–1391 | 5.5 |
| *β*-Damascenone | 1386 | 1383[e] | 1370–1397 | 0.2 |
| *β*-Elemene | 1394 | 1389[e] | 1374–1402 | 0.1 |
| *α*-Gurjunene | 1413 | 1409[e] | 1394–1421 | 0.2 |
| (*E*)-Caryophyllene | 1424 | 1417[e] | 1405–1440 | 8.4 |
| *β*-Gurjunene | 1433 | 1431[e] | 1408–1459 | 0.2 |
| *α*-Guaiene | 1441 | 1437[e] | 1424–1454 | 0.7 |
| Geranyl acetone | 1451 | 1453[e] | 1435–1461 | 0.2 |
| *α*-Humulene | 1459 | 1452[e] | 1435–1470 | 18.7 |
| 9-*epi*-(*E*)-Caryophyllene | 1466 | 1464[e] | 1456-1472 | 1.4 |
| *γ*-Gurjunene | 1474 | 1475[e] | 1455–1485 | 0.5 |
| *β*-Chamigrene | 1479 | 1476[e] | 1471–1496 | 1.9 |
| *β*-Selinene | 1492 | 1489[e] | 1473–1496 | 12.8 |
| Aciphyllene | 1501 | 1501[e] | 1501 | 10.7 |
| (*E*,*E*)-*α*-Farnesene | 1507 | 1505[e] | 1472–1516 | 1.1 |
| *γ*-Cadinene | 1510 | 1513[e] | 1498–1531 | 0.5 |
| *δ*-Cadinene | 1526 | 1522[e] | 1508–1539 | 3.6 |
| *α*-Cadinene | 1541 | 1537[e] | 1503–1541 | 0.1 |
| *α*-Calacorene | 1547 | 1544[e] | 1522–1549 | 0.6 |

| **Compound** [a] | **RI** [b] | **RI** [c] | **RI range** [d] | **%** |
|---|---|---|---|---|
| (*E*)-Nerolidol | 1563 | 1561[e] | 1539–1570 | 2.4 |
| Caryophyllene oxide | 1589 | 1582[e] | 1563–1595 | 4.3 |
| Gleenol | 1596 | 1586[e] | 1572–1589 | 0.2 |
| Widdrol | 1609 | 1599[e] | 1573-1648 | 0.2 |
| Humulene epoxide II | 1616 | 1608[e] | 1592–1610 | 4.9 |
| *α*-Corocalene | 1627 | 1622[e] | 1583-1627 | 0.1 |
| *τ*-Cadinol | 1636 | 1638[e] | 1624–1648 | 1.1 |
| *α*-Muurolol | 1645 | 1644[e] | 1620–1656 | 0.9 |
| Neointermedeol | 1660 | 1658[e] | 1654–1677 | 1.8 |
| (*E*)-10-Hydroxycalamenene | 1672 | 1668[e] | 1675,1676 | 0.5 |
| Cadalene | 1679 | 1675[e] | 1652–1680 | 0.2 |
| Pentadecanal | 1711 | 1715[f] | 1703–1728 | 1.0 |
| Tetradecanoic acid | 1758 | 1758[e] | 1749–1782 | 1.0 |
| (5*E*,9*E*)-Farnesyl acetone | 1916 | 1913[e] | 1918–1921 | 0.2 |
| Isophytol | 1943 | 1946[e] | 1939–1951 | 0.1 |
| Hexadecanoic acid | 1965 | 1959[e] | 1939–1996 | 2.9 |
| Ethyl hexadecanoate | 1989 | 1992[e] | 1975–2000 | tr |
| Phytol | 2110 | 1942[e] | 2104–2136 | 2.2 |
| Linoleic acid | 2131 | 2132[e] | 2097–2158 | 0.1 |
| Oleic Acid | 2139 | 2141[e] | 2102–2161 | 0.2 |
| Oxygenated monoterpenes | | | | 3.5 |
| Sesquiterpene hydrocarbons | | | | 67.7 |
| Oxygenated sesquiterpenes | | | | 16.5 |
| Oxygenated diterpenes | | | | 2.3 |
| **Total identification** | | | | **97.1** |

[a] Compounds are listed in order of their elution from a HP-5MS column; [b] **RI:** Relative retention indices calculated against *n*-alkanes ($C_7$-$C_{30}$); [c] **RI:** Retention indices data from the Adams (2017) and/or Andriamaharavo (2014). e) [7], f) [8]; [d] **RI range:** 90% confidence retention index range reported by Kelmendi et al. (2011) [9] or the data of NIST Standard Reference Database [10]; Tr: trace (<0.1).

Antibacterial activity of the essential oil of *M. repandus* were estimated in regard to antibacterial activity using the microdilution technique [11]. The effect of *M. repandus* essential oil on the studied bacterial strains is demonstrated in Table 2. The essential oil possessed certain antibacterial effect on all of the tested pathogens, with the MICs ranged from 0.05-0.8 mg/mL. For gram-positive bacteria, the EO showed bactericidal activity against all tested bacteria at the concentrations similar to their corresponding MICs. *M. repandus* EO showed the most significant activity against *P. larvae* (MIC = MBC = 0.05 mg/mL) among all tested bacteria, whereas it showed a weak activity against gram-negative bacteria. According to the results, gram-positive bacteria were more sensitive to this EO compared to gram-negative bacteria. It might be attributed to the presence of the outer membrane in gram-negative bacteria (composed of polysaccharides and lipopolysaccharides), which provides a hydrophilic surface that acts as a penetration barrier that preventing macromolecules and hydrophobic compounds from penetrating into the target cell membrane [12]. The major components sesquiterpenoids and oxygenated sesquiterpenes of the *M. repandus* EO, which are reported to possess significant antibacterial activity, could be responsible for the antibacterial effect [13]. The main compounds (α-humulene [14], aciphyllene [15], (*E*)-caryophyllene and caryophyllene oxide [16, 17]) of the oil were also reported to have antibacterial activities against diverse bacterial strains.

**Table 2**. Antibacterial activity of essential oil of *M. repandus*

| **Microorganism** | **MIC [a]** | | **MBC [b]** | |
|---|---|---|---|---|
| | **EO (µg/mL)** | **Ch (µg/mL)** | **EO (µg/mL)** | **Ch (µg/mL)** |
| Gram positive | | | | |
| *Bacillus subtilis* ATCC 6633 | 100 | 4 | 100 | 4 |
| *Staphylococcus aureus* ATCC 6538 | 100 | 4 | 100 | 32 |
| *Paenibacillus larvae* ATCC 9545 | 50 | 2 | 50 | 4 |
| Gram negative | | | | |
| *Escherichia coli* ATCC 25922 | 800 | 4 | 800 | 32 |
| *Pseudomonas aeruginosa* ATCC 27853 | 800 | 128 | 1600 | 512 |

Positive control: Ch, chloramphenicol; MIC [a]: Minimal inhibitory concentration; MBC [b]**:** Minimal bactericidal concentration.

The checkerboard microdilution method was performed to evaluate the synergistic interactions between the *M. repandus* EO and Streptomycin [18]. The results, as shown in Table 3, confirmed the synergistic effects of *M. repandus* EO in combination with Streptomycin against all tested bacterial strains (FICI ≤ 0.5). The strong synergy observed between Streptomycin and the EO against the gram-positive bacteria is worthy of note. In particular, the MICc value for Streptomycin was found to be 64-fold lower than that normally required to achieve the direct inhibition of bacterial growth. The use of essential oils and synthetic antibiotics combined has been demonstrated to increase the antimicrobial effects, to expand the antimicrobial spectrum, to inhibit drug resistance, and to reduce adverse/toxic side effects [19].

**Table 3.** Fractional inhibitory concentrations indices (FICIs) of streptomycin combined with EO against tested bacterial strains

| Microorganism | | $MIC_a$ (µg/mL) | $MIC_c$ (µg/mL) | FICI |
|---|---|---|---|---|
| *Bacillus subtilis* | EO | 100.00 | 25.00 | 0.27 (S) |
| ATCC 6633 | SM | 4.00 | 0.06 | |
| *Staphylococcus aureus* | EO | 100.00 | 12.50 | 0.14 (S) |
| ATCC 6538 | SM | 4.00 | 0.06 | |
| *Escherichia coli* | EO | 800.00 | 200.00 | 0.50 (S) |
| ATCC 25922 | SM | 4.00 | 1.00 | |
| *Pseudomonas aeruginosa* | EO | 800.00 | 200.00 | 0.38 (S) |
| ATCC 27853 | SM | 2.00 | 0.25 | |

$MIC_a$: MIC of the sample alone; $MIC_c$: MIC value of the most effective combination; FICI: The fractional inhibitory concentration index; SM: Streptomycin. S, synergy (FICI ≤ 0.5).

Biofilm formation of *S. aureus* was also evaluated in the presence of the essential oil by the crystal violet method [20]. As shown in Figure 1, the *M. repandus* EO was able to inhibit the biofilm formation in all tested concentrations (5-160 µg/mL). *Staphylococcus aureus* is among the leading pathogenic microorganism causing bloodstream infections capable of developing biofilms on host tissue and indwelling medical devices [21, 22]. Once biofilm formed, it will become resistance to antibacterial treatment [23]. Previous studies have demonstrated the promising clinical applications of *α*-humulene for inhibiting the formation of *Bacteroides fragilis* biofilms [14]. However, the antibiofilm effect of essential oil is thought to be determined by interaction effects of all the components of the oil, whereas the use of a single compound is generally not effective enough for inhibition of biofilm growth [24-26].

Overall, the results revealed that the *M. repandus* essential oil has a considerable potential for gram-positive pathogens control. *M. repandus* EO could emerge as a potential alternative for the prevention of contamination related to biofilm formation.

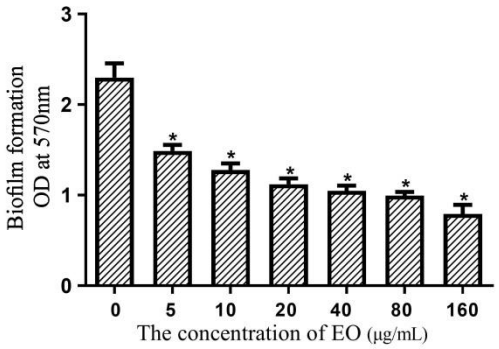


**Figure 1**. Inhibition of *Staphylococcus aureus* biofilm formation. Differences were statistically significant in relation to the control for P < 0.05 (*).

## Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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