Antimicrobial Activities of Sesquiterpene-Rich Essential Oils of Two Medicinal Plants, *Lannea egregia* and *Emilia sonchifolia*, from Nigeria

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**Abstract:** *Lannea egregia* (Anacardiaceae) and *Emilia sonchifolia* (Asteraceae) are plants used in traditional medicine in southwestern Nigeria. The essential oils from the leaves of *L. egregia* and *E. sonchifolia* were obtained by hydrodistillation and analyzed by gas chromatography–mass spectrometry. Both essential oils were dominated by sesquiterpenoids. The major components in *L. egregia* leaf essential oil were α-panasinsen (34.90%), (E)-caryophyllene (12.25%), α-copaene (11.39%), and selina-4,11-diene (9.29%), while *E. sonchifolia* essential oil was rich in γ-himachalene (25.16%), (E)-caryophyllene (15.72%), and γ-gurjunene (8.58%). The essential oils were screened for antimicrobial activity against a panel of bacteria and fungi and displayed minimum inhibitory concentrations ranging from 156 µg/mL to 625 µg/mL. Based on these results, either *L. egregia* or *E. sonchifolia* essential oil may be recommended for exploration as complementary antibacterial or antifungal agents.

**Keywords:** α-panasinsen; γ-himachalene; (E)-caryophyllene; α-copaene; selena-4,11-diene; antibacterial; antifungal

1. Introduction

Medicinal plants are widely used in treatment of diseases, and this has encouraged researchers to investigate plants that are of pharmacological value with potential therapeutic application in the management of human health [1]. Many ethnomedicinal plants have been investigated and reported to possess antiviral [2], anticancer [3], antiprotozoal [4], antibacterial [5], antifungal [6], anti-inflammatory [7], antioxidant [8] and other biocidal activities [9–11]; hence, their usefulness in folk medicine for treatment of various diseases has given credence to the application of the ethnopharmacological approaches for drug discovery.

The genus *Lannea* is in the family Anacardiaceae, which consists of nearly 800 species in 82 genera. There are around 40 *Lannea* species distributed across the savanna region of the West African tropics from Guinea through Ghana to Nigeria [12,13]. Several important members of *Lannea* species include *L. kerstingii*, *L. welwitschii*, *L. schimperii*, *L. egregia*, *L. acida*, *L. microcarpa*, and *L. fruticosa* [14]. *Lannea egregia* Engl. and K. Krause, locally called “ekudan” in Yoruba in Nigeria, “sambituliga” in Ivory Coast, and “tiuko” in Guinea [15], is a tropical woody perennial plant about 13 m in height with alternate leaves growing in the savanna region and shares the same local name as *L. barteri* (Oliv.) Engl., in Ivory Coast, Benin, and Guinea [16].
Ethnomedicinally, *L. egregia* has seen traditional use in treatment of various ailments in humans. The roots and bark are used externally for ulcers, sores, and leprosy [12]. The plant decoction is taken as a remedy for diarrhea, edema, epilepsy, rheumatism, insanity, paralysis, and gastric pains [17,18], as well as to improve the hemoglobin level and as part of vermifuge medicine [16]. The macerated roots have been used to treat wounds [19]. Traditionally, leaves of *L. egregia*, boiled with fermented corn water, were used for treatment of hemorrhoids [19] and to manage cancer [20]. The leaf, stem bark, and root extracts of *L. egregia* from Olokemeji Forest, Nigeria, were shown to have antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Escherichia coli* using a zone-of-inhibition assay, but showed only weak activity with minimum inhibitory concentrations (MIC) ranging from 6.3 to 25 mg/mL [21]. In this work, a phytochemical screening was carried out, but individual components were not identified.

*Emilia sonchifolia* (L.) DC. (Asteraceae) is a bushy annual herb distributed mainly in Asian countries, but naturalized throughout the tropics [13]. It has been traditionally used as an important medicinal plant in most tropical and subtropical countries [22], including in the South-South region of Akwa Ibom State, Nigeria [23,24]. The plant has been used to treat diarrhea, night blindness, sore throat, chest pain, liver disease, eye inflammation, stomach tumor, rashes, measles, earache, inflammation, convulsions, fever, muscular aches, and asthma [24–26]. There have been several studies reported in the literature on the biological activities and phytochemical screening of extracts of *E. sonchifolia* (Table 1). The plant extracts have shown anti-inflammatory, antioxidant, cytotoxic, analgesic, wound-healing, antimetastatic, immunomodulatory, and antiangiogenic activities [27].

| Emilia sonchifolia Extract (Geographical Source) | Phytochemicals Identified | Biological Activity | Ref. |
|-----------------------------------------------|---------------------------|---------------------|------|
| Methanol plant extract (Kerala, India)        | None identified           | In vitro cytotoxicity (L-929 murine lung fibroblast, IC$_{50}$ = 15 µg/mL) | [28] |
| Aqueous leaf extract (Nsukka, Nigeria)        | None identified           | Anti-inflammatory (mouse paw edema assay, ED$_{50}$ = 780 mg/kg) | [23] |
| ethanol plant extract (Kerala, India)         | None identified           | Inhibition of perchlorate oxidative stress (rat model) | [29] |
| Methanol leaf extract (Ibiono, Nigeria)       | None identified           | Analgesic (acetic acid writing, formalin hind paw, and hot plate assays, mouse model) | [25] |
| CH$_3$OH/CH$_2$Cl$_2$ (1:1) extract of aerial parts (Nsukka, Nigeria) | Quercetin, chlorophyll, caffeic acid derivative | Anti-inflammatory (inhibition of pro-inflammatory cytokines, mouse model) | [30] |
| Ethanol extract of aerial parts (Liuzhou, China) | Emiline (pyrrolidine alkaloid) | Neuroprotective (in vitro PC12 cells) | [31] |
| Aqueous HCl (0.5 N) plant extract (Taiwan)    | Pyrrolizidine alkaloids: senecionine, seneciphylline, integerrimine, senkirkine, otoisenine, neosenkirkine, petasitenine, acetylsenkirkine, desacyldororine, acetylpetasitenine, and dororine | None carried out, but pyrrolizidine alkaloids known to be hepatotoxic. | [32] |
| Aqueous plant extract (Kerala, India)         | None identified           | Wound-healing activity (rat model) | [33] |
| Ethanol leaf extract (Abraka, Nigeria)        | None identified           | Antifungal activity (Curvularia lunatus, MIC = 72 mg/mL) | [34] |
| Methanol leaf extract (Uyo, Nigeria)          | None identified           | Antioxidant (FRAP and DPPH assays) | [26] |

IC$_{50}$ = Median inhibitory concentration. ED$_{50}$ = Median effective dose. FRAP = Ferric ion Reducing Antioxidant Power. DPPH = 2,2-diphenyl-1-picrylhydrazyl.

We report herein our investigation into the collection of *L. egregia* leaf essential oil and the essential oil from the leaves of *E. sonchifolia* from southwest Nigeria, the analysis of the essential oil compositions, and antimicrobial screening of the essential oils. This
investigation is part of our ongoing research aimed at the characterization of the bioactivity and the compositions of the essential oils from Nigerian medicinal plants for potential exploitation in pharmaceutical applications.

2. Results and Discussion

2.1. Essential Oil Compositions

2.1.1. Lannea egregia

Hydrodistillation of the leaves of L. egregia collected from Agbegi-Odofin Village, Ikire, Osun State, Nigeria, yielded a pale-yellow essential oil with an average yield of $0.68 \pm 0.2\%$ on a weight-to-weight basis. The essential oil was analyzed by gas chromatography—mass spectrometry (GC-MS) (Table 2, Figure 1). The essential oil showed monoterpene hydrocarbons (1.53%), oxygenated monoterpenoids (2.86%), sesquiterpene hydrocarbons (86.43%), oxygenated sesquiterpenoids (1.15%), and non-terpenoids (6.50%). The predominant sesquiterpene hydrocarbons include $\alpha$-panasinsen (34.90%), (E)-caryophyllene (12.25%), $\alpha$-copaene (11.39%), and selina-4,11-diene (9.29%). The major oxygenated monoterpenoid was linalool (1.12%).

As far as we are aware, there have been no published reports on essential oils from Lannea species, so essential oil compositional comparisons at the genus level are not possible. Both $\alpha$-copaene and (E)-caryophyllene are common essential oil components, including the Anacardiaceae (see, for example [35,36]). Selin-4,11-diene, on the other hand, is relatively uncommon in the family, but has been observed in Sclerocarya birrea leaf essential oil [37] and Haematostaphis barteri leaf essential oil [38]. Likewise, $\alpha$-panasinsen is a rare volatile component in the Anacardiaceae, but detected as an aroma component of Mangifera indica cv. Alphonso [39] and Sclerocarya birrea subsp. caffra [40] fruits.
Table 2. The chemical constituents of *Lannea egregia* leaf essential oil.

| Sr. No. | RT   | RI(calc) | RI(db) | Compound                        | Ave % | St Dev |
|---------|------|----------|--------|---------------------------------|-------|--------|
| 1       | 15.838 | 977      | 978    | β-Pinene                        | 0.21  | 0.06   |
| 2       | 16.152 | 983      | 986    | 6-Methylhept-5-en-2-one         | 0.38  | 0.04   |
| 3       | 16.494 | 989      | 991    | 2-Pentylfuran                   | 0.25  | 0.02   |
| 4       | 18.725 | 1024     | 1025   | p-Cymene                        | 0.34  | 0.03   |
| 5       | 19.044 | 1028     | 1030   | Limonene                        | 0.24  | 0.02   |
| 6       | 19.149 | 1030     | 1031   | β-Phellandrene                  | 0.11  | 0.01   |
| 7       | 20.961 | 1057     | 1058   | γ-Terpinene                     | 0.22  | 0.01   |
| 8       | 21.811 | 1070     | 1069   | cis-Linalool oxide              | 0.12  | 0.02   |
| 9       | 22.81  | 1085     | 1086   | Terpinol                        | 0.08  | 0.02   |
| 10      | 23.099 | 1089     | 1091   | p-Cymenone                      | 0.32  | 0.04   |
| 11      | 23.752 | 1099     | 1099   | Linalool                        | 1.12  | 0.01   |
| 12      | 23.979 | 1102     | 1104   | Hotrienol                       | 0.13  | 0.02   |
| 13      | 24.116 | 1104     | 1104   | Nonanal                          | 0.30  | 0.02   |
| 14      | 24.759 | 1113     | 1112   | (E)-2,4-Dimethylhepta-2,4-dienal| 0.26  | 0.04   |
| 15      | 26.577 | 1139     | 1139   | (Z)-3-Ethylidene-1-methyl-1,4-cycloheptadiene | 0.22  | 0.02   |
| 16      | 27.252 | 1149     | —      | Unidentified                    | 0.44  | 0.02   |
| 17      | 28.064 | 1160     | 1169   | p-Dimethoxybenzene              | 0.54  | 0.06   |
| 18      | 29.26  | 1177     | 1172   | Lavandulol                      | 0.12  | 0.02   |
| 19      | 29.416 | 1180     | 1180   | Terpinen-4-ol                   | 0.44  | 0.05   |
| 20      | 30.132 | 1190     | 1192   | Methyl salicylate               | 0.30  | 0.00   |
| 21      | 30.415 | 1194     | 1195   | α-Terpineol                     | 0.26  | 0.05   |
| 22      | 32.05  | 1218     | 1219   | β-Cyclocitril                   | 0.42  | 0.03   |
| 23      | 32.783 | 1228     | —      | Unidentified                    | 0.47  | 0.05   |
| 24      | 34.591 | 1255     | 1257   | Carvenone                       | 0.25  | 0.01   |
| 25      | 40.81  | 1347     | 1349   | α-Cubebene                      | 0.20  | 0.02   |
| 26      | 42.245 | 1369     | 1367   | Cycloasativene                  | 0.61  | 0.06   |
| 27      | 42.704 | 1376     | 1375   | α-Copaene                       | 11.39 | 0.22   |
| 28      | 43.231 | 1384     | 1382   | β-Bourbonone                    | 1.25  | 0.06   |
| 29      | 44.584 | 1404     | —      | Unidentified                    | 0.61  | 0.05   |
| 30      | 45.524 | 1419     | 1417   | (E)-Caryophyllene               | 12.25 | 0.10   |
| 31      | 46.156 | 1429     | 1430   | β-Copaene                       | 0.57  | 0.02   |
| 32      | 47.186 | 1446     | 1447   | Geranyl acetone                 | 0.62  | 0.06   |
| 33      | 47.774 | 1455     | 1454   | α-Humulene                      | 1.85  | 0.03   |
| 34      | 48.052 | 1460     | 1457   | allo-Aromadendrene              | 0.65  | 0.04   |
| 35      | 48.978 | 1474     | 1478   | γ-Muurolene                     | 0.57  | 0.03   |
| 36      | 49.235 | 1478     | 1479   | α-Amorphene                     | 0.41  | 0.06   |
| 37      | 49.533 | 1483     | 1476   | Selina-4,11-diene               | 9.29  | 0.03   |
| 38      | 49.853 | 1488     | 1492   | β-Selinene                      | 4.26  | 0.04   |
| 39      | 50.065 | 1492     | 1492   | Valencene                       | 3.86  | 0.09   |
| 40      | 50.295 | 1495     | 1497   | α-Selinene                      | 1.24  | 0.08   |
| 41      | 50.425 | 1497     | 1497   | α-Muurolene                     | 1.35  | 0.06   |
| 42      | 51.313 | 1512     | 1512   | γ-Cadinene                      | 0.88  | 0.05   |
| 43      | 51.704 | 1519     | 1521   | α-Panasinse                     | 34.90 | 0.25   |
| 44      | 52.716 | 1536     | 1538   | α-Cadinene                      | 0.37  | 0.05   |
| 45      | 52.96  | 1540     | 1541   | α-Calacore                     | 0.54  | 0.02   |
| 46      | 55.41  | 1581     | 1577   | Caryophyllene oxide             | 0.60  | 0.05   |
| 47      | 61.503 | 1688     | 1694   | Acorenone B                     | 0.55  | 0.06   |
| 48      | 62.926 | 1714     | 1715   | Pentadecanal                    | 1.82  | 0.12   |
| 49      | 69.609 | 1840     | 1841   | Phytone                         | 1.81  | 0.06   |

Total identified: 98.48

RT = retention time (min); RI(calc) = retention index determined with respect to a homologous series of *n*-alkanes on a ZB-5 ms column; RI(db) = retention indices from the databases. Monoterpene hydrocarbons (Sr. Nos. 1, 4–7, 9, 10), 1.53%; oxygenated monoterpenoids (Sr. Nos. 8, 11, 12, 18, 19, 21, 22, 24), 2.86%; sesquiterpene hydrocarbons (Sr. Nos. 25–28, 30, 31, 33–45), 86.43%; oxygenated sesquiterpenoids (Sr. Nos. 46, 47), 1.15%; others (Sr. Nos. 2, 3, 13–15, 17, 20, 32, 48, 49), 6.50%. Sr. No. 16 MS(EI): 152(7%), 137(37%), 119(27%), 109(100%), 93(16%), 91(28%), 81(33%), 79(26%), 77(21%), 67(91%), 55(23%), 43(71%), 41(30%). Sr. No. 23 MS(EI): 152(2%), 137(18%), 125(29%), 82(15%), 70(18%), 69(13%), 56(16%), 55(20%), 54(26%), 43(19%), 42(17%), 41(21%).
2.1.2. *Emilia sonchifolia*

Hydrodistillation of the leaves of *E. sonchifolia* yielded a pale-yellow essential oil (0.46%). A total of 62 constituents, 97.60% of *E. sonchifolia* volatile oil, were identified by GC-MS. The volatile oil composition is displayed in Table 3 and visualized in Figure 2. The leaf oil was dominated by sesquiterpenoids: γ-himachalene (25.16%), (E)-caryophyllene (15.72%), γ-gurjunene (8.58%), (E)-β-farnesene (3.96%), germacrene D (3.53%), and caryophyllene oxide (3.05%), in addition to the fatty acid palmitic acid (5.24%), and the monoterpene β-pinene (4.87%).

**Table 3. Chemical composition of *Emilia sonchifolia* leaf essential oil.**

| Sr. No. | RT   | RI(calc) | RI(db) | Compound                  | Ave %   | St Dev |
|---------|------|----------|--------|---------------------------|---------|--------|
| 1       | 13.703 | 924      | 921    | Tricyclene                | 1.08    | 0.16   |
| 2       | 16.285 | 971      | 974    | β-Pinene                  | 4.87    | 0.04   |
| 3       | 19.510 | 1027     | 1024   | Limonene                  | 0.25    | 0.01   |
| 4       | 41.005 | 1343     | 1345   | 7-epi-Silphiperfol-5-ene  | 0.07    | 0.00   |
| 5       | 41.195 | 1346     | 1345   | α-Cubebeene               | 0.11    | 0.00   |
| 6       | 41.445 | 1350     | 1350   | α-Longipinene             | 0.04    | 0.00   |
| 7       | 42.700 | 1369     | 1369   | Cyclosativene             | 1.19    | 0.02   |
| 8       | 43.105 | 1375     | 1374   | α-Copaene                 | 1.46    | 0.01   |
| 9       | 43.305 | 1378     | 1374   | Isoledene                 | 0.18    | 0.00   |
| 10      | 43.640 | 1384     | 1387   | β-Bourbonene              | 0.50    | 0.01   |
| 11      | 43.865 | 1387     | 1385   | α-Bourbonene              | 0.08    | 0.00   |
| 12      | 43.985 | 1389     | 1389   | β-Elemene                 | 2.38    | 0.05   |
| 13      | 44.170 | 1392     | 1390   | Sativene                  | 0.04    | 0.01   |
| 14      | 44.895 | 1403     | 1398   | Cyperene                  | 0.06    | 0.00   |
| 15      | 45.140 | 1407     | 1409   | α-Gurjunene               | 0.20    | 0.01   |
| 16      | 45.960 | 1420     | 1417   | (E)-Caryophyllene         | 15.72   | 0.35   |
| 17      | 46.585 | 1430     | 1430   | β-Copaene                 | 0.23    | 0.01   |
| 18      | 46.725 | 1432     | 1432   | trans-α-Bergamotene       | 0.10    | 0.01   |
| 19      | 47.485 | 1444     | 1447   | Isogermacrene D           | 0.05    | 0.00   |
| 20      | 47.580 | 1446     | 1445   | Myltyl-4(12)-ene          | 0.14    | 0.00   |
| 21      | 47.700 | 1448     | 1444   | 6,9-Guaiadiene            | 0.09    | 0.00   |
| 22      | 47.925 | 1452     | 1454   | (E)-β-Farnesene           | 3.96    | 0.03   |
| 23      | 48.205 | 1456     | 1452   | α-Humulene                | 2.97    | 0.03   |
| 24      | 48.490 | 1461     | 1464   | 9-epi-(E)-Caryophyllene   | 0.42    | 0.05   |
| 25      | 48.620 | 1463     | 1465   | cis-Muurola-4(14),5-diene | 0.04    | 0.02   |
| 26      | 49.285 | 1474     | 1476   | Selina-4,11-diene         | 0.49    | 0.03   |
| 27      | 49.410 | 1476     | 1479   | γ-Muurolene               | 0.41    | 0.01   |
| 28      | 49.540 | 1478     | 1475   | γ-Gurjunene               | 8.58    | 0.36   |
| 29      | 49.605 | 1479     | 1483   | α-Amorphene               | 2.81    | 0.57   |
| 30      | 49.820 | 1482     | 1484   | Germacrene D              | 3.53    | 0.10   |
| 31      | 49.945 | 1484     | 1481   | γ-Himachalene             | 25.16   | 0.78   |
| 32      | 50.040 | 1486     | 1487   | Aristolochene             | 0.61    | 0.13   |
| 33      | 50.140 | 1488     | 1492   | δ-Selene                  | 0.52    | 0.03   |
| 34      | 50.305 | 1490     | 1489   | β-Selene                  | 0.81    | 0.02   |
| 35      | 50.455 | 1493     | 1496   | Valencene                 | 0.97    | 0.03   |
| 36      | 50.745 | 1498     | 1498   | α-Selene                  | 1.19    | 0.03   |
| 37      | 50.855 | 1499     | 1500   | α-Muurolene               | 2.11    | 0.04   |
| 38      | 50.965 | 1501     | 1505   | α-Cuprenene               | 0.16    | 0.03   |
| 39      | 51.120 | 1504     | 1505   | (E,E)-α-Farnesene         | 0.38    | 0.05   |
| 40      | 51.375 | 1508     | 1505   | β-Bisabolene              | 0.13    | 0.04   |
| 41      | 51.590 | 1512     | 1509   | Tridecanal                | 0.06    | 0.01   |
| 42      | 51.755 | 1514     | 1513   | γ-Cadinene                | 0.35    | 0.01   |
| 43      | 51.925 | 1517     | 1514   | Cubebol                   | 0.08    | 0.01   |
| 44      | 52.030 | 1519     | 1522   | δ-Cadinene                | 1.29    | 0.04   |
| 45      | 52.140 | 1521     | 1520   | 7-epi-α-Selene            | 0.41    | 0.03   |
Table 3. Cont.

| Sr. No. | RT   | RI(calc) | RI(db) | Compound                | Ave % | St Dev |
|---------|------|----------|--------|-------------------------|-------|--------|
| 46      | 52.255 | 1523     | 1521   | trans-Calamenene         | 0.05  | 0.01   |
| 47      | 52.345 | 1524     | 1521   | β-Sesquiphellandrene     | 0.16  | 0.02   |
| 48      | 53.165 | 1538     | 1537   | α-Cadinene              | 0.10  | 0.01   |
| 49      | 53.445 | 1543     | 1544   | α-Calacorene            | 0.18  | 0.01   |
| 50      | 53.680 | 1544     | 1545   | trans-Cadinene ether    | 0.11  | 0.00   |
| 51      | 53.845 | 1547     | 1548   | α-Elemol                | 0.04  | 0.00   |
| 52      | 54.680 | 1561     | 1564   | β-Calacorene            | 0.05  | 0.01   |
| 53      | 55.580 | 1576     | 1577   | Spathulenol             | 0.32  | 0.02   |
| 54      | 55.890 | 1581     | 1582   | Caryophyllene oxide     | 3.05  | 0.03   |
| 55      | 57.510 | 1609     | 1608   | Humulene epoxide II     | 0.39  | 0.01   |
| 56      | 59.300 | 1641     | 1638   | trans-Muurolool         | 0.21  | 0.01   |
| 57      | 60.055 | 1654     | 1652   | Himachalol              | 0.13  | 0.00   |
| 58      | 59.980 | 1653     | —      | Unidentified            | 0.49  | 0.05   |
| 59      | 60.255 | 1658     | 1658   | neo-Intermedeol         | 0.32  | 0.00   |
| 60      | 69.900 | 1839     | 1841   | Phytone                 | 0.27  | 0.01   |
| 61      | 75.845 | 1960     | 1959   | Palmitic acid           | 5.24  | 1.25   |
| 62      | 80.780 | 2066     | 2071   | Dibenzyl disulfide      | 0.23  | 0.00   |

RT = retention time (min); RI(calc) = retention index determined with respect to a homologous series of \( n \)-alkanes on a ZB-5 ms column; RI(db) = retention indices from the databases. Monoterpenoids and hydrocarbons (Sr. Nos. 1–3), 6.20%; sesquiterpene hydrocarbons (Sr. Nos. 4–40, 42, 44–49, 52), 80.47%; oxygenated sesquiterpenoids (Sr. Nos. 43, 50, 51, 53–57, 59, 60), 5.13%; others (Sr. Nos. 41, 61, 62, 63), 5.80%. Sr. No. 59 MS(EI): 206(4%), 191(5%), 173(3%), 163(7%), 149(9%), 136(14%), 135(13%), 124(22%), 123(32%), 121(19%), 109(100%), 95(39%), 93(28%), 81(33%), 79(28%), 69(24%), 67(46%), 55(30%), 53(18%), 41(47%).

Figure 2. Gas chromatogram of *Emilia sonchifolia* leaf essential oil. Major compounds are indicated by Sr. Nos. from Table 3.
The essential oil of the aerial parts of *E. sonchifolia* from Belagavi, Karnataka, India, has been reported [41]. The essential oil from India was also dominated by sesquiterpene hydrocarbons (67.6%), but with a remarkably different composition. The major components in the essential oil from India were γ-muurolene (32.1%) and (E)-caryophyllene (22.7%). γ-Muurolene was not observed in the essential oil from Nigeria, while γ-himachalene, γ-gurjunene, and germacrene D were not reported in the essential oil from India. Both caryophyllene oxide and palmitic acid were found in the essential oil from India (1.1% and 1.2%, respectively). Apparently, the geographical separation of these two samples has a profound effect on the phytochemistry.

Both *L. egregia* and *E. sonchifolia* essential oils were dominated by sesquiterpene hydrocarbons, with (E)-caryophyllene abundant in both oils. α-Copaene, abundant in *L. egregia* essential oil, was found to be only 1.5% in *E. sonchifolia* oil. Selina-4,11-diene and α-panasinsen were major components in *L. egregia* essential oil but were not detected in the essential oil of *E. sonchifolia*. Likewise, γ-himachalene, abundant in *E. sonchifolia* essential oil, was not detected in the essential oil of *L. egregia*.

### 2.2. Antimicrobial Activity

The leaf essential oils of *L. egregia* and *E. sonchifolia* were screened for antibacterial and antifungal activity against a panel of microorganisms (Table 4). It has been suggested that essential oils having MIC values < 100 µg/mL show very strong activity, those with MIC of 101–500 µg/mL show strong activity, 500 µg/mL < MIC < 1000 µg/mL are moderately active, and above 1000 µg/mL are inactive [42,43]. Thus, the essential oils in this study can be considered strongly active. It is not readily apparent which essential oil components are responsible for the activities; most sesquiterpenes have not been individually screened for antimicrobial activity. However, three of the major components, β-pinene, linalool, and (E)-caryophyllene, were also screened in this work and these compounds showed activities similar to the essential oils themselves. The observed antimicrobial activities are consistent with some of the ethnobotanical uses of these two plants, and based on these results, either *L. egregia* or *E. sonchifolia* essential oil may be recommended for exploration as antibacterial or antifungal agents.

| Organism            | *Lannea egregia* EO | *Emilia sonchifolia* EO | (±)-β-Pinene | (±)-Linalool | (E)-Caryophyllene | Caryophyllene Oxide | Positive Control a |
|---------------------|---------------------|-------------------------|--------------|--------------|-------------------|---------------------|-------------------|
| **Bacteria**        |                     |                         |              |              |                   |                     |                   |
| *Bacillus cereus*   | 312.5               | 625                     | 312.5        | 312.5        | 312.5             | 312.5               | 1.22              |
| *Staphylococcus aureus* | 312.5               | 1250                    | 256.3        | 312.5        | 312.5             | 78.1                | 0.61              |
| *Staphylococcus epidermidis* | 312.5               | 156.3                   | 312.5        | 312.5        | 312.5             | 312.5               | <19.5             |
| *Streptococcus pyogenes* | 625                 | 312.5                   | 625          | 312.5        | 312.5             | 625                 | <19.5             |
| **Molds**           |                     |                         |              |              |                   |                     |                   |
| *Aspergillus fumigatus* | 156.3               | 156.3                   | 156.3        | 156.3        | 156.3             | 156.3               | <19.5             |
| *Aspergillus niger*  | 156.3               | 156.3                   | 78.1         | 1250         | 1250              | 156.3               | 1.56              |
| *Cryptococcus neoformans* | 312.5               | 625                     | 312.5        | 312.5        | 312.5             | 312.5               | 0.78              |
| *Microsporum canis*  | 312.5               | 312.5                   | 312.5        | 312.5        | 312.5             | 312.5               | <19.5             |
| *Microsporum gypseum* | 312.5               | 312.5                   | 312.5        | 312.5        | 312.5             | 312.5               | <19.5             |
| *Trichophyton mentagrophytes* | 156.3               | 312.5                   | 156.3        | 625          | 625               | 156.3               | <19.5             |
| *Trichophyton rubrum* | 312.5               | 312.5                   | 312.5        | 312.5        | 312.5             | 312.5               | <19.5             |
| **Yeast**           |                     |                         |              |              |                   |                     |                   |
| *Candida albicans*   | 156.3               | 312.5                   | 156.3        | 156.3        | 156.3             | 312.5               | 1.56              |

MIC = minimum inhibitory concentration (µg/mL). a Gentamicin was the positive control for bacteria, amphotericin B was the positive control for fungi.
3. Materials and Methods

3.1. Plant Materials

Leaves of *Lannea egregia* and *Emila sonchifolia* were collected directly from source plants in two locations in southwestern states in Nigeria in the month of August, 2019. *Lannea egregia* was collected from Agbegi-Odofin Village, Ikire (Osun State, 7° 22’20.68” N, 4° 11’14.60” E), and *E. sonchifolia* was obtained from the campus of Lagos State University, Ojo (Lagos State, 6° 28’1.20” N, 3° 10’58.80” E). Botanical identification of the two plants was done by Mr. S. A. Odewo at the Herbarium, Forest Research Institute of Nigeria (FRIN), Jericho, Ibadan, Nigeria, where their voucher specimens (Voucher Numbers FHI 112544 and FHI 112546, respectively) have been deposited. The leaves of *L. egregia* and *E. sonchifolia* were manually removed, chopped, air-dried in the laboratory for 7–10 days, pulverized using an electric blender, and stored in polyethene containers until ready for use.

3.2. Isolation of Essential Oils

A sample (450 g each) of *L. egregia* leaves and *E. sonchifolia* leaves was subjected to hydrodistillation thrice in an all-glass Clevenger-type apparatus. Each sample of *L. egregia* and *E. sonchifolia*, respectively, was mixed with water in a ratio of 2:6. The mixture was hydrodistilled for 3–4 h with constant stirring until no additional oil was observed to be distilled. For each plant species, the essential oils were combined, dried over anhydrous sodium sulfate to eliminate traces of water, and stored in a sealed vial under refrigeration (4 °C) prior to analysis.

3.3. Gas Chromatography–Mass Spectrometry

The leaf essential oils of *L. egregia* and *E. sonchifolia* were analyzed using gas chromatography–mass spectrometry (GC-MS) as previously described by us [38]: Shimadzu GCMS-QP2010 Ultra, ZB-5 ms GC column, GC oven temperature 50 °C–260 °C (2 °C/min), 1-µL injection of 5% solution of each essential oil dissolved in CH₂Cl₂ (split mode, 30:1). Each essential oil sample was injected three times. Retention indices (RI) were calculated in comparison with a homologous series of *n*-alkanes. Compounds were identified by comparison of the MS fragmentation and retention indices with those in the databases [44–47] and with matching factors >90%. Quantification was done by external standard method. Calibration curves of representative compounds from each class were drawn and used for quantification.

3.4. Antibacterial and Antifungal Screening

The essential oils were screened for antimicrobial activity against a panel of bacteria (*Bacillus cereus* (ATCC No. 14579), *Staphylococcus aureus* (ATCC No. 29213), and *Staphylococcus epidermidis* (ATCC No. 12228), *Streptococcus pyogenes* (ATCC No. 19615), and fungi (*Aspergillus fumigatus* (ATCC No. 96918), *Aspergillus niger* (ATCC No. 16888), *Cryptococcus neoformans* (ATCC No. 32045), *Microsporum canis* (ATCC No. 11621), *Microsporum gypseum* (ATCC No. 24102), *Trichophyton mentagrophytes* (ATCC No. 18748), *Trichophyton rubrum* (ATCC No. 28188), and *Candida albicans* (ATCC No. 18804)) using the microbroth dilution technique [48,49] as previously reported by us [38]. Serial dilutions of the essential oils (2500, 1250, 625, 312.5, 156.3, 78.1, 39.1, and 19.5 µg/mL) in appropriate media (cation-adjusted Mueller Hinton broth for bacteria and yeast-nitrogen base growth medium for fungi) were carried out in 96-well microtiter plates. Microorganisms (1.5 × 10⁸ CFU/mL for bacteria and 7.5 × 10⁷ CFU/mL for fungi) were added to the 96-well plates, which were incubated for 24 h at 37 °C for bacteria and 35 °C for fungi. Minimum inhibitory concentrations (MIC) were determined to be the lowest concentrations without turbidity. Gentamicin (Sigma-Aldrich, St. Louis, MO) was the positive antibacterial control, amphotericin B (Sigma-Aldrich, St. Louis, MO) was the positive antifungal control, and dimethylsulfoxide (DMSO) was used as the negative control (50 µL DMSO diluted in 50 µL broth medium, and then serially diluted as above). (−)-β-Pinene, (±)-linalool, (E)-caryophyllene, and...
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caryophyllene oxide (Sigma-Aldrich, St. Louis, MO) were also individually screened for activity.

4. Conclusions

The essential oils of Laneea egregia and Emilia sonchifolia, medicinal plants collected from southwestern Nigeria, were found to be rich in sesquiterpenoids. Both essential oils exhibited antibacterial and antifungal activities that are consistent with traditional uses of the plants. While sesquiterpene hydrocarbons were the predominant chemical class in both essential oils, it is not apparent which individual components may be responsible for the antimicrobial activity. It is likely, however, that synergistic effects are also responsible for the activities of the components. Nevertheless, the essential oils may be recommended for further exploration as complementary antimicrobial agents.

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Abbreviations

ATCC: American Type Culture Collection; CFU, colony forming units; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ED\textsubscript{50}, median effective dose; EI, electron impact; FRAP, ferric reducing antioxidant power; GC, gas chromatography; IC\textsubscript{50}, median inhibitory concentration; MIC, minimum inhibitory concentration; MS, mass spectrometry; RI, retention index/retention indices; RT, retention time.

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