Chemical composition, and antibacterial and antioxidant activities of essential oils from *Laggera tomentosa* Sch. Bip. ex Oliv. et Hiern (Asteraceae)

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**Abstract:** *Laggera tomentosa* Sch. Bip. ex Oliv. et Hiern (Asteraceae), an endemic Ethiopian medicinal plant, is traditionally used to treat various ailments. Previously, the chemical constituents of the essential oil (EO) of its leaves and inflorescence were documented. However, no data about the chemical compositions of other parts of the EOs of the plant have been reported to date. Moreover, there are no previous biological activity reports on any parts of the EOs of this plant. Thus, in this study, the EOs were isolated from the stem bark and roots of this plant by hydrodistillation and analyzed using gas chromatography-mass spectrometry to identify their components. In addition, antibacterial potentials of the oils were evaluated using the disc diffusion and minimal inhibitory concentration (MIC) methods. 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydrogen peroxide methods were also employed to assess their antioxidant properties. Oxygenated monoterpenes (71.82% and 77.51%), of which 2,5-dimethoxy-\(p\)-cymene (57.28% and 64.76%) and thymol methyl ether (9.51% and 8.93%) were identified as major components in the EOs of stem bark and roots of *L. tomentosa* and the oils, were the most potent in the DPPH (IC\(_{50}\), 0.33 ± 1.10 and 0.39 ± 0.97 mg/mL) assay, respectively. Moreover, the EOs demonstrated appreciable activity towards the gram+ (*S. aureus* and *B. cereus*) bacteria. Among these oils, the oil of the stem bark showed the greatest activity to the gram+ (MIC = 0.625 mg/mL) bacteria. Therefore, the overall results suggested that the EOs of *L. tomentosa* may be a promising prospect for pharmaceutical, food, and other industrial applications.

**Key words:** *Laggera tomentosa*, essential oils, 2,5-dimethoxy-\(p\)-cymene, thymol methyl ether, antibacterial and antioxidant activities

1. Introduction

The genus *Laggera* Sch. Bip. ex Benth. & Hook., belonging to the Asteraceae (Compositae) plant family, has about 20 species, distributed mainly in sub-Saharan Africa and southeastern Asia [1]. Most of its species are often used in folk and traditional medicines for the treatment of inflammation, jaundice, leukemia, bronchitis, removing phlegm, and bacterial diseases, and their leaves, as well as aerial parts, have been reported to have antiinflammatory, antibacterial, antiviral, antioxidant, hepatoprotective, insecticidal, antifungal, anthelmintic, sedative, antituberculosis, and antidiarrheal properties [2]. The essential oils (EOs) of *Laggera* species have also been reported to exhibit antibacterial, antifungal, antioxidant, larvicidal, and insecticidal activities [3–5]. To date, the chemical constituents of the extracts and/or EOs of only 8 plants of the genus *Laggera* have been reported from 13 countries [2]. However, many bioactive compounds with many-sided activities have been identified from these plants, which has drawn researchers to further investigate *Laggera* species [1,2]. The genus is rich in flavonoids, cyclitols, monoterpens, and sesquiterpenes (eremophilanes and eudesmanes). Flavones, fatty acids, and their derivatives, sterols (stigmasterol and \(\beta\)-sitosterol), phenolic acids like 2-hydroxybenzoic acid, and phytotoxic thymol derivatives, such as 3-hydroxythymoquinone and 5-acetoxy-2-hydroxythymol, have also been reported from extracts of the plants of the genus *Laggera* [1,2,6,7]. 2,5-dimethoxy-\(p\)-cymene, \(\alpha\)-humulene, \(\beta\)-caryophyllene, \(y\)-eudesmol, 10-\(epi\)-\(y\)-eudesmol, and juniper camphor are the most frequent chemical compounds in the EOs of these plants, and the former (oxygenated monoterpene) is the most abundant, as well as the most dominant constituent of many of the EOs [2].

*Laggera tomentosa* Sch. Bip. ex Oliv. et Hiern, an annual fragrant subshrub or bushy herb, is an endemic medicinal plant of Ethiopia. Its leaves are used to treat various diseases, including the common cold, cough, flu, rabies, leech infestation, dysentery, and febrile illness and headaches, while the aerial parts are used for the treatment of toothache, swelling, and

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ringworm, and the roots are used for the treatment of evil eye [2]. The plant is also reported to be used against migraine, as a fumigant, as a treatment for stomachache, and for cleansing milk containers [8]. It is also used to treat skin infections and external parasites [9]. Previously, the chemical constituents of the EO of the leaves and inflorescence of *L. tomentosa* were documented [10]. However, to the best of our knowledge, and according to a literature survey, no data about the chemical compositions of other parts of the EOs of the plant have been reported to date. Moreover, there are no previous biological activity reports on any parts of the EOs of this plant. Therefore, in this paper, various chemical constituents of the stem bark and roots of *L. tomentosa* EOs were isolated and analyzed, respectively, by hydrodistillation and gas chromatography-mass spectrometry (GC-MS). Moreover, the antioxidant and antibacterial potentials of the oils, which may be useful in foods, pharmaceuticals, and other industries, were also assessed and reported.

2. Experimental

2.1. Plant materials

Fresh stem bark and roots of *L. tomentosa* were collected from Daletti, about 26 km southwest of Addis Ababa, Ethiopia, near the town of Alemgena, in April 2019. The plant materials were authenticated by Professor Legesse Negash and a voucher specimen (No. 00L1) was deposited at the Ethiopian National Herbarium of the Addis Ababa University.

2.2. Extraction and isolation of the essential oils

EOs were obtained from the dried and powdered samples (150 g each) of the stem bark and roots of *L. tomentosa* by hydrodistillation using a Clevenger-type apparatus for about 3 h. The obtained oils were separated completely from the condensed water using a separation funnel to afford 0.17% w/w of light yellow and 0.12% w/w of pale yellow EOs from the stem bark and roots of *L. tomentosa*, respectively. The separated oils were then dried by anhydrous Na$_2$SO$_4$ and kept in sterilized dark glass bottles in the refrigerator at 4 °C prior until the analyses.

2.3. GC-MS analysis of the oils

Samples of each of the EOs, extracted as described above, were diluted in n-hexane (1:10) and analyzed using GC-MS (Thermo Fisher Scientific Inc., Waltham, MA, USA), which was equipped with a DB-5 (30 m × 0.25 mm i.d., 0.25-µm film thickness) capillary separation column. GC/MS operating conditions comprised the following: oven temperature, from 50 to 250 °C, at increments of 5 °C/min, and held for 5 min; injector temperature, 220 °C; transfer line, 250 °C; carrier gas, He at 1.0 mL/min constant flow rate; injection volume of the sample, 1 µL; split ratio, 1:20. Ionization of the components of the EO samples was performed in electron impact (70 eV) mode, at an m/z range of 40–450. The identity of the components of the EOs was achieved by visual interpretation and comparing their retention indices (RIs) relative to the n-alkanes of the C$_7$–C$_{50}$ and mass (m/z) spectra with those from the literature [11–13], and NIST and Wiley libraries.

2.4. Antioxidant activities

Antioxidant activities of the EOs of the stem bark and roots of *L. tomentosa* were determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydrogen peroxide (H$_2$O$_2$) assays at final concentrations within the range of 0.03 to 1.0 mg/mL. Ascorbic acid (AA) was used as a positive control in both assays.

2.4.1. 2,2-diphenyl-1-picrylhydrazyl assay

The method described by Lesjak et al. [14], with some modifications, was used to test antioxidant activity of the EOs by DPPH. Three milliliters of standard solution of each of the concentrations, from 0.03 to 1.0 mg/mL, was mixed with 1.0 mL of 90 µM of DPPH solution in methanol (MeOH) to make the test solutions. AA was prepared in same way as the test samples. A mixture of 3 mL of MeOH and 1 mL of DPPH solution was used as the negative control. Each assay was performed 3 times and the prepared samples were incubated in the dark at 37 °C for about 30 min. Next, the absorbance for each was determined at a wavelength of 515 nm using a spectrophotometer. The antioxidant activity of all of the oils was expressed as the percentage of DPPH radical scavenging and the 50% inhibitory concentration (IC$_{50}$) (mg/mL).

2.4.2. Hydrogen peroxide assay

The H$_2$O$_2$ scavenging activity of the EOs was investigated 3 times using the method clearly described by Gülçin [15]. The concentrations, from 0.03 to 1.0 mg/mL, of each of the oils and AA in deionized water were dissolved in 3.4 mL of 0.10 M phosphate buffer at a pH of 7.4 and mixed with H$_2$O$_2$ (40 mM, 0.60 mL) solution. After few minutes, the absorbance of the mixture was determined at 230 nm using a UV/Vis spectrophotometer. The negative control was prepared by replacing the test samples with distilled water. The antioxidant activity of all of test samples was expressed as the percentage of inhibition of the H$_2$O$_2$ and as the IC$_{50}$ (mg/mL).

2.5. Bactericidal activities

Disc diffusion analysis was performed to assess the antibacterial activity of the oils against 2 gram+ (*Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 10876) and 2 gram– (*Klebsiella pneumoniae* ATCC 70063 and *Escherichia coli* ATCC 10031) bacteria. The bacteria were cultured in nutrient broth at 37 °C for 24 hours. Bacteria were removed, and the bacteria suspension was prepared by adjusting the 0.5 MacFarland standard turbidity. A 1 µL of bacterial suspension of each of the bacteria concentration was spotted on a Muller-Hinton agar plate. The oils were tested at final concentrations within the range of 0.03 to 1.0 mg/mL. The plates were incubated at 37 °C for 24 hours. The diameter of the inhibition zones was measured. The experiments were performed in triplicate.
Growing in Cameroon [21]; the whole EOs of the herb (44.2%) [19] and leaves (29.17%) [20] of most dominant chemical compound of many of these oils [2]. For example, it was the predominant compound of the oil and those of the stem bark and roots was similar, as the oil was rich in oxygenated monoterpenes (78%) [10]. When stem bark of L. oloptera this oil were chrysanthenone (57.5%), isochrysanthenone (6.8%), filifolone (5.2%), and (Z)-isogeranic acid (4.5%), which differed from that of the EO isolated from the leaf and inflorescence of the same plant [10]. The dominant components of the 2 compounds were not identified. Surprisingly, the constituents of both oils (especially their dominant compounds) the base peaks of the angelates or tiglates. However, due to the lack of RTs, RIs, and mass (m/z) spectra in the literature, as well as their RIs, were different. From their mass spectra, the base peak m/z 82 of L. tomentosa, were not identified. The mass spectra of the 2 compounds were similar, but their GC retention times (RTs), as well as their RIs, were different. From their mass spectra, the base peak m/z 82 of I and m/z 83 of II may have been due to the base peaks of the angetales or tiglates. However, due to the lack of RTs, RIs, and mass (m/z) spectra in the literature, the 2 compounds were not identified. Surprisingly, the constituents of both oils (especially their dominant compounds) differed from that of the EO isolated from the leaf and inflorescence of the same plant [10]. The dominant components of this oil were chrysanthenone (57.5%), isochrysanthenone (6.8%), filifolone (5.2%), and (Z)-isogeranic acid (4.5%), which were not identified as major compounds from L. pterodonta, L. alata, L. crispata, L. decurrens, L. aurita, L. gracilis, and L. oloptera. These great variations in the compositions of the EOs of the leaf and inflorescence, as well as the roots and stem bark of L. tomentosa and their contents, may have been due to environmental conditions, agronomic management, postharvest technology, storage conditions, genetic factors, and analysis conditions [2]. However, the chemical class of this oil and those of the stem bark and roots was similar, as the oil was rich in oxygenated monoterpenes (78%) [10]. When compared with the EO profiles of the other investigated Laggera species mentioned above, the oxygenated monoterpenes, 2,5-dimethoxy-p-cymene, detected as the dominant compound in the present study was most abundant, as well as the most dominant chemical compound of many of these oils [2]. For example, it was the predominant compound of the EOs of the herb (44.2%) [19] and leaves (29.17%) [20] of L. alata in Nigeria; the leaves (34.1%) of L. alata var. montana growing in Cameroon [21]; the whole L. alata plant (24.4%) in Kenya [5]; the fresh leaves (28.2%) and winged stems
Table 1. Chemical composition of the EOs from the stem barks and roots of *L. tomentosa*.

| No. | RP | Constituents | Area% | SBEO | REO |
|-----|----|--------------|-------|------|-----|
|     |     | Oxygenated monoterpenes |       | 71.82 | 77.51 |
| 1   | 1104 | Linalool | 0.09 | 0.05 |
| 2   | 1126 | *cis*-2-menthenol | 0.05 | tr |
| 3   | 1145 | *trans*-2-menthenol | tr | tr |
| 4   | 1149 | *trans*-verbenol | 0.73 | 0.09 |
| 5   | 1162 | *cis*-chrysanthenol | 0.08 | 0.23 |
| 6   | 1183 | 4-terpineol | 0.06 | tr |
| 7   | 1192 | *p*-cymen-8-ol | 0.07 | 0.05 |
| 8   | 1199 | *cis*-piperitol | tr |     |
| 9   | 1212 | *trans*-piperitol | tr | tr |
| 10  | 1229 | 8,9-dehydrothymol | tr |     |
| 11  | 1231 | *cis*-carveol | 0.08 | tr |
| 12  | 1234 | *cis*-geraniol | 0.05 | tr |
| 13  | 1243 | Thymol methyl ether | 9.51 | 8.93 |
| 14  | 1251 | Carvacrol methyl ether | tr | tr |
| 15  | 1258 | 8,9-dehydrothymol methyl ether | tr |     |
| 16  | 1291 | Thymol | 0.09 | 1.58 |
| 17  | 1297 | Carvacrol | 1.74 | 0.07 |
| 18  | 1308 | 6-ethyl-3,4-dimethylphenol | 0.11 | tr |
| 19  | 1316 | 4-terpinenyl acetate | tr | tr |
| 20  | 1327 | 6-hydroxy-2-tert-butylbenzene | 0.61 | 0.88 |
| 21  | 1333 | *trans*-piperitol acetate | tr | tr |
| 22  | 1339 | Piperitenone | 0.05 | 0.14 |
| 23  | 1358 | Eugenol | 0.20 | 0.31 |
| 24  | 1405 | 3-(2-methoxyphenyl)-3-pentanol | tr |     |
| 25  | 1427 | 1,4-dimethoxy-2-tert-butylbenzene | tr |     |
| 26  | 1434 | 2,5-dimethoxy-phenol | 57.28 | 64.76 |
| 27  | 1442 | *cis*-geranyacetone | 0.06 | 0.26 |
| 28  | 1447 | 2,4-diisopropylphenol | 0.11 | tr |
| 29  | 1453 | Δ8,9-dehydro-4-hydroxythymol dimethyl ether | 0.42 | 0.16 |
| 30  | 1461 | *trans*-geranyacetone | tr |     |
| 31  | 1501 | 3-tert-butyl-4-methoxyphenol | 0.24 |     |
| 32  | 1558 | Methyl-3-(2,6,6-trimethyl-1-cyclohexen-1-yl)propionate | tr |     |
| 33  | 1576 | Neryl (S)-2-methylbutanoate | 0.19 |     |
Table 1. (Continued).

|   |   | Oxygenated sesquiterpenes |   |   |
|---|---|----------------------------|---|---|
| 34 | 1536 | Nerolidol | tr | 0.12 |
| 35 | 1563 | 7-epi-cis-sesquisabinene hydrate | tr |   |
| 36 | 1584 | Humulene epoxide I | 0.09 |   |
| 37 | 1587 | (-)-Spathulenol | 0.18 | 0.06 |
| 38 | 1593 | Caryophyllene oxide | 0.35 | 1.89 |
| 39 | 1623 | Humulene epoxide II | 5.96 | 1.12 |
| 40 | 1641 | Caryophylla-4(12),8(13)-dien-5α-ol | 0.13 | 0.96 |
| 41 | 1648 | Isoaromadendrene epoxide | 2.02 | tr |
| 42 | 1662 | Hinesol | tr | 0.62 |
| 43 | 1672 | Agarospirol | 0.99 | 1.37 |
| 44 | 1684 | -α-bisabolol | tr |   |
| 45 | 1702 | Ledene oxide | 0.14 |   |
| 46 | 1710 | Germacr-4(15),5,10(14)-trien-1α-ol | 0.12 | 0.43 |
| 47 | 1745 | 10-epi-γ-eudesmol acetate | 0.11 | 1.06 |
| 48 | 1753 | α-sinensal | 0.19 | 0.21 |
|   |   | Sesquiterpene hydrocarbons | <1.5 | 4.47 |
| 49 | 1849 | Hexahydrofarnesyl acetone | tr | tr |
| 50 | 1424 | β-caryophyllene |   | 1.17 |
| 51 | 1451 | α-humulene | 1.23 | 2.34 |
| 52 | 1464 | 9-epi-β-caryophyllene | tr |   |
| 53 | 1486 | Germacrene D |   | 0.96 |
| 54 | 1491 | α-selinene | 0.08 |   |
| 55 | 1514 | δ-cadinene | 0.05 | tr |
|   |   | Alkanes | <0.5 | <0.5 |
| 56 | 1400 | Tetradecane | 0.08 | tr |
| 57 | 2000 | Eicosane | tr | 0.09 |
| 58 | 2100 | Heneicosane | tr | tr |
| 59 | 2200 | Docosane | 0.09 | 0.07 |
| 60 | 2300 | Tricosane | 0.07 | tr |
| 61 | 2400 | Tetracosane | tr | tr |
|   |   | Fatty acids | 0.08 | 5.32 |
| 62 | 1279 | Pelargonic acid | 0.08 |   |
| 63 | 1569 | Lauric acid |   | 0.14 |
| 64 | 1966 | Palmitic acid |   | 5.18 |
(50.5%) of *L. pterodonta* in Cameroon [22]; the leaves (36.75%) of *L. pterodonta* in China [23]; the leaves (43.3%) of *L. pterodonta* in Côte d’Ivoire [24]; the leaves (27.7%) of *L. aurita* in Burkina-Faso [25]; the leaves (33.4%) of *L. gracilis*, and the roots (67.8%) and stems (36.5%) of *L. oloptera* in Cameroon [21]; and the aerial parts (43.2%) [26] and (32.2%) [27], leaves (22.28%), flowers at bud stage (48.99%), flowers at full bloom/shattering stage (64.01%), stems (62.81%), and roots (75.55%) [28] of *L. crispata* in India. However, the EOs of the studied *Laggera* species showed different chemical classes of compounds [2]. These variations may have been due to the factors mentioned earlier.
3.2. Antioxidant activities of the essential oils

Assessed EOs of *L. tomentosa* were able to reduce stable purple DPPH radical to the yellow DPPH-H with IC$_{50}$ values in the range of 0.33 ± 1.10 to 0.43 ± 1.12 mg/mL (Table 2). As shown in Table 2, the EO of the stem bark of *L. tomentosa* had slightly higher radical scavenging activity (lowest IC$_{50}$ value, 0.33 ± 1.10 mg/mL) than the root EO (IC$_{50}$ value, 0.39 ± 0.97). When compared with the H$_2$O$_2$ assay, the EOs exhibited better antioxidant activities in DPPH than that in H$_2$O$_2$ (Table 2). The overall inhibitory activity of the EOs against H$_2$O$_2$ can be presented in the following order: AA (0.14 ± 0.56 mg/mL) > stem barks EO (0.36 ± 1.05 mg/mL) > roots EO (0.43 ± 1.12 mg/mL). However, the oils showed potent antioxidant activities in all of the tested concentrations when compared with AA. This observed activity of the oils was most likely related to their dominant constituents, such as 2,5-dimethoxy-p-cymene and thymol methyl ether (oxygated monoterpenes). Linalool, terpineol, δ-cadinene, and phenolic chemical compounds, such as eugenol, thymol, and carvacrol, which were also present in the oils, may also have had a significant contribution to the antioxidant activity of the oils [29–31].

To the best of our knowledge, and according to literature survey, there have been no previous antioxidant activity reports for the EOs and extracts of any parts of *L. tomentosa*. However, among the 20 *Laggera* species, antioxidant activities of the EOs of the aerial parts of only 2 plants, namely *L. decurrens* and *L. aurita*, were reported. For the oils of these plants, good antioxidant activity values that were slightly higher than the current results, were reported [3,32]. At a concentration of 1 mg/mL, the EO of *L. decurrens*, with high content of 3-methoxythymoquinone (28.1%), showed strong antioxidant activity (93.1%), which was comparable to that of AA (96%). However, the EO of *L. aurita*, with a dominant compound of hexadecanoic acid (21.2%), showed moderate antioxidant activity (81.40 ± 1.98% at 4 mg/mL) in the DPPH assay.

3.3. Bactericidal activities of the essential oils

Results from the disc diffusion tests for the in vitro antibacterial activity of the EOs of the roots and stem bark of *L. tomentosa* are presented in Table 3. These oils displayed slightly variable activity towards the investigated bacterial strains. As shown in Table 3, the DIZs of the studied oils ranged from 2.79 ± 0.97 to 9.81 ± 0.70 mm for the different (0.10, 0.25, and 0.5 mg/mL) concentrations tested. The gram− pathogen, *E. coli*, was the most resistant, while the gram+ bacterium, *S. aureus*, was the most sensitive microorganism to the EOs. The DIZs of the stem bark EO were 9.81 ± 0.70 and 8.15 ± 1.32 mm, while those of the root EO were 8.47 ± 0.93 and 7.42 ± 1.26 mm for *S. aureus* and *B. cereus*, respectively, at a concentration of 0.5 mg/mL. The results indicated the susceptibility of the gram− bacteria to the EOs. However, the oils showed no inhibitory activity towards *E. coli* at any of the concentrations and *K. pneumoniae* at 0.10 and 0.25 mg/mL (Table 3). The results of the MIC values (Table 4) also indicated that the oils were more sensitive against gram+ microorganisms than gram− ones, which were similar to the results obtained by the disc diffusion method. The EO of the stem bark showed the greatest activity against the gram+ (MIC = 0.625 mg/mL) bacteria, while that of the roots demonstrated moderate activity (MIC = 1.25 mg/mL) against the same bacterial strains. However, both oils were least active against the gram− bacteria (MIC = 2.5 mg/mL bacteria).

These results were the first antibacterial activity results for *L. tomentosa* EOs. From the overall results, it can be concluded that if the concentration of these oils increases, their inhibitory effect against the bacteria will also increase. The high activity of the oils of *L. tomentosa* (especially the stem bark oil) against the gram+ (S. aureus and B. cereus) bacteria was most likely due to the chemical compounds having antibacterial properties in them. EOs that contain 2,5-dimethoxy-p-cymene as a major component of the oil have been reported to have antibacterial properties [33]. Therefore, the bactericidal activity of the *L. tomentosa* oils may have mainly been related to this compound. Thymol methyl ether, which represented 9.51% and 8.93%, respectively, of the oils of the stem bark and roots of *L. tomentosa*, and a major constituent of the stem bark oil, humulene epoxide II (5.96%), may also have had a significant contribution to the antibacterial activity of the oils. Other compounds, including terpineol, δ-cadinene, α-humulene, caryophyllene oxide, eugenol, thymol, carvacrol, linalool, and geraniol, which were also present in the oils, have been reported to have antibacterial activities [29,31,34–36], and may
also have collectively had a remarkable contribution to the bactericidal activities of the oils. According to a literature survey, a detailed list of the in vitro bactericidal activities against a broad set of gram+ and gram– bacteria for different EOs of plants of the genus *Laggera* was reported [2,3,32]. The antibacterial activity results of the oils of these plants, and those obtained in the current study, showed variations that may have been due to factors such as the composition and concentration of the EOs, and the type and concentration of the target organisms [37,38], in addition to the factors stated earlier in Section 3.1. However, among the investigated oils of these plants, the EO of *L. decurrens*, which has high 3-methoxythymoquinone (28.1%) content, showed the highest antibacterial activity (MIC = 0.13 mg/mL) against *S. aureus* [32]. The EO of *L. pterodonta*, which has high 2,5-dimethoxy-p-cymene (36.75%) content, displayed moderate activity against some gram– bacteria, such as *Enterobacter aerogenes* (MIC = 0.13 mg/mL), *Enterococcus faecalis* (MIC = 0.13 mg/mL), *Salmonella typhi* (MIC = 0.5 mg/mL), and *Pseudomonas aeruginosa* (MIC = 0.5 mg/mL) [23]. The EO of *L. crispata*, which has a major compound of 2,5-dimethoxy-p-cymene (43.2%), also demonstrated

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### Table 2. Antioxidant effect of standard and EOs from *L. tomentosa* by DPPH and H$_2$O$_2$ assays.

| Assay             | *L. tomentosa* EOs | Standard |
|-------------------|--------------------|----------|
|                   | REO                | SBEO     | AA       |
| DPPH (IC$_{50}$, mg/mL) | 0.39 ± 0.97 | 0.33 ± 1.10 | 0.09 ± 0.89 |
| H$_2$O$_2$ (IC$_{50}$, mg/mL) | 0.43 ± 1.12 | 0.36 ± 1.05 | 0.14 ± 0.56 |

Values are expressed as the mean ± SD (n = 3); *IC$_{50}$*: 50% inhibitory concentration; DPPH: 2,2-diphenyl-1-picrylhydrazine, H$_2$O$_2$: hydrogen peroxide, AA: ascorbic acid (positive control).

### Table 3. Antibacterial activity of the investigated EOs of *L. tomentosa*.

| EOs      | Concentration (mg/mL) | DIZ (mm) | Gram+ | Gram– |
|----------|-----------------------|----------|-------|-------|
|          |                       |          | *S. aureus* | *B. cereus* | *E. coli* | *K. pneumoniae* |
| REO      | 0.10                  | 2.92 ± 1.63 | 2.79 ± 0.97 | NI | NI |
|          | 0.25                  | 5.33 ± 1.21 | 5.25 ± 0.88 | NI | NI |
|          | 0.50                  | 8.47 ± 0.93 | 7.42 ± 1.26 | NI | 1.61 ± 1.59 |
| SBOE     | 0.10                  | 3.45 ± 1.41 | 2.58 ± 1.13 | NI | NI |
|          | 0.25                  | 5.84 ± 1.02 | 5.04 ± 0.94 | NI | NI |
|          | 0.50                  | 9.81 ± 0.70 | 8.15 ± 1.32 | NI | 2.34 ± 1.16 |

Results are presented as the mean ± SD (n = 3), DIZ: diameter of inhibition zones, NI: no inhibition.

### Table 4. Minimal inhibitory concentrations (MICs) of the EOs isolated from the stem barks and roots of *L. tomentosa*.

| EOs     | MIC (mg/mL) | Gram+ | Gram– |
|---------|-------------|-------|-------|
|         |             | *S. aureus* | *B. cereus* | *E. coli* | *K. pneumoniae* |
| REO     | 1.25        | 1.25 | 2.5 | 2.5 |
| SBEO    | 0.625       | 0.625 | 2.5 | 2.5 |
moderate antibacterial activity against *Klebsiella pneumoniae* (ZI = 6 mm) and *Staphylococcus aureus* (ZI = 8 mm), but no activity against *E. coli* [26].

### 4. Conclusion

The compositions of the EOs of endemic Ethiopian *L. tomentosa* stem bark and roots were analyzed and their antioxidant and bactericidal activities were investigated for the first time herein. The oxygenated monoterpenes, 2,5-dimethoxy-\(\beta\)-cymene, as well as thymol methyl ether, were identified as the dominant compounds of both oils, which differed from the previously reported chemical profile of the EOs obtained from the leaves and inflorescence of the same plant. Furthermore, the 2 oils demonstrated strong DPPH and \(\text{H}_{2}\text{O}_{2}\) scavenging activities and bactericidal activity against the gram+ (*S. aureus* and *B. cereus*) bacteria, which might have been due to their high oxygenated monoterpene content. Thus, these activities suggest that the oils may be a promising prospect for pharmaceutical, food, and other industrial applications.

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