Antibacterial and Antioxidant Activity of *Cirsium englerianum* (Asteraceae), an Endemic Plant to Ethiopia

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**Abstract**

**Background and objectives:** *Cirsium englerianum* (Asteraceae) is an endemic medicinal plant to Ethiopia. It is used to treat skin infection, snake bite and cough. The aim of the present study was to evaluate the bioactivity of root extracts of *C. englerianum*. **Methods:** Phytochemical screening tests were employed by standard protocols to identify the phytocemicals. Column chromatographic separation was used to isolate the compounds and the spectroscopic techniques (IR, NMR and ESMS) were used to elucidate structures of the compounds. Disc diffusion technique was used to evaluate antibacterial activity. In vitro antioxidant activity was assessed by 1, 1-diphenyl-2-picrylhydrazyl (DPPH), and phosphomolybdenum assays. The total flavonoids content was determined by aluminium chloride method. **Results:** Phytochemical screening tests revealed presence of alkaloids, steroids, terpenoids, tannins, and flavonoids in the acetone root extract. Column chromatographic separation of chloroform/methanol (1:1) extract offered stigmasterol (1), and stigmasteryl stearate (2). The acetone extract was potentially effective against the tested bacterial strains (*Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*) at all concentrations (25, 50 and 100 mg/mL). In vitro antioxidant activity attributed that the acetone extract showed DPPH scavenging (IC₅₀ = 154.44±74 µg/mL) and total antioxidant activity (8.24±0.9 mg of ascorbic acid equivalent per gram of dry extract). The total flavonoid content was observed in the range from 5.88 ±0.21 to 8.24±0.9 milligrams of catechin equivalents per gram of dry plant extract. **Conclusion:** Stigmasterol and stigmasteryl stearate were reported for the first time from this plant. The results proved that acetone extract exhibited potential antibacterial and antioxidant activity which correlated with inhibition zone diameter, and free radical scavenging activity.

**Keywords:** antibacterial activity; antioxidant activity; *Cirsium englerianum*; phytochemical

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**Introduction**

The emergence of drug resistant human pathogenic organisms against the currently used chemotherapeutic agents [1,2], and the toxicity of synthetic antioxidants [3], are on the increase and require global attention. Natural source antioxidants like phenolic compounds have been playing an important role in human health due to their broad biological activity and high efficiency to trap/scavenge free radicals [4]. Similarly, the resistance of several bacteria to certain antibiotics leads to the search for new active molecules with a broad spectrum of actions [5]. Indigenous medicinal plants can be sources of new bioactive compounds to fight antibiotic resistance. Consequently, screening indigenous medicinal plants for antibacterial and antioxidant activity as

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well as determination of the structure of the active chemical constituents may help in sustaining the use of natural products as sources of new antibacterial and antioxidant agents [6].

*Cirsium englerianum* O. Hoffm (Asteraceae) is an endemic medicinal plant to Ethiopia. It is used to treat skin infections, haematuria, snake bite, sterility, anthrax, respiratory tract problems, stomach-ache and headache, scabies, influenza virus, and cough and diarrhoea for livestock’s [7,8]. However, despite the broad ethno-medicinal uses, the phytochemicals, and bioactivity of this plant have not been investigated yet. We herein report chemical constituents, antibacterial and antioxidant activities from the root extracts of the species first time.

**Material and Methods**

**Ethical considerations**
The Ethics Committee of Wolaita Sodo University approved this research with the code of RCS/CNS-12/18.

**Instruments**

Electron ionization mass spectrometry (EI-MS) and high-resolution electron ionization mass spectrometry (HREIMS) were determined by a Triple TOF 5600 LC/MS/MS system (AB SCIEX, Framingham, MA, USA) with electron ionization (EI) method in the positive mode. Melting point was determined with Mettler Toledo Model FP62 machine. IR spectra was measured by KBr pellets on Perk-Elmer BX Infrared Spectrometer (USA) in the range 400-4000 cm\(^{-1}\). \(^1\)H and \(^13\)C NMR spectra were recorded on a Bruker Instrument (Darmstadt, Germany) using CDC13 (δ 7.26 for \(^1\)H and δ 77.0 for \(^13\)C) as the solvent with TMS as the internal standard.

**Chemicals**

DPPH (1, 1-diphenyl-2-picrylhydrazyl), sodium phosphate, ammonium molybdate, butylated hydroxytoluene (BHT), L-ascorbic acid (AA), catechin and aluminium chloride were purchased from Sigma-Aldrich (USA). Other solvents and reagents used in the experiment were of analytical grade. Analytical TLC plate with silica gel 60 F254 TLC (Merck, Germany) was used to determine the TLC profile. Column chromatographic separation was performed on silica gel (60-120 mesh).

**Plant material**
The roots of *Cirsium englerianum* were collected from Wolaita Zone, Sodo Zuriya woreda, located around 330 km from Addis Ababa, Ethiopia, in October 2019. The collected plant specimen was identified in comparison with the specimens at the Herbarium in the Department of Biology, Addis Ababa University, Ethiopia, given voucher code (CeMA-2/19). At room temperature, air-dried roots were chopped into small pieces and powdered by grinding mill.

**Phytochemical screening**

In order to investigate phytochemicals such as alkaloids, steroids, terpenoids, tannins, and flavonoids in the crude extracts, standard methods were employed [9,10] with minor modifications. The results were reported as (+) for presence and (-) for absence.

**Extraction and isolation**

Root powder (300 g) was extracted with chloroform/methanol (1:1), acetone, and methanol (1.5 L) separately, three times for 24 h while shaking by electronic shaker at room temperature. The solutions were filtered by suction, and concentrated by vacuum rotary evaporator at a reduced temperature of 40 °C. Chloroform/methanol (1:1) extract (21 g) was subjected to column chromatography (CC), and eluted with an increasing gradient of 5% ethyl acetate in n-hexane, then 1-10 % methanol in ethyl acetate. A total of 78 fractions (25 mL each) were collected.

**Antibacterial activity**

The antibacterial activity was evaluated by disc diffusion method as previously described [11], with minor modifications, using four human pathogenic bacterial strains, such as two Gram-positive (*Bacillus cereus NCTC 7464* and *Staphylococcus aureus Staphylococcus aureus 25923*), and two Gram-negative (*Escherichia coli 20922* and *Salmonella typhi DT104*). The microorganisms were obtained from Ethiopian public health institution and deposited in the

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Microbiology Laboratory stocks in Wolaita Sodo University Teaching Hospital. The bacterial strains were grown in nutrient agar plates at 37 °C. The acetone, chloroform/methanol (1:1) and methanol extracts were solubilized in 10% dimethyl sulfoxide (DMSO). The extracts were prepared at the concentrations of 25, 50 and 100 mg/mL. Petri dishes containing Mueller Hinton agar were swabbed with Mueller Hinton agar were swabbed at room temperature for 30 min in dark. The prepared samples were incubated at 95 °C for 90 min, then cooled to room temperature. The absorbance was measured at 695 nm by spectrophotometry. Three mL of methanol was used as blank. The total antioxidant activity was expressed as milligrams of ascorbic acid equivalent/gram of dried extract (mg AAE/g) according to the calibration curve.

Total flavonoids content
Total flavonoids content was evaluated using spectrophotometric method as described before [14]. The plant extract (1 mL, 1 mg/mL) was diluted with 1.25 mL distilled water, and 75 μL 5% NaNO₂ was added to the mixture. After 6 min, 150 μL 10% AlCl₃ was added, and after another 5 min, 1 mL 1M NaOH was added to the mixture. Then, the absorbance of the pink colour mixture was was determined at 510 nm versus water as the blank. A standard curve was developed with 5 to 120 μg/mL of catechin. The results were expressed in milligrams of catechin equivalents per gram of dry plant extract (mg CE/g of extract). Three readings were taken for each sample and the results were averaged.

Statistical analysis
The antibacterial and antioxidant activity tests were presented as mean±SD of three measurements. The comparisons between the control group and the test groups (plant extracts) were statistically performed using one way analysis of variance (ANOVA). Statistical significance p-level was selected at a 5% significance.

Results and Discussion
Root extracts of C. englerianum in different solvent system were subjected to various qualitative tests such as alkaloids, flavonoids, steroids, tannins, and terpenoids. The results were attributed the presence alkaloids, triterpenoids, flavonoids, tannins, and steroids in the acetone extract. However, tannins and terpenoids were not detected in the chloroform/methanol (1:1) extract and the methanol extract (Table 1). Extraction of the root with chloroform/methanol (1:1), acetone, and methanol offered 31.8 g
(10.6%), 29.5 g (9.8%), and 21 g (7%) yield, respectively. Silica gel column chromatography furnished compounds 1 and 2 reported for the first time from the species. The detailed characterizations of these compounds are presented below.

Compound 1 (a white amorphous powder) was eluted with 15% ethyl acetate in n-hexane. Its melting point was 170-176 °C. IR spectra analysis showed broad bands at 3360 cm⁻¹, characteristic of O-H stretching.

The absorption peak around 3000 cm⁻¹ was due to cyclic olefinic –HC=CH-, peaks at 2936 cm⁻¹ and 2865 cm⁻¹ were assigned for SP³ C-H stretch, at 1462 cm⁻¹ was a bending frequency for cyclic (CH₂)n, and at 1382 cm⁻¹ for –CH₂ (CH₃)2γ. In addition, the absorption frequency at 1056 cm⁻¹ was indicative of cycloalkane. ¹H-NMR showed the presence of two methyl singlets at δ 0.67 and 0.69; three methyl doublets appeared at δ 0.79, 0.83, and 0.86; and a methyl triplet at δ 0.91. In addition, the protons at δ 5.00, 5.14, and 5.34 suggest the presence of three protons, which were a trisubstituted and a disubstituted olefinic bond. Moreover, there was a peak at δ 3.52 of a sterol moiety of H-3 as a triplet of a doublet of doublets. ¹³C-NMR spectra were well resolved. Briefly, 21 carbons, at δ 140.8 and 121.7 for C-5 and C-6 double bond, at δ 71.8 for C-3 β-hydroxyl group, at δ 19.4 and 11.9 for angular methyl carbon atoms for C-19 and C-18, were assigned respectively. In addition, DEPT-135 experiment revealed the presence of three quaternary carbons including olefinic carbon at 140.8 ppm, elven methine, nine methylene, and six methyl carbons. Based on the spectroscopic data as well as comparison with literature [15,16], the isolated compound 1 was identical to stigmasterol (Figure 1) and reported for the first time from this plant.

Compound 2 was obtained as white amorphous needles. Its melting point was 101-106 °C. IR spectra showed the absorption band around 3000 cm⁻¹ because of cyclic olefinic –HC=CH- moiety. The absorption peaks at 2926 and 2853 cm⁻¹ were assigned to SP³ -C-H stretch, at 1739 cm⁻¹ to carbonyl carbon -C=O stretching, at 1462 cm⁻¹ to a bending frequency of cyclic (CH₃)n, and at 1382 cm⁻¹ for –CH₂ (CH₃)2γ. The absorption frequency at 1056 cm⁻¹ signified the presence of cycloalkane. Electrospray mass spectrometry (ESMS): m/z 678.9988 [M+H]⁺. The calculated m/z value =678.6315, suggested the molecular formula C₃₀H₅₂O₃. ¹H-NMR spectra showed the presence of two methyl singlets at δ 0.69 and 0.71, and three methyl doublets at δ 0.82, 0.84, and 0.88; and a methyl triplet at δ 0.69. A peak at δ 3.54 was assigned to the H-3 of a sterol moiety.

Peaks at δ 5.08, 5.17, and 5.30 suggests a trisubstituted and a disubstituted olefinic bond. Moreover, peaks at δ 2.31, 2.18, 1.64, and 1.14– 1.21 were clearly correlated with long-chain aliphatic groups of fatty acid moieties, and peak at δ 0.90 suggested fatty acid terminal methyl group. ¹³C-NMR spectra revealed the presence of 47 carbons, and vinylic carbons at δ 121.7 (C-6), 129.2 (C-22), and 138.3 (C-23), and de-shielded methine carbons at 71.9 (C-3). Moreover, Dept-135 spectra clearly showed the presence of twenty-five methylene carbons, of which, nine methylenes for stigmasterol, and the remaining methylene groups resonated around δ 25.2-29.6, suggesting the presence of long-chain aliphatic groups of fatty acid moieties. In addition, it revealed the presence of three quaternary carbons including olefinic carbon at δ 140.7, and seven methyl carbons at δ 12.5 (C-18), 19.8(C-19), 21.1 (C-21), 18.98(C-21), 11.86(C-21), and 14.1(C-29).

Table 1. Phytochemical screening of Cirsium englerianum root extracts

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Reagents</th>
<th>Plant extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Mayer reagent</td>
<td>ChME + + +</td>
</tr>
<tr>
<td>Steroids</td>
<td>CHCl₃ and conc. H₂SO₄</td>
<td>+ + +</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>CHCl₃ and conc. H₂SO₄</td>
<td>- + -</td>
</tr>
<tr>
<td>Tannins</td>
<td>FeCl₃</td>
<td>- + -</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Dilute NH₃ solution</td>
<td>+ + +</td>
</tr>
</tbody>
</table>
ChME: chloroform/methanol (1:1) extract; AE: acetone extract; ME: methanol extract

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Based on the spectra and the literature [17], the isolated compound (2) was identical to stigmasteryl stearate (Figure 1), reported for the first time from this plant species. Stigmasterol (1): white amorphous powder; melting point (170-176 °C); IR (cm⁻¹), 3360, 3000, 2936, 2865, 1462, 1382; ¹H-NMR (400 MHz, CDCl₃, δ in ppm, and J values in Hz), 0.69 (3H, s), 0.69 (3H, d, J=6.6 Hz), 0.87 (3H, d, J = 6.6 Hz), 0.82 (3H, m), 0.86 (3H, s), 0.91 (3H, t), 3.52 (1H, m), 5.00 (1H, dd, J = 8.5, 16.0 Hz), 5.14 (1H, dd, J = 8.5, 16.0 Hz), 5.34 (1H, d, J = 4.2 Hz).¹³C-NMR (400 MHz, CDCl₃, δ in ppm), 37.3 (C-1), 28.9 (C-2), 71.8 (C-3), 39.7 (C-4), 140.8 (C-5), 121.7 (C-6), 31.7 (C-7), 31.9 (C-8), 51.2 (C-9), 36.5 (C-10), 21.1 (C-11), 39.8 (-12), 42.3 (C-13), 56.9 (C-14), 24.3 (C-15), 25.4 (C-16), 56.7 (C-17), 19.4 (C-18), 18.9 (C-19), 40.5 (C-20), 21.2 (C-21), 129.3 (C-22), 138.3 (C-23), 55.9 (C-24), 50.2 (C-25), 12.0 (C-26), 12.1 (C-27), 24.49 (C-28), 11.9 (C-29).

Stigmasteryl stearate (2): white amorphous needles; melting point (101-106 °C); IR (cm⁻¹), 3000, 2926, 2853, 1739, 1462, 1382, 105; ¹H-NMR (400 MHz, CDCl₃, δ in ppm, and J values in Hz), 0.69 (3H, d, J=6.6 Hz), 0.71 (3H, s), 0.82 (3H, m), 0.87 (3H, d, J = 6.6 Hz), 0.88 (3H, s), 0.90 (3H, t, J = 6.5 Hz) 0.91 (3H, d), 1.25 (m, 36H) 1.29 (m, 2H), 1.62 (2H, m), 2.31 (2H, d, J = 7.8 Hz), 4.12 (1H, m), 5.17 (1H, dd, J = 8.5, 16.0 Hz), 5.02 (1H, dd, J = 8.5, 16.0 Hz), 5.30 (1H, d, J = 4.2 Hz).¹³C-NMR (300 MHz, CDCl₃, δ in ppm), 37.3 (C-1), 31.9 (C-2), 71.9 (C-3), 42.2 (C-4), 140.7 (C-5), 121.7 (C-6), 31.8 (C-7), 31.9 (C-8), 51.2 (C-9), 36.5 (C-10), 21.2 (C-11), 39.8 (C-12), 42.3 (C-13), 56.8 (C-14), 22.5 (C-15), 29.1 (C-16), 56.9 (C-17), 19.4 (C-18), 19.0 (C-19), 40.5 (C-20), 18.9 (C-21), 129.3 (C-22), 138.3 (C-23), 55.2 (C-24), 45.5 (C-25), 12.0 (C-26), 12.1 (C-27), 24.4 (C-28), 11.9 (C-29), 173.5 (C-1’), 34.0 (C-2’), 39.7 (C-3’), 31.6 (C-4’), 29.2-29.9 (5’-12’), 26.1 (C-13’), 25.4 (C-14’), 24.7 (C-15’), 24.3 (C-16’), 23.1 (C-17’), 14.1 (C-18’). Electrospray mass spectrometry (ESMS): m/z = 678.9988 [M+H]+. The calculated m/z = 678.6315; Molecular formula, C₇₃H₁₁₂O₂.

The root extracts of *C. englerianum* were evaluated in vitro for antibacterial activity via the disc diffusion method. The result showed that the maximum zone of inhibition ranged from 6.7-19.6 mm for acetone extract against *B. cereus* strain in all concentration, which was comparable at the concentration 100 mg/mL with standard chloramphenicol (22.6±0.57 mm). The ANOVA analysis clearly showed that the acetone extract at the concentration of 100 mg/mL had no significant effect (p<0.05) on *Bacillus cereus* and *Staphylococcus aureus* on the level of inhibition with respect to the reference antibiotic (chloramphenicol); however, there was a significant difference in all crude extracts at the concentration below 100 mg/mL with respect to the broad-spectrum antibiotic. On the contrary, the methanol extract showed no effect in *S. aureus* in any of the concentrations (Table 2).

The reaction between DPPH radicals and antioxidants has been employed as a measure of antioxidant activity, and a high percentage DPPH radical scavenging of a compound indicates excellent activity [18]. In the present study, the acetone extract showed relatively greater radical scavenging activity with IC₅₀ value of 154.44±74 μg/mL, compared to those methanol and chloroform/methanol (1:1) extracts in exhibiting IC₅₀ values of 177.37±12.34 and 183.92±10.12 μg/mL, respectively (Table 3).
Comparing the DPPH scavenging potential of extracts with synthetic antioxidants, it is found that the extracts showed potential scavenging activity with respect to synthetic BHT and AA (Table 3).

Table 2. Antibacterial activity and inhibition zones (mm±SD) of Cirsium englerianum root extracts

<table>
<thead>
<tr>
<th>Extracts (mg/mL)</th>
<th>ME</th>
<th>ChME</th>
<th>CHL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus cereus</td>
<td>25</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>6.7±0.68</td>
<td>13.4±0.93</td>
<td>19.6±0.51*</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>n</td>
<td>9±0.15</td>
<td>18.3±0.58*</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>n</td>
<td>7±0.59</td>
<td>12.4±0.35</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>n</td>
<td>7.5±0.40</td>
<td>11.5±0.51</td>
</tr>
</tbody>
</table>

Values of IC50 are the average result of three independent assays expressed as mean±SD. ChME: chloroform/methanol (1:1) extract; AE: acetone extract; ME: methanol extract. *no significant difference (p>0.5)

Total antioxidant potential of acetone, methanol, and chloroform/methanol (1:1) root extracts at concentrations of 0.5 and 1.0 mg/mL were measured spectrophotometrically by phosphomolybdenum method.

The method is based on the reduction of Mo (IV) to Mo (V) by the sample analyte ensuing formation of greenish phosphate/Mo (V) compounds with maximum absorption at 695 nm. The antioxidant capacities of C. englerianum extracts were found to be in the order of acetone extract> methanol>chloroform/methanol (1:1) extract (Figure 2).

Table 3. IC50 values of DPPH scavenging activity of Cirsium englerianum root extracts

<table>
<thead>
<tr>
<th>Extracts</th>
<th>IC50 (µg/mL±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChME</td>
<td>183.92±10.12</td>
</tr>
<tr>
<td>AE</td>
<td>154.44±0.00</td>
</tr>
<tr>
<td>ME</td>
<td>177.37±12.34</td>
</tr>
<tr>
<td>BHT</td>
<td>34.27±±2.24</td>
</tr>
<tr>
<td>AA</td>
<td>23.94±1.33</td>
</tr>
</tbody>
</table>

Values of IC50 are the average result of three independent assays expressed as mean±SD. ChME: chloroform/methanol (1:1) extract; AE: acetone extract; ME: methanol extract, AA: ascorbic acid; a: no significant difference (p>0.5)

Total flavonoids content of the extracts was expressed as catechin equivalent, ranging from 6.10±0.52 to 25.75±7.42 mg catechin equivalent/g extract (Table 4). The more considerable flavonoids content was achieved in the acetone extract with 25.75±7.42 mg CE/L. There was no significant difference (p<0.05) in total flavonoids content among methanol, acetone and chloroform in methanol (1:1) extract.

Table 4. Total flavonoids content of Cirsium englerianum root extracts

<table>
<thead>
<tr>
<th>Extract</th>
<th>Total flavonoids content (mg CE/g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE</td>
<td>8.24±0.90</td>
</tr>
<tr>
<td>ME</td>
<td>5.88±0.21</td>
</tr>
<tr>
<td>ChME</td>
<td>7.82±3.38</td>
</tr>
</tbody>
</table>

ChME: chloroform/methanol (1:1) extract; AE: acetone extract; ME: methanol extract

Preliminary phytochemical screening of extracts of the present study revealed that the major chemical constituents were phenolic compounds and steroids in all solvent systems. We reported the known plant steroids stigmasteryl (1) and its derivative stigmasteryl stearate (2) from this plant for the first time. In vitro antibacterial activity attributed that the acetone extract showed potential antibacterial activity against all tested human isolated photogenic bacterial strains. This might be correlated with the phenolic compounds such as flavonoids, alkaloids and tannins in the acetone extract. While comparing the efficacy of extracts against Gram positive and Gram-negative bacteria...
bacterial strains, it was observed that the extracts were more effective on the Gram-positive bacterial strains. Conversely, *Staphylococcus aureus* resistant to various concentrations of the methanol extract. The observed comparative resistant of Gram-negative bacterial strains to the current extracts might be due to the reason that, in general, the Gram-negative bacteria are more resistant than the Gram-positive ones [20].

The free radical scavenging activity is evaluated by the ability of antioxidants present in an extract to reduce purple coloured 1, 2-diphenyl 2-picryl hydrazyl (DPPH) radical to yellow coloured diphenylpicrylhydrazine [18]. The smaller decrease in absorbance of the extracts indicated a lower rate of oxidation of DPPH and higher antioxidant activity in the presence of extract. Our finding revealed the acetone extracts exhibited high antioxidant activity compared to the extracts in methanol and chloroform/methanol (1:1), respectively. A higher DPPH radical-scavenging activity is associated with a lower IC50 value [21]. Therefore, the acetone extract had the highest DPPH reducing activity based on its relatively low IC50 values (Table 3). A positive result by the acetone and methanol extracts in this study indicated that they contained antioxidants that can scavenge free radicals. Methanol extract was followed by the acetone extract, and the chloroform/methanol (1:1) extract showed the least DPPH reducing activity due to its relatively high IC50 values. This result was also supported by the total flavonoid content, in which the acetone extract exhibited high flavonoid content among the extracts. Acetone extracts from *C. englerianum* have high concentration of total flavonoids, which is in correlation with the high antioxidant activity. Flavonoids are class of secondary metabolites with significant antioxidant properties. Antioxidant activity of flavonoids depends on the structure and substitution pattern of hydroxyl groups [22].

**Conclusion**

In conclusion, to promote Ethiopian herbal drugs and the traditional use of medicinal plants, there is an urgent need to evaluate their therapeutic potentials as per the standard guidelines. Despite the rich biodiversity of Ethiopian flora, there is limited information about the type of secondary metabolites present in most of the medicinal plants and their biological activity. We herein reported stigmasterol and stigmasteryl stearate from the root extract of *C. englerianum*, and it’s in vitro antibacterial and antioxidant activity for the first time. The results proved that acetone extract possess potential antibacterial and antioxidant activity that might be due to the presence of high content of phenolic compounds such as flavonoids, and tannins. Finally, the results of this study support the use of this plants as traditional medicines by local people in Ethiopia.

**Acknowledgments**

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**Author contributions**

The authors have made substantive intellectual contributions to this study. Mesfin Bibiso participated in plant material collection, experimental work, preparation of the manuscript, and proof reading; Mathewos Anza participated in plant material collection, experimental work, and preparation of the manuscript; Bereket Alemayehu participated in data collection, identification of plants, and proof reading.

**Declaration of interest**

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

**References**


**Abbreviations**

DPPH: 1,1-diphenyl-2-picrylhydrazl; EIMS: electron ionization mass spectrometry; HREIMS: high resolution electron ionization mass spectrometry; EI: electron ionization; BHT: butylated hydroxy toluene; DMSO: dimethyl sulfoxide; ANOVA: analysis of variance; ChME: chloroform methanol extract; AE: acetone extract; ME: methanol extract.