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Original article

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 evalute the bioactivity of root extracts of *C. englerianum*. **Methods**: Phythochemical screening tests were employed by standard protocols to identifiy the phythochemicals. Column chromatographic separation was used to isolate the compounds and the spectroscopic techniques (IR, NMR and ESMS) were used to elucidtae structures of the compounds. Disc diffusion technique was uesd to evalute antibacterial activity. In vitro antioxidant activity was assessed by 1, 1-diphenyl-2-picrylhydrazyl (DPPH), and phosphomolybdenun 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 (IC_{50} =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 Univeristy approved this research with the code of RCS/CNS-12/18.

Instruments

Electron ionization mass spectrometry (EIMS) 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 CDCl3 (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 hydroxyltoluene (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 F_{254} 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

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 a suspension (approximately 10^8 CFU/mL) at the Mac Farland scale, which originated from a young bacterial culture. After drying the plates, the disks (6 mm in diameter) were soaked in the extracts, and deposited on the surface of an agar medium (Mueller-Hinton). Incubated at 37 °C for 24 h, the zone inhibition was determined for antibacterial activity of the extracts. The experiments were triplicated. DMSO (10%) was used as the negative control, whereas chloramphenicol as the positive control.

Antioxidant activity

The antioxidant activities of plant extracts were evaluated by using their ability to scavenge the activity of the free radicals of the stable DPPH [12]. The extracts were prepared in concentrations 50-100 µg/mL, separately. Then DPPH in methanol (2 mL, 0.006%, w/v) was added in each test tube, containing 1 mL of the extract solutions. Then the reaction mixtures and the reference standards (AA and BHT), were vortexes and left to at room temperature for 30 min in dark. The absorbances of the solutions were then determined at 520 nm. Methanol was used as blank. Finally, the free radical scavenging ability was calculated using the equation below. IC_{50} values were used to express the ability of the extracts to scavenge DPPH. IC_{50} which meant the concentration of the extract needed to scavenge 50% of DPPH radical, was calculated using the graph of scavenging activity plotted against sample concentration using Microsoft excel software. Antioxidant potential is inversely proportional to inhibitory concentration (IC_{50}) value, its high value indicates less antioxidant capacity.

$$\text{Scavenging activity (\%)} = \frac{Ab_C - Ab_{ex}}{Ab_C} \times 100$$

Where Ab_c is the absorbance of control and Ab_{ex} is the absorbance of extract.

Total antioxidant activity

The total antioxidant activity of the crude extracts was evaluated by the phosphomolybdenum method as previously described [13]. An amount of 0.3 mL of plant extracts (0.5 and 1 mg/mL) was mixed with 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). 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% $NaNO_2$ was added to the mixture. After 6 min, 150 µL 10% $AlCl_3$ 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 SP^3 C-H stretch, at 1462 cm^{-1} was a bending frequency for cyclic $(CH_2)_n$, and at 1382 cm^{-1} for $–CH_2\ (CH_3)2\gamma$. In addition, the absorption frequency at 1056 cm^{-1} was indicative of cycloalkane. ^{1}H-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. ^{13}C-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 SP^3 -C-H stretch, at 1739 cm^{-1} to carbonyl carbon -C=O stretching, at 1462 cm^{-1} to a bending frequency of cyclic $(CH_2)n$, and at 1382 cm^{-1} for –CH2 $(CH_3)_2\gamma$. 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 $C_{47}H_{82}O_2$. ^{1}H-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.

Table 1. Phytochemical screening of *Cirsium englerianum* root extracts

Phytochemicals	Reagents	Plant extracts		
		ChME	AE	ME
Alkaloids	Mayer reagent	+	+	+
Steroids	$CHCl_3$ and conc. H_2SO_4	+	+	+
Terpenoids	$CHCl_3$ and conc. H_2SO_4	-	+	-
Tannins	$FeCl_3$	-	+	-
Flavonoids	Dilute NH_3 solution	+	+	+

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

Peaks at δ 5.08, 5.17, and 5.30 suggested 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. ^{13}C-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).

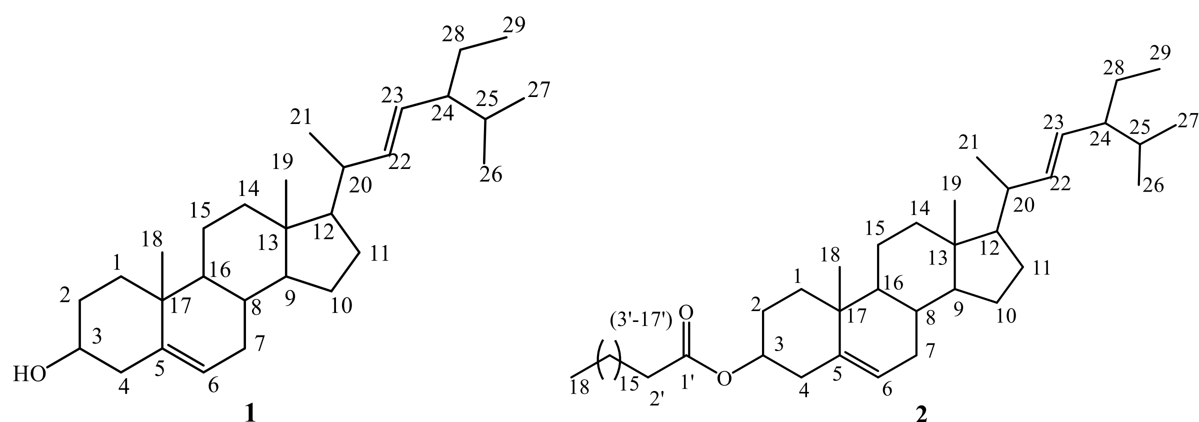

Figure 1. The structures of isolated compounds from *Cirsium englerianaum*

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; ^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm, and J values in Hz), 0.69 (3H, s), 0.69 (3H, d, J=6.6HZ), 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). ^{13}C-NMR (400 MHz, $CDCl_3$, 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;, ^{1}H-NMR (400 MHz, $CDCl_3$ δ in ppm, and J values in Hz), 0.69 (3H, d,J=6.6HZ), 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). ^{13}C-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, $C_{47}H_{82}O_2$.

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 IC_{50} value of 154.44±74 µg/mL, compared to those methanol and chloroform/methanol (1:1) extracts in exhibiting IC_{50} values of 177.37±12.34 and 183.92±10.12 µg/mL, respectively (Table 3).

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

	Extracts (mg/mL)									
	AE			ME			ChME			CHL (μg/mL)
Bacillus cereus	25	50	100	25	50	100	25	50	100	50
Staphylococcus aureus	6.7±0.68	13.4±0.93	19.6±0.51*	n	n	6.4±0.69	n	8.6±0.58	14.5±0.55	22.6±0.57*
Salmonella typhi	n	9.0±0.15	18.3±0.58*	nd	nd	nd	n	5.5±0.50	8.6±0.32	23.3±0.58*
Escherichia coli	n	7.3±0.59	12.4±0.35	n	7.8±0.51	9.3±0.07	n	n	13.3±0.71	31.4±0.14
Bacillus cereus	n	7.5±0.40	11.5±0.51	n	n	10.2±0.64	n	7.3±0.26	13.4±0.67	28.5±0.51

CHL: chloramphenicol; n≤ 6: null; n >: sensitive, nd: not determined [19]; ChME: chloroform/methanol (1:1) extract; AE: acetone extract; ME: methanol extract; *no significant difference (p>0.5)

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 3. IC_{50} values of DPPH scavenging activity of *Cirsium englerianum* root extracts

Extracts	IC_{50} (μg/mL±SD)
ChME	183.92±10.12
AE	154.44±74.00
ME	177.37±12.34
BHT	34.27 ± 2.24[a]
AA	23.94 ± 1.33[a]

Values of IC_{50} 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 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).

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.

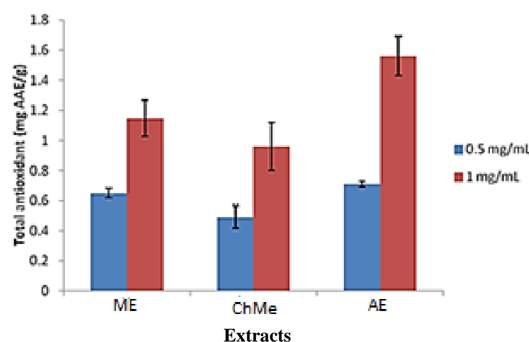

Figure 2. Total antioxidant activity of extracts of *Cirsium englerianum*; values are average of triplicate measurements (mean±SD). ChME: chloroform/methanol (1:1) extract; ME: methanol extract; AE: acetone extract

Table 4. Total flavonoids content of *Cirsium englerianum* root extracts

Extract	Total flavonoids content (mg CE/g extract)
AE	8.24±0.90
ME	5.88±0.21
ChME	7.82±2.38

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 stigmasterol (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

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 IC_{50} value [21]. Therefore, the acetone extract had the highest DPPH reducing activity based on its relatively low IC_{50} values (Table 3). A positive result by the acetone and methanol extracts in this study indicated that they contained antioxwidants 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 IC_{50} values. This result was also supported by the total flavonoidss content, in which the acetone extract exhibited high flavonoid content among the extracts. Acetone extracts from *C. engelerianum* 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.

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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

[1] Blaser MJ. Indigenous microbes and the ecology of human diseases. *Embo Rep*. 2006; 7(10): 956–960.

[2] Cassell GH, Mekalanos J. Development of antimicrobial agents in the era of new and reemerging infectious diseases and increasing antibiotic resistance. *J Am Med Assoc.* 2001; 285(5): 601–605.

[3] Yu R, Mandlekar S, Kong ANT. Molecular mechanisms of butylated hydroxylanisole-induced toxicity: induction of apoptosis through direct release of cytochrome C. *Mol Pharmacol.* 2000; 58(2): 431–437.

[4] Xu DP, Li Y, Meng X, Zhou T, Zhou Y, Zheng J, Zhang JJ, Li HB. Natural antioxidants in foods and medicinal plants: extraction, assessment and resources. *Int J Mol Sci.* 2017; 18(1): 1–32.

[5] Haddouchi F, Chaouche TM, Zaouali Y,

Ksouri R, Attou A, Benmansour A. Chemical composition and antimicrobial activity of the essential oils from four *Ruta* species growing in Algeria. *Food Chem*. 2013; 141(1): 253–258.

[6] Chekole G, Asfaw Z, Kelbessa E. Ethnobotanical study of medicinal plants in the environs of Tara-gedam and Amba remnant forests of Libo Kemkem district, northwest Ethiopia. *J Ethnobiol Ethnomed.* 2015; 11(4): 1–38.

[7] Mesfin F, Demissew S, Teklehaymanot T. An ethnobotanical study of medicinal plants in Wonago Woreda, SNNPR, Ethiopia. *J Ethnobiol Ethnomed.* 2009; 5: 1–18.

[8] Mirutse G, Zemede A, Zerihun W. Medicinal plants of the Meinit ethnic group of Ethiopia: an ethnobotanical study. *J Ethnopharmacol*. 2009; 124(3): 513–521.

[9] Kaur GJ, Arora DS. Antibacterial and phytochemical screening of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi*. *BMC Complement Altern Med.* 2009; 9(30): 1–10.

[10] Olayinka AA, Anthony IO. Preliminary phytochemical screening phytochemical screening and in vitro antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. *BMC Complement Altern Med*. 2010; 10(21): 1–8.

[11] Laouer H, Meriem EK, Parado S, Baldovini N. An antibacterial and antifungal phenylpropanoid from *C. monataum*. *Phytother Res*. 2009; 23(12): 1726–1730.

[12] Ebrahimzadeh MA, Hosseinimehr SJ, Hamidinia A, Jafari M. Antioxidant and free radical scavenging activity of *Feijoa sallowiana* fruits peel and leaves. *Pharmacologyonline*. 2008; 1: 7–14.

[13] Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem*. 1999; 269(2): 337–341.

[14] Ghasemi K, Ghasemi Y, Ali M. Antioxidant activity, phenol and flavonoid contents of 13 *Citrus* species peels and tissues. *Pak J Pharm Sci.* 2009; 22(3): 277–281.

[15] Mathewos A, Mesfin B, Bereket A, Engida D. Phytochemical analysis, in vitro antioxidant and antibacterial activities of root extracts of *Carduus macracanthus*. *J Coast Life Med*. 2015; 5(11): 486–491.

[16] Pierre LL, Moses MN. Isolation and characterisation of stigmasterol and β -sitosterol from *Odontonema Strictum* (Acanthaceae). *J Innov Pharm Biol Sci*. 2015; 2(1): 88–96.

[17] Tofiana FA, Immaculata I, Elfahmi M, Kartasasmita RE. Synthesis of stigmasteryl oleate, palmitate and stearate applying ethyl chloroformate as activator. *J Chem Pharm Res*. 2016; 8(2): 371–378.

[18] Gul MZ, Bhakshu LM, Ahmad F, Kondapi AK, Qureshi IA, Ghazi IA. Evaluation of *Abelmoschus moschatus* extracts for antioxidant, free radical scavenging, antimicrobial and antiproliferative activities using in vitro assays. *BMC Complement Altern Med*. 2011; 11: 1–12.

[19] Dandena T, Milkyas E. Antibacterial activity of coumarins and carbazole alkaloid from roots of *Clausena anisate*. *Adv Pharmacol Pharm Sci.* 2019; Article ID 5419854.

[20] Zeinab B, Buthaina J, Rafik K. Resistance of gram-negative bacteria to current antibacterial agents and approaches to resolve it. *Molecules*. 2020; 25(6): 1–23.

[21] Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *Afr J Biotechnol.* 2006; 5(11): 1142–1145.

[22] Sharififar F, Dehghn-Nudeh G, Mirtajaldini M. Major flavonoids with antioxidant activity from *Teucrium polium* L. *Food Chem.* 2009; 112(4): 885–888.

Abbreviations

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