Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Original article https://doi.org/10.12980/jclm.5.2017J7-110

Phytochemical analysis, *in vitro* antioxidant and antibacterial activities of root extracts of *Carduus macracanthus*

Mathewos Anza[1*], Mesfin Bibiso[1], Bereket Alemayehu[2], Engeda Desalegn[3]

[1]*Department of Chemistry, College of Natural and Computational Sciences, Wolaita Sodo University, P. O. Box 138, Wolaita Sodo, Ethiopia*

[2]*Biomedical Science Division, Department of Biology, College of Natural and Computational Sciences, Wolaita Sodo University, P. O. Box 138, Wolaita Sodo, Ethiopia*

[3]*School of Nutrition, Food Science and Technology, College of Agriculture, Hawassa University, P. O. Box 5, Hawassa, Ethiopia*

ARTICLE INFO

Article history:
Received 29 Jun 2017
Received in revised form 28 Aug 2017
Accepted 20 Sep 2017
Available online 21 Oct 2017

Keywords:
Carduus macracanthus
Phythochemicals
Antioxidant
Antibacterial

ABSTRACT

Objective: To investigate phytochemical constituents, antibacterial and antioxidant activity of the root extracts of *Carduus macracanthus* (*C. macracanthus*).

Methods: Phytochemical screening tests were conducted as per of the standard procedure to identify the classes of compounds present in the root extract of *C. macracanthus*. Silica gel column chromatographic technique was applied to separate the constituents from chloroform/methanol (1:1) root extracts. Various spectroscopic techniques were applied to determine the structure of the isolated compound. *In vtiro* antibacterial activities of crude extracts were evaluated via disc diffusion method. Minimum inhibitory concentration and minimum bacterial concentration were determined by broth dilution method. Moreover, antioxidant activities of extracts were determined by DPPH assay, the phosphomolybdenum method and total flavonoid contents were determined as per of the standard procedures.

Results: Phytochemical screening of root extracts of *C. macracanthus* revealed the presence alkaloids, flavonoids, triterpenes, tannins and steroids. Silica gel column chromatography separation of root extract using chloroform/methanol (1:1 ratio) yielded a known plant steroid stigmasterol. The result revealed that methanol extract had the greatest DPPH scavenging (IC_{50} = 97.50 ± 4.38 μg/mL) and total antioxidant (1.92 ± 0.24 mg AAE/g of dried extract) activities. Chloroform/methanol (1:1), and methanol extracts showed that growth suppression of *Bacillus cereus* and *Staphylococcus aureus* at a concentration above 25 mg/mL, and minimum inhibitory concentration was found to be 25 mg/mL. Methanol extract showed growth suppression against *Escherichia coli* at a concentration of 50 mg/mL. The antibacterial activities of the extracts were compared with a commercially available antibiotic (chloramphenicol) and showed moderate antibacterial activities, with inhibition zones ranging between 18–28 mm. However, the extracts had no antibacterial effects on *Salmonella typhi*.

Conclusions: The present study found that polar extracts of the root of *C. macracanthus* possessed antibacterial and antioxidant activities.

1. Introduction

Medicinal plants have played a pivotal role in the primary healthcare and formed the basis of traditional systems of medicines. They are effective in the treatment of infectious diseases while simultaneously mitigating many side effects that are often associated with synthetic antimicrobials. A disease that caused through pathogenic microorganisms is the major cause of deaths across the world. Increasing cases of drug resistance, unwanted side effects of existing antibiotics and the reappearance of earlier known infections lead to the need for new, safe and effective antimicrobial agents[1-3]. Thus, phytoconstituents such as phenolics are being considered to be safe and provide lesser chances for microbes to develop drug resistance.

Oxidative stress is mainly caused by the reactive oxygen species (ROS) such as hydroxyl radical (·OH), peroxide (ROO·) and superoxide radicals ($O_2 \cdot^-$). These reactive oxygen species are classes of compounds formed from oxygen metabolism, and are able to cause severe damage to cells and tissues. The effect of oxidative stress is linked to cardiovascular diseases, cancer, inflammatory diseases and many other health issues. Antioxidants are significant agents in reducing oxidative stress which damages cells and biological molecules[4].

Natural antioxidants such as bioactive flavonoids are of great

*Corresponding author: Mathewos Anza, Department of Chemistry, College of Natural and Computational Sciences, Wolaita Sodo University, P. O. Box 138, Wolaita Sodo, Ethiopia.
Tel: +251913348379
E-mail: mathewosanza@gmail.com
The journal implements double-blind peer review practiced by specially invited international editorial board members.

importance due to their indigenous origin and strong efficiency to scavenge free radicals. Because of synthetic antioxidants like butylated hydroxyanisole (BHA), propyl gallate (PG) and butylated hydroxytoluene (BHT) have been used to prevent oxidation, they are found to cause internal and external bleeding in rats and guinea pigs when taken at high dose[5,6]. One such example is tea (black or green) which is frequently used as beverage all over the world and is a rich source of polyphenolic compounds[5,7]. Hence, herbal medicinal plants rich in polyphenolic compounds are playing paramount role in antioxidant activity[5].

Ethiopia has a flora that is extremely rich in its diversity. The flora of Ethiopia is very heterogeneous with an estimated number between 6 500 and 7 000 species of higher plants, of which about 12% is endemic[8]. About 1 000 identified medicinal plant species have been reported to the Ethiopian flora; however, many others are not yet identified. About 300 of these species are frequently mentioned in many sources[9].

In Asteraceae family, there are several genera, commonly known as thistles. They are more accurately known as plume thistles. *Carduus* (Asteraceae) is widely used herbal medicinal plants throughout the world[10]. Among the species in *Carduus*, *Carduus macracanthus* (*C. macracanthus*) is endemic to Ethiopia, and it is used for various ethnomedical values. The phytoconstituents in the plants *C. macracanthus* have not been isolated, purified and investigated for antimicrobial and antioxidant activities. Therefore, the present study aimed at investigating phytochemicals, *in vitro* antibacterial and antioxidant activities of the root extract of *C. macracanthus*.

2. Materials and methods

2.1. Materials and chemicals

2.1.1. Materials

IR spectrum was recorded by KBr pellets on Perk-Elmer BX Infrared Spectrometer in the range 4 000–400 cm^{-1}. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker avance 400 MHz spectrometer with tetramethylsilane as internal standard. Analytical TLC plate with silica gel 60 F254 (Merck, Germany) was used to determine TLC profile. Column chromatography was used to perform isolation of pure compounds on silica gel (60–120 mesh).

2.1.2. Chemicals

DPPH, sodium phosphate, ammonium molybdate, BHT, L-ascorbic acid, catechin, and aluminium chloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals and solvents used in this experiment were of analytical grade reagents.

2.2. Plant material

Roots of *C. macracanthus* were collected from Sodo Zuriya woreda, Wolaita zone. The zone is located at 330 km from Addis Ababa, the capital of Ethiopia. The collected plant specimens were identified and compared with the specimens of the Herbarium in the Department of Biology, Addis Abba University, Ethiopia.

2.3. Preparation of extraction

At room temperature air dried root of *C. macracanthus* were powdered by using a mechanical grinder. 200 g of plant powder was socked into cold percolation with 1 L different solvent system (chloroform/methanol (1:1 ratio), acetone, and methanol) three times for 48 h while shaking at room temperature. The solution was filtrated by using suction filtration, and then the filtrate concentrated by using a rotary evaporator at a temperature 40 ˚C to yield 21.6 g (10.8%) brown crude extracts of *C. macracanthus*. The dried crude extract was checked for TLC profile by using various solvent systems such as *n*-hexane in ethyl acetate (7:3 and 5:5) ratio, and *n*-hexane, ethyl acetate and methanol (6:3:1) ratio to separate bioactive constituents in different polarity. Among various polarities, *n*-hexane in 7:3 ratio of in ethyl acetate showed good separation, and then it was collected in labeled sterile evaporating dish, finally put in deep freezer until the next experiment.

2.4. Isolation with column chromatography

About 16 g of (chloroform/methanol) 1:1 ratio crude extract was subjected to normal column chromatography and eluted with increasing gradient of ethyl acetate in *n*-hexane. A total of 30 fractions (30 mL each) were collected. Among 30 fractions, fraction 12 (30% ethyl acetate in *n*-hexane) formed a white precipitate. The precipitate and filtrate were separated by using suction filtration. The precipitate was checked for TLC profile in (7:3) *n*-hexane in the ethyl acetate solvent system, and showed single spot with a yellow fluorescence on F254 ($R_f = 0.62$). Finally, the precipitate was further washed with *n*-hexane to give 17 mg of compound (1).

2.5. Phytochemical analysis

2.5.1. Preliminary phytochemical screening test

Phytochemical screening tests were done to determine the classes of secondary metabolites present in the crude extract as per standard protocols[11,12].

2.5.2. Structure elucidation

The white crystalline powder of compound (1) was analyzed IR, NMR (^{1}H, ^{13}C, DEPT-135, COSY, HSQC and HMBC experiments. Then the structure was elucidated by using spectroscopic methods and further confirmed by literature data.

2.5.3. Determination of total flavonoid content (TFC)

Total flavonoid was estimated using the method described by Ebrahimzadeh *et al*.[13]. The 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 1mol/L NaOH was added to the mixture. Immediately, the absorbance of the mixture, pink in color, was determined at 510 nm versus prepared water blank. A standard curve was prepared using 5–120 µg/mL of catechin. Results were expressed as milligram of catechin equivalents per milligram of dry extract (mg CE/mg) of the plant extract. ($Y = 0.022x + 0.041$, $R^2 = 0.99$, $P < 0.001$).

2.6. Determination of antioxidant activity

2.6.1. DPPH assay

The DPPH free radical scavenging activity of the extracts of seed of *C. macracanthus* was assayed according to the method of Szollosi and Szollosi[14] with slight modification. Different concentrations (50–1 000 µg/mL) of the extracts were taken in different test tubes. Freshly prepared DPPH solution (2 mL, 0.006%, w/v) in methanol was added in each of the test tubes containing 1 mL of the extract. The reaction mixture and the reference standards (ascorbic acid and BHT) were vortexed and left to stand at room temperature in the dark

for 30 min. The absorbance of the resulting solution was then taken at 520 nm. Methanol was used as blank. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenged (\%)} = \frac{Ac–As}{Ac} \times 100$$

where *Ac* is the absorbance of the control and *As* is the absorbance in presence of the sample of the extracts. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (in μg/mL) of extracts that scavenges the DPPH radical by 50%. High IC_{50} value indicates less antioxidant capacity.

2.6.2. Total antioxidant using phosphomolybdenum assay

The total antioxidant activities of the crude extracts were evaluated by the phosphomolybdenum method reported by Pierre *et al.*[15] with slight modification. 0.3 mL plant extract (0.5 and 1 mg/mL) was mixed with 3 mL of reagent solution (0.6 mol/L sulphuric acid, 28 mmol/L sodium phosphate and 4 mmol/L ammonium molybdate). The samples were incubated at 95 ˚C for 90 min and cooled to room temperature and the absorbance was measured at 695 nm. 3 mL of methanol was used as a blank. The total antioxidant activity was expressed as milligrams ascorbic acid equivalent/gram of dried extract (mg AAE/g) ($Y = 0.321X + 0.019$; $R^2 = 0.996$, $P < 0.001$) based on the calibration curve.

2.7. Antibacterial activity tests

2.7.1. Microorganism strain

Four human pathogenic bacterial strains [two Gram-positive *Staphylococcus aureus* (*S. aureus*) and *Bacillus cereus* (*B. cereus*) and two Gram-negative *Escherichia coli* (*E. coli*) and *Salmonella typhi* (*S. typhi*)] were used to test the antibacterial activity of *C. macracanthus* crude extract with respect to standard antibiotic drug (chloroamphenicol). These microorganism strains were fresh cultured and isolated in the Food Science Laboratory, Hawassa University, Ethiopia, following standard laboratory procedures.

2.7.2. Evaluation of antibacterial activity

Antibacterial activities of the extracts were evaluated by the disc diffusion method in accordance with the guidelines of the Clinical and Laboratory Standards Institute[16] with minor modification. Chloramphenicol was used as a standard antibacterial agent. With a loop, touched the top of 24 h old culture, individual bacteria were selected and transferred to a tube of saline (0.85%) where the turbidity was adjusted to the standard inoculums of MacFarland scale 0.5 [~10^6 colony forming units (CFU) per milliliter]. To avoid further growth before inoculation, the medium was sterilized in a flask and cooled to 40–45 ˚C. Petri dishes containing 20 mL of Mueller-Hinton agar were used to inoculate bacterial suspensions, and then the medium was distributed in Petri dishes homogeneously. Filter paper discs (Whatman No. 1, 6 mm diameter) was impregnated with the extract solution. The solution of extracts prepared in DMSO (100, 50, 25 and 12.5 mg/mL) was placed on the inoculated plates, and Petri dishes were incubated for 24 h at 37 ˚C. The filter paper disc was also impregnated with chloroamphenicol used as positive control and DMSO as negative control. The inhibition zone diameters were measured in millimeters. The experiments were carried out in triplicate, and the results of antibacterial activity of extracts were analyzed in terms of inhibition zones with respect to a standard antibiotic drug.

2.7.3. Minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) determination procedure

Overnight dextrose broth cultures of *S. aureus*, *E. coli*, *B. cereus* and *S. typhi* at 37 ˚C were prepared. A drop plate method was used to analyze MIC and MBC and a culture media of mannitol salt agar for *S. aurous*, Salmonella-Shigella agar for *S. typhi*, ECD agar for *E. coli* and Bacillus cereus agar for *B. cereus* were used respectively. After inoculated in the appropriate media, the four microorganisms were incubated at 37 ˚C for 24 h and all microorganisms also inoculated into dextrose broth in order to confirm the MIC and MBC after incubating for 24 h, the MIC was measured and recorded. To determine MBC, the positive Petri dishes were taken and inoculated into the standard plate count agar and incubated for 24 h. The Petri dishes with no colony were recorded as MBC.

2.8. Statistical analysis

The quantitative data of antibacterial and antioxidant activity tests were presented as mean ± SD of triplicate measurements. The comparisons for the tests between the control group (chloramphenicol) and the test groups (plant extracts) were performed by One-way analysis of variance (ANOVA). Statistical significance was considered when the *P*-values were less than or equal to 0.05 at 95% confidence level.

3. Results

3.1. Phytochemical screening

The root extracts of *C. macracanthus* were subjected to various qualitative tests for the phytoconstituents such as alkaloids, steroids, flavonoids, tannins, and triterpenoids. Thus, as indicated in Table 1, phytochemical screening of root extracts of *C. macracanthus* revealed the presence of alkaloids, triterpenoids, flavonoids, tannins, and steroids in methanol extract. However, tannins were absent in the chloroform/methanol (1:1) root extracts and alkaloids in acetone extract.

Table 1
Preliminary phytochemical screening of root extracts from *C. macracanthus*.

Chemical constituents	Reagents	Extracts		
		Chloroform/ methanol (1:1)	Acetone	Methanol
Alkaloids	Mayer reagent	+	–	+
Steroids	CCl_3 and conc. H_2SO_4	+	+	+
Triterpenoids	CCl_3 and conc. H_2SO_4	+	+	+
Tannins	$FeCl_3$	–	+	+
Flavonoids	Dilute NH_3 solution	+	+	+

+: Presence; –: Absence.

3.2. Characterization of compound (1)

The chloroform/methanol (1:1 ratio) root extract of *C. macracanthus* was subjected to solvent partition in column chromatography to afford a compound (1). The structure of compound (1) was elucidated by spectroscopic techniques. IR spectra of compound (1) showed that the absorption bands at 3390 cm^{-1} are characteristic of O-H stretching. Absorption at the right of 3000 cm^{-1} is due to cyclic olefinic –HC= CH- structure, and 2930 cm^{-1} assigned to SP^3 C-H stretch, 1462 cm^{-1} is a bending frequency for cyclic (CH_2) n and 1382 cm^{-1} for $–CH_2$ (CH_3) 2γ. The absorption

frequency at 1 056 cm^{-1} signifies cycloalkane.

^{1}H NMR spectra of compound (1) showed that the presence of two methyl singlets' at δ 0.71 and 1.06; three methyl doublets' that appeared at δ 0.80, 0.82, and 0.91; and a methyl triplet at δ 0.83. Compound (1) also showed protons at δ 5.02, 5.17, and 5.37 suggesting the presence of three protons corresponding to that of a trisubstituted and a disubstituted olefinic bond. The proton corresponding to the H-3 of a sterol moiety was appeared as a triplet of doublet of doublets at δ 3.54.

^{13}CNMR has given signal at 140.8 and 121.7 ppm for C5 and C6 double bond respectively, 71.8 for C3 β-hydroxyl group, 19.4 and 11.9 for angular methyl carbon atoms for C19 and C18 respectively. In addition, DEPT-135 spectra revealed that the presence of three quaternary carbon signals including olefinic carbon at 140.8 ppm, elven methine, nine methylene, and six methyl carbons.

The above spectral data supported by the key COSY and HMBC correlations clearly indicated the presence of sterol skeleton, having a hydroxyl group at C-3 position with two double bonds at C-5/C-6 and C-20/C-21 with six methyl groups. Thus, the structure of compound (1) was assigned as the known compound stigmasterol.

Compound (1) white crystalline needles (melting point 144–145 °C with R_f value 0.62), IR (KBr) υmax/cm^{-1}: 3 390, 2 935, 1 651, and 1 062, 894 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ0.71, 0.80, 0.82, 0.83, 0.91 and 1.06 (each 3H, -CH_3 × 6), 3.54 (1H, m, H-3), 5.37 (1H, t, H-6), 5.15 (1H, s, H-22), 5.01 (1H, S, H-23) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ140.8 (C-5), 138.3 (C-22), 129.3 (C-3), 121.7 (C- 6), 71.8 (C-3), 56.9 (C-14), 56.8 (C-17), 55.9 (C-24), 51.2 (C-9), 50.2 (C-25), 42.3 (C-13), 40.5 (C-20), 39.8 (C-12), 39.7 (C-4), 37.3 (C-1), 36.5 (C-10), 31. 9 (C-8), 31.7 (C-7), 25.4 (C-16), 28.9 (C-2), 24.4 (C-28), 24.3 (C-15), 21.2 (C-21), 21.1 (C-11), 19.4 (C-26), 19.4 (C-27), 18.9 (C-19), 11.9 (C-29), 12.05 (C-18).

3.3. TFC

The TFC (Table 2) followed the order: methanol > acetone > chloroform: methanol (50:50, v/v). There was significant difference ($P < 0.05$) in TFC among methanol, acetone and chloroform: methanol (50:50, v/v).

Table 2

Total flavonoid content of various solvent extracts of *C. macracanthus*.

Plant extract in different solvent system	TFC (mg CE/g)
Acetone	12.54 ± 1.50^{b}
Methanol	25.75 ± 7.42^{c}
Chloroform: methanol (50:50, v/v)	6.10 ± 0.52^{a}

Values are expressed as mean ± SD (n = 3) from triplicate experiments. Means with different letters in a column were significantly different at the level of $P < 0.05$.

3.4. Antioxidant activity

3.4.1. DPPH assay

The DPPH radical scavenging effects of extracts of *C. macracanthus* in different solvent systems showed in (Figure 1). At the concentration of 1 000 μg/mL used, the scavenging effect of L-ascorbic acid, BHT, and *C. macracanthus* extracts, on the DPPH radical decreased in the order of L-ascorbic acid > methanol > BHT > acetone > chloroform: methanol (50:50, v/v) > acetone, which were (97.30 ± 3.50)%, (96.92 ± 2.42)% > (94.70 ± 14.60)%, (93.92 ± 3.01)% > (80.61 ± 3.45)%, respectively.

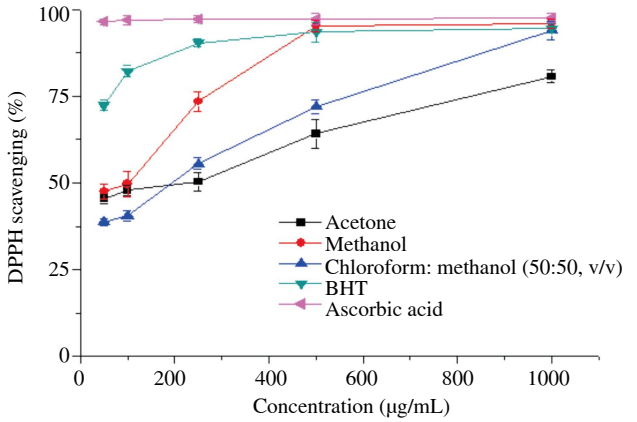

Figure 1. DPPH radical scavenging activity (%) of root extracts of *C. macracanthus* and controls (L-ascorbic acid and BHT).
Values are average of triplicate measurements (mean ± SD).

The IC_{50} values of all the extracts were calculated from the graph of percentage scavenging activity against concentration of the extracts in (Table 3). This suggested that dried root extract of *C. macracanthus* contained compounds that can donate electron/hydrogen easily and stabilize free radicals.

Table 3

IC_{50} values of DPPH scavenging activities in various solvent extracts from *C. macracanthus*.

Plant extracts in different solvent	IC_{50} (μg/mL)
Chloroform: methanol (50:50, v/v)	195.76 ± 6.20^{c}
Acetone	248.17 ± 19.23^{c}
Methanol	97.50 ± 4.38^{b}
BHT	34.27 ± 2.24^{a}
Ascorbic acid	23.94 ± 1.33^{a}

3.4.2. Total antioxidant capacity by phosphomolybdenum method

Total antioxidant capacity was reported as milligram ascorbic acid equivalents (mg AAE/g of dried extract). The results revealed that the methanol extract of *C. macracanthus* had also the highest total antioxidant activity followed by chloroform: methanol (50:50, v/v) and the lowest total antioxidant activity was found in the acetone extract (Figure 2).

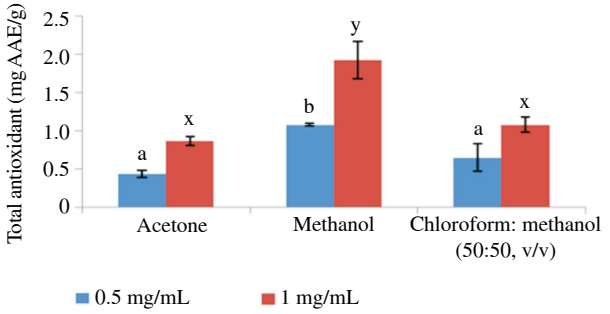

Figure 2. Total antioxidant activity (mg AAE/g of dw) of extracts of *C. macracanthus* at concentrations of 0.5 and 1.0 mg/mL.
Values are average of triplicate measurements (mean ± SD). Values within the same concentration with different letters in the histogram bar are significantly different at $P < 0.05$.

3.5. In vitro antibacterial activity

3.5.1. Disc diffusion method

As indicated Table 4, antibacterial activities were determined

via disc diffusion method measuring the 'inhibition zone' for root extract of *C. macracanthus* in different solvent system against two Gram-positive (*B. cereus*, *S. aureus*), and two Gram-negative (*S. typhi* and *E. coli*) bacteria strains. The activities of root extracts of *C. macracanthus* have also been compared with the broad spectrum commercially available antibiotic (chloramphenicol). Chloramphenicol showed the inhibition zone for all tested bacteria strains in a range of 18–28 mm at the concentration 50 μg/mL; while methanol/chloroform (1:1) extract of *C. macracanthus* recorded for *B. cereus* (16.00 ± 0.10), *S. aureus* (17.03 ± 0.30) and *E. coli* (11.03 ± 0.90) at 100 mg/mL concentration. However, in a concentration of 50 mg/mL, the values were decreased as *B. cereus* (8.00 ± 0.10), *S. aureus* (12.00 ± 0.10) and *E. coli* (8.00 ± 0.20). The present result showed that the root extract of *C. macracanthus* did not inhibit the growth of *S. typhi* in all solvent systems.

Table 4
Antibacterial activities of root extracts of *C. macracanthus*.

Strains	Concentration (mg/mL)	Zone of inhibition (mm)		
		Chloroform/methanol (1:1) extract	Acetone extract	Methanol extract
B. cereus	100	16.00 ± 0.10[a]	11.03 ± 0.90[b]	15.02 ± 0.62[a]
	50	8.00 ± 0.10[b]	–	–
	25	–	–	–
S. aureus	100	17.03 ± 0.30[a]	15.00 ± 0.95[a]	14.02 ± 0.27[a]
	50	12.00 ± 0.10	10.00 ± 0.23[b]	10.03 ± 0.15[b]
	25	–	–	–
E. coli	100	11.03 ± 0.90[b]	10.00 ± 0.17[b]	14.00 ± 0.00[a]
	50	8.00 ± 0.20[b]	–	7.01 ± 0.80[b]
	25	6.03 ± 0.30[b]	–	–
S. typhi	100	–	–	–
	50	–	–	–
	25	–	–	–
Chloramphenicol	50 μg/mL	18–28[a]		

The data represented as mean ± SD, $n = 3$. Means with same letters with reference antibiotics no significantly differences were observed at 0.05 level of Dunnett test ($P > 0.05$). –: Not detected.

3.5.2. MIC and MBC

The chloroform/methanol (1:1), acetone, and methanol root extract of *C. macracanthus* were examined MIC and MBC. As indicated Table 5, the results revealed that chloroform/methanol (1:1) and methanol extracts of *C. macracanthus* showed growth suppression at a concentration above 25 mg/mL, and MIC and MBC of the extracts against *B. cereus* and *S. aureus* were found to be 25 mg/mL and 100 mg/mL, respectively. MIC and MBC were found to be 50 mg/mL and 100 mg/mL, respectively against *S. aureus*, *E. coli* in chloroform/methanol (1:1 ratio) extracts. A high antibacterial activity was obtained against *B. cereus* and *S. aureus* (MIC 25 mg/mL) in comparison with the activity obtained against *E. coli* (MIC 50 mg/mL) chloroform/methanol (1:1) root extracts *C. macracanthus*.

4. Discussion

The phytochemical screening test of *C. macracanthus* revealed the presence bioactive phenolic compounds such as flavonoids, tannins and alkaloids. Physical and spectral data of compound (1) were consistent to the reported literature values[17,18] of stigmasterol. Thus, antioxidant and antibacterial activity of *C. macracanthus* extracts might be attributed to the presence of these phenolic compounds. The total flavonoids content of methanol extract of *C. macracanthus* obtained from this study was higher than the TFC obtained from different *Carduus* species[19,20]. DPPH radical was scavenged by antioxidants through the donation of hydrogen forming the reduced DPPH. The color changes from purple 2, 2-diphenyl-1-picrylhydrazyl radical to reduced yellow diamagnetic 2, 2-diphenyl-1-picrylhydrazine molecule, which can be quantified by its absorbance reduction at wavelength 520 nm[21]. As the concentration of sample increased, the percent inhibition of DPPH radical also increased[22]. Thus, the DPPH scavenging activity of root extract of *C. macracanthus* conducted under this study was lower than the study conducted by Zheleva-Dimitrova *et al*.[19] on different *Carduus* species collected from Bulgaria but greater than the DPPH scavenging activity of *Carduus acicularis* Bertol[20].

The lower the IC_{50} value, the higher is the scavenging potential. Stronger scavenging activity (lower IC_{50} values) was recorded for methanol extract which appeared nearly two times stronger than that of the chloroform: methanol (50:50, v/v) and two and a half times stronger than that of acetone extract ($P < 0.05$). The IC_{50} values of L-ascorbic acid and BHT tested as references were found to be significantly lower (stronger DPPH scavenger) ($P < 0.05$) than that of methanol, chloroform: methanol (50:50, v/v) and acetone extracts. The method is utilized for the spectrophotometric quantification of total antioxidant capacity, based on the reduction of Mo(VI) to Mo(V) in presence of antioxidant compound and subsequent formation of a green phosphate/Mo(V) complex at acidic pH and at higher temperature[23]. No significant difference ($P > 0.05$) was found in the total antioxidant activity of acetone and chloroform: methanol (50:50, v/v) ($P > 0.05$). However, these values were

Table 5
MIC and MBC value of root extracts of *C. macracanthus*.

Concentration (mg/mL)	Root extract of *C. macracanthus* in chloroform/methanol (1:1) ratio				Root extract of *C. macracanthus* in acetone				Root extract of *C. macracanthus* in methanol			
	B. cereus	*S. aureus*	*S. typhi*	*E. coli*	*B. cereus*	*S. aureus*	*S. typhi*	*E. coli*	*B. cereus*	*S. aureus*	*S. typhi*	*E. coli*
100	+	+	–	+	+	+	–	+	+	+	–	+
50	+	+	–	+	+	–	–		+	+	–	+
25	+		–	–	–	–	–	–	–	+	–	–
12.5	–	–	–	–	–	–	–	–	–	–	–	–
6.25	–	–	–	–	–	–	–	–	–	–	–	–
3.125	–	–	–	–	–	–	–	–	–	–	–	–
1.563	–	–	–	–	–	–	–	–	–	–	–	–
MIC	25 mg/mL	50 mg/mL	–	50 mg/mL	50 mg/mL	100 mg/mL	–	100 mg/mL	50 mg/mL	25 mg/mL	–	50 mg/mL
MBC	100 mg/mL	100 mg/mL	–	–	–	–	–	–	–	–	–	–

+: Presence; –: Not detected.

significantly lower ($P < 0.05$) than the total antioxidant activity of methanol extract.

The root extract of *C. macracanthus* in chloroform/methanol (1:1) showed strong antibacterial activity comparable at a concentration of 100 mg/mL to that of standard chloramphenicol (50 μg/mL). ANOVA test of the antibacterial activity of crude extracts in all solvent systems of *C. macracanthus* at a concentration 100 mg/mL on *S. aureus* had no significant effect ($P > 0.05$) on the level of inhibition respect to reference antibiotic (chloramphenicol). Chloroform/methanol and methanol extract on *B. cereus*, and methanol extract on *E. coli* at a concentration 100 mg/mL showed no significant effect ($P > 0.05$) with respect to control antibiotic. However, the extracts showed a significant difference in all solvent system crude extracts at the concentration below 100 mg/mL with respect to commercially available antibiotic.

Therefore, the finding of the present study showed that plant extracts under *in vitro* study had broad spectrum antibacterial activity, though *S. typhi* was resistant to various concentrations of the extracts in all solvent systems. 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.

Known plant sterol was isolated from the root extract of *C. macracanthus*. The structures of the isolated compound were identified as stigmasterol on the basis of spectroscopic data and by comparing its physical properties reported in the literature. The complete ^{1}H and ^{13}C NMR spectral assignments of the isolated compound was made based on COSY, HSQC, HMBC and spectroscopic data. The compound (1) was isolated for the first time from the *C. macracanthus*. These results proved that polar extracts of the root of *C. macracanthus* possess antibacterial and antioxidant activity.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This work was supported by Wolaita Sodo University, Ethiopia. Department of Chemistry, Addis Ababa University is duly acknowledged for access to IR and NMR spectrometers.

References

[1] Blaser MJ. Indigenous microbes and the ecology of human diseases. *EMBO Rep* 2006; **7**: 956-60.

[2] Jones KE, Patel NG, Levy MA, Storeygard AD, Balk JL, Daszak P. Global trends in emerging infectious diseases. *Nature* 2007; **451**: 990-3.

[3] Cassell GH, Mekalanos J. Development of antimicrobial agents in the era of new and reemerging infectious diseases and increasing antibiotic resistance. *JAMA* 2001; **285**: 601-5.

[4] Farhat MB, Landoulsi A, Chaouch-Hamada R, Sotomayor JA, Jordán MJ. Characterization and quantification of phenolic compounds and antioxidant properties of *Salvia* species growing in different habitats. *Ind Crops Prod* 2013; **49**: 904-14.

[5] Borneo R. Leon AE, Aguirre A, Ribotta P, Cantero JJ. Antioxidant capacity of medicinal plants from the Province of Cordoba (Argentina) and their *in vitro* testing in a model food system. *Food Chem* 2009; **112**: 664-70.

[6] Lee SE, Hwang HJ, Ha JS, Jeong H, Kim JH. Screening of medicinal plant extracts for antioxidant activity. *Life Sci* 2003; **73**: 167-79.

[7] Katalinic V, Milos M, Kulisic T, Jukic M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chem* 2006; **94**: 550-7.

[8] Dawit K, Afework B. Species composition, abundance, distribution and habitat association of rodents of Wondo Genet, Ethiopia. *Sinet Ethiop J Sci* 2008; **31**: 141-6.

[9] Endashaw B. Study on actual situation of medicinal plants in Ethiopia. Tokyo: Japan Association for International Collaboration of Agriculture and Forestry; 2007. [Online] Available from: http://www.jaicaf.or.jp/publications/ethiopia_ac.pdf [Accessed on 17th June, 2017]

[10] Rose F. *The wild flower key*. London: Frederick Warne & Co.; 1981, p. 377-80.

[11] Pradeep A, Dinesh M, Govindaraj A. Vinothkumar D, Ramesh NG. Phytochemical analysis of some important medicinal plants. *Int J Biol Pharm Res* 2014; **5**: 48-50.

[12] Saleem M, Karim M, Qadir MI, Ahmed B, Rafiq M, Ahmad B. *In vitro* antibacterial activity and phytochemical analysis of hexane extract of *Vicia sativa*. *Bangladesh J Pharmacol* 2014; **9**: 189-93.

[13] 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.

[14] Szollosi R, Szollosi IV. Total antioxidant power in some species of Labiatae (Adaptation of FRAP method). *Acta Biol Szeged* 2002; **46**: 125-7.

[15] Pierre BK, Pierre SH, Tatjana S. Study of polyphenol content and antioxidant capacity of *Myrianthus arboreus* (Cecropiaceae) root bark extracts. *Antioxidants* 2015; **4**: 410-26.

[16] Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from animals; Approved standard–3rd ed. Wayne: Clinical and Laboratory Standards Institute; 2002. [Online] Available from: http://www.dbt.univr.it/documenti/OccorrenzaIns/matdid/matdid485539.pdf [Accessed on 17th June, 2017]

[17] Anjoo K, Ajayku M. Isolation of stigmasterol and β-sitosterol from petroleum ether extract of aerial parts of *Ageratum conyzoides* (Asteraceae). *Int J Pharm Pharm Sci* 2011; **3**: 94-6.

[18] Pierre LL, Moses MN. Isolation and characterisation of stigmasterol and B-sitosterol from *Odontonema strictum* (Acanthaceae). *J Innov Pharm Bio Sci* 2011; **2**: 88-95.

[19] Zheleva-Dimitrova D, Zhelev I, Dimitrova-Dyulgerova I. Antioxidant activity of some *Carduus* species growing in Bulgaria. *Free Rad Antiox* 2011; **1**: 15-20.

[20] Slavov IZ, Dimitrova-Dyulgerova IZ, Mladen R. Phenolic profile and antioxidant activity of methanolic extract of *Carduus acicularis* Bertol. (Asteraceae). *Ecologia Balkanica* 2016; **8**: 41-6.

[21] Kailaspati PC, Sharada LD, Tushar AD. Free radical scavenging activity of plant extracts of *Chlorophytum tuberosum*. *Der Pharmacia Lettre* 2016; **8**: 107-11.

[22] Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. *J Agric Food Chem* 2005; **53**: 1841-56.

[23] Mohamed I, Mohamed M, Basith JA, Asarudeen A. Determination of total phenol, flavonoid and antioxidant activity of edible mushrooms *Pleurotus florida* and *Pleurotus eous*. *Int Food Res J* 2011; **18**: 579-82.