Journal of Biology and Today's World
ISSN 2322-3308
Journal home page: http://journals.lexispublisher.com/jbtw/

Received: 01 August 2015 • Accepted: 30 September 2015

Research

doi:10.15412/J.JBTW.01040901

Evaluation of Biological Activity and Phenolic Compounds of *Cardaria draba* (L.) Extracts

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ABSTRACT

The antibacterial, antioxidant, anti-inflammatory and scolicidal activities, as well as phenolic compounds from various leaf and seed extracts of *Cardaria draba* (L.) Desv. (whitetop) were examined. The antibacterial activity against two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) was evaluated by the agar well diffusion method. Antioxidant activity was assayed by using the seed and leaf extracts on synthetic DPPH free radicals and assessing their capacity to inhibit the peroxidation of linoleic acid. Anti-inflammatory activity was investigated by protein denaturation. The viability of *Echinococcus granulosus* protoscolices was investigated by evaluating their motility under a light microscope. Phenolic compounds in leaf and seed extracts were analyzed by HPLC. Our result showed that the MICs of various *C. draba* extracts against bacterial strains ranged from 3 to 134 µg/mL. The maximum and minimum MIC values for *S. aureus* were 3 µg/mL for the ethanolic leaf extract (ELE) and 86 µg/mL for the aqueous seed extract (ASE), respectively. Results of antioxidant assays showed that the ethanolic seed extract (ESE) had a significantly higher antioxidant capacity than ELE, and that both extracts had significantly lower antioxidant capacity than BHA and ascorbic acid. Results of the anti-inflammatory assay showed that as the concentration of extracts and control increased, there was a concomitant increase in the percentage inhibition of protein denaturation. ELE (500 µg/mL) and ESE (100 µg/mL) showed maximum and minimum percentage inhibition of protein denaturation (90% and 48%, respectively). Scolicidal activity and percentage mortality increased with exposure time, but the level depended on the extract concentration. In total, 16 compounds were identified from both extracts. Isorhamnetin (13.85%), quercetin (12.9%), and kaempferol (11.5%) were most abundant compounds in ELE, while the most abundant compounds in ESE were caffeic acid (13.3%), *p*-coumaric acid (7.9%), sinapic acid (7.9%), and ellagic acid (7.9%). These results support the therapeutic potential of *C. draba*.

Key words: 1,1-diphenyl-2-picrylhydrazyl (DPPH), high performance liquid chromatography (HPLC), minimum inhibitory concentration (MIC), protein denaturation, whitetop

1. INTRODUCTION

Natural medicines, especially medicinal plants, are a fundamental mainstay for the pharmaceutical industry, and serve as alternatives for and the basis of modern medicines, including those against infectious diseases (1). Since synthetic antioxidants are toxic at higher concentrations (2-4), mining plants for natural antioxidants has increased over the years. A disease that causes morbidity and mortality in various parts of the world is human cystic echinococcosis (hydatid disease) (5). Scolicidal solutions, which include benzimidazole carbamate derivatives such as albendazole and mebendazole, are absolutely necessary in the treatment of hydatid disease. However, surgeons need less harmful and more effective drugs to treat this disease. Many attempts have been made to find new antimicrobial compounds from different biotic sources such as plants, animals and

microorganisms having scolicidal activity against the hydatid cyst of *Echinococcus granulosus* (6). Infectious diseases and other microbial pathogenicities are typically treated with chemically synthesized antimicrobial drugs (7). Excessive use of antibiotics has resulted in the development of multiple drug resistance in numerous bacterial pathogens rendering some antibiotics virtually useless (8, 9). Thus, new drugs are required for the treatment of bacterial infections. Natural products are an important source of new drugs and have served as an optional medicine for the therapy of various diseases for decades (10-14). Herbal therapy is one suitable way to treat diseases caused by multidrug-resistant bacteria (15-18). The use of plant extracts and phytochemicals, which contain antibacterial attributes, may be of therapeutic importance, as has been tested on multiple microorganisms in different countries (19-22). *Cardaria draba* (L.) Desv. (Brassicaceae; syn. *Lepidum draba* (L). Link), commonly known as whitetop or hoary cress, is a perennial plant that reproduces by seed and by horizontal creeping roots (23). *C. draba* is native to western Asia, including Iran and eastern Europe, and is an invasive species in North America, introduced by contaminated seeds in the early 1900s (23). *C. draba* can be found in most parts of Iran, in fields and adjacent to water sources and in gardens and bare lands. It can be found in a wide diversity of soil types where moisture is adequate. It typically grows in a wide range of disturbed habitats involving cultivated land, rangeland, pastures, along roadsides, waste areas, and is known to prosper in riparian or irrigated areas (24). Infusions of *C. draba* leaf and seeds have purgative and expectorant effects. A decoction of the whole *C. draba* plant and seed is used as a diuretic in ethnomedicine in Iran (25). This study is the first comprehensive *in vitro* evaluation of aqueous and ethanolic *C. draba* leaf and seed extracts aimed at understanding the antibacterial potential against drug-resistant human pathogenic bacteria and scolicidal activity against *E. granulosus* hydatid cysts. In this study, the phenolic compounds from both extracts, as well as their antioxidant and anti-inflammatory activities, were also investigated.

2. MATERIALS AND METHODS

2.1. Plant preparation

The leaves of several plants at the flowering stage (Figure 1 A, B) were collected in March 2013 from the area surrounding Hamun Lake, Zabol (31° 1′ 43″ N, 61° 30′ 4″ E), in Sistan and Baluchestan Provinces of Iran.

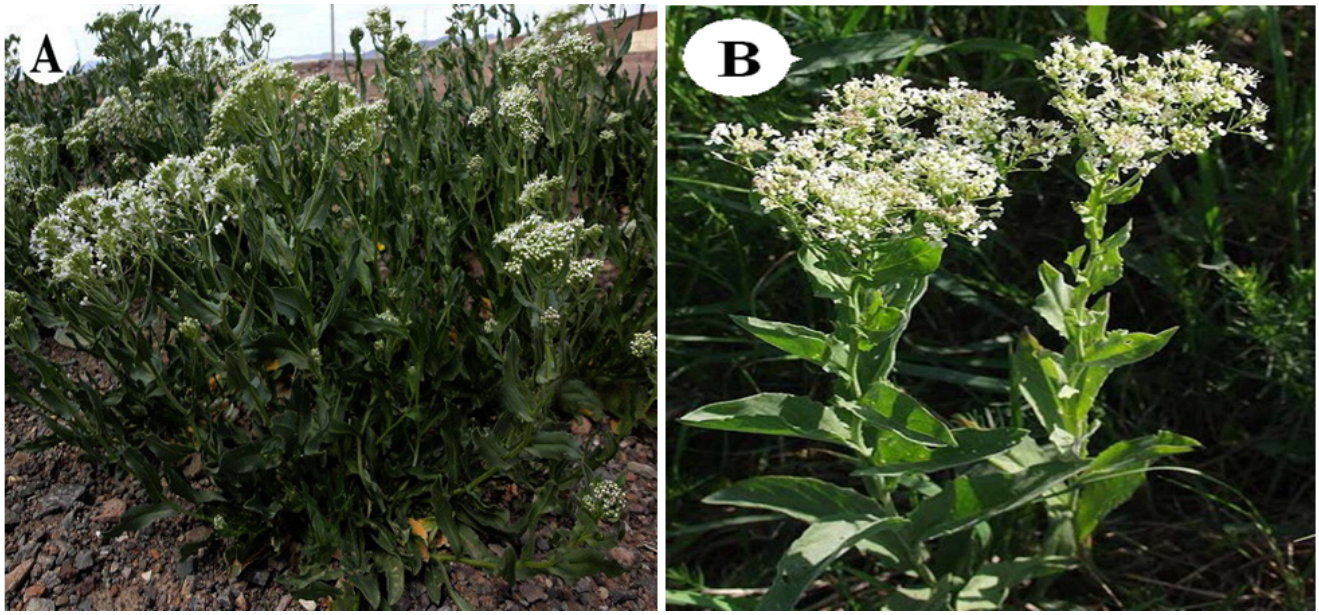

Figure 1. *Cardaria draba* in the flowering stage used for extract analyses in this study. (A) Wild population; (B) close-up of plant

The taxonomy was confirmed by the Zabol Medicinal Plants Research Center, Zabol University of Medical Sciences, Iran and a voucher specimen (no. 26577) was deposited in the herbarium. Seeds were collected from plants at the same location in May, 2013. The leaves and seeds were dried on paper towels in a laboratory at 25°C for 96 h.

2.2. Preparation of extracts

Leaves and seeds were ground to a powder in the laboratory, after drying for 72 h in the shade to avoid the loss of active constituents and 200 ml of 85% ethanol was mixed with 60 g of each of the powders. The mixtures were stored for 24 h in tightly sealed vessels at room temperature (25°C), sheltered from sunlight and mixed several times with a sterile glass rod. Each mixture was filtered through Whatman no. 1 filter paper and standardized at 1 mL/g of powdered plant material. The clear, colorless supernatant was evaporated on a rotary vacuum evaporator (Laborota 4000, Heidolph, Germany) to remove the ethanol. The semi-solid extracts were stored in a freezer at -80°C overnight then freeze dried for 24 h at -70°C in 200 mL under vacuum. The extract was stored in an airtight container at 4°C until use. The aqueous extracts, prepared by weighing 60 g of leaves and seeds and soaking each separately in 200 mL of distilled water in a conical flask, were left for 24 h. Each crude aqueous extract was filtered through sterile Whatman no. 1 filter paper into a clean conical flask. Water was evaporated in a 100°C water bath and the concentrated aqueous extracts were

stored at 4°C until use. Different concentrations of ethanolic leaf extract (ELE), ethanolic seed extract (ESE), aqueous leaf extract (ALE) and aqueous seed extract (ASE) were used in various assays, as outlined next.

2.3. Investigation of antibacterial activity

Four bacterial strains were tested (purchased from the Persian Type Culture Collection, Tehran, Iran): Gram-positive bacteria (*Staphylococcus aureus* PTCC 1112 and *Bacillus subtilis* PTCC 1023) and Gram-negative bacteria (*Pseudomonas aeruginosa* PTCC 1074 and *Escherichia coli* PTCC 1330). The bacteria were grown in nutrient broth (Himedia, M002) at 37°C and preserved on nutrient agar slants at 4°C. An antibiogram was prepared by the disc diffusion method (26, 27) using common antibiotics (all in µg/L; Padtan-Teb Co., Tehran, Iran):

Ampicillin (20); Amikacin (20); Cotrimoxazole (20); Ciprofloxacin (10); Cloxacillin (25); Cefadroxil (20); Cefuroxime (20); Doxycycline (20); Erythromycin (10); Gentamycin (10); Kanamycin (20); Nalidixic acid (20); Norfloxacin (10); Penicillin-G (10); Sparfloxacin (10); Tobramycin (10); Tetracyclin (25).

The surface of the Muller Hinton agar was inoculated with bacteria from each broth culture and then different antibiotic disks were located on the culture surface. After 18 h of incubation at 30 ± 1°C, the diameters of the inhibition zones were measured to the closest millimeter. Screening was conducted by the agar well diffusion method (28). The bacterial strains growing on nutrient agar at 37°C for 18 h were suspended in a saline solution (0.8% NaCl) and adjusted to a turbidity of 0.5 McFarland standard equivalents to 10^8 CFU/ml. The suspension (60 µL of 2000 µg/mL extracts) was inoculated into the 6 mm diameter wells on 90 mm diameter Petri dishes that had been punched in the agar. Dimethyl sulfoxide (DMSO) was added at 5% (v/v) to the ethanolic and aqueous extracts. Initial trials indicated that DMSO concentrations up to 1% (v/v) did not affect microbial activity. Plates were incubated at 37°C for 24 h. Antibacterial activity was assessed by measuring the diameter of the zone of inhibition (ZOI). Assays were conducted in triplicate. DMSO served as the control for the ethanolic and aqueous extracts. The minimum inhibitory concentration (MIC) of *C. draba* extracts was determined by the Natta et al. (29) method, with some modifications. Extracts were diluted to 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 or 0.19 µg/mL, and were added to the microtiter plates and incubated at 37°C for 24 h. MIC was visually detected with the lowest concentration showing > 95% growth inhibition. Assays were conducted in triplicate.

2.4. Evaluation of antioxidant activity

2.4.1. Antiradical capacity

Antiradical capacity was measured by method of Shimada et al. (30) based on the scavenging capacity of the plant extract on the synthetic 1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma-Aldrich, St. Louis, MO, USA) free radicals. Each extract sample (0.5-40.0 mg/mL) in methanol (5 mL) was mixed with 1 mL of DPPH methanolic solution until a final concentration of 0.2 mM DPPH was reached. The mixture was shaken vigorously, left to stand for 35 min in the dark, and the absorbance was then measured at 517 nm against a blank. Antiradical capacity was determined as follows:

Antiradical capacity (%) = [(ΔA_{517} of control–ΔA_{517} of sample)/ΔA_{517} of control] ×100.

The IC_{50} value (mg/mL), which is the efficient concentration at which DPPH radicals are scavenged by 50%, was obtained by interpolation from linear regression analysis. α-Tocopherol, butylated hydroxyanisole (BHA) and ascorbic acid (all Sigma-Aldrich) were used as standard controls.

2.4.2. Antioxidant activity

Antioxidant capacity, which was determined by the paired diene method (31), represents the capacity of a plant extract to inhibit the peroxidation of linoleic acid, in which the double bond is changed to a paired diene. Each extract sample (0.01-30.0 mg/mL) in ethanol (100 µL) was blended with 3 mL of 10 mM linoleic acid (Sigma-Aldrich) in test tubes to form an emulsion in 0.2 M sodium phosphate buffer (pH 6.6), and then placed in the dark at 37°C to stimulate oxidation. After incubation for 17 h, 7 mL of 70% ethanol in deionized water was added, and the absorbance of the mixture was measured at 234 nm against a blank in a Hitachi U-2001 spectrophotometer (Tokyo, Japan). Antioxidant capacity was measured as follows:

Antioxidant capacity (%) = [(ΔA_{234} of control–ΔA_{234} of sample)/ΔA_{234} of control] ×100.

The IC_{50} value was determined as for the antiradical assay using the same controls.

2.5. Anti-inflammatory activity

The anti-inflammatory activity of the ethanolic leaf and seed extracts was assayed by the protein denaturation method (32). Briefly, 1 mL of three concentrations (100, 200, and 500 µg/mL) of both extracts or standard (diclofenac sodium, a powerful non-steroidal anti-inflammatory drug) and 3 mL of phosphate buffered saline (pH 6.5) was blended with 2 mL of egg albumin and incubated at 25°C for 15 min. The mixture was denatured in a 65°C water bath for 12 min. After cooling, the absorbance was measured at 660 nm (A_{660}) by using double distilled water as the blank. The percentage inhibition of protein denaturation was estimated by using the following formula:

$$\% \text{ inhibition} = A_s - A_c / A_c \times 100$$

where A_s and A_c are sample absorbance and control absorbance, respectively. Each assay was performed in triplicate.

2.6. Scolicidal activity

To study scolicidal activity, *E. granulosus* protoscolices were obtained from the infected livers of calves killed in an abattoir. Animals were treated humanely according to the Helsinki Convention. Hydatid fluid was collected together with protoscolices using the Smyth and Barrett (33) method. Briefly, hydatid fluid was transferred to a glass cylinder. Protoscolices, which settled at the bottom of the cylinder after 40 min, were washed three times with normal saline and their viability was verified by motility under a light microscope (Nikon Eclipse E200, Japan). Protoscolices were transferred into a dark receptacle containing normal saline and stored at 4°C. Three concentrations of ELE and ESE (2.5, 5, and 10 mg/mL) were tested for 10, 20, 30, and 60 min. To prepare these concentrations, 25, 50 and 100 µL of extracts, added to test tubes, were dissolved in 9.7 mL of normal saline supplemented with 0.5 mL of Tween-80 (Merck, Darmstadt, Germany) under constant stirring. For each assay, one drop of protoscolex-rich solution was added to 3 mL of each extract solution, mixed slowly, and incubated at 37°C. After each incubation period (10, 20, 30, and 60 min), the upper phase was carefully removed so as not to disturb the protoscolices, then 1 mL of 0.1% eosin stain was added to the remaining colonized protoscolices and mixed slowly. After incubating for 20 min at 25°C, the supernatant was discarded. The remaining uncentrifuged pellet of protoscolices was then smeared on a manually scaled glass slide, covered with a cover glass, and evaluated under a light microscope. The percentage of dead protoscolices was determined after counting a minimum of 600 protoscolices. In the control, protoscolices were treated only with normal saline and Tween-80. Assays were carried out in triplicate.

2.7. High performance liquid chromatography analysis

The phenolics of ELE and ESE were analyzed by high performance liquid chromatography (HPLC) according to Shyu (34). HPLC analysis was performed on a Knauer Gradient Model 8300 HPLC pump system (Knauer, Bad Homburg, Germany) and an S-3210 photodiode-array detector (PDA) (Schambeck SFD GmbH, Bad Honnef, Germany) with a Waters (150 × 4.6 mm i.d.) Eclipse XDR-C18 column, using a binary solvent system: solvent A, dd H_2O/CH_3COOH, 97:3, v/v; solvent B, MeOH. The following gradient program was used: 100–90% A from 0 to 10 min, 90–30% A from 10 to 32 min, 30–0% A from 32 to 45 min at a flow rate of 1.0 mL/min.

2.8. Statistical analysis

All values represent the mean± standard deviation (SD) of three replicates. Each replicate represents an independent leaf and seed sample from different plants in a completely random design. Data was subjected to analysis of variance (One-way ANOVA) and significant differences between means were determined by the least significant difference (LSD) test at $P < 0.05$ using SPSS v. 11.5 (IBM SPSS, New York, USA).

3. RESULTS AND DISCUSSION

3.1. Antibacterial potential against drug-resistant human pathogenic bacteria

An antibiogram of the Gram-positive and Gram-negative bacteria (Table 1) indicates that all bacterial strains were resistant to some widely used broad-spectrum antibiotics. All bacteria were sensitive to the new generation antibiotics except for *B. subtilis* due to its complex growth requirements. Consequently, definitive Clinical and Laboratory Standard Institute (CLSI) (35) cut-off values for susceptibility to antibiotics and resistance could not be found (Table 1).

Table 1. Susceptibility of four reference bacterial strains to antibiotics in nutrient agar

	Diameter of the inhibitory zones (mm)			
Antibiotics (µg/mL)	*Bacillus subtilis*	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
Ampicillin (20)	28	0	0	19
Amikacin (20)	13	11	19	24
Cotrimoxazole (20)	28	12	16	0
Ciprofloxacin (10)	20	0	0	6
Cloxacillin (25)	0	0	0	0
Cefadroxil (20)	0	0	0	0
Cefuroxime (20)	0	0	0	0
Doxycycline (20)	12	11	10	23
Erythromycin (10)	26	0	0	0
Gentamycin (10)	14	15	0	21
Kanamycin (20)	26	12	0	17
Nalidixic acid (20)	0	0	18	0
Norfloxacin (10)	0	11	7	16
Penicillin-G (10)	0	0	0	0
Sparfloxacin (10)	14	0	0	22
Tobramycin (10)	28	14	15	18
Tetracyclin (25)	27	20	12	0

The antibacterial activity of the *C. draba* leaf and seeds ethanolic extracts was significantly more efficacious than the aqueous extract against all the reference bacterial strains (Table 2).

Table 2. Antimicrobial sensitivity measured as diameter (mm) inhibition zone of aqueous and ethanol extracts of seeds and leaf of Cardaria *draba*

Microorganism	ASE	ESE	ALE	ELE
Staphylococcus aureus	14 ± 0.25 d	15 ± 1.04 c	20± 0.32 b	26 ± 1.21 a
Bacillus subtilis	4 ± 0.55 d	9 ± 0.54 c	13 ± 0.12 b	15 ± 0.35 a
Pseudomonas aeruginosa	8 ± 1.02 d	11 ± 0.24 c	16 ± 1.09 b	18 ± 0.85 a
Escherichia coli	5 ± 1.65 d	12 ± 0.00 c	13 ± 0.00 b	14 ± 0.27 a

Values are mean of ± SD of three replicates. ASE: aqueous seeds extract; ESE: ethanol seeds extract; ALE: aqueous leaf extract; ELE: ethanol leaf extract. Different letters show significant differences (LSD) across microorganisms for each extract type at $P < 0.05$.

ELE and ASE showed maximum and minimum ZOIs, respectively against all microorganisms tested. In contrast, *S. aureus* and *E. coli* showed maximum and minimum ZOIs, respectively among all extracts. The ZOIs for *S. aureus* in ASE, ESE, ALE and ELE were 14, 15, 20, and 26 mm, respectively. The ZOIs for *E. coli* were 5, 12, 13, and 14 mm in ASE, ESE, ALE and ELE, respectively. The MICs of different *C. draba* extracts against bacterial strains ranged from 3 to 134 µg/mL (Figure 2).

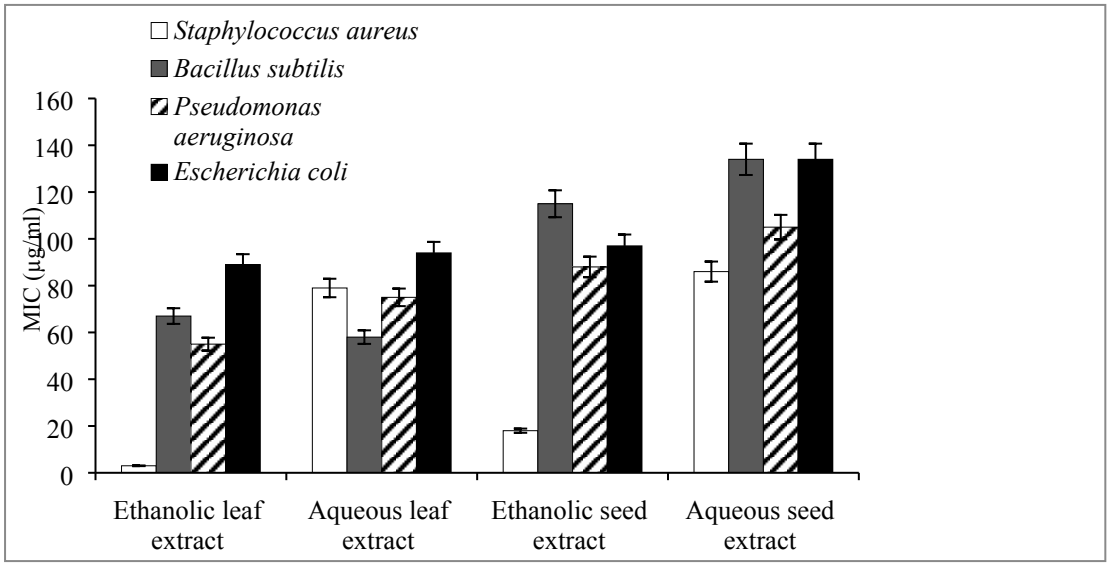

Figure 2. The MIC of different *Cardaria draba* extracts against tested bacterial isolates. Different letters show significant differences (LSD) across microorganisms for each extract type at $P < 0.05$.

The maximum and minimum MIC values for *S. aureus* were 3 µg/mL (ELE) and 86 µg/mL (ASE), respectively. The maximum and minimum MIC values for *B. subtilis* were 67 µg/mL (ELE) and 134 µg/mL (ASE), respectively. The maximum and minimum MIC values for *P. aeruginosa* were 55 µg/mL (ELE) and 105 µg/mL (ASE), respectively while those for *E. coli* were 89 µg/mL (ELE) and 134 µg/mL (ASE), respectively. In this study, all tested strains were susceptible to the ethanolic and aqueous extracts from seeds and leaves but the most effective was ELE. The lowest MIC values were for *S. aureus* by ELE (3 µg/mL). The antibacterial effect of *C. draba* may be related to the presence of phenolic compounds, which have proven antimicrobial activity (36). Gull et al. (37) studied the antimicrobial potency of garlic (*Allium sativum* L.) and ginger (*Zingiber officinale* Roscoe) against eight local clinical bacterial isolates, namely *E. coli*, *P. aeruginosa*, *B. subtilis*, *S. aureus*, *K. pneumoniae*, *Shigella sonnei*, *S. epidermidis*, and *Salmonella typhi*. All tested bacterial strains were most susceptible to the garlic aqueous extract but showed poor susceptibility to the ginger aqueous extract. The MICs of different bacterial species varied from 0.05 to 1.0 mg/mL. These results differed considerably with our results, possibly as a result of different compounds or secondary metabolites or different amounts of the same compounds in garlic and ginger relative to *C. draba*. Future studies should involve analytic techniques to analyze such compounds in detail. Prasad (38) investigated the antimicrobial potential of members of the Brassicaceae against clinical bacterial isolates. In their study, leaf extracts of *Brassica oleracea* L., *Raphanus sativus* L. and *B. rapa* L. showed significant antimicrobial activity against *S. aureus*, *E. coli* and *P. aeruginosa* while the ELE showed higher antimicrobial activity than methanolic, chloroform and diethyl ether extracts. In the present study, the ethanolic extracts of *C. draba* leaves and seeds showed much stronger antibacterial activity than the aqueous extracts whereas in a traditional method of treating bacterial infections, a decoction made by boiling plant parts in water is generally used (39). The observations in this study may be related to the nature of biologically active components whose activity may increase in the presence of ethanol; the stronger extraction capacity of ethanol could have extracted many active components responsible for antibacterial activity. Both Gram-positive and Gram-negative bacteria were sensitive

to all leaf and seed extracts of *C. draba* but *S. aureus* (Gram-positive bacterium) was most sensitive to bacteria (Table 2 and Figure 2). A large number of food products are perishable by nature and require protection from spoilage during preparation, storage, and distribution to bestow them a desired shelf life. Microbial growth is a major concern since some microorganisms can potentially bring about food-borne illnesses (40). In the food industry, to prevent growth of pathogenic microorganisms and spoilage in foods, many preservation techniques, such as acidification, drying, heat treatment, and salting have been used (41). Today, due to concerns regarding synthetic chemical additives, and in response to increasing consumer consciousness, it has become popular to preserve foods with natural additives. Antimicrobials can be added directly into a food product formulation, coated on its surface or incorporated into the packaging material, to inhibit the growth of undesirable microorganisms (40). Since *C. draba* inhibited the growth of food pathogens such as *S. aureus*, *B. subtilis*, *P. aeruginosa* and *E. coli*, the use of this plant or its extracts could decrease the incidence of food poisoning and increase the shelf life of foods.

3.2. Antioxidant activity

The results of antiradical capacity and antioxidant activity are shown in Table 3. ESE had significantly higher antioxidant capacity than ELE, and both extracts had significantly lower antioxidant capacity than BHA and ascorbic acid. The antiradical capacity of both *C. draba* ELE and ESE was the same, but significantly lower than BHA, ascorbic acid, and α-tocopherol.

Table 3. IC50 values (mg/mL) of the *Cardaria draba* leaf and seed ethanolic extract of two assays

	Antioxidant capacity (mg/mL)	Antiradical capacity (mg/mL)
C. draba leaf extract	0.01 ± 0.00 d	0.1 ± 0.01 d
C. draba seed extract	0.03 ± 0.00 c	0.1 ± 0.04 d
Butylated hydroxyanisole (BHA)	0.5 ± 0.01 b	0.25 ± 0.00 c
Ascorbic acid	3.10 ± 0.00 a	4.01 ± 0.01 a
α-Tocopherol	0.03 ± 0.01 c	1.00 ± 0.07 b

Values are mean ± SD of three replicates; means with different letters within a column are significantly different ($P < 0.05$; LSD, least significant difference).

Natural antioxidants of higher plants, particularly representative compounds such as phenolics, carotenoids and vitamins demonstrate antioxidant activity that tend to decrease disease-associated chronic health problems (42). There is an inverse association between antioxidative status and the occurrence of human illnesses such as aging, cancer, atherosclerosis and neurodegenerative diseases (43). Kaur et al. (44) reported that *Lepidium latifolium* leaf extract contained a high content of phenols and flavonoids with antioxidant activity ranging from 41.3% to 83.9%, and can serve as a dietary substitute if consumed as a leafy vegetable. Sandoval et al. (45) investigated the antioxidant activity of the aqueous extract of a cruciferous vegetable, maca (*Lepidium meyenii* Walp.), which at 0.03–3 mg/mL, quenched 100 mM DPPH in a dose-dependent manner, i.e., it had the ability to scavenge free radicals *in vitro* during oxidative stress. Zhao et al. (46) also evaluated the *in vitro* antioxidant activity of the ethanolic leaf extract of three coloured maca types (white, yellow and purple), all of which were able to scavenge hydroxyl radicals, with a scavenging ratio of more than 90% at a dose of 200 µL. Yellow maca, cultivated at 3000 m, had the best hydroxyl free radical scavenging activity. Results from our antioxidant assays indicate that *C. draba* leaf extract had higher antioxidant capacity than the seed extract. Plant phenolic compounds act as primary antioxidants (47). Phenolics can play a protective function by acting as electron donors or donating an H atom; the bond dissociation enthalpy of the O-H bond and ionization potential are of special significance in deciding the preferred mechanism during radical scavenging activity (48).

3.3. Anti-inflammatory activity

The results of anti-inflammatory activity are shown in Table 4. There was a significant difference among all concentrations of seed and leaf extracts and various concentrations of the control (100, 200, and 500 µg/mL) for ELE (100 µg/mL) and ESE (200 µg/mL). As the concentration of extracts and control increased, there was a concomitant increase in the percentage inhibition of protein denaturation. ELE (500 µg/mL) and ESE (100 µg/mL) showed maximum and minimum percentage inhibition of protein denaturation (90% and 48%, respectively).

Table 4. Anti-inflammatory effect of *Cardaria draba* leaf and seeds ethanolic extracts

Treatments/ concentrations (µg/mL)	Inhibition of protein denaturation (%)
C. draba leaf extract/ 100	61.45 ± 3.3 f
C. draba leaf extract/ 200	84.22 ± 2.7 e
C. draba leaf extract/ 500	90.12 ± 3.2 c
C. draba seed extract/ 100	48.41 ± 1.4 g
C. draba seed extract/ 200	60.22 ± 1.1 f
C. draba seed extract/ 500	84.91 ± 2.1 e
Diclofenac sodium/ 100	89.01 ± 1.1 d
Diclofenac sodium/ 200	93.22 ± 2.1 b
Diclofenac sodium/ 500	96.14 ± 2.7 a

Values are mean ± SD of three replicates; means with different letters within a column are significantly different ($P < 0.05$; LSD, least significant difference).

In chronic diseases, inflammation is a very common symptom, serving as a usual protective response to tissue injury caused by noxious chemicals, microbial agents and physical trauma (49). Anti-inflammatory drugs that are commonly used for the management of inflammatory conditions are non-steroidal and are associated with abundant unwanted side effects such as ulcers and gastric irritation, so finding a new compound (or extract) with fewer side effects is desired (50). The use of medicinal plants in traditional medicine to treat anti-inflammatory conditions is a practical and reasonable alternative when seeking new, safe and effective anti-inflammatory agents (51). *C. draba* is one such candidate plant. In this study, *C. draba* leaf and seed extracts (100, 200, and 500 µg/mL) significantly inhibited protein denaturation in a dose-dependent manner and had anti-inflammatory effects, confirming that phenolic compounds have anti-inflammatory activities in plants (52). Raval et al. (53) evaluated the anti-inflammatory activity of *Lepidium sativum* seed powder on Charles Foster albino rats. The powder had a strong inhibitory effect on fibroblast proliferation and a modulation effect on connective tissue. Reddy et al. (54) investigated the anti-inflammatory activity of the methanolic extract of *L. sativum* seeds against the denaturation of egg albumin, a protein. They reported a concentration-dependent inhibition of protein denaturation in the range of 25-1000 µg/mL while Diclofenac sodium at 50-2500 µg/mL served as the reference drug.

3.4. Scolicidal activity

The mortality rates of *E. granulosus* protoscolices exposed to different concentrations of C. draba ethanolic leaf and seed extracts for various exposure periods are shown in Table 5.

Table 5. Scolicidal activity of ethanolic extracts from leaf and seeds of *Cardaria draba* against *Echinococcus granulosus* at 2.5, 5 and 10 mg/mL following various exposure periods (mean±SD)

Concentrations (mg/mL)	Exposure time (min)	Protoscolices	Dead protoscolices	Mortality (%)
Leaf extract				
2.5	10	942.00 ± 44.35	209.00 ± 20.12	22.18
	20	1223.22 ± 54.14	296.49 ± 10.48	24.23
	30	1321.00 ± 22.98	369.00 ± 24.32	27.93
	60	928.00 ± 55.91	298.00 ± 12.33	32.11
	Control	998.00	94.00	9.41
5	10	880.22 ± 77.11	439.00 ± 29.11	49.87
	20	986.72 ± 55.22	494.00 ± 33.81	50.06
	30	867.94 ± 82.13	502.00 ± 23.12	57.86
	60	997.84 ± 82.00	590.00 ± 12.89	59.12
	Control	998.00	94.00	9.41
10	10	894.00 ± 0.00	541.00 ± 0.00	60.51
	20	976.29 ± 22.79	610.12 ± 28.88	62.49
	30	998.00 ± 65.27	666.46 ± 71.23	66.77
	60	1027.00 ± 49.71	694.32 ± 32.22	67.60
	Control	998.00	94.00	9.41
Seed extract				
2.5	10	832.00 ± 40.11	200.00 ± 54.00	24.03
	20	962.22 ± 44.18	242.00 ± 29.19	25.15
	30	861.12 ± 34.15	248.00 ± 13.77	28.79
	60	999.00 ± 41.00	292.00 ± 22.11	29.22
	Control	998.00	94.00	9.41
5	10	942.00 ± 33.21	460.43 ± 82.51	48.87
	20	1023.00 ± 42.00	505.00 ± 22.86	49.36
	30	987.00 ± 42.99	540.00 ± 40.48	54.71
	60	892.00 ± 32.00	534.00 ± 45.32	59.86
	Control	998.00	94.00	9.41
10	10	992.48 ± 48.00	598.00 ± 14.32	60.25
	20	1078.13 ± 98.00	664.31 ± 00.00	61.61
	30	1098.00 ± 79.12	685.00 ± 21.00	62.38
	60	927.49 ± 23.00	615.16 ± 48.19	66.33
	Control	998.00	94.00	9.41

Values are mean of ± SD of three replicates. In the control, protoscolices were treated only with normal saline + Tween-80.

As exposure time increased, the percentage mortality increased, but the level depended on the extract concentration. Thus, exposure to ELE for 60 min at 2.5, 5, and 10 mg/mL resulted in 32.11%, 59.12%, and 67.60% inhibition, respectively. Similarly, exposure to ESE for 60 min at 2.5, 5, and 10 mg/mL resulted in 29.22%, 59.86%, and 66.33% inhibition, respectively. The percentage mortality in the control was 9.41%, significantly less than in all concentrations of ELE and ESE. Herbal therapy is widely used to treat animal and human diseases. One such disease, hydatidosis, causes considerable deaths in humans and animals around the world. Gangwar et al. (55) evaluated the scolicidal potential of the methanolic fruit extract (10 and 20 mg/mL) of *Mallotus philippinensis* Muell. At 10 and 20 mg/mL, the mortality rate was 97% and 99%, respectively for 60 min treatment while up to 93% mortality was observed with 20 mg/mL for only 10 min treatment. When more than 20 mg/mL was applied for more than 2 h, 100% mortality resulted. The *M. philippinensis* extract had a significantly higher scolicidal activity than the standard anti-parasitic drug praziquantel with no associated side effects. Moazeni and Mohseni (56) investigated the scolicidal activity of *Rhus coriaria* L. on hydatid cyst protoscolices. They reported 16.93% death of protoscolices in the control group, but when protoscolices were exposed to *R. coriaria* extract at 10 mg/mL, the percentage increased to 94.13%, 97.67% and 100% after 10, 20, and 30 min, respectively. The level of mortality of protoscolices increased to 98.89% and 100% when they were exposed to 30 mg/mL of extract for 10 and 20 min, respectively and 100% mortality was observed at 50 mg/mL after 10-min exposure. In our study, *C. draba* leaf and seed extracts exhibited a dose- and time-dependent scolicidal effect on the protoscolices of hydatid cysts. Both *C. draba* extracts displayed scolicidal activity and could thus be used as a scolicidal agent.

3.5. HPLC analysis of phenolic compounds

The percentage of phenolic compounds obtained in ELE and ESE ranged from 1.1% to 13.8% (Table 6).

Table 6. HPLC profile of major phenolic compounds from *Cardaria draba* leaf and seed ethanolic extracts

Compound	RT* (min)	Leaf extract (%)	Seed extract (%)
Phloroglucinol	2.5	1.2	3.1
Gallic acid	2.9	4.2	4.8
Tannic acid	5.2	2.7	3.1
Quercetin	7.2	12.9	7.8
Catechol	7.8	3.1	3.9
Resorcinol	8.1	5.6	4.3
Vanillin	8.5	6.4	6.7
Ellagic acid	9.4	7.5	7.9
Kaempferol	11.5	11.5	6.5
Isorhamnetin	12.7	13.8	6.4
Caffeic acid	20.1	7.2	13.3
p-Coumaric acid	22.4	5.1	7.9
Sinapic acid	23.7	6.7	7.9
p-Anisic acid	31.7	7.2	6.6
Myricetin	33.8	2.3	4.2
Luteolin	39.4	1.1	4.5
Total		98.5	98.9

*RT: retention time; % values represent absolute percentages

In total, 16 compounds were identified from both extracts. Isorhamnetin, quercetin and kaempferol were the most abundant compounds in ELE (13.85%, 12.9%, and 11.5%, respectively) while the most abundant compounds in ESE were caffeic acid, *p*-coumaric acid, sinapic acid and ellagic acid (13.3%, 7.9%, 7.9%, and 7.9%, respectively). Phenolics are produced in plants as secondary metabolites via the shikimic acid pathway amounting to more than 8000 compounds that are widely dispersed throughout the plant kingdom. Phenolics possess at least one aromatic ring with one or more hydroxyl groups attached. Phenolic compounds are present in high concentrations in the epidermis of leaves and fruits and have different roles as secondary metabolites, and are associated with procedures such as UV protection, pigmentation, disease resistance, stimulation of nitrogen-fixing nodules, attracting insects, seed dispersion and pollination (57-62). Phenolic compounds also form part of a plant's natural defense system against bacteria, viruses, fungi and insects, and they can control plant hormones. *Brassica* species contain a wide and diverse range of polyphenols, namely the flavonoids and hydroxycinnamic acids, which serve as biochemical markers to differentiate members within different genera or even within the same species (52). Quercetin, isorhamnetin and kaempferol, the main flavonols in *Brassica* species, are most usually found as *O*-glycosides (52). In our study, these compounds were more abundant than other phenolic compounds assayed by HPLC. Gallic acid and its derivatives are polyphenyl natural products with a wide range of biological activities, including antimicrobial, antioxidant, anti-inflammatory and anticancer (63-65). It is plausible that the phenolic compounds discovered in *C. draba* leaf and seed extracts accounted for the antibacterial, antioxidant, anti-inflammatory and scolicidal activities. Quercetin can assist in the prevention of particular diseases, such as chronic inflammation, atherosclerosis and cancer by decelerating oxidative degradation and activating enzymes that detoxify carcinogens and block the formation of cancer by deactivating at least 30 types of agents that may induce

cancer (66-68). Kim et al. (66) reported that kaempferol has strong antioxidant potential while Braca et al. (69) reported that great stability to kaempferol's radical scavenging capacity was related to presence of an *O*-dihydroxy structure. Ayaz et al. (70) reported that phenolic fractions extracted from *Brassica oleracea* leaves, rich in quercetin and kaempferol derivatives, effectively inhibited the growth of Gram-positive bacteria *Bacillus subtilis*, *Enterobacter faecalis*, and *S. aureus* as well as the Gram-negative bacterium *Moraxella catarrhalis*, which is a serious respiratory pathogen in humans (70). Garcia-Lafuente et al. (71)showed that quercetin, kaempferol and isorhamnetin had an anti-inflammatory effect on activated macrophages.

4. CONCLUSION

In conclusion, the antimicrobial, antioxidant, anti-inflammatory and scolicidal activities of *C. draba* can be increased if the active constituents are purified and suitable dosages are determined for correct management of diseases, thus offering assistance to traditional medical practitioners and providing a simple solution in parts of the world where there are sanitary problems related to human hygiene and foodborne diseases while also offering one practical solution to reducing the clinical burden associated with the development of drug resistance. This study provides a theoretical and experimental explanation for the therapeutic potential of *C. draba*. Despite these findings, *in vivo* experiments are needed to appraise *C. draba* as a low-cost therapy, and to assess its side effects in a bid to prevent reappearing infections.

Funding/Support

Not mentioned any funding/ support by authors.

ACKNOWLEDGMENT

The authors are very grateful to Department of Range and Watershed Management, Faculty of Natural Resources, University of Zabol for supports this study.

AUTHORS CONTRIBUTION

JSR and SMHAi designed the study. JSR, SMHA, MSR, MSR and MR carried out the experiments and analyzed the results. MSR wrote the paper. JATdS provided scientific advice. JSR and JATdS critically reviewed the data, results and manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

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