

Antioxidant and Antibacterial Activity of Various Extracts of *Malcolmia africana* (L.) R. Br

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Article information	Abstract
<p>Article history: Received: 29 Sep 2012 Accepted: 11 Nov 2012 Available online: 28 Jan 2013 ZJRMS 2014; 16(3): 6-11</p> <p>Keywords: Radical scavenging DPPH Methanol Plant extracts</p> <p>*Corresponding author at: Department of Chemical Engineering, North Tehran Branch, Islamic Azad University, Tehran, Iran. E-mail: akbaresmaeili@yahoo.com</p>	<p>Background: Antioxidants are compounds that protect the body against cell membrane injury or damage to the cell's genetic material from free radical activity. The objective of this research was to study the antioxidant and antibacterial activities of various extracts from the seeds and aerial parts of <i>Malcolmia africana</i> (L.) R. Br.</p> <p>Materials and Methods: The overall results of <i>M. africana</i> tests allowed us to study the various extracts [hexane extract (HE), methanol extract (ME) and chloroform extract (CE)] of the test total phenolic, ABTS [(2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid))] and DPPH (2, 2-diphenyl-1-picryl hydrazyl) and also to test β-carotene, largest property antioxidant. The antimicrobial activity of the extracts of both samples was determined against seven Gram-positive and Gram-negative bacteria.</p> <p>Results: The amount of total phenolics was highest in the methanol extract (ME) of the seeds (225.19 ± 0.02) and aerial parts (208.52 ± 0.012 mg) Gallic acid/gr sub-fractions. The property antioxidant shows both of the mechanisms of electron transfer and the hydrogen transfer which the extracts has gone through.</p> <p>Conclusion: This investigation showed that ME has the highest antioxidant behavior. The antimicrobial activities of the extracts of both samples were determined against seven Gram-positive and Gram-negative bacteria. The bioassay shows that the seeds and aerial parts exhibit moderate antimicrobial activity. Therefore, the extracts could be suitable as antimicrobial and anti-oxidative agents in the food industry.</p>

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Introduction

The Cruciferae family of plants are abundant in the northern hemisphere [1]. The *Malcolmia* species include *M. grandiflora*, *M. behboudiana*, *M. karelinii*, *M. scorpiodes*, *M. crenata*, *M. strigosa* and *M. africana*, on four species *M. behboudiana*, *M. scorpiodes*, *M. strigosa* and *M. africana* of phylogenetic katen [1]. It has been reported that oleic acid, linoleic acid monohydroxy and eicosenoic compound. Today, antioxidant and antimicrobial activity are major research areas in medicine and biochemistry [2]. So antioxidants, which can oxidize substrates in a chain reaction, appear to be very important in the prevention of many diseases [2]. The phenolic content and composition of plants and the products produced from them depend on genetic and environmental factors, as well as post-harvest processing conditions [3, 4].

The relationship between the phenolic content and antioxidant activity alongside the total phenolic are the other areas of investigating antioxidant activities with different mechanisms, such as, hydrogen-donation, singlet oxygen quenching, metal ion-chelation, and acting as substrates for radicals such as superoxide and hydroxyl [5-8]. The study of antimicrobial activity can be important in compounds of natural products. In a previous study, the effect of the microbial transformation of several monoterpenes produced from various fungi (*Aspergillus niger*, *Pseudomonas sp.*, and *Penicillium sp.*) was studied

on the production of a number of compounds [9-15]. In this study, we could not find any further information, and we therefore examined the antioxidant and antibacterial activities of various extract of ME, HE and CE from the seeds and aerial parts of *M. africana*.

Materials and Methods

Plant material: Samples of *Malcolmia africana* (L.) R. Br. was collected during the flowering stage in August 2010 from the Karaj province of Iran. Voucher specimens have been deposited at the Herbarium of the Research Institute of Forests and Rangelands (TARI), Tehran, Iran.

Preparation of extracts: The seeds and aerial parts were mixed with hexane macerated and extracted to get the hexane extract. They were again extracted with chloroform and concentrated by means of vacuum evaporation in order to obtain the chloroform extracts. The remaining macerated was then treated with methanol to get the methanol extract. The extracts were then subjected to preliminary phytochemical evaluation [13].

Preparations of the extract (ME, CE, HE): A portion of the plant material (40 g) was successively extracted with 400 ml of methanol and chloroform and hexane (Merck, Darmstadt, Germany) by using a Soxhlet extractor (Isolab, Wetheim, Germany) for 72 h at a temperature not exceeding the boiling point of the

solvent. The methanol extracts were filtered using Whatman filter paper (No: 1) and then concentrated in vacuum at 40°C using a Rotary Evaporator (Buchi, Flawil, Switzerland). The residue obtained was lyophilized in a Modulyo freeze-dryer (Edwards, Crawley, Sussex, UK) and the resulting powdered material was stored at -80°C until testing.

DPPH assay: The hydrogen atom -or- electron donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of a purple colored methanol solution of DPPH. This spectrophotometer assay uses the stable radical, DPPH as a reagent [16]. Fifty microliters of various concentrations of the extracts in methanol were added to 5 mL of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical, DPPH (I %), was calculated in following way:

$$I \% = (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the plotted graph of inhibition percentage against extract concentration. Tests were carried out in triplicate.

β -Carotene-linoleic acid assay: In this assay antioxidant capacity was determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation [17]. A stock solution of β -carotene/linoleic acid mixture was prepared as follows: 0.5 mg of β -carotene was dissolved in 1 ml of chloroform (HPLC grade); 25 μ l linoleic acid and then, 200 mg of Tween 40 were added. Chloroform was completely evaporated using a vacuum evaporator. One hundred milliliters of distilled water, saturated with oxygen (30 min 100 ml/min), was then added while vigorously shaking. Two thousand five hundred microliters of this reaction mixture was dispensed to test tubes and 350 μ l portions of the extracts prepared at 2 g/l concentrations were added and the emulsion system was incubated for up to 48 h at room temperature. The same procedure was repeated with the synthetic antioxidant, butylated hydroxytoluene (BHT) as the positive control, and blank. After this incubation period, the absorbance of the mixtures was measured at 490 nm. Anti-oxidative capacities of the extracts were compared with those of BHT and blank.

Assay for total phenolics: The total phenolic constituent of *M. africana* in ME was determined by literature methods involving FCR and gallic acid as standards [18]. Extract solution (0.1 ml) containing extract was taken in a volumetric flask; 46 mL of distilled water and 1 ml of FCR were added and the flask was thoroughly shaken. After 3 min, 3 ml of a solution of 7% Na_2CO_3 was added and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 765 nm. The same procedure was repeated for all standard Gallic acid solutions (0-1000 mg/0.1 ml⁻¹)

and a standard curve was obtained with the equation given below:

Absorbance=(0.0012×Gallic acid (μg)+0.0033 involving FCR and Gallic acid (both Sigma-Aldrich) as standards [18]. An aliquot (0.1 ml) of the extract solution containing 1 mg of extract was transferred to a volumetric flask, 46 ml of distilled water and 1 ml of FCR were added and the flask was shaken thoroughly. After 3 min, 3 ml of a 7% Na_2CO_3 solution was added and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 765 nm. The same procedure was repeated for all standard Gallic acid solutions (0-1000 g in 0.1 ml) and a standard curve was obtained according to the equation.

ABTS radical cation-scavenging assay: The assay was performed by a slightly modified protocol. ABTS solution (7 mm) was reacted with an ammonium persulphate (2.45 mm) solution and kept for 12-16 h in the dark, to produce a dark colored solution containing ABTS radical cation. The initial absorbance was measured at 734 nm.

This stock solution was diluted with ethanol to give a final absorbance value of about 0.7 (± 0.02) [18] and equilibrated at 30°C. Different concentrations of the sample (50-250 g/ml) were prepared by dissolving the extracts in water. About 0.3 ml of the sample was mixed with 3 ml of ABTS working standard in a microcuvette. The decrease in absorbance was measured exactly one minute after mixing the solution, and then re-measured up to six minutes later. The final absorbance was noted. The inhibition percentage was calculated according to the formula:

$$\% \text{ inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100\%$$

Total flavonoid content: Total flavonoid content was assayed using aluminum chloride. The plant extracts were added in 2 ml HCl in round bottom flasks and were refluxed at 100°C for 30 min. The hydrophilic extract will be increased to 5 ml by using distilled water. 0.3 ml of 5% (w/v) $NaNO_2$ and 3 ml of 10% $AlCl_3$ were then added to the mixture. At the six min mark, 2 ml of 1 M NaOH was added to the mixture. The mixture was mixed well and the absorbance at 510 nm was read using a spectrophotometer. Total flavonoid content was expressed as mg catechin CE/g dry weight of sample. The experiment was replicated for three independent assays [19].

Antimicrobial assay: The antibacterial activity was evaluated by using the broth dilution method [20, 21]. Nine bacteria species were selected as representative of Gram-positive or Gram-negative and were tested: Gram-positive bacteria *Bacillus anthracis* (PTCC 1274), *Bacillus cereus* (PTCC 1247), *Bacillus subtilis* (PTCC 1023), *Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus aureus* (ATCC 25923) and the Gram-negative bacteria *Escherichia coli* (ATCC 25922), *Pseudomonas* sp. (ATCC 85327), *Salmonella thifi* (ATCC 1231), and *Shigella fleksheneri* (ATCC 1042), were identified by the Research Centre of Science and Industry, Tehran, Iran.

Microorganisms (obtained from enrichment cultures of the microorganisms in 1 ml of Mueller-Hinton broth,

incubated at 37°C for 12 h) were cultured on a Mueller-Hinton agar medium. The following method was used to measure antibacterial activity: 40 µl of diluted essential oil (40 µl oil in 2 ml DMSO (Dimethyl sulfoxide) 10%) was added to a 200 µl microbial suspension (1 loop from the medium in physiological serum was compared with a 0.5 McFarland standard) in well 1 in a microplate, and 100 µl from this well was added to a 100 µl microbial suspension in well 2, and this continued until 8 wells in the microplate were filled. The microplates were then incubated at 37°C for 24 h [14, 22].

Results

The objective of this research was to study the antioxidant and antibacterial activities of obtained extracts from the stems and roots of cultivated *M. africana*, particularly against Gram-positive bacteria. In a β-carotene-linoleic acid test, the aerial parts and seeds, ME of *M. africana* had the highest antioxidant effects. The antioxidant activity measured with DPPH assays was 39.54 and 42.39 µg/ml, respectively. As shown in Figure 1, (DPPH), the ME, CE and HE were able to reduce the stable radical, DPPH to a yellow-colored diphenyl picryl hydrazine where the IC₅₀ value of the Methanol extracted from the aerial parts was 39.5±0.005 µg/ml.

The IC₅₀ value of the methanol extracted from the seed was 42.4±0.004 µg/ml. For all samples studied, the ME of the aerial parts had the strongest free radical-scavenging activity. The concentration of the positive control BHT required to scavenge 50% of the free radical (IC₅₀) was 18.3±0.006 µg/ml. In the case of the linoleic acid system, ME from the seeds and aerial parts inhibit the oxidation of linoleic acid which is an important issue in food processing and preservation (Fig. 2).

Antioxidants minimize the oxidation of the lipid components in cell membranes or inhibit the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation that are known to be carcinogenic. In general, a similar activity pattern to that seen in the first system was observed. Among the extracts prepared with various solvents, the strongest effect was supplied by methanol sub-fraction (78.58%). Based on the absorbance values of the various extract solutions, the solutions were reacted with Folin-Ciocalteu reagent (FCR) and compared with standard solutions of Gallic acid equivalents. As described above, total phenolics are shown in figure 3.

The total amount of phenolics was highest in the methanol of aerial parts extract (MW) (225.19±0.02 mg Gallic acid/g). The lowest value was exhibited by the hexane aerial parts extract (11.09±0.02 mg Gallic acid/g). Indeed, when the results given in figures 1-3 are compared, it can be seen that the phenolic content was high in polar extracts

Table 1. Antioxidative capacities of the seeds and aerial parts of *M. africana*^a

Seeds	IC ₅₀ (µg/mL)	Aerial parts	IC ₅₀ (µg/mL)
HE ^b (10000 ppm)	865.3	HE (10000 ppm)	168.6
CE ^c (10000 ppm)	173.1	CE (10000 ppm)	201.5
ME ^d (1000 ppm)	42.4	ME (1000 ppm)	39.5

a) Results are means of three different experiments

b) Methanol Extract (ME), c) Chloroform Extract (CE), d) Hexane Extract (HE)

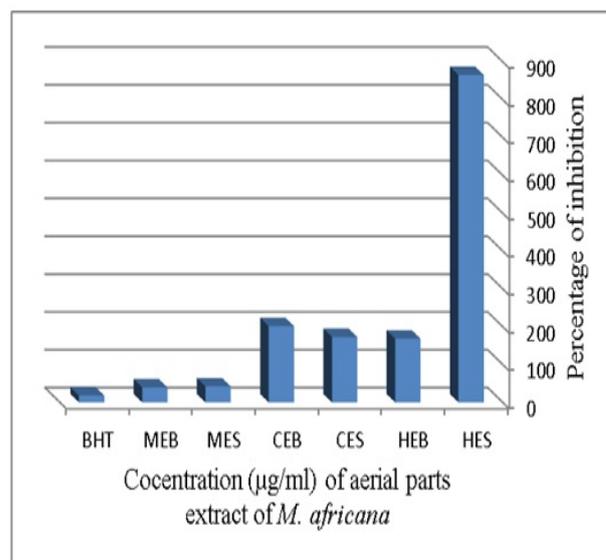


Figure 1. Free radical-scavenging capacities of the aerial parts extract of *M. africana* measured in DPPH assay

MEB=methanol extract of aerial parts; MES= methanol extract of seeds; CEB=chloroform extract of aerial parts; CES=chloroform extract of seeds; HEB=Hexane extract of aerial parts; HES=Hexane extract of seeds; BHT=Butylated hydroxytoluene

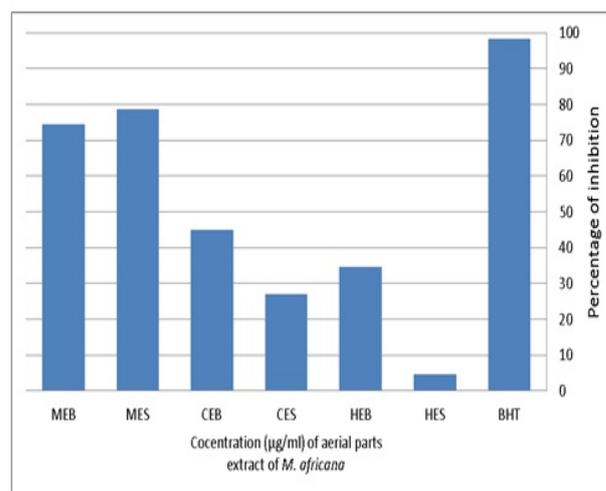


Figure 2. Antioxidant activity of aerial parts extract of *M. africana* defined as inhibition percentage in β-carotene-linoleic acid assay

MEB=methanol extract of aerial parts; MES=methanol extract of seeds; CEB=chloroform extract of aerial parts; CES=chloroform extract of seeds; HEB=Hexane extract of aerial parts; HES=Hexane extract of seeds; BHT=Butylated hydroxytoluene

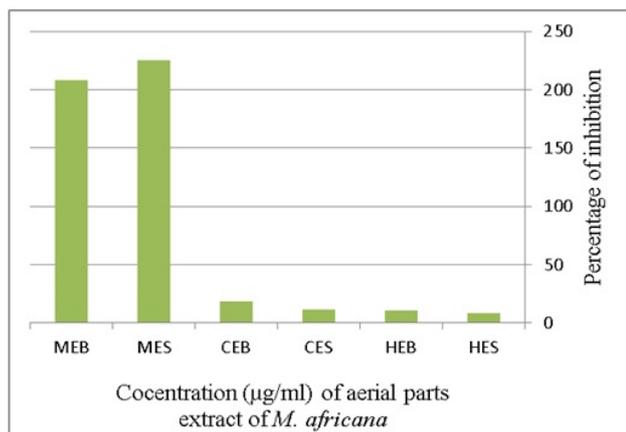


Figure 3. Antioxidant activity of aerial parts extract of *M. africana* defined as the inhibition percentage in total phenolic-Gallic acid assay

MEB=methanol extract of aerial parts; MES=methanol extract of seeds; CEB=chloroform extract of aerial parts; CES=chloroform extract of seeds; HEB=Hexane extract of aerial parts; HES=Hexane extract of seeds; BHT=Butylated hydroxytoluene.

Discussion

In this research, we could not find further information, and we studied the antioxidant and antibacterial activities of various extracts of ME, HE and CE from the seeds and aerial parts of *M. africana* using stable radical scavenging 2, 2- Diphenyl-1-picrylhydrazyl (DPPH), phenolic compounds measurement, hydrogen peroxide sweeper measurement, measurement of flavonoids, ferric reducing, trapping nitric acid and chelation of metal and activity through FTC method. The IC_{50} value of the methanol extracted from aerial parts was 39.5 ± 0.005 µg/ml. The IC_{50} value of the Methanol extracted from the seeds was 42.4 ± 0.004 µg/ml. For all samples studied, the ME of the aerial parts had the strongest free radical-scavenging activity. The concentration of the positive control BHT required to scavenge 50% of the free radical (IC_{50}) was 18.3 ± 0.006 µg/ml.

The results of recent studies on free radicals confirms the fact that antioxidant-rich foods play a vital role in the prevention of cardiovascular disease, cancer [23] and neurodegeneration diseases, including Parkinson's and Alzheimer's disease [23, 24] as well as the prevention from inflammation and problems caused by cellular and skin aging [22-28]. The antimicrobial and antioxidant activity of plant *Salvia glutinosa* has been previously reported. In the plant *S. glutinosa* is the main substance of Sesquiterpene (D germakern) [29].

In previous studies, the desirable antimicrobial and antioxidant activity of the *Tanacetum pinnatum* plant was confirmed. In this study, the antioxidant activity of thymol has also been investigated using three methods including thymol antioxidant activity investigation using stable radical scavenging DPPH, Phenolic compounds measurement and measurement of beta-carotene linoleic acid. The value of IC_{50} in this test was equal to 1205.0 ± 722.8 µg/ml. The comparison with previous studies shows that the aromatic compounds and

monoterpenes with –OH functional groups have a good antioxidant effect [20].

Studies on the sex of *Malcolmia*, have reported their phenolics and acids as follows: oleic acid (5-31.3%), linoleic acid (2-24.8%), linolenic acid (1.7-64.1%), and erucic acid (3-55.1%) [13]. Zyrdr *Malcolmia crenata* chemicals have also been reported. Glucoiberin, Glucocheirolin and Olefin [30], are among the cruciferae oil plants under study; descourainia specially collected from Hongyuan, had a high oil content of 44.17% and a linoleic acid content of 40.9%. All the data suggest that this oil plant species can be utilized for industrial purposes. Zhang et al. [31] have emphasized the fact that *M. africana* and *Arabis hirsuta* have high linoleic acid content with 57.37% and 51.38% respectively. These two species are also worthy of further inspection.

As shown in figure 4, Terpenoids have been found to possess antioxidant and antimicrobial properties in various studies [30-32]. The ME, CE and HE extracts were individually tested against a panel of microorganisms, including *Staphylococcus aureus* (for minimum inhibitory concentration test), *Staphylococcus*, *Bacillus cereus*, *Bacillus subtilis*, *Salmonella thifi*, *Escherichia coli*, *Pseudomonas* sp. *Shigella flexneri* and *Bacillus antracila*.

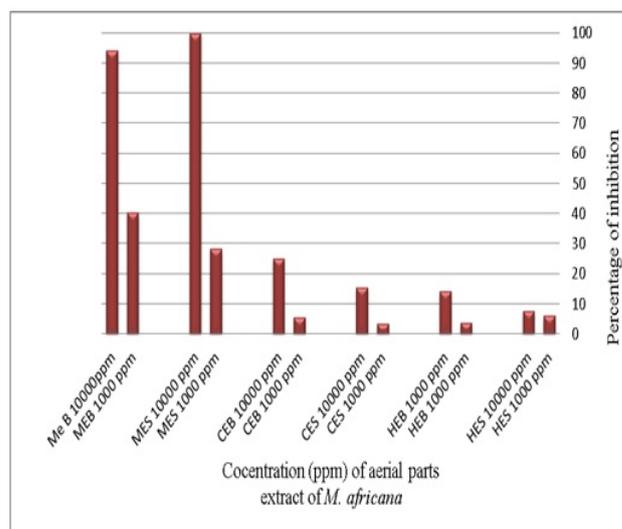


Figure 4. ABTS test for aerial parts and seeds of *M. Africana*

MEB= methanol extract of aerial parts; MES= methanol extract of seeds; CEB= chloroform extract of aerial parts; CES= chloroform extract of seeds; HEB= Hexane extract of aerial parts; HES= Hexane extract of seeds; BHT= Butylated hydroxytoluene.

The bacterial growth was indicated by the presence of a white “pellet” on the well bottom. The results of this work have shown that *M. africana* possesses compounds with antimicrobial and antioxidant properties. A further study in vivo condition is also necessary to confirm the antimicrobial and antioxidant activities of *M. africana* which may be used for the preservation and/or extension of the shelf-life of raw and processed foods as well as pharmaceuticals and natural therapies of infectious diseases in humans, and the management of plant diseases.

Table 2. Antimicrobial activity of methanol extract, hexane extract and chloroform extract of *M. africana*, using agar well diffusion

Microorganisms	Gram stain	ME ^a		HE ^b		CE ^c	
		Seeds	Aerial parts	Seeds	Aerial parts	Seeds	Aerial parts
Bacillus antracila (PTCC 1274)	+	-	5	20	5	-	60
Bacillus cereus (PTCC 1247)	+	-	-	10	3	3	-
Bacillus subtilis (PTCC 1023)	+	10	20	45	20	5	10
Staphylococcus epidermidis (ATCC 12228)	+	-	-	20	-	-	10
Staphylococcus aureus (ATCC 25923)	+	-	-	20	4	5	60
Escherichia coli (ATCC 25922)	-	-	-	5	5	5	-
Pseudomonas sp.(ATCC 85327)	-	10	10	30	5	15	-
Salmonella thifi (ATCC 1231)	-	-	-	-	-	-	-
Shigella flexneri (ATCC 1042)	-	-	-	20	5	5	20

Agar disc diffusion method Diameter of inhibition zone (mm) including disk diameter of 100 mm.

a)Methanol Extract (ME); b)Hexane Extract (HE); c)Chloroform Extract (CE)

In addition, the present study provides additional data for supporting the use of *M. africana* plants as tea or additives in food, and traditional remedies for the treatment of infectious diseases and cancer. Total flavonoid content in the seeds and aerial parts extracts showed different activities. The activities of total flavonoid content in seed extracts showed that it can withstand high temperatures. Talik et al. [32] studied total flavonoid and total phenolic contents in *Curcuma longa* using different extractions such as boiled aqueous for 10 min, 30 min, boiled ethanol for 10 min, stirring aqueous for 1 h and stirring ethanol for 1 h.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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