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Chemical composition by nano scale injection and antioxidant activity of *Cleome iberica* DC

A. BAMONIRI[a] , A. MAZOOCHI[a], A. H. EBRAHIMABADI[a], B. F. MIRJALILI[b], M. BEHPOUR[a], J. SAFAEI-GHOMI[a], H. BATOOLI[c]
[a]*Essential Oils Research Center, University of Kashan, Kashan, I. R. Iran*
[b]*Department of Chemistry, College of Science, Yazd University, Yazd, I. R. Iran*
[c]*Isfahan Research Center of Natural Sources, Kashan Station, Kashan, I. R. Iran*

The chemical composition by nano scale injection and antioxidant activity of the essential oil and the methanolic extract from aerial parts of *Cleome iberica* have been studied. GC and GC-MS analyses of the essential oil resulted in the identification of 14 compounds, representing 99.97% of the oil. lavandulyl acetate (26.63%), *p*-cymene (13.73%), geranyl acetate (11.99%) and neryl acetate (9.27%) were the main components, comprising 61.62% of the oil. In vitro antioxidant properties of the essential oil and the methanolic extract were determined by DPPH, β-carotene-linoleic acid assay methods and compared to those of the synthetic antioxidant BHT. Results of both assay methods indicated that the methanolic extract has superior antioxidant activity to essential oil. Also, total phenolic content of the extract was determined as gallic acid equivalent.

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1. Introduction

Cleome is a genus of flowering plants traditionally included in the family Capparidaceae. This genus is distributed in the tropical and warm temperate regions of the world with about 170 species of herbaceous annual or perennial plants [1]. Sixteen species of the genus *Cleome* are known wild in moderate and subtropical areas of southern Iran [2]. Several species of *Cleome* have been target of phytochemical studies, *e.g* triterpenes and flavonoids have already been isolated [3].

Moreover, several pharmacological activities such as anti-inflammatory [4,5], antioxidant [6,7], antineoplasic [8], antioxidative stress [9], antimicrobial [10,11], antipyretic [12], diuretic [13] and analgesic effects [14,15] have already been demonstrated for crude extracts or compounds obtained from different *Cleome spp*. On the other hand, some species of this genus are used as folk medicine in the treatment of scabies, rheumatic fever and inflammation [16,17], bronchitis and diarrhea [18], stomachache, headaches, neuralgia and other localized pains [19].

Meanwhile, the oil of just one species of genus *Cleome* (*C. hirta* Oliv.) is repellent to livestock tick and maize weevil[20].

C. iberica DC., is grassy, annual plant, with exalted and branching stems, thick and vertical roots, white - pink colored convened flowers and three leaflet leaves and endemic in Iran, grows in Northern provinces, west regions and central parts of Iran [21,22].

Antimicrobial and antioxidant properties are cause of the many active phytochemicals including flavonoids, terpenoids, carotenoids, coumarins, curcumines etc. Thus, there is at present growing interest, in naturally-occurring antioxidants that can be used to protect human beings from oxidative stress damage for survey of aromatic and medicinal plants [23]. Hence, they are very important for investigating in diets or medical therapies for biological tissue deterioration due to free radicals. Herbs and species are amongst the most important targets to search for natural antimicrobials and antioxidants for the safety considering [24,25].

A literature survey revealed no reports on the chemical composition, and antioxidant activity of the essential oil and extract of this plant in Kashan area, thus, this is the first such study on it. For this reason, *C. iberica* was collected and then its essential oil and methanolic extract were investigated for composition and in vitro antioxidant activities.

2. Experimental

2.1. Plant materials

The aerial parts (leaves and flowers/inflorescences) of *C. iberica* DC. were collected during the fruition period in July 2008 in Qamsar, around Kashan (Isfahan province, Iran) at an altitude of 1850 m. The voucher specimens of the plant were deposited in the herbarium (voucher No.KBGH 1352) of Research Institute of Forests and Rangelands, Kashan, Iran.

2.2. Preparation of the extract

A portion (50g) of air-dried and ground plant material was Soxhlet-extracted, with 500 mL of methanol for 8 h at

a temperature not exceeding the boiling point of the solvent [26]. The extract was concentrated using a rotary evaporator (Buchi, Flawil, Switzerland) at a maximum temperature of 45ºC, and dried extract was stored in a refrigerator before further analysis. The yield of dried methanolic extract for aerial parts was 15.76%.

2.3. Extraction of the essential oil

The air-dried and ground aerial parts (24g) of *C. iberica* were subjected to hydrodistillation for 3.5 h using a Clevenger-type apparatus [27]. After decanting and drying over anhydrous sodium sulfate, colorless oil was recovered from the aerial parts in a yield of 0.04% (v/w) and stored at low temperature (4ºC) further analysis.

2.4. GC-MS analysis conditions

2.4.1. GC

GC analysis of the oil was performed on an Agilent HP-6890 gas chromatograph equipped with flame ionization detector (FID) and an HP-5MS capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 µm). The oven temperature was programmed as follows: 50ºC (2 min), 50-130ºC (5ºC min^{-1}), 130ºC (2 min), 130-200ºC (3ºC min^{-1}), 200ºC (2 min) and 200-260ºC (20ºC min^{-1}). Injector and detector temperatures were maintained at 220ºC and 290ºC, respectively. The amount of the sample injected was 1.0 nL (diluted 1.0 µL of sample in 1000 ml of *n*-pentane, v/v) in the splitless mode. Helium was used as carrier gas with a flow rate of 1 mL min^{-1}.

2.4.2. GC-MS

GC-MS analysis of the oil was performed on a Agilent HP-5973 mass selective detector coupled with a Agilent HP-6890 gas chromatograph, equipped with a cross-linked 5% PH ME siloxane HP-5MS capillary column (30 m × 0.25 mm i.d, film thickness, 0.25 µm) and operating under the same conditions as above was described. The flow rate of helium as carrier gas was 1 mL min^{-1}. The MS operating parameters were as follows: ionization potential, 70 eV; ionization current, 2 A; ion source temperature, 200ºC; resolution, 1000.

2.4.3. Compound identification

Essential oil was analyzed by GC and GC/MS systems using a non-polar column and identification of components in the oil was based on retention indices (RI) relative to *n*-alkanes and computer matching with the WILEY 275.L library, as well as by comparison of the fragmentation pattern of the mass spectra with data published in the literature [28,29]. The percentage composition of the samples was computed from the GC-FID peak areas without the use of correction factors.

2.5. Antioxidant activity

2.5.1. Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH, 95%), β-carotene, linoleic acid, 2,6-di-tert-butyl-4-methylphenol (butylated hydroxyl toluene, BHT) and gallic acid were from Sigma–Aldrich Chemie (Steinheim, Germany). Analytical grade methanol, ethanol and dimethyl sulfoxide (DMSO), HPLC grade chloroform, standard Folin–Ciocalteu's phenol reagent, anhydrous sodium sulfate, Na_2CO_3 and Tween 40 were from Merck (Darmstadt, Germany). Ultra pure water was used for the experiments.

2.5.2. DPPH assay

The hydrogen atom or electron donation ability of the essential oil and methanolic extract was measured using stable free radical 2,2-diphenylpicrylhydrazyl (DPPH) by a published DPPH radical scavenging activity assay method [30] with minor modifications. It is a widely used reaction based on the ability of antioxidant molecule to donate hydrogen to DPPH which consequently turns into an inactive form. Briefly, stock solutions (10 mg mL^{-1}) of the essential oil, extract and synthetic standard antioxidant butylated hydroxyl toluene (BHT) in methanol were prepared. Dilutions are made to obtain concentrations ranging from 2 to 5×10^{-4} ng mL^{-1}. Two mililiters of various concentrations of each sample in methanol were added to 2 mL of a freshly prepared 80 µg mL^{-1} DPPH methanol solution. After a 30 min incubation period at room temperature, the absorbencies were read against a blank at 517 nm. Inhibition of free radical DPPH in percent (I %) was calculated in the following way:

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

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 form the graph plotted from inhibition percentage against extract concentration. Tests were carried out in triplicate.

2.5.3. β-Carotene/linoleic acid assay

In this assay, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and conjugated diene hydroperoxides arising from linoleic acid oxidation. The method described by Tepe et al. [31] was used with slight modifications 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 µl linoleic acid and 200 mg Tween 40 were added. Chloroform was completely evaporated using a vacuum evaporator. Then, 100 mL of distilled water saturated with oxygen (30 min, 100 mL min^{-1}) were added with vigorous shaking.

The samples (2 g L^{-1}) were dissolved in DMSO and 350 µL of each sample solution was added to 2.5 mL of the previous mixture in test tubes and the emulsion system was incubated in hot water (50°C) for 2 h. The same procedure was repeated with the synthetic antioxidant butylated hydroxytoluene (BHT) as positive control and a blank. After this incubation period, absorbencies of the mixtures were measured at 470 nm. Antioxidant capacities (Inhibition percentages, I %) of the tested solutions were calculated using the following equation:

$$I\% = (\beta\text{-carotene content after 2 h assay/initial } \beta\text{-carotene content}) \times 100$$

Tests were carried out in triplicate. Percent inhibitions of the samples were compared with that of positive and negative standards.

2.5.4. Assay for total phenolics

The total phenolic compounds content in the methanolic extract of *C. iberica* was determined by employing the methods given in the literature [32,33] involving Folin-Ciocalteu reagent and gallic acid as standard. An aliquot (0.1 mL) of extract solution containing 1000 µg extract was added to a volumetric flask, 46 mL distilled water and 1 mL Folin-Ciocalteu reagent was added and the flask was shaken thoroughly. After 3 min, a 3 mL solution of Na_2CO_3 (2% w/v) was added and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 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:

$$\text{Absorbance} = 0.0012 \times \text{Gallic acid (µg)} + 0.0033$$

Total phenolic constituent of each extract as gallic acid equivalent was determined by putting its measured absorbance at 760 nm in this standard curve and equation. Tests were carried out in triplicate.

3. Results and discussion

3.1. Chemical composition of oil

Air dried aerial parts of the plant were subjected to hydrodistillation using a Clevenger-type apparatus and the colorless oil was obtained in the yield of 0.04% (v/w). Fourteen components were identified in *C. iberica* oil constituting 99.97% of the total oil and listed in Table 1 with their percentages. Constituents are listed in order of their elution from HP-5MS column. The main components comprising 61.62% of the oil were lavandulyl acetate (26.63%), *p*-cymene (13.73%), geranyl acetate (11.99%) and neryl acetate (9.27%). Other components were present in amounts less than 8%.

Table 1. Percentage composition of the aerial parts oil of C. iberic.

Compound [a]	**RI** [b]	**%** [c]
P-Cymene	1018	13.73
(E)-β-Ocimene	1039	3.56
Linalool	1095	3.43
IsoPentyl isovalerate	1102	2.90
Lavandulyl acetate	1288	26.63
Neo-verbanol acetate	1314	1.51
Presilphiperfol-7-ene	1335	3. 53
Neryl acetate	1355	9.27
α–Copaene	1367	4.70
Geranyl acetate	1376	11.99
Cubebol	1506	4.11
Presilphiperfolan-8-ol	1574	7.36
Geranyl isovalerate	1600	2.07
α-Bisabolol	1677	5.18
Total identified		99.97

[a] Compounds listed in order of those RI.
[b] RI, (retention index) measured relative to n-alkanes (c_8-c_{32}) on the non-polar HP-5MS column.
[c] %, Relative percentage obtained from peak area.

Although phytol [20], 1-isocyano-4-methyl benzene [10], terpenolene [34], ethyl palmitate [35], piperitone [36] and Carvacrol [37] have been reported in the oil of the other *Cleome* genus such as *C. hirta*, *C. chrysantha*, *C. monophylla*, *C. viscosa* and *C. coluteoides* as the major component, did not find in this oil.

Otherwise, *p*-cymene and *α*–Copaene which were found of this oil have been reported in the oil of *Cleome iberica* growing in Touchal, north of Tehran province. Meanwhile, the presence of carotol (21.77%), germacrene D (15.8%) and *β*-cubebene (15.46%), have been defined in *C. iberica* from Tehran area in high concentrations [38].

By far, qualitative and quantitative variations between our results and previous reports, for the constituents of the *Cleome* genus oil, attributed to the differences in environmental conditions such as local, climatic, seasonal and differences in oil extraction methods.

3.2. Antioxidant activity

In This work, we have investigated the free radical scavenging activity and lipid oxidation inhibition of *C. iberica* essential oil and methanolic extract. Free radical scavenging activities of the both essential oil and extract were measured in DPPH assay and the reaction followed a concentration dependent pattern. Free radical scavenging increases with increasing extract concentration and the later providing 50% inhibition (IC_{50}) are given in Table 2. Results of the DPPH assay method indicated that the free radical scavenging activity of the methanolic extract (IC_{50} = 120.25 ± 0.90 µg mL^{-1}) was superior to oil (less than 17% inhibition in 10 mg mL^{-1} concentration), but not as well as that of BHT (IC_{50} = 19.72 ± 0.8 µg mL^{-1}).

Table 2. Effects of C. iberica essential oil, methanolic extract and positive control on the in vitro free radical (DPPH) scavenging

Sample	[$IC_{50,}$ µg/mL]
Methanolic extract	120.25 ± 0.90
Essential oil	ND [a]
BHT	19.72 ± 0.8

[a] Less than 17% inhibition at 10 mg mL^{-1} (ND = Not Determined).

Due to the major contribution of phenolic compounds in antioxidant activity [39,40], total phenolic constituent of the methanolic extract was determined as gallic acid equivalent. Relatively low antioxidant activity of the plant extract may be a consequence of its low phenolic compounds content which was reflected in its Folin-Ciocalteu test (15.29 ± 0.7 µg mg^{-1}, 1.529% w/w as gallic acid equivalent).

In the case of inhibition of linoleic acid assay, methanolic extract of the plant was showed an inhibition percentage (89.11% ± 0.047) comparable to that of synthetic standard BHT (98.13% ± 0.026), since, the essential oil was indicated an inhibition percentage (16.32% ± 0.062), are given in figure 1. Peroxy radicals usually initiate lipid peroxidation by abstraction of an allylic or benzylic hydrogen atom from the molecule under oxidation [41]. Thus, the possible presences of allylic and/or benzylic hydrogen containing secondary metabolites in the above mentioned extract, establishes considerable antioxidant activity in the *β*-carotene/linoleic acid test.

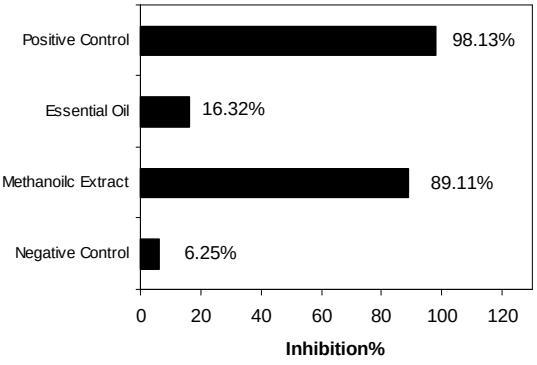

Fig 1. Antioxidant activity of C. iberica extract and essential oil defined as inhibition percentage in β-carotene/linoleic acid assay.

Whenever, there are many reports on the antioxidant activity of the *Cleome* genus plant species such as *C. gynandra* [42,43,44], *C. Arabica* [6], *C. rutidosperma* [45] and *C. viscose* [46] but no reports on the antioxidant activity of the *C. iberica*.

4. Conclusions

This study is the first to evaluate the essential oil composition in Kashan area (lavandulyl acetate as the major component), antioxidant activities employing a variety of DPPH, β-carotene-linoleic acid assay methods and total phenolic content by Folin-Ciocalteu test of the *C. iberica*. The methanolic extract of this plant has potent antioxidant property on inhibition of linoleic acid assay and may have potential as natural preservative ingredients in food and/or pharmaceutical industries.

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*Corresponding author: bamoniri@kashanu.ac.ir