Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI:10.26479/2019.0501.69**

ESSENTIAL OIL COMPOSITION AND BIOLOGICAL ACTIVITIES OF EXTRACT OF *ACINOS GRAVEOLENS* FROM IRAN

Reza Dehghani Bidgoli[1*], Abdolrasoul H. Ebrahimabadi[2], Nahid S. HaghighiQamsari[2], Hossein Batooli[3]

1. Department of Rangeland and Watershed Management, University of Kashan, Iran.
2. Essential Oils Research Institute, University of Kashan, Kashan, Iran.
3. Associate Prof., Kashan Botanical Garden, Research Institute of Forests and Rangelands, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran.

ABSTRACT: Determination of chemical constituents of the essential oil of *Acinos graveolens* and evaluation of biological activities of this plant methanol extract were performed in this study. Essential oil of *A. graveolens* was extract using simultaneous distillation extraction (SDE) method and have been analyzed by GC/FID and GC/Mass systems. Twenty-seven constituents representing 93.09% of the oil were identified both qualitatively and quantitatively. Germacrene-D (56.65%), bicyclogermacrene (8.69%) and caryophyllene oxide (7.46%) were detected as the plant major essential oil components. The plant extract was also tested for its antioxidant activities using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and b-carotene/linoleic acid assays. Extract of *A. graveolens* showed a good activity in DPPH assay with IC_{50} value of (50.34±5.33 µg/ml) and a moderate activity in *β*-carotene-linoleic acid assay with inhibition percentage of 46.1±5.0%. Total phenolic compounds content of the extracts was recorded as 51.05±0.71 µg/mg. The plant also showed weak anticancer and antimicrobial activities in brine shrimp lethality and antimicrobial assays, respectively.

KEYWORDS: *Acinos graveolens* , Antioxidant, *β*-carotene, Essential oil, Extract

Corresponding Author: Dr. Reza Dehghani Bidgoli* Ph.D.

Department of Rangeland and Watershed Management, University of Kashan, Iran.

Email Address: dehghanir@kashanu.ac.ir

1. INTRODUCTION

The inhibition of oxidative reactions in food, pharmaceutical and cosmetic products and the prevention of oxidative stress related diseases in the human body are some of the useful potential

Peer review under responsibility of Life Science Informatics Publications

functions of antioxidants [19], [2]. Plant phenols and polyphenols, with their potential antioxidant activities, play a major role in the prevention of various pathologic conditions such as cancer, cardiovascular and neurodegenerative diseases believed to be originated from oxidative stress [15], [4]. Therefore, during recent years, interest in finding naturally occurring antioxidants with potential protecting effects on human beings has been intensified [22], [5]. Microbial contamination is another important issue in the field of food, beverage, cosmetic, and pharmaceutical industries. The resistance of many bacterial strains against antibiotics is substantially increasing. Therefore, search for finding new substances with antibacterial activity has become an urgent necessity. Medicinal plants are frequently used in popular medicine as remedies for many infectious diseases [27], [6]. Also, plants have been seen to possess the potential to be excellent lead structures and to serve as a basis of promising therapeutic agents for cancer treatment. Many successful anti-cancer drugs currently in use or their analogues are plant-derived and much more are under clinical trials [13], [7]. Accordingly many of biologically active compounds with great therapeutic potential and providing the molecular basis for most of the drugs currently in clinical use are natural products isolated from plant spices. *Acinos* is one of the smallest genera of the Lamiaceae family native to Europe, Mediterranean region, central Asia, northern Africa and North America. The name *Acinos* comes from the Greek word *akinos* meaning a small aromatic plant. *Acinos* species are annual evergreen perennial woody plants. They are small, tufted, bushy or spreading plants growing to 10 -45 cm tall which propagate by division, softwood cuttings or from seed in the spring [25], [14]. *Acinos* is represented by10 species distributed over Mediterranean regions toward central Asia and Iran [28],[16]. Some of them are traditionally used in Mediterranean countries. Some species of genus *Acinos* are employed in folk medicine as antiseptic, stimulant, tonic, and antispasmodic due to their beneficial effects on melancholy, coughs, toothache, sciatica, neuralgia, and gastrointestinal disorders [11], [12]. *A. suaveolens* is the most examined species and also has the greatest number of uses in traditional medicine while some of the species weren't examined at all. In Iran, two species of this genus exist, *A.arvensis* (Lam.) Dandy and *A. graveolens* (M. B.) Link. *Acinos graveolens*is an annual, dwarf herb which is widely distributed in Iran and neighboring countries [10], [8].

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Plant material

Plant materials for extraction were aerial parts of *A. graveolens* collected during spring 2015 from Ghamsar area (Isfahan Province, Iran). An authenticated specimen of the plant was deposited in the herbarium of the Kashan Research Botanical Garden, Research. Institute of Forests and Rangelands, Kashan, Iran.

2.1.2. Solvents and chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH, 95%), β-carotene, linoleicacid, 2,6-di-tert-butyl-4-methylphenol (butylated hydroxyl toluene,BHT), and Gallic acid were procured from Sigma–Aldrich Chemie (Steinheim, Germany). Analytical grade methanol, ethanol, anddimethyl sulphoxide (DMSO), HPLC grade chloroform, standard Folin–Ciocalteu's phenol reagent, anhydrous sodium sulphate, sodium carbonate, and Tween 40 were obtained from Merck (Darmstadt, Germany). Ultra-pure water was used for thee xperiments.

2.2. Preparation of the extract

2.2.1. Isolation of the essential oil

Essential oil of *A. graveolens* (30g of air dried plant) was extract using simultaneous distillation extraction (SDE) method and have been studied by GC and GC-MS systems. The obtained essential oil was dried over anhydrous sodium sulphate and after filtration, stored in an amber vial at low temperature (4ºC) prior to analysis.

2.2.2. Preparation of methanol extract

Ten grams of the powdered aerial parts of *A. graveolens* were Soxhlet-extracted with 400 ml methanol for 8 h. Solvent removal by rotary evaporator and drying the residue in the vacuum oven at 50 ˚C was yielded 2.04 g (20.4%) of dried extract. Extract was kept in the dark at 4 ˚C until tested.

2.3. Chromatographic analysis

2.3.1. Gas chromatography (GC) analysis

Oil obtained from aerial parts of *A. graveolens* was analysed using an Agilent HP-6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with HP-5MS 5% phenylmethyl siloxane capillary column (30 m _ 0.25 mm, 0.25 lm film thickness; Restek, Bellefonte, PA) equipped with an FID detector. Oven temperature was maintained at 40˚C for 3 min initially and then raised at the rate of 3˚C/min to 280˚C. Injector and detector temperatures were set at 220˚C and 290˚C, respectively. Helium was used as carrier gas at a flow rate of 1 ml/min, and diluted samples (1/1000 in n-pentane, v/v) of 1.0 µl were injected manually in the split less mode. Peaks area percents were used for obtaining quantitative data.

2.3.2. Gaschromatography/mass spectrometry (GC/MS) analysis

GC/MS analysis of the oil was carried out on an Agilent HP-6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with a HP-5MS 5% phenylmethyl siloxane capillary column (30 m _0.25 mm, 0.25 lm film thickness; Restek, Bellefonte, PA) equipped with an Agilent HP-5973 mass selective detector in the electron impact mode (Ionization energy: 70 eV) operating under the same conditions as described above. Retention indices were calculated for all components using a homologous series of n-alkanes injected in conditions equal to samples ones. Identification of components of essential oil was based on retention indices (RI) relative to n-

alkanes and computer matching with the Wiley275.L and Wiley7n.L libraries, as well as comparisons of the fragmentation pattern of the mass spectra with data published in the literature [21], [1].

2.4. Antioxidant activity

2.4.1. DPPH assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay usually involves hydrogen atom transfer reaction but, based on kinetic data, an electron transfer mechanism has also been suggested for this assay (Foti et al., 2004; Huang et al., 2005). Radical-scavenging activity (RSA) of extracts was determined using a published DPPH radical-scavenging activity assay method (Sarker et al.,2006) with minor modifications. Briefly, stock solutions (10 mg/ml each) of the extracts and the synthetic standard antioxidant BHT were prepared in methanol. Dilutions are made to obtain concentrations ranging from 1 to 5×10^{-10}mg/ml. Diluted solutions (2 ml each) were mixed with 2 ml of freshly prepared 80 µg/ml DPPH methanol solution and allowed to stand for 30 min in the dark at room temperature for any reaction to take

place. Ultraviolet (UV) absorbencies of these solutions were recorded on a spectrometer (Cintra 6, GBC, Australia) at 517 nm using a blank containing the same concentration of extracts or BHT without DPPH. Inhibition of free radical DPPH in percent (I%) was calculated as follow:

$$I\% = [A_{blank} \_ A_{sample}/A_{blank}] \times 100$$

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. The sample concentration providing 50% inhibition (IC_{50}) was calculated by plotting inhibition percentages against concentrations of the sample. All tests were carried out in triplicate and IC_{50} values were reported as means ± SD of triplicates.

2.4.2. β-Carotene/linoleic acid bleaching assay

In this assay, antioxidant activity was determined by measuring the inhibition of volatile organic compounds and conjugated diene hydro peroxides arising from linoleic acid oxidation. The method described by Miraliakbari and Shahidi (2008) was used with slight modifications [23], [17]. A stock solution of β-carotene and linoleic acid was prepared with 0.5 mg of β-carotene in 1 ml chloroform, 25 µl of linoleic acid and 200 mg Tween 40. The chloroform was evaporated under vacuum and 100 ml of aerated distilled water was then added to the residue. The samples (2 g/l) were dissolved in DMSO and 350 µl of each sample solution was added to 2.5 ml of the above mixture in test tubes. The test tubes were incubated in a hot water bath at 50˚C for 2 h, together with two blanks, one contained the antioxidant BHT as a positive control and the other contained the same volume of DMSO instead of the extracts. The test tube with BHT maintained its yellow color during the incubation period. The absorbencies were measured at 470 nm on an ultraviolet spectrometer (Cintra 6, GBC, Australia). Antioxidant activities (inhibition percentage, I%) of the

samples were calculated using the following equation:

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

Where $A_{\beta\text{-carotene after 2 h assay}}$ is the absorbance of β-carotene after 2 h assay remaining in the samples and $A_{\text{initial}\beta\ -\text{carotene}}$is the absorbance of β-carotene at the beginning of the experiments. All tests were carried out in triplicate and inhibition percentages were reported as means ±SD of triplicates.

2.4.3. Assay for total phenolics

Total phenolics constituent of the methanol extract of *A. graveolens* determined by literature methods involving Folin–Ciocalteu reagent and Gallic acid standard [26], [24]. Solution of the extract (0.1 ml) containing1000 µg of the extract was taken in a volumetric flask, 46 ml of distilled water and 1 ml Folin–Ciocalteu reagent were added, and the flask was thoroughly shaken. After 3 min, 3 ml of 2% Na_2CO_3 solution 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 the standard Gallic acid solutions (0–1000 mg/0.1 ml) and a standard curve obtained with the following equation:

$$\text{Absorbance} = 0{:}0012 \times \text{Gallic acid } (\mu g) + 0{:}0033$$

Total phenols of the extract, as Gallic acid equivalent, was determined by using the absorbance of the extract measured at 760 nm as input to the standard curve and the equation. All tests were carried out in triplicate and Gallic acid equivalent values were reported as means ±SD of triplicates.

2.5. Antimicrobial activity

2.5.1. Microbial strains

The extracts of *A. graveolens* were individually tested against a set of 11 microorganisms. Following microbial strains were provided by Iranian Research Organization for Science and Technology(IROST) and used in this research: Pseudomonas aeruginosa (ATCC27853), Escherichia coli (ATCC 10536), Bacillus subtilis (ATCC6633), Staphylococcus aureus (ATCC 29737), Klebsiella pneumonia (ATCC 10031), Staphylococcus epidermidis (ATCC 12228), Shigella dysenteriae (PTCC 1188), Proteus vulgaris (PTCC 1182), Salmonella paratyphi-A serotype (ATCC 5702), Candida albicans (ATCC 10231)and Aspergillus niger (ATCC 16404). Bacterial strains were cultured overnight at 37˚C in nutrient agar (NA) and Fungi were cultured overnight at 30˚C in sabouraud dextrose agar (SDA).

2.5.2. Disk diffusion assay

Determination of antimicrobial activities of dried *A. graveolens* was accomplished by agar disk diffusion method (National Committee for Clinical Laboratory Standard) [29], [20]. The dried plant extract were dissolved in DMSO to a final concentration of 30 mg/ml and filtered by 0.45 µm Millipore filters for sterilization. Antimicrobial tests were carried out using the disk diffusion method reported by Murray et al (1995)and employing 100 µl of suspension containing 108

CFU/ml of bacteria,106 CFU/ml of yeast and 104 spore/ml of fungi spread on the nutrient agar (NA), sabouraud dextrose (SD) agar and potato dextrose(PD) agar mediums, respectively. The disks (6 mm in diameter) impregnated with 10 µl of the extracts solutions in dividedly (300 µg/disk) and DMSO (as negative control) were placed on the inoculated agar. The inoculated plates were incubated for 24 h at37 ˚C for bacterial strains and 48 h and 72 h at 30 ˚C for yeast and mold isolates, respectively. The diameters of inhibition zones were used as a measure of antimicrobial activity and each assay was repeated twice [30],[19].

2.5.4. MIC agar dilution assay

MIC values of the extract of *A. graveolens* were evaluated based on the agar dilution method described by Gul, Ojanen, and Hanninen (2002). Appropriate amounts of this extract was added aseptically to sterile molted SDA medium containing Tween 20 (0.5%, v/v) to produce the concentration range of 7.8–500 µg/ml. The resulting SDA agar solutions were immediately mixed and poured into petri plates. The plates were spot inoculated with 5 µl of Bacterial suspension. The inoculated plates were incubated at 30 ˚C for 72 h. At the end of incubation period, the plates were evaluated for the growth presence or absence. The MIC was defined as the lowest concentration of the compounds needed to inhibit the growth of microorganisms. Each test was repeated at least twice.

2.6. Brine shrimp cytotoxicity assay

Brine shrimp lethality test is a rapid, reliable, inexpensive and convenient bioassay for primary evaluation of natural products cytotoxicity (Colegate and Molyneux) [3] and have been widely used since its proposal by Meyer et al., 1982 [19]. Brine shrimp (*Artemia salina*) eggs were hatched and converted to larvae (napulii) in a glass rectangular vessel (5 L) containing sterile artificial seawater prepared using water (2 L), NaCl (46 g), $MgCl_2.\cdot 6H_2O$ (22 g), Na_2SO_4(8 g), $CaCl_2.\cdot 2H_2O$ (2.6 g), and KCl (1.4 g) with a pH of 9.0 adjusted with Na_2CO_3, under constant aeration for 48 h. After hatching, active nauplii free from egg shells were collected from the brighter part of the hatching chamber and used for the assay. Serial concentrations (10, 100, 300, 500, 700 and 1000 μg/ml) of the extracts solutions were prepared in sterile artificial seawater. Dimethyl sulfoxide (DMSO, 1%) was used for complete dissolution of the extracts. Ten active nauplii were carefully drawn through a glass capillary and placed in each vial containing 5 ml of test solution. Sterile artificial seawater (5 ml) containing DMSO (1%) was used as negative control. The vials were maintained at room temperature for 24 h under light and the surviving larvae were counted. Experiments were conducted along with control and different concentrations in a set of three tubes per dose. The percentage lethality was determined by comparing the mean surviving larvae of the test and control tubes. Half maximum lethal concentration (LC_{50}) values were obtained from the best-fit line plotting concentration versus percentage lethality.

3. RESULTS AND DISCUSSION

3.1. Chemical composition of the essential oil

Simultaneous distillation extraction with organic solvent (pentane) of dried aerial parts of *A. graveolens* for 3.5 h yielded its essential oil (yield:0.1%, w/w). GC and GC/MS analysis of the plant's essential oil led to the identification and quantification of 27 components (Table 1), which accounted for 93.09% of the total oil. Germacrene-D(56.65%), Bicyclogermacrene (8.69%), and Caryophyllene oxide (7.46%) were the most abundant components of the oil. The oil was characterized by a high percentage of sesquiterpene hydrocarbons (75.12%), followed by oxygenated sesquiterpenes (11.51%). The major compounds obtained in this study were also in similarity with the major components of the essential oil obtained from aerial parts of *A. graveolens* growing in Shiraz (a province at the south east of Iran),(Javidnia, Miri, Soltani, and Khosravi, 2010) and from its aerial parts in Serbia(Golubovic et al.,2010). The main components of the oil in study in Shiraz were Dillapiole (32.7%) and Germacrene-D (16.5%) and in the other study in Serbia were germacrene D (58.65%) and bicyclogermacrene (7.14%). The high Germacrene-D content (56.65%) ofthe plant oil seen in our study is in good agreement with its levels in other Acinos species (Stojanovic et al.,2009).The results of this literature showed Germacrene D is widespread main sesqiuterpene among examined *Acinos* species. However, there are two exceptions: Jovanovic_ et al. (2002) reported caryophylleneoxide as the dominant constituent for *A. hungaricusoil* and Skaltsa et al. (1999) found germacrene as a major compound in *A. alpinus* oil. Also, caryophylleneoxide was the major component in *A. alpinus* and *A. hungaricus* with the amount of 12.2%, 16.8% respectively.

Table 1: Chemical composition of *A. graveolens* volatile materials

No	Compound[a]	Composition (%)	RI[b]	RI[c]
1	Sabinene	0.2	981	969
2	Decane	0.14	1002	1000
3	α-phellandrene	0.05	1009	1002
4	dl-Limonene	3.38	1033	1024
5	Acetophenone	0.11	1073	1059
6	trans-Sabinene hydrate	0.18	1105	1098
7	trans-Verbenol	0.39	1152	1140
8	Terpinen-4-ol	0.2	1185	1174
9	β-Bourbonene	0.63	1394	1387
10	β-Elemene	2.47	1401	1389
11	trans-Caryophyllene	4.46	1430	1417
12	β-Gurjunene	0.41	1439	1431
13	(E)- β–Farnesene	1.03	1465	1454
14	Germacrene-D	56.65	1498	1484

15	Bicyclogermacrene	8.69	1509	1500
16	α-Farnesene	0.78	1517	1505
17	Caryophyllene oxide	7.47	1597	1582
18	Salvial-4(14) en-1- one	0.37	1607	1594
19	Megastigmatrienone[d]	0.83	1638	1472
20	α-Cadinol	1.93	1657	1652
21	Caryophyllen<14-hydroxy-9- epi- (E)->	0.91	1688	1668
22	Germacera-4(15),5,10(14)-trien-1-α-ol	0.84	1703	1685
23	Mint sulfide	0.18	1753	1741
24	n-Nonadecane	0.13	1911	1900
25	n-Eicosane	-	2003	2000
26	n-Octadecanol	-	2068	2077
27	n-Heneicosane	-	2093	2100
Total	Total	93.09		

[a]Compounds listed in order of elution from HP-5MS column.

[b]Relative retention indices to C8–C24 n-alkanes on HP-5MS column.

3.2. Amount of total phenolic constituents

Based on the measured absorbance value of the plant extract reacting with Folin–Ciocalteu reagent, and in comparison with absorbance values of Gallic acid solutions in the standard curve, the number of total phenolics in the extract of *A. graveolens* were estimated at 51.05±0.71µg/mg.

3.3. Antioxidant activity

Methanol extract of plant was subjected to screening for their possible antioxidant activities using 2,2-diphenyl- 1-picrylhydrazyl (DPPH) and β-carotene/linoleic acid assay methods. DPPH is a stable free radical which can readily experience reduction in the presence of an antioxidant. It shows a maximum ultraviolet and visible (UV–Vis) absorbance at 517 nm. The reduction in the intensity of absorption at 517 nm of methanol solutions of DPPH radical in the presence of antioxidants is usually taken as a measure of their antioxidant activity. In this study, the ability of samples to scavenge DPPH radical was determined on the bases of their concentrations providing 50% inhibition (IC50). Plant extract and positive control (BHT) IC50 values are given in Table 2. Extract of *A. graveolens* showed good activity with an IC50 value of 50.34±5.33 µg/ml. Various results were obtained from radical scavenging activity evaluation of other *Acinos* species. In addition, *Acinos* species are promising source of natural antioxidants because of high content of flavonoids and linolenic acid in their extracts. Flavonoids are known as high-level antioxidants because of their ability to scavenge free radicals and reactive oxygen species such as singlet oxygen, superoxide anion radical and hydroxyl radicals [25]. In β-carotene/linoleic acid test, the antioxidant transfers hydrogenatom(s) to the peroxyl ($R1R2CHOO^{-}$) radicals formed from the oxidation of linoleic acid and converts them to hydroperoxides(R1R2CHOOH) leaving β-carotene molecules intact [31],[9]. Percent inhibition of linoleic acid oxidation of the extracts of *A. graveolens* listed in Table 2. Compounds containing hydrogen atoms in the allylic and/or benzylic

positions may show better activity in this test because of relatively easy abstraction of hydrogen atom from these functional groups by peroxy radicals formed in the test circumstances [14]. Literature is poor about the antioxidant activity of *A. graveolens.*

3.4. Cytotoxic activity

Lethal concentrations (LC_{50}) recorded in the brine shrimp lethality bioassay performed on methanol extracts of *A. graveolens* was 800±100μg mL^{-1} indicating a weak cytotoxicity for the plant in this test.

Table 2: Antioxidant activity, total phenolic compounds contents and cytotoxic activity of methanol extract of A. *graveolens*

Sample	Plant organ	DPPH IC_{50} (µg/ml)	β-carotene/linoleic acid Inhibition (%)	Total phenolics (µg/mg)	Brine shrimp bioassay LC_{50} (µg/ml)
A.graveolens	Aerial part	50/34±5/33	46/1±5	51/05±0/71	800±100
BHT		16/13±0/51	94/1±5	NA	NA

NA (Not applicable)

3.5. Antimicrobial activity

The antimicrobial activity of the extract of *A. graveolens* was evaluated against a set of 11 microorganisms and their potency were assessed qualitatively and quantitatively by the presence or absence of inhibition zones, zone diameters and MIC values. The results indicate that, at tested concentrations, the plant has no considerable antimicrobial activity against tested microorganisms. Antimicrobial activity of methanol extract from aerial parts of *A. graveolens* just showed little activity against three bacteria. The results are given in Table 3

Table 3: Antimicrobial activities of the methanol extract of *A. graveolens*

Plant extract	Antibiotics							
			Rifampn		Gentamicin		Tetracycline	
	DD	MIC	DD	MIC	DD	MIC	DD	MIC
Gram-positive bacteria								
B. subtilis	-	-	13	15.62	21	500	21	7/8
S. epidermidis	9	1000	40	250	35	500	35	250
S. aureus	-	-	10	250	21	500	21	250
Gram-negative bacteria								
E. coli	-	-	11	500	20	500	20	31/25
k.pneumonia	8	>1000	7	250	22	250	22	250
S. dysenteriae	-	-	8	250	18	500	18	250
P. vulgaris	-	-	10	125	23	500	23	125
S. paratyphi-Aserotype	8	>1000	-	NT	21	500	21	-
C. albicans	-	-	NA	NA	NA	NA	NA	NA
A. niger	-	-	NA	NA	NA	NA	NA	NA
A. brasilienis	-	-	NA	NA	NA	NA	NA	NA

A dash (-) indicate no antimicrobial activity.

NT (Not tested), NA (Not applicable).

4. CONCLUSION

Plant secondary metabolites generally display remarkable biological activities such as antioxidant and antimicrobial properties which are useful for preserving foods from decay and contamination and/or preventing living tissues from various diseases. According to literature data, reports are weak about *A. graveolens*. Extract of *A. graveolens* showed good antioxidant activity for the plant. These result encourage complementary and more in-depth studies on the chemical composition of the plant extract with the aim of separation and structure elucidation of its active components and evaluation of biological activity of each compound separately.

ACKNOWLEDGEMENT

Financial support made by the Research Chancellor of the University of Kashan, Kashan, and Islamic Republic of Iran is gratefully acknowledged.

CONFLICT OF INTEREST

No conflict of interest between authors and all authors is fully satisfied with this project and its publication.

REFERENCES

1. Adams R.P. Identification of essential oil components by gas chromatography/ mass spectroscopy. Carol Stream, IL.: Allured Publishing Co. 2001; 550pp.
2. Batooli H. Biodiversity and species richness of plant elements in Qazaan Reserve of Kashan.Pajouhesh –va-Sazandeghi. 2004. 16(4), 85-104. [in Persian].
3. Colegate S.M, Molyneux R.J. Bioactive Natural Products: Detection, Isolation, and Structural Determination, second ed. CRC Press, Taylor & Francis group, New York. 2008; 250p.
4. Foti M.C, Daquino C, Geraci C. Electron-transfer reaction of cinnamicacids and their methyl esters with the DPPH radical in alcoholic solutions. J Organic Chem. 2008; 69(7), 2309–2314.
5. Ghahreman A. Flora of Iran, Research institute of Forest and Rangelands and Tehran University press. Tehran. 1995; 1260 p.
6. Golubovic T, et al. Chemical composition and antimicrobial activity of the essential oil of *Acinos graveolens*. J Khim Pri Soed. 2010; 4, 542-544.
7. Gul H.I, Ojanen T, Hanninen O. Antifungal evaluation of bisMannichbases derived from acetophenones and their corresponding piperidinols and stability studies. Biological and Pharmaceutical Bulletin. 2002; 25, 1307–1310.
8. Hedge I.C. *Acinos*, in: K. H. Rechinger (ed.), *Labiatae*, in: K. H. Rechinger (ed.) Flora Iranica. 1982; Vol. 150, Akademische.
9. Huang D, Ou B, Prior R.L. The chemistry behind antioxidant capacity assays. J Agr and Food Chem. 2005; 53, 1841–1856.
10. Javidnia K, Miri R, Soltani M, Khosravi A.R. Essential oil composition of *Acinos graveolens* from iran. J chem natural com. 2010; 46(1): 130-131.
11. Jovanovic T, Kitic D, Palic R, Stojanovic G, Ristic M. Chemical composition of the essential oil of *Acinos bhungaricus*(Simonkai) Silic.J. Essent. Oil Res. 2002; 14: 29-30.
12. Karousou R, Balta M, Hanlidou E, Kokkinos S. Essential oil composition of *Acinos graveolens* J. Ethnopharmacol. 2007; 107, 248.
13. Khazir J, Ahmad Mir B, Pilcher L, Riley D.L. Role of plants in anticancer drug *discovery.Phytochemistry Letters*. 2014; 7: 173-181.
14. Larson R.A. Naturally occurring antioxidants (p. 70). Boca Raton, FL, USA:CRC Press.1997; 210p.
15. Losso J.N, Shahidi F, Bagchi D. Anti-angiogenic functional and medicinal foods. Boca Raton, FL: Taylor & Francis. 2007; 250p.
16. Mabberley D.J. The Plant Book, a Portable Dictionary of the Higher Plants, Cambridge

University Press, Cambridge. 1990; P. 6.

17. Miraliakbari H, Shahidi F. Antioxidantactivity of minor components of tree nut oils. J Food Chem. 2008; 111(2), 421–427.
18. Moure A, et al. Natural antioxidants from residual sources. J Food Chem, 2001; 72:145–171.
19. Murray P.R, Baron E.J, Pfaller M.A, Tenover, F.C, Yolke R.H. Manual of clinical microbiology (7th ed., pp. 1773). Washington, DC: ASM. 1995; 230p.
20. National Committee for Clinical Laboratory Standard. InPerformancestandards for antimicrobial disk susceptibility test. Sixth approved standard.M2-A6, Wayne, PA. 1997; 250pp.
21. Sarker S.D, Latif Z, Gray A.I. Natural products isolation. New Jersey, USA:Humana Press Inc. 2006; p. 20.
22. Scalbert A, Manach C, Morand C, Remesy C. Dietary of polyphenols and the prevention of diseases. Crit Rev in Food Scie and Nut. 2005; 45(4), 287–306.
23. Skaltsa H, Lazaris D, Loukis A. Composition of the essential oil of *Acinos alpinus*(L.) Moench.from Greece. J Essential Oil Res. 1999; 11: 35-37.
24. Slinkard K, Singleton V.L. Total phenol analysis: Automation and comparison with manual methods. American J Eno and Viti. 1997; 28(1), 49–55.
25. Stojaovic G, Golubovic T, Kitic D, Palic R. *Acinos*species: Chemical composition, antimicrobial and antioxid ativeactivity of *Acinos alpinus*(L.) J Med Plants Res. 1999; 3(13): 1240-1247.
26. Tayoub Gh, et al. Chemical composition, antimicrobial and antioxid ativeactivity of *Rosmarinus officinalis* J of .Flavors Frag; 2018; 21: 809-812.
27. Tekwu E.M, Pieme A.C, Beng V.P. Chemical composition, antimicrobial and antioxid ativeactivity of *Medicago sativa* J .Ethno. 2007; 142:245-273.
28. Uahimi H, Akkbari D, Loukis M. Composition of the essential oil of *Acinos alpinus*(L.) Moench.from Greece. J Essential Oil Res. 2016; 15: 30-37.
29. Uramin, Ozako D, Loukis S. Composition of the essential oil of *Lippa spp*(L.) Moench.from Turkey. J Essential Oil Res. 2016; 12: 42-50.
30. Varon H, Mizad D, Fatti J. Composition of the essential oil of *Acinos alpinus*(L.) Moench.from Greece. J Essential Oil Res. 2014; 18: 55-63.
31. Zaloti H, Barina D, Milosin B. Natural products isolation of *Sisyphus arinda*. J Essential Oil Res. 1999; 11: 35-37.