



GC-MS Analysis, Antioxidant and Antimicrobial Screening of Volatile Oil of *Lepidium vesicarium*

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ABSTRACT

Background: *Lepidium vesicarium* (Cruciferae), one of the important medicinal plants with a long history of medicinal use. The current study was designed to evaluate the free radical scavenging and antimicrobial activities of the *L. vesicarium* EO as one of the Iranian plant species.

Methods: The compositions of the EO from the aerial parts of *L. vesicarium* were analyzed by GC-MS and GC-FID. Furthermore, anti-oxidant and anti-microbial potentials were investigated via DPPH reagent and disk diffusion procedure, respectively.

Results: A total of 18 compounds amounting 97.70% of the oil have been identified, while Benzyl cyanide (43.94%), Isothio cyanic acid (22.69%) and Benzyl isothio cyanate (20.69%) were the main constituents. The EO showed no activity against the free radicals and studied microbial strains (gram positive and gram negative and also fungi species).

Conclusion: On the whole, the presence of cyanide derivatives in studied EO revealed the rational use of this plant in medicine. Comparing with other species of *Lepidium*, anti-oxidant and anti-microbial properties of *L. vesicarium* essential oil were not noticeable.

Introduction

Many edible and perennial herbaceous species of Cruciferae family such as rapeseed, broccoli sprouts and common radish were used for medicinal uses and also flavor agents in folk medicine. Brassicaceae family is famous to have different types of glucose inolates in volatile parts which play a major role to illustrate important pharmacological properties, such as anti-microbial, anti-oxidant and insecticidal.¹ Hence, the substances of these plants can be considered candidate for developing novel anti-microbial and radical scavenging agents. Furthermore, numerous studies have been reported the main pharmacological properties of different species of this genus. Sexual performance boost (both in animals and humans), enhancing the female fertility, decreasing menopausal symptoms, improving the memory, anti-cancer, osteoporosis prophylaxis and anti-depressant characteristic are some of the pharmacological properties.²⁻⁵ Some secondary metabolites like alkaloids, glucosinolates, isothiocyanates are probably responsible for mentioned characteristic.^{6,7} *Lepidium vesicarium* as a weed species is abundantly distributed in the rangelands of East Azerbaijan, Iran¹. Furthermore, this species is contradistinguished by nodal swellings in stem.⁸ Information about the volatile composition of the different species of *Lepidium*, available to date. All those previous

investigations revealed, phenyl acetonitriles, benzaldehyde and benzyl isothio cyanate as the major constituents in the oil.^{6,7} With the exception of the reports on the effect of environmental factors on seed germination of *L. vesicarium*,¹ to the best of our knowledge since now, there are no scientific data available in the literatures on any volatile chemicals or bioactivity study on this species. Since part of our mission at the Pharmacognosy Department of Faculty of Pharmacy, Tabriz, is to discover plant non-volatile,⁹⁻¹⁶ and volatile compositions,^{17,18} which can be used as an anti-oxidant,¹⁰⁻¹⁷ and anti-microbial agents,^{9,11,12,15,17} volatile constituents as well as anti-microbial and anti-oxidant, properties of this plant were also evaluated. Other investigations on the non-volatile compositions of *L. vesicarium* are in progress.

Materials and Methods

Plant material

The aerial parts (leaves, stems and flowers) of *L. vesicarium* were collected from Moghan East Azarbaijan province, Tabriz in August 2016. A voucher specimen (Tbz-fph 1554) after identification was stored at the Herbarium of the East Azerbaijan natural resources research center, Faculty of Pharmacy, Tabriz University of Medical Science, Tabriz, Iran after identification.

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Distillation of plant materials

The 100 g of chopped and powdered of *Lepidium vesicarium* sample was subjected to hydro-distillation for 4h using a Clevenger type apparatus successively. The yield of EO was determined (V/W) and dried via anhydrous sodium sulphate, then stored in a sealed vial for further analysis.

GC-MS and GC-FID analysis

The EO of the aerial parts of *L. vesicarium* was evaluated by a Shimadzu GC-MS QP5050A at the Drug Applied Research Center, Tabriz, Iran equipped with a methyl silicon DB-1 column (60m × 0.25 mm). The carrier gas was helium at a flow rate of 1.3 ml/min. The multi-step oven temperature program was raised at a rate of 3 °C min from 50 °C (kept for 2min) to 250 °C (kept constantly for 7 min). In addition, the injector temperature and split ratio were adjusted at 260 °C and 1:33 correspondingly. On the other hand, the mass spectral data yielded in these condition: Ionization voltage 70 ev, Ion source temperature 210 °C, quadropole temperature 120 C. For identification of the components of EO, Kovats indices (KI), retention time (RT) and mass spectra (MS) were compared directly with standard compounds as well as computer matching with NIST and Willey/NBS library along with published mass spectra.¹⁹⁻²² In order to carry out the quantification of the constituents of EO (relative percentage amount (area %)) Flame Ionization Detector (FID) chromatograms at the same GC-MS operational conditions was used.

Determination of in-vitro radical scavenging activity

The free radical scavenging potential of EO was assessed on the basis of reduction of DPPH (Sigma-Aldrich) according to our previous works.^{15,23-25} DPPH (8 mg) was dissolved in chloroform (100 ml) to get a concentration of 80 mcg/ml. The various concentrations of the EO were prepared from stock solution (1mg/ml). Correspondingly, DPPH solution was added to all concentrations. Subsequently, the mixture was shaken and incubated at room temperature for 30 min. After the incubation period, the reduction in the number of free radicals was measured against a blank (methanolic solution of DPPH) using Shimadzu spectrophotometer at 517 nm. All tests were took place in triplicate. The percentage bleaching of DPPH by EO was calculated by following Equation:

$$I\% = 100 \times [(A_{\text{blank}} - A_{\text{EO}}) / A_{\text{blank}}] \quad \text{Eq. (1)}$$

where A_{blank} in $t = 30 \text{ min}$ A_{EO} in $t = 30 \text{ min}$

RC_{50} (50% scavenging activity of EO was extrapolated from dose response curve (inhibition percentage against various concentration). The same procedure was practiced for the reference standard, Quercetine and the radical scavenging capacity of the tested sample was compared with it.²⁵

Anti- microbial activity

In this project, four lyophilized strains of gram-positive bacteria (*Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis*

(ATCC 9372) and *Listeria monocytogenes* (ATCC 1163), two gram negative bacteria *Escherichia coli* (ATCC 8739) and *Salmonella typhi* (ATCC 1230) and one lyophilized fungi *Candida albicans* (ATCC 10231) were obtained from the Institute of Pasture, Iran, respectively. Subsequently, the anti-microbial property of the sample was evaluated via disc diffusion method based on standard NCCIs methodology (with some modification).¹¹ For this aim, Muller-Hinton Agar plates, impregnated with a 0.5 McFarland standard bacterial strain which were prepared previously. A 10 microliter of the EO was applied to each sterilized Filter paper discs (Whatman No 6 mm diameter) and allowed to disperse on discs. Subsequently, inoculated discs placed on the seeded Muller-Hinton Agar then incubated overnight at 37 °C for any reaction to occur between EO and bacterial species. The assay were done in triplicate with 4 discs on one plate. Amikacin and DMSO were used as a positive and negative controls for microbial species respectively. Degrees of the sensitivity of the studied microbial strains against EO were expressed by measuring the diameter of the inhibition zone (mm) and compared with the diameter of growth inhibition zone of positive control.^{11,17}

Results and Discussion

In the present study, the chemical constituents of odorous green liquid EO of the aerial parts of *L. vesicarium* (1.7% v/w) were determined by the GC-MS analysis and the Koats index, molecular formula and relative area percent are listed in Table 1. Furthermore, the anti-oxidant and anti-microbial potentials of EO were evaluated. 19 compounds were detected in volatile part of the plant, representing about over 98% of the total constituents of oil. The components are compatible with the crucifera family ingredients,^{26,27} but at different area percents of individual components which in 64.63% of compounds were phenolic compounds including Benzyl cyanide (43.94%) and Benzyl isothio cyanate (20.69%) as main compounds. These two mentioned phenolics were produced in degradation process of benzyl glucosinolate.⁷ The other major ingredient was isothio cyanic acid as a straight chain hydrocarbon (HSCN) (22.69%).^{19,20,28} Additionally, Methallyl cyanid (3.48%) (N-compound) and spathulenol (2.18%) (Sesquiterpen) were found in a lesser amounts. Interestingly, there is a considerable difference between the chemicals of other species of *Lepidium* growing in difference locations. While, Benzyl cyanide was found to be the major component of the *L. vesicarium* oil, the oil of *L. meyenii* contained phenylacetone nitril as a main constituent.^{19,20,28} Additionally, Delta-cadinen, spathulenol and caryophyllene oxidie as a sesquiterpens compounds of *L. vesicarium*, were not detected in other species to date. Among all of the studied species, Benzyl iso-thiocyanate is the single common phenolic compounds up to now.^{19,20,28} Furthermore, the anti-bacterial and anti-oxidant activity of the EO were screened by disk diffusion and DPPH methods respectively.

Table 1. Chemical compositions of the essential oil of *Lepidium vesicarium*.

No.	Compound	KI ^a	literature KI	Area	Identification method
1	Methylal cyanide	–	–	3.48	GC-MS, Is
2	Hexane nitrile	911.10	881	0.19	GC-MS, Is
3	Isotio cyanic acid	947.46	942	22.69	GC-MS, Is
4	Acetyl butyryl	961.52	959	0.18	GC-MS, Is
5	Nonyl aldehyde	1083.88	1084	0.86	GC-MS, Is
6	Benzyl cyanide	1094.87	1096	43.94	GC-MS, Is
7	Bicyclo[3.1.1]hept-2-en-4-ol, 2,6,6-trimethyl-acetate	1247.07	1287	0.18	GC-MS, Is
8	2H-1-Benzopyran, 3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl	1283.51	1296	0.56	GC-MS, Is
9	Benzyl isotio cyanate	1324.81	1317	20.69	GC-MS, Is
10	Geranyl acetone	1430.86	1455	0.36	GC-MS, Is
11	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)	1466.94	1486	0.27	GC-MS, Is
12	Delta_cadinene	1518.01	1530	0.32	GC-MS, Is
13	Methoxy benzyl isotio cyanate	1558.79	1560	0.69	GC-MS, Is
14	Iso spathulenol	1565.64	1562	0.32	GC-MS, Is
15	Spathulenol	1569.30	1570	2.18	GC-MS, Is
16	Caryophyllene oxide	1575.99	1578	0.36	GC-MS, Is
17	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	1673.11	1690	0.27	GC-MS, Is
18	Hexahydro farnesyl acetone	1831.93	1858	0.16	GC-MS, Is
Total identified	97.70%				
Non_terpenoids	94.25%				
Terpenoids	3.45%				
Sesquiterpenes	0.32%				
Oxygenated sesquiterpenes	3.13%				

Compounds listed in order of elution from a DB-1 column, Identification Method (Is = Kovats retention index according to authentic standard. KI is the Koats Index relative to C8–C20 n-alkanes on the DB-1 column.

The EO showed no inhibitory effect on microorganisms (Mean Inhibition Zone Diameter \pm SD (MIZD) 6 ± 0.2 mm) in comparison to Amikacin (MIZD 15 ± 0.1 mm) and also on free radicals (RC₅₀ 0.957 ± 0.13 mg/ml) in comparison to Quercetin (RC₅₀ 0.003 ± 0.000 mg/mL) respectively. The fact that, among the compounds, phenolics possess anti-oxidant and anti-microbial activities.²⁹ In our study, lack of these secondary metabolites may be led to weak radical scavenging potential. Since the amount of the obtained EO is low, further supplementary biological tests (such as various antioxidant tests) were not performed.

Conclusion

Overall, according to the results, EO of *L. vesicarium* had no noticeable anti-oxidant and anti-microbial activity. Moreover, the findings of this investigation revealed that main constituents of EO of *L. vesicarium* are cyanide derivatives which were in line with the previous studies.

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Conflict of Interests

The authors claim that there is no conflict of interest.

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