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RESEARCH ARTICLE

Antioxidant Compounds from *Levisticum officinale* Koch

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ABSTRACT

Levisticum officinale, known as Lovage, belongs to Apiaceae family and has antioxidant and anti-inflammatory effects in traditional medicine. In this study, the antioxidant activity of different extracts including n-hexane, ethyl acetate, and methanol of both aerial parts and roots was evaluated. The antioxidant activity of the samples including (extracts and fractions) was studied by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and Dot blot assay. Finally, the active compounds were isolated through column chromatography and then identified by NMR spectroscopy. Among tested extracts, ethyl acetate extract of the root had a high antioxidant activity. Thus, this extract became a candidate to isolate its active compounds. Finally, three compounds, namely, bergapten, ferulic acid, and vanillin were isolated as active compounds from one of the active fractions. This study indicated that vanillin, bergapten, and ferulic acid are responsible for antioxidant activity in some fractions of *L. officinale*. Further studies are suggested to evaluate the components of other active fractions.

Keywords: 2,2-Diphenyl-1-picrylhydrazyl assay, Active fraction, Antioxidant activity, *Levisticum officinale*

INTRODUCTION

An antioxidant is described as a substrate capable of inhibiting the oxidation of other substrates.[1] Oxidative stress is associated with some chronic conditions including neuronal disease, cataracts, various types of cancerm and cardiovascular disease. Hence, antioxidant agents can be positive in reducing the risk of these diseases.[2] Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylhydroxyanisole have potentially health hazards (2), so natural antioxidants such as plant extracts and their components are suggested.

Levisticum officinale, known as lovage, is a perennial medicinal plant growing wildly in Kerman province. This plant belongs to Apiaceae family and used as anti-asthmatic, diuretic, carminative, and antispasmodic agents. Phytochemistry studies have shown that *L. officinale* is a rich source ofphthalide, furanocoumarin, and polyacetylene compounds.[3-7]

L. officinale, a well-known species, is accepted by the German Commission E for use in infections of the urinary tract and kidney and bladder stones. Although *L. officinale* is accepted as a safe comestible plant, its use in pregnancy, renal failure, and renal inflammation is contraindicated.[8,9]

L. officinale has been identified as antioxidant agent and its some effects such as the improvement of learning and memory could be attributed to its antioxidant activity.[10]

The antioxidant activity of polar extracts (including hydroalcoholic and aqueous) of *L. officinale* has been studied by some researchers, but there is

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no report on the antioxidant activity of non-polar extracts. Therefore, in this present work, we discuss the antioxidant activity of both non-polar (including n-hexane and ethyl acetate) and polar (methanol) extracts of *L. officinale* and isolation of its effective compounds.

MATERIALS AND METHODS

Plant materials

The roots of *L. officinale* were collected from the Hezar Mountain located inKerman province in July 2015. The plant material was identified by Prof. Farideh Attar and voucher specimen (46553-TUH) is kept at the herbarium of Faculty of Sciences, University of Tehran, Iran.

Extraction and isolation

The powdered roots of *L. officinale* (3 kg) were extracted with n-hexane, ethyl acetate, and methanol by maceration method, respectively. Finally, ethyl acetate extract was dried by rotary evaporation and 100 g dried extract was obtained. The obtained ethyl acetate extract was fractionated by silica gel-column chromatography (CC) (230–400 mesh, 1 kg). The silica gel CC was eluted with gradient from 100% n-hexane to 100% ethyl acetate, followed by increasing concentration of methanol (up to 20%) in ethyl acetate. Finally, 16 fractions were obtained. As shown in Figure 1, Fractions 6–11 have similar antioxidant activity. Therefore, Fraction 8 was selected for isolation effective compounds. To isolate active compound, 2 g of Fraction 8 was subjected on normal silica gel CC and was washed by n-hexane-chloroform-acetone (80-15-5) as isocratic and4 subfractions (F_8A-F_8D) were obtained together 17 mg ferulic acid.[3-5]

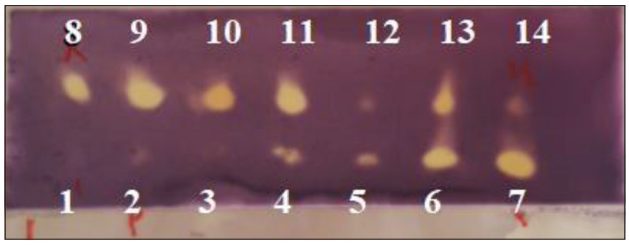

Figure 1: Antioxidant activity of the fractions (1-14) from *Levisticum officinale* on dot blot assay

Subfraction F_8B (650 mg) was loaded on silica gel CC and washed with a gradient of n-hexane-acetone. Finally, 3.5 mg vanillin (as white crystals) and 13 mg bergapten were obtained.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging assay

Free radical scavenging activity of the compounds was calculated, using the DPPH assay. Reaction between the samples (dissolved in methanol) and 200 μM DPPH was occurred in a 96-well microtiter plate (incubated at 37°C) for 30 min. After the end of the reaction, absorbance was measured at 520 nm,[11] and percent inhibition was calculated using the following formula:

$$\%DPPH\ radical\ scavenging = \frac{Absorbance\ of\ blank - Absorbance\ of\ sample}{Absorbance\ of\ blank} \times 100$$

The experiment was done in triplicate.

Dot blot assay

Dot blot assay is a simple method for screening the biological activity of plant extracts.[12] In the present study, we used dot blot assay to evaluate the antioxidant activity of different extracts from *L. officinale*. To do this, the sample was dissolved in the suitable solvent (in concentration of 3mg/mL). Then, the samples were loaded on to the thin layer chromatography plate after which DPPH reagent was sprayed on it.

Nuclear magnetic resonance (NMR) procedure

NMR spectra were recorded at a target temperature of 18°C on a Bruker Avance III 500 MHz spectrometer operating at 500.13 MHz for ^{1}H, and 125.77 MHz for ^{13}C. A1 mm TXI-microprobe with z-gradient was used for ^{1}H-detected experiments. ^{13}C NMR spectra were recorded with a 5 mm BBO probe head with z-gradient. Spectra were analyzed using Bruker Top Spin 3.1 software. Deuterated solvents for NMR (100 at% D) were purchased from Armar Chemicals.

^{1}H and ^{13}C-NMR information of the isolated compounds

Ferulic acid (**1**) – white amorphous powder (17 mg); ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 6.39 (1H, d, H-2'), 6.81 (1H, d, H-6), 7.14 (1H, dd, H-5), 7.34 (1H, d, H-3), 7.60 (1H, d, H-1').

Bergapten (**2**) – white amorphous powder (13 mg); ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.30 (3H, s, O-CH_3), 6.37 (1H, d, H-3), 6.82 (1H, d, H-3'), 7.35 (1H, s, H-8), 7.69 (1H, d, H-2'), 7.76 (1H, d, H-4); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 62.6 (O-CH_3), 106.7 (C-3'), 112.9 (C-8), 114.9 (C-3), 116.4 (C-4a), 126.2 (C-6), 142.6 (C-8a), 144.3 (C-4), 146.6 (C-2'), 147.7 (C-7), 161.4 (C-2).

Vanillin (**3**) – white crystals (3.5 mg); ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 9.83 (1H, s, H-1), 7.45 (1H, d, H-2), 7.05 (1H, d, H-6), 7.46 (1H, d, H-7), 4.02 (3H, s, O-CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 191.0 (C-1), 108.8 (C-2), 147.2 (C-3), 151.7 (C-4), 114.4 (C-5), 127.6 (C-6), 56.9 (O-CH_3).

Ethical considerations

Ethical Committee of Ardabil University of Medical Sciences approved this study with the code of IR.ARUMS.REC.1397.212.

RESULTS AND DISCUSSION

The aerial parts and root extracts of *L. officinal*e including *n*-hexane, ethyl acetate, and methanol extracts were investigated for their antioxidant activities using DPPH assay. As shown in Table 1, the root ethyl acetate extract has potent antioxidant capacity. Hence, the antioxidant activity of the obtained fractions (1–14) from the ethyl acetate extract was studied using Dot blot assay. Figure 1 shows Dot blot assay to evaluate antioxidant activity of the fractions obtained from *L. officinale* thyl acetate extract. In Dot blot assay, antioxidant areas are visible as yellow circular patches surrounded by pink area. Yellow area diameter is associated with antioxidant activity, so that yellow area diameter of fractions with high antioxidant activity is large. Finally, ferulic acid (**1**), bergapten (**2**), and vanillin (**3**) were obtained from Fraction 8 and their structures were identified by NMR spectral optical as well as through comparison with the literature data.[13-15] Figure 2 shows structure of the isolated compounds from active fractions of *L. officinale*.

To evaluate the antioxidant activity of the isolated compounds, DPPH was used. As shown in Table 2, the antioxidant activity of ferulic acid and bergapten has been compared to that of BHT (IC_{50} = 25.3 ± 0.4) with IC_{50} = 18.7 ± 0.3 and 25.4 ± 0.8 μg/mL, respectively. Based on the present results, it can be stated, ferulic acid, bergapten, and relatively vanillin are responsible for the antioxidant activity of the ethyl acetate extract of the roots from *L. officinale*.

In general, *L. officinale* has strong antioxidant effects.[16] In reported studies, *L. officinal*e has been used as an antioxidant, anti-inflammatory, and anticholinesterase agent.[10]

The antioxidant activity of *L. officinale* as well as its poral extracts has been confirmed by several studies. For example, Tomsone *et al.* showed the leaf ethanol extract of *L. officinal*e has high antioxidant activity.[17] In another study, polar extract of *L. officinale* indicated relatively good antioxidant activity.[18] Apart from the extract, the essential oil of *L. officinale* has also antioxidant activity. For instance, Mohamadi *et al.* indicated

Table 1: Antioxidant activity of the extracts from *Levisticum officinale* on DPPH assay

Sample	IC_{50} (μg/mL)
Hexane extract of aerial parts	>500
Hexane extract of roots	>500
Ethyl acetate extract of aerial parts	280±3.5
Ethyl acetate extract of roots	200±3.3
Methanol extract of aerial parts	400±4.7
Methanol extract of roots	400±4.7
BHT	23.6±0.4

DPPH: 2,2-Diphenyl-1-picrylhydrazyl, BHT: Butylated hydroxytoluene

Table 2: Antioxidant activity of the isolated compounds from the root ethyl acetate extract of *Levisticum officinale* on DPPH assay

Sample	IC_{50} (μg/mL)
Vanillin	189±1.9
Bergapten	25.4±0.8
Ferulic acid	18.7±0.3
BHT	23.6±0.4

DPPH: 2,2-Diphenyl-1-picrylhydrazyl, BHT: Butylated hydroxytoluene

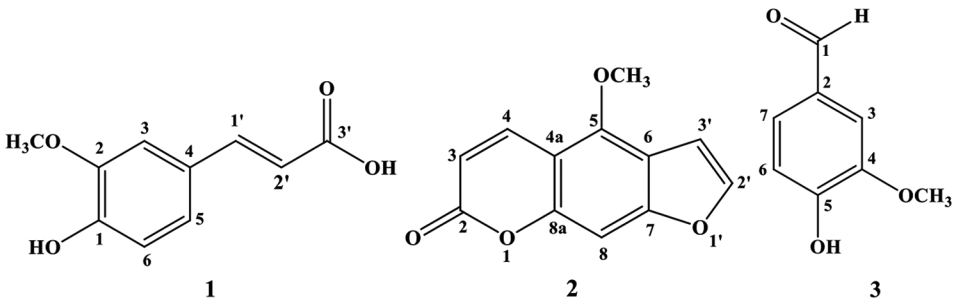

Figure 2: Structure of the isolated compounds (1-3) from *Levisticum Officinale*

that the essential oil of *L. officinale* in the flowering stage had a high antioxidant activity than vegetative and seed stages.[19]

In the previous studies, antioxidant activity of *L. officinale* was reported which was attributed to polar extract. However, in this study, the high antioxidant activity of non-polar extract (including ethyl acetate extract) has been confirmed. Finally, phytochemical investigation of ethyl acetate extract led to isolation of ferulic acid, bergapten, and vanillin as antioxidant compounds. Hence, non-polar extract of *L. officinale* (including ethyl acetate extract) can be candidate as a new and rich resource in antioxidant phytochemicals. In this study, antioxidant activity of isolated compounds was studied by DPPH method. However, in further study, it is better that other methods such as ABTS and FRAP to be used for evaluating antioxidant activity.

CONCLUSION

The result of this study indicated that the ethyl acetate extract of the root and its some fractions has antioxidant activity in DPPH and Dot blot assay. Finally, three compounds, namely, vanillin, bergapten, and ferulic acid were isolated as antioxidant compounds from *L. officinale*. In further studies, isolation of active compounds from other effective fractions of the root ethyl acetate is suggested.

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

REFERENCES

1. Moon JK, Shibamoto T. Antioxidant assays for plant and food components. J Agric Food Chem 2009;57:1655-66.
2. Gu L, Wu T, Wang Z. TLC bioautography-guided isolation of antioxidants from fruit of *Perilla frutescens* var. *acuta*. LWT Food Sci Technol 2009;42:131-6.
3. Miran M, Esfahani HR, Jung JH, Aliahmadi A, Skropeta D, Abbas-Mohammadi M, *et al*. Characterization and antibacterial activity of phthalides from the roots of the medicinal herb *Levisticum officinale* W.D.J. Koch. Iran J Pharm Res 2020;19:182-6.
4. Miran M, Esfahani HR, Farimani MM, Ahmadi AA, Ebrahimi SN. Essential oil composition and antibacterial activity of *Levisticum officinale* Koch at different developmental stages. J Essent Oil Bear Plants 2018;21:1051-5.
5. Esfahani HM, Farimani MM, Ebrahimi NS, Jung JH, Aliahmadi A, Abbas-Mohammadi M, *et al*. Antibacterial components of *Levisticum officinale* Koch against multidrug-resistant *Mycobacterium tuberculosis*. Pharm Sci 2020;26:441-7.
6. Schinkovitz A, Stavri M, Gibbons S, Bucar F. Antimycobacterial polyacetylenes from *Levisticum officinale*. Phytother Res 2008;22:681-4.
7. Guzman JD, Evangelopoulos D, Gupta A, Prieto JM, Gibbons S, Bhakta S. Antimycobacterials from lovage root (*Ligusticum officinale* Koch). Phytother Res 2013;27:993-8.
8. Yarnell E. Botanical medicines for the urinary tract. World J Urol 2002;20:285-93.
9. Cohen JS, Insel PA. The physicians' desk reference. Problems and possible improvements. Arch Intern Med 1996;156:1375-80.
10. Amraie E, Pouraboli I, Rajaei Z. Neuroprotective effects of *Levisticum officinale* on LPS-induced spatial learning and memory impairments through neurotrophic, anti-inflammatory, and antioxidant properties. Food Funct 2020;11:6608-21.

11. Kweon MH, Hwang HJ, Sung HC. Identification and antioxidant activity of novel chlorogenic acid derivatives from bamboo (*Phyllostachys edulis*). J Agric Food Chem 2001;49:4646-55.
12. Kale AS, Patil AS, Paikrao HM. A modified TLC bioautographic technique for the detection of antilithiatic potential of therapeutic plants from indigenous ayurvedic system. Pharm Methods 2017;8:209-12.
13. Aarabi A, Mizani M, Honarvar M. The use of sugar beet pulp for production of vanillin. Int J Biol Macromol 2016;94:345-54.
14. Chunyan C, Bo S, Ping L, Jingmei L, Ito Y. Isolation and purification of psoralen and bergapten from *Ficus carica* L. leaves by high-speed countercurrent chromatography. J Liq Chromatogr Relat Technol 2009;32:136-43.
15. Jain PG, Surana SJ. Isolation, characterization and hypolipidemic activity of ferulic acid in high-fat-diet-induced hyperlipidemia in laboratory rats. EXCLI J 2016;15:599-613.
16. Tajner-Czopek A, Gertchen M, Rytel E, Kita A, Kucharska AZ, Sokół-Łętowska A. Study of antioxidant activity of some medicinal plants having high content of caffeic acid derivatives. Antioxidants (Basel) 2020;9:412.
17. Tomsone L, Kruma Z, Talou T, Tian-Ming Z. Natural antioxidants of horseradish and lovage extracted by accelerated solvent extraction. J Hyg Eng Des 2015;10:16-24.
18. Wojdyło A, Oszmiański J, Czemerys R. Antioxidant activity and phenolic compounds in 32 selected herbs. J Food Chem 2007;105:940-9.
19. Mohamadi N, Rajaei P, Amiri MS. Essential oil composition and antioxidant activity of *Levisticum officinale* Koch. At various phenological stages. J Med Plant 2017;1:45-55.