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Effect of Acid Pre-treatment on the Yield, Chemical Compounds, and Biological Activities of *Oliveria Decumbens* Essential Oil

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Abstract: Oliveria decumbens Vent. (Apiaceae family) is an annual herb, and distributes in Syria, Iraq, south-east Anatolia, and Iran. In this study, the effect of acid pre-treatment before hydro-distillation was investigated on the yield and biological properties of the O. decumbens essential oil. Hydro-distillation method was used for the extraction of essential oils from the air-dried powdered flowers (10 g) which were treated with acid solution. Then, the yield as well as antioxidant and insecticidal properties of the essential oils were calculated. The obtained results indicated that the pre-treatment with high acid concentration (0.6 mM HCl) increased the essential oil yield (7.16%) compared to the conventional method (4.4%). Acid solution could hydrolyze the cell walls, and then release secondary metabolites out of the cells. Also, carvacrol and thymol were the major constituents with up to 60% obtained through both methods, and the acid pre-treatment could not change the amount of them, significantly. However, no significant changes were detected in the antioxidant and insecticidal properties of the essential oil, which were pretreated with 0.6 mM HCl, comparing to hydro-distillation method. Therefore, it may be suggested that if high yield is required, the O. decumbens flowers could be treated with acid solution.

Keywords: Antioxidant, Essential oil, Oliveria decumbens, Pre-treatment

1. Introduction

Oliveria decumbens Vent. (Apiaceae family) is an endemic medicinal plant of Iran. This plant is the only species of *Oliveria* genus, which grows in subtropical areas of Iran including Ilam, Kermanshah, Khuzestan, Fars, and Kohgiluyeh and Boyer-ahmad provinces.[1]

Oliveria decumbens has been applied as remedies for inflammation, febrifuge and digestive, cancer, infections, abdominal pains, fever, indigestion, and diarrhea.[2,3] Furthermore, its insecticidal, anti-tumour, anti-cholinesterase, anti-helicobacter pylori, anti-oxidant, and anti-bacterial activities has been studied.[4]

O. decumbens is a good source of essential oil. The flowering stages of this plant begin in June and change with three color phases as white, pink-purple, and green. The highest amount of essential oil and its major constituents have been studied, and the results indicated that thymol, carvacrol, γ-terpinene, and *p*-cymene were the major constituents. Also, the biological activities of the essential oil of *O. decumbens* can be related to the presence of their major constituents.[5,6] Hajimehdipoor et al. identified ten constituents in the *O. decumbens* essential oil, and the main compounds were carvacrol, myristicin, ρ-cymene, γ-terpinene, and thymol.[7]

The pre-treatment of the plant is one of the important methods for the breaking of cell walls to release secondary metabolites. An efficient pre-treatment method could reduce the production cost and increase energy efficiency.[8-10] Some researchers have been revealed that the pre-treatments of the plants with chemical or physical methods could increase the quality and quantity of essential oils. Naufalin et al.[8] demonstrated that the pre-treatment of *Cymbopogun citratus* by microwave heating before hydro-distillation improved the antimicrobial and antioxidant activities of essential oil. The effect of enzymatic pre-treatment before hydro-distillation method was evaluated on the yield and chemical compounds of *Rosmarinus officinalis* essential oil. The pre-treatment with enzyme improved the yield of essential oil yields in comparison with non-enzymatic pre-treatment. Also, no significant changes was observed in the composition of obtained essential oils.[9] Gupta and Guha[10] showed that ultrasonic pre-treatment of *Piper betle* leaves significantly increased the essential oil yield, as compared to classic methods. Moreover, the essential oil analysis indicated that the ultrasonic pretreatment could change the chemical composition of some compounds such as hydrindane,

Copaene, and Lemairamin. Also, the ultrasonic pre-treatment increased the antioxidant activity of essential oil.

Up to now, the effect of acid pre-treatment before hydro-distillation (A-HD) has not been studied on the yield, chemical compounds as well as antioxidant and insecticidal properties of *O. decumbens* flowers essential oil. Thus, the current work aimed to isolate the essential oil of *O. decumbens* flowers by A-HD, and then its yield, chemical compounds, and antioxidant activity were evaluated.

2. Results and Discussion

Effect of Acid pre-treatment on the yield

Several pre-treatment methods such as physical (ultrasounds, microwaves, and mechanical) and chemical (ionic liquid, acid-alkali, alkali, and acid) have been applied for the extraction of essential oils from plants.[8-11] In our previous study, we concluded that ultrasonic pre-treatment before hydro-distillation method was clearly an effective method to increase the yield of essential oil, then to decrease the extraction time and energy consumption, and also to improve insecticidal and antioxidant properties, comparing to hydro-distillation method.[12] Acid is usually applied for the degradation of cellulose from plant cell walls, and to increase the subsequent release of essential oil.[13,14] In this study, acid pre-treatment before hydro-distillation method was applied for the isolation of essential oil from *O. decumbens*. Figure 1 indicates the effect of kind (HCl, HNO_3 and H_2SO_4) and concentration (0.30, 0.45, 0.60, and 0.75 mM) of acids on the essential oil yield of *O. decumbens*.

According to the results, the increment of HCl concentration from 0. 30 to 0.60 mM increased the essential oil yield from 5.3 to 7.1%, and then the yield was decreased. Acid solution could hydrolyze the cell wall and then releases secondary metabolites out of the cells. But, high concentration of acid could degrade the essential oil compounds, and so decrease the essential oil yield.[15] In the cause of HNO_3, acid concentration had no significant effect on the yield. The effect of H_2SO_4 concentration on the yield revealed that 0.45 mM had the highest effect, and at this concentration, the yield was 3.9%. Also, the increment of H_2SO_4 concentration from 0.45 to 0.60 mM decreased the essential oil yield.

Figure 2 indicates the yield of *O. decumbens* essential oil at optimum condition of acid concentration. The experiments were performed in three repetitions and the columns with the same letters had no significant difference at the 5% probability level. As shown, the maximum yield belonged to 0.60 mM HCl, followed by control (non-treated essential oil) (4.4%). Also, 0.75 mM HNO_3 and 0.45 mM H_2SO_4 had lowest yield in compared to control.

The yield of extracted oil obtained with acid pre-treatment were compared with the other extraction methods. Mollaei et al.[12] applied ultrasonic pre-treatment for the extraction of essential oil from *O. decumbens* flowers. The optimal conditions for the extraction of essential oil were ultrasonic power of 149.71 W, ultrasonic temperature of 38.63 °C, ultrasonic duration of 25.20 min, and water/plant ratio of 10:1 mL/g. At this condition, the essential oil yield was 5.82%.[12] it confirms that acid pre-treatment was effective than ultrasonic pre-treatment for the extraction of *O. decumbens* essential.

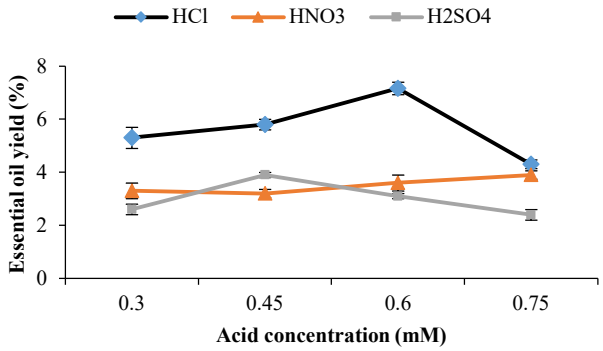

Figure 1. The effect of kind and concentration of acids on the essential oil yield of *O. decumbens*. The experiments were performed in three repetitions.

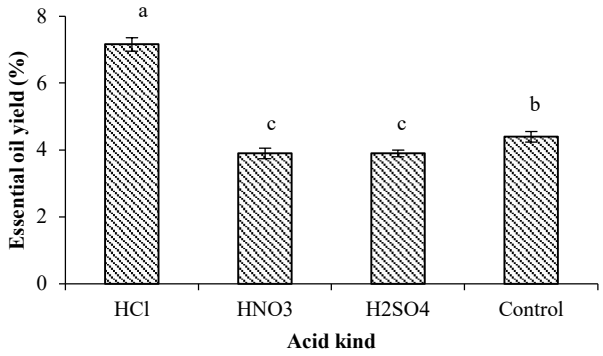

Figure 2. The yield of *O. decumbens* essential oil at optimum condition of acid concentration. The experiments were performed in three repetitions and the columns with the same letters had no significant difference at the 5% probability level.

Effect of acid pre-treatment on the antioxidant activity

Free radicals are compounds that have single electron and can irreversibly react with some molecules (free amino acids, proteins, nucleic acids, carbohydrates, lipids, and lipoproteins). This process damages and causes some diseases such as cardiovascular diseases, cataracts, cancer, etc. Enzymatic and non-enzymatic factors are the antioxidant defense system. Enzymatic factors such as catalase, superoxide dismutase, and glutathione peroxidase inhibit hydrogen peroxide, superoxide, and peroxide radicals, respectively.[17,18] Non-enzymatic factors such as phenolic compounds prevent free radicals and numerous reactive oxygen species. These compounds are divided into various groups such as flavonoids, phenolic acids, quinones, coumarins, lignans, lignins, stilbenes, and tannins.[19,20] In this study, the antioxidant properties of the essential oil which was

treated with 0.60 mM HCl was compared with control (non-treated essential oil). Based on the results, the inhibitory activity of the essential oil was concentration dependent, and the inhibitory percentage increased with increasing concentration (Figure 3).

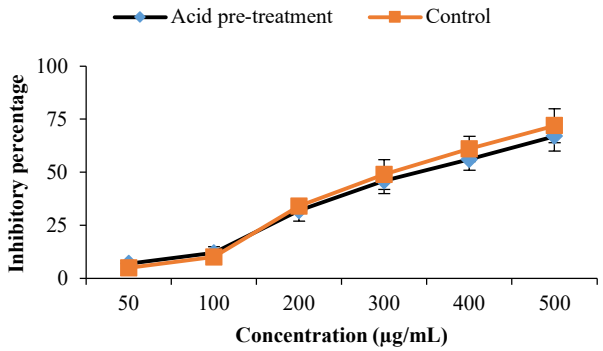

Figure 3. The inhibitory percentage of DPPH at different concentrations of the essential oil. Acid pre-treatment: 0.60 mM HCl; Control: non-treated essential oil.

Figure 4 illustrates the IC_{50} values of essential oil obtained by conventional hydro-distillation and acid pre-treatment before hydro-distillation methods. The results were compared with the IC_{50} value of ascorbic acid as positive control. According to the results, no significant changes were observed in the antioxidant property of the essential oils obtained by hydro-distillation and acid pretreatment hydro-distillation. But, the antioxidant activity was low compared with control.

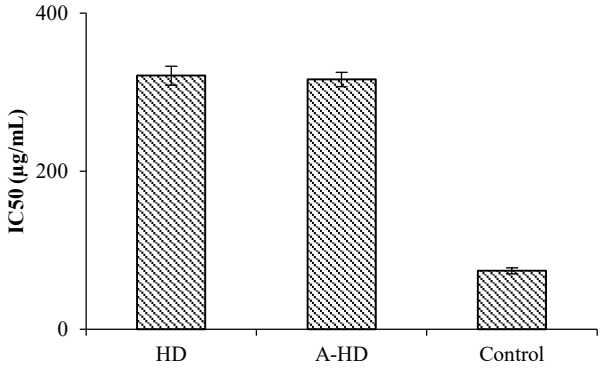

Figure 4. The effect of different methods on the antioxidant activity of essential oil. HD: conventional hydro-distillation; A-HD: acid pretreatment before hydro-distillation (0.60 mM HCl); Control: Ascorbic acid.

Effect of acid pre-treatment on the insecticidal activity

Insecticidal property of the essential oil which was treated with 0.60 mM HCl was evaluated against cabbage looper larvae, and then compared with non-treated essential oil.

The results indicated that the percentage of larvae mortality was concentration dependent, and the percentage improved with increasing concentration (Fig. 5).

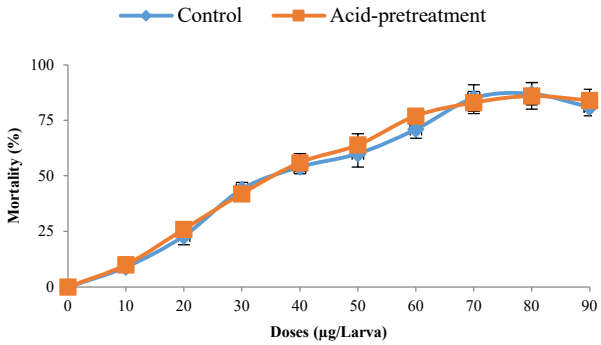

Figure 5. The percentage of larvae mortality at different concentrations of essential oil. Acid pre-treatment: 0.60 mM HCl; Control: non-treated essential oil.

Also, the essential oil obtained with acid pre-treatment before hydro-distillation had not significantly high insecticidal activity in comparison with non-pretreatment method (Figure 6). Also, the insecticidal property of control was higher than the other samples.

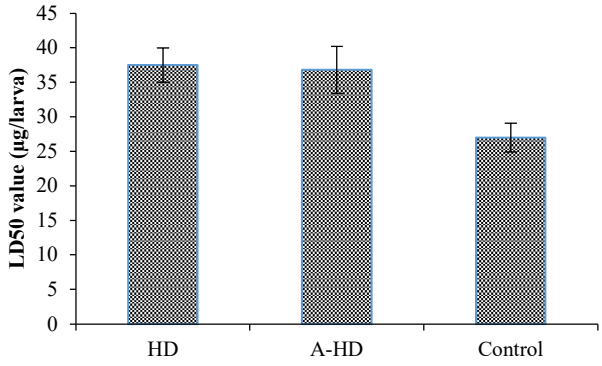

Figure 6. The effect of different methods on the insecticidal activity of essential oil. HD: conventional hydro-distillation; A-HD: acid pretreatment-hydro-distillation. Control: Imidacloprid solution of 20% (2.5 mL/L).

Effect of acid pre-treatment on the essential oil compounds

In the essential oils extracted using HD and A-HD methods, eight major constituents were identified, yielding up to 96% of the essential oils (Table 1). The results showed that the main constituents were myristicin (3.1-4.3%), carvacrol (30.6-32.5%), thymol (29.4-31.1%), γ-terpinene (12.4-12.6%), and p-cymene (11.8-14.0%). Carvacrol and thymol were the major constituents with up to 60% obtained by both methods. Also, the acid pre-treatment could not change the amount of them, significantly.

Thymol (2-isopropyl-5-methylphenol) and Carvacrol (5-isopropyl-2-methylphenol) are two phenolic compounds that belong to monoterpene, and can be found as major constituents of essential oil in members of the Ranunculaceae, Verbenaceae, and Laminaceae. Biosynthesis of carvacrol and thymol is thought to involve hydroxylation of p-cymene and γ-terpinene precursors. They are famous as biocides, with a

wide spectrum of anti-tumoral, hepato-protective, antioxidant, anti-leishmanial, anti-inflammatory, and antimicrobial activities, which have been the subject of some studies in vitro and in vivo.[21-23]

Investigation of carvacrol and thymol modes of action such as biological properties, health benefits, nutritional, and pharmacological can play serious role in their beneficial applications in various industries by providing more understanding of the health usages and improving performance parameters in agriculture species. They have moreover had applications in positively affected human health, influenced food quality, and functional food formulations.[24]

Table 1. The chemical constituents of essential oils from *O. decumbens* extracted by HD, and A-HD methods

No	Components	[a]Retention index	Methods	
			HD (%)	A-HD (%)
1	β-Pinene	975	1.5	1.1
2	β-Myrcene	990	1.2	2.2
3	p-Cymene	1023	14.0	11.8
4	Limonene	1026	2.5	2.4
5	γ-Terpinene	1057	12.4	12.6
6	Thymol	1292	29.4	31.1
7	Carvacrol	1298	32.5	30.6
8	Myristicin	1522	3.1	4.3
	Monoterpene hydrocarbons		30.4	30.1
	Oxygenated monoterpenes		61.9	61.7
	Phenyl propene		3.1	4.3
	Total		96.6	96.1

HD: Hydro-distillation.
US-HD: Ultrasonic pre-treatment before hydro-distillation
[a]Retention index of each constituent calculated in DB-5 column by retention time with that of n-alkanes (C8–C26).

Also, the essential oil compounds were categorized into three groups comprising hydrocarbon monoterpenes, oxygenated monoterpenes, and phenyl propens (Figure 7). The results showed that oxygenated monoterpenes were the main groups, followed by hydrocarbon monoterpenes, and using acid pre-treatment significantly not decreased the percentage of oxygenated monoterpenes (i.e. carvacrol and thymol).

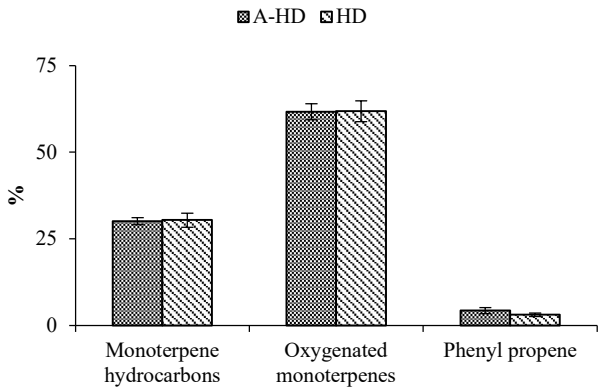

Figure 7. The percentage of essential oil compounds in three main groups.

3. Experimental

Plant materials

The flowering aerial parts of *O. decumbens* Vent were gathered from its wild habitat in July 2022 in the northen regions of Kermanshah province, Iran. After identifying, voucher specimen was placed in the Azarbaijan Shahid Madani University Herbarium. After drying of this plant in a shade at room temperature, it was kept at 4 °C until further analysis.

Acid pre-treatment before hydro-distillation (A-HD)

200 mL distilled water were mixed with 1 mL of 0.6 mM HCl, and then the obtained mixture were added to a one-liter round flask. Ten grams of powdered *Oliveria decumbens* flowers was added to above solution and refluxed for 10 minutes.

Classic Hydro-distillation (HD)

After acid pre-treatment of the *O. decumbens* flowers, the flask coupled to a Clevenger apparatus, and hydro-distillation method was applied for the essential oil extraction from the air-dried powdered flowers (10 g, 200 mL of distilled water, 3 hour) according to the method mentioned in British Pharmacopoeia.[25] The isolate essential oil was dehydrated by sodium sulfate and kept at -25 °C for further analysis.

Antioxidant property

The antioxidant properties of the *O. decumbens* essential oils were evaluated using DPPH assay. 200 microliter of the DPPH solution (1.0 mM) was added to various amounts of essential oil. The obtained mixture was incubated for 30 min in the dark, at 25 °C. Then, the absorbance of the sample was read at 517 nm. The radical scavenging activity (%) was calculated, and finally, the IC_{50} values of the samples were measured.[26]

Insecticidal activity

The Insecticidal activities of *O. decumbens* essential oils were evaluated by fumigant test against cabbage loopers. After diluting of the essential oils with acetone, 1 µL of essential oils was added to every larva. The larvae were located at small boxes with artificial diet (0.5 g). After 24 hours, the larvae mortality (%) was read, and the LD_{50} values were reported. An imidacloprid solution of 20% (2.5 mL /L) was used as a positive control. The tests were replicated three times with at least 5 larvae in each replicate.[12]

GC and GC–MS chromatography

The isolation of essential oil constituents was done by GC-FID (Agilent 6890 N) equipped with a fused capillary column DB-5 (30 m × 0.25 mm, 0.25 µm). The injector temperature of the GC was set at 250 °C. The oven temperature was constant at 70 °C for 5 min, and then was increased to 240 °C (5 °C/min). Finally, it was kept constant for 4 min. The carrier gas was nitrogen, and its flow rate was adjusted at 1.0 mL/min. The ionization voltage and splitting ratio were 70 eV and

1:100, respectively. In addition to GC, GC-MS (Model: Agilent 7890 AG Chromatograph and Agilent 5975 c Mass) was used. The oven temperature program, column, and other settings were similar to GC. The scan time was 30 m/z, and the compounds were analyzed at a range of 600 m/z. The essential oil components were identified by Kovats Indices (KI), NIST and Wiley libraries, and moreover according to the earlier studies. The percentage of constituents was obtained by electronic integration of FID peak areas without any correction factors.

4. Conclusions

In this study, the effect of acid pre-treatment before hydro-distillation on the yield and biological properties of the *O. decumbens* essential oil was investigated. The obtained results showed that the pre-treatment with 0.60 mM HCl increased essential oil yield, compared to the conventional method. Also, thymol and carvacrol were the major constituents, and the acid pre-treatment not changed the amount of them, significantly. However, no significant changes were observed in the antioxidant and insecticidal properties of the essential oil, comparing to hydro-distillation method. Therefore, it may be suggested that, if high yield is required, the *O. decumbens* flowers could be treated with acid solution.

Declaration of Interests

The authors declare that there exists no conflict of interest.

Author Contributions

Saeed Mollaei: Supervision, Conceptualization, Writing-Reviewing and Editing. Mina Mohammadi: Investigation, Methodology, Data curation, Writing-Original draft preparation.

Supporting Information

The Supporting Information is available free of charge athttp://www.org.chem.res./doi: XXXX

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