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Chemical Composition, Antibacterial and Antioxidant Activities of the Essential Oil Isolated from Verbena officinalis

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ABSTRACT: The essential oil of Verbena officinalis growing in Iran was extracted using hydro-distillation method and the antibacterial and antioxidant activities were evaluated. The antibacterial activity of the essential oil against a Gram-positive (S. aureus ATCC25923) and Gram negative (E. coli ATCC 25922) were examined. The results obtained revealed that the essential oil exhibited variable levels of antibacterial activities against tested bacterial strains and highest sensitivity to essential oil of V. officinalis was observed by E. coli ATCC 25922. The antioxidant and free radical scavenging activity of the obtained oil was tested by means of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) assay and β-carotene bleaching test. In the DPPH assay, while the free radical scavenging activity of the HD oil showed inhibitions of 51.4, 55.00 and 66.30% at 0.25, 0.5 and 1.00 mg/ml, respectively, in the β- carotene bleaching assay, the percentage inhibition increased with increasing the concentration of the oil. The results have shown that the essential oil of Verbena officinalis possess antioxidant and antibacterial activities, and therefore it could be used as a natural preservative ingredient in food and/or pharmaceutical industries.

Keywords: *Antioxidant, Essential Oils, Verbena officinalis.*

Introduction

Essential oils are complex mixtures of volatile compounds with strong odor that are synthesized in several plant organs, including buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood or bark and stored in secretory cells, cavities, canals, epidermic cells or glandular trichomes (Franz & Novak, 2010; Bakkali *et al*., 2008). These volatile compounds have diverse ecological functions, acting as defensive substances against microorganisms and herbivores, but can also be important to attract the insects for the dispersion of pollens and seeds (Bakkali *et al*., 2008).

Many plants are sources of compounds with antioxidant activity that might be used as natural preservatives. In addition, antioxidants play a special role in human healthcare particularly prevention of cardiovascular diseases and cancer that has been associated with the intake of fruits and vegetables, rich in natural antioxidants (Zhang *et al*., 2006; López *et al*., 2007).

Essential oils have been studied most from the viewpoint of their flavour and fragrance chemistry only for flavouring foods, drinks and other goods. The essential oils and their components are gaining increasing interest due to their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional applications (Ormancey *et al*., 2001).

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Verbena officinalis grows in all temperature regions of the globe and is cited in the traditional medicine of many countries. It has been one of the most mentioned plants in a recent ethnobotanic study carried out in which 108 informants (20% of the total) have indicated 175 different uses belonging to 9 therapeutic categories (Akerreta *et al*., 2007). The main components of V. officinalis L. are iridoids, phenylpropanoids, flavonoids, luteolin and terpenoids (Bilia *et al*., 2008)

In this research chemical and biological evaluations concerned with phytochemical characterization, antibacterial and antioxidant activities of the essential oil obtained from V. officinalis are investigated.

Materials and Methods

All the solvents and reagents used in this experiment namely methanol, ethanol, DMSO, 2, 2-diphenyl-2-picylhydrazyl hydrate (DPPH), 3,5-ditert-butyl-4-hydroxytoluene (BHT), β-carotene, linoleic acid and Tween 40, were purchased from Sigma Aldrich .

- *Plant Material*

The leaves of V. officinalis were collected from the local area of Fars, Iran in spring 2014. The Fresh leaves were shade dried at room temperature. Dried leaves were made into fine powder using an electric grinder.

- *Isolation of the Essential Oils*

The dried plant samples of V. officinalis (500 g) were subjected to hydro-distillation using a Clevenger-type apparatus for 4 h. The oil was extracted with $CHCl_3$ and then were dried over anhydrous sodium sulphate and stored under nitrogen at 20°C in a sealed vial for further application.

- *Antibacterial Activity*
- *Disc diffusion method*

The antibacterial activity of the essential oil was determined with the disc diffusion method (NCCLS, 2002). Briefly, bacterial suspensions were adjusted to 1×10^7 CFU mL^{-1} and spread in TSA or PCA using sterile cotton swabs. Subsequently, filter paper discs (6 mm; Whatman #1) were placed on the surface of Petri dishes and impregnated with 20 µL of essential oil at different concentrations (diluted in DMSO). Negative controls were prepared only with DMSO. After staying at 4 °C (2 h), all the Petri dishes were incubated at 30 °C. Antibacterial activity was evaluated by measuring the radius of the inhibition zones to the nearest millimetre, and the minimum inhibitory concentration (MIC) was defined as the lowest concentration that inhibited the growth of bacteria.

- *Antioxidant Activity*
- *Free radical scavenging effect*

The DPPH free radical scavenging effect was assessed by the method of Kondo and co-authors (Kondo et al., 2002). Briefly, 0.1 mL of essential oil at different concentrations were added to 2 mL DPPH (0.21 mM in 95% ethanol). The mixture was shaken, left for 60 min at room temperature in the dark, and the absorbance was measured at 517 nm in a spectrophotometer. The percentage of DPPH inhibition was calculated using the following equation:

$$\text{Percentage of inhibition} = \text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}} / \text{Abs}_{\text{control}} \times 100$$

Where, $\text{Abs}_{\text{control}}$ is the absorbance of the control reaction (blank with 0.1 mL ethanol and DPPH) and $\text{Abs}_{\text{sample}}$ is the absorbance of the sample reaction (0.1 mL essential oil diluted in ethanol and DPPH).

The sample concentration (in 1 mL reaction mixture) estimated the percentages of inhibition against essential oil concentrations. All determinations were performed in triplicate order. As a positive control the synthetic antioxidant BHT was

employed. To standardize DPPH results, the antioxidant activity index (AAI), proposed by Scherer and Godoy (Scherer and Godoy, 2009), was calculated in the following order:

AAI = DPPH concentration in reaction mixture (_g mL−1) EC50 (_g mL−1)

Samples were classified as showing poor antioxidant activity (AAI < 0.5), moderate (0.5 < AAI < 1.0), strong (1.0 < AAI < 2.0) and very strong (AAI > 2.0) (Scherer and Godoy, 2009).

- *β- Carotene bleaching assay*

This method evaluates the capacity of the oil to reduce the oxidative loss of β-carotene in a β-carotene linoleic acid emulsion (Taga *et al*., 1984). β-Carotene (10 mg) was dissolved in 10 ml of chloroform (CHCl3). An aliquot (0.2 ml) of this solution was added into a boiling flask containing 20 mg of linoleic acid and 200 mg of Tween 40. The chloroform was removed using a rotary evaporator at 40°C for 5 min. Distilled water (50 ml) was slowly added to the residue with vigorous agitation, to form an emulsion. The emulsion was added to a tube containing 0.2 ml of essential oil. The absorbance was immediately measured at 470 nm and the test emulsion was incubated in a water bath at 50°C for 5 min, after which the absorbance was measured again. BHT was used as the positive control. In the negative control, the essential oils were substituted with an equal volume of ethanol. The antioxidant activity (%) of the oil was evaluated in terms of the bleaching of the β- carotene using the following formula:

$$\% \text{ inhibition} = \{(At\text{-}Ct) / (CO\text{-}Ct)\} \times 100$$

where, At and Ct are the absorbances measured for the oil and control, respectively, after incubation for 5 min, and CO is the absorbance values for the control measured at zero time during the incubation. The oil concentration providing 50% antioxidant activity (EC_{50}) was calculated by plotting antioxidant percentage against oil concentration.

- *GC–MS analysis*

The essential oil was analyzed on an Agilent 6890 gas mass selective detector (Agilent Technologies, Palo Alto, USA). A vaporization injector operating in the split mode at 250°C and a fused silica capillary column (dimethyl polydimethylsiloxane, Agilent Technologies) were used. The oven temperature was programmed at 45°C per 1 min, raised to 250°C at 5°C min-1 and maintained at 250°C for 5 min. Helium was used as carrier gas at 30 cm s-1 and the injection volume was 1-L. The temperatures of transfer line, ion source, and quadrupole analyzer were maintained at 280°C, 230°C, and 150°C, respectively. A turbo molecular pump (10−5 Torr) was used. A solvent delay of 3 min was selected. The acquisition data and instrument control were performed by the MSD Chem- Station. The identity of each compound was assigned by comparison of their relative retention time relative to a standard mixture of n-alkanes (Adams, 2001), as well as by comparison with the mass spectra characteristic features obtained with the Wiley's library 275 spectral data bank (G1035B; Rev D.02.00; Agilent Technologies, Santa Clara, CA, USA) (Teixeiraa *et al*., 2013).

- *Statistical analysis*

Statistical analysis involved the use of SPSS software Ver: 20. Analysis of variance was performed by anova procedures. Significant differences between means were determined by Duncan's multiple-range test. All determinations were carried out in triplicate order.

Results and Discussion

- *Chemical composition of the essential oil*

Dried leaves of V. officinalis were subjected to hydrodistillation using a Clevenger apparatus that resulted in a pale

yellow-colored essential oil. GC–MS analysis of the essential oil was carried out according to the standard method and the results are presented in Table 1 and Figure 1.

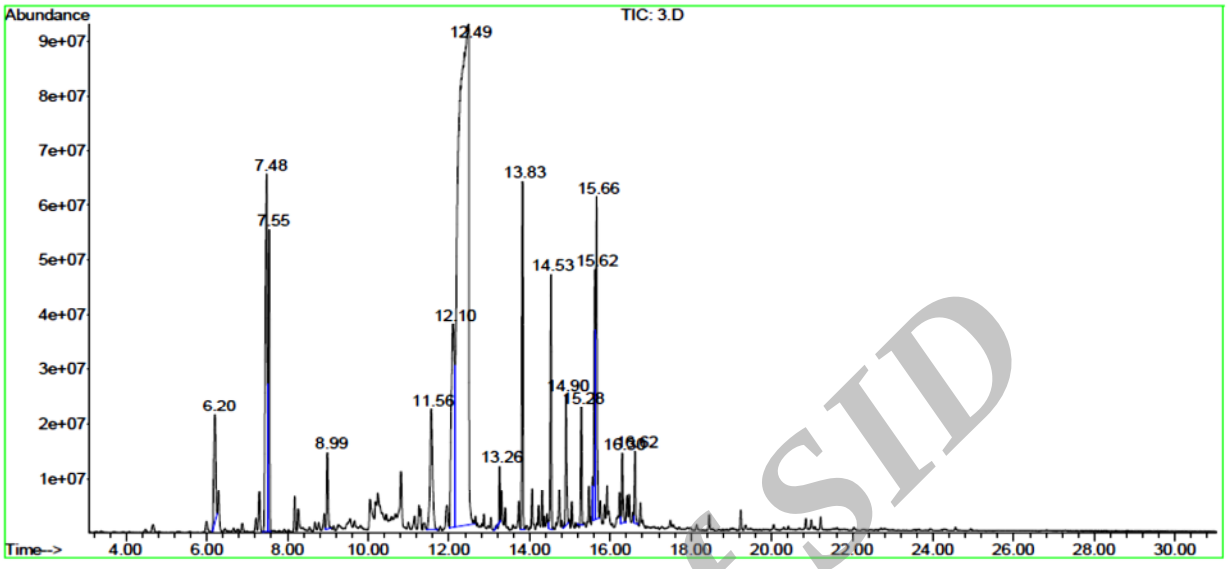

Fig. 1. GC-Mass Chromatograph of V. officinalis essential oil

Table 1. Chemical composition of V. officinalis essential oils

Peak no	Component	Composition (%)	RT(min)
1	6-Methyle-5-Hepten-2-One	2.21	6.20
2	1,8-Cineole Eucalyptol	7.44	7.48
3	l-Limonene	4.33	7.55
4	Linaloole	1.12	8.99
5	Z-Citral	3.24	11.56
6	Thymol	7.30	12.10
7	4-(1-Methylethyl)-Benzylic Alcohol Safranal	53.80	12.49
8	Lavandulyl Acetate NEROL	0.47	13.26
9	Beta.-Caryophyllene	4.47	13.83
10	AR-Curcumene	2.89	14.53
11	Beta.-Bisabolene	1.73	14.91
12	CIS-.Alpha.-Bisabolene	1.37	15.28
13	7,8,8a-Hexahydr Onaphthalene-1-Methanol	3.44	15.62
14	(-)-Caryophyllene Oxide	4.34	15.66
15	Alpha.-Cadinol	0.93	16.30
16	Heptan-7-One	0.92	16.62

- *Antimicrobial Activity*

The antibacterial activity of the essential oil against Gram-positive (*S. aureus* ATCC 25923) and Gram negative (*E. coli* ATCC 25922) is presented in Table 2. The results revealed that the essential oil exhibited variable levels of antibacterial activity against tested bacterial strains. According to the literature data Gram-positive bacteria seemed to be more sensitive to different examined essential oils than Gram negative bacteria. This study indicated a notable susceptibility of examined Gram-negative pathogenic bacteria such Escherichia coli, where the highest sensitivity to the essential oil of V. officinalis was observed.

- *Antioxidant Activity*

The principle of the antioxidant activity is based on the availability of electrons to neutralize free radicals. In this study, the antioxidant activity of V. officinalis oil was evaluated by two complementary tests: scavenging of DPPH free radicals and the β-carotene bleaching test.

The antioxidant activity of the V. officinalis essential oil has been evaluated in a series of in vitro tests. In the DPPH test, the ability of the essential oil to act as the donor for hydrogen atoms or electrons in transformation of DPPH into its reduced form DPPH-H was measured spectrophotometrically.

The free radical scavenging activity of essential oil of V. officinalis obtained by the essential oil showed percentage inhibitions of 51.4, 55.00 and 66.30% at concentrations of 0.25, 0.5 and 1.0 mg/ml, respectively. These results indicated that the activity increases as the concentrations of the oils were increased, at least within the limit of the test concentrations of the oils (Figure 2). The essential oil showed DPPH-radical scavenging (IC_{50} = 0.66 mg/ml), whereas for BHT, IC_{50} was 0.2 mg/ml at concentration of 0.25 mg/ml.

Assessed essential oil was able to reduce the stable radical DPPH to the yellow-colored DPPH-H reaching 50% of reduction. Comparison of DPPH scavenging activity of essential oil to those expressed by BHT pointed out very similar IC_{50} values (for BHT, IC_{50}). It appears that for the neutralization of DPPH radicals the most active compounds were monoterpene aldehydes and ketones (citrals, citronellal, isomenthone, and menthone) and the mixture of mono- and sesquiterpene hydrocarbons (first E-caryophyllene). These findings are in correlation with earlier published data on antioxidant activities of different essential oils and selected essential oil components (Mimica-Dukic *et al*., 2003).

The result of lipid peroxidation inhibitory activity of the essentials oils, assessed by the β-carotene bleaching test are shown in Figure 3. β-Carotene usually undergoes rapid discoloration in the absence of an antioxidant. This is because the oxidation of β-carotene and linoleic acid generates free radicals (Jayaprakasha *et al*., 2001).

Table 2. Antibacterial Activity (Inhibition Zone Measured in mm, Including Hole of 5 mm Diameter) of *V. officinalis* Essential Oil [a]

Bacteria strain	Essential oil	
	DD [b]	MIC [c]
S. aureus (ATCC25923)	23.2±0.44	64
E. coli (ATCC25922)	29.4± 0.84	32

[a] Results are means of three different experiments.
[b] Diameter of inhibition zone including disc diameter of 6 (mm).
[c] MIC, minimum inhibitory concentration (as mg/ml).

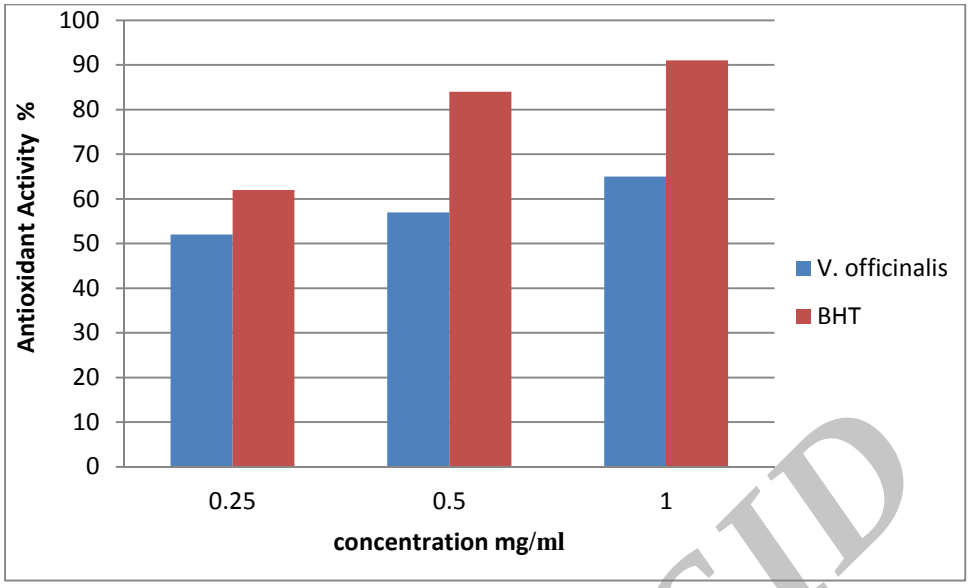

Fig. 2. Free radical-scavenging activity of *V. officinalis* essential oil evaluated by the 1,1-diphenyl-2-picrylhydrazyl (DPPH).

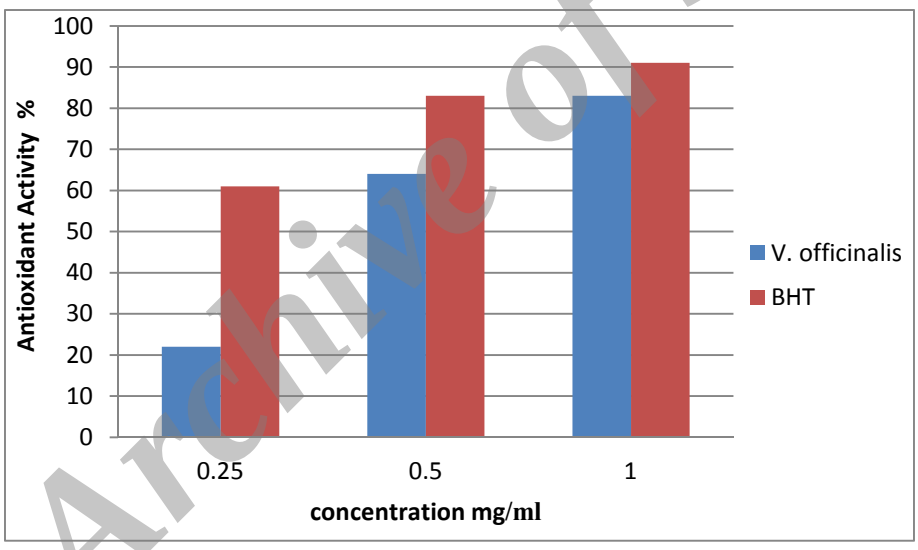

Fig. 3. Antioxidant activity of V. officinalis L. essential oil determined by β-carotene bleaching test.

The results obtained from this assay are similar to the data obtained from DPPH test. The percentage inhibitions were 28.62, 62.03 and 78.5% for essential oil at concentrations of 0.25, 0.5 and 1.0 mg/ml, respectively.

These results are in agreement the results obtained from DPPH test. It was noted that the antioxidant activities of the tested samples were dependent on their concentrations.

In a research carried out by Rehecho *et al*. the extracts from Verbena officinalis L. were obtained and characterized. The analysis by HPLC-DAD and LCeMS allowed the detection and identification of three iridoids, fifteen flavonoids and four phenolic acid derivatives. In addition, three new flavonoids have been isolated. To our knowledge, these flavonoids have not been reported as natural products. The extracts showed significant antioxidant activity using

three in vitro model systems and the results have been correlated with total phenolic and total flavonoid contents (Rehecho *et al*., 2010).

Many reports on the investigations of the activity of V. officinalis have shown that there are biologically active compounds in V. officinalis essential oil that exhibit cytotoxic, antioxidant and anti-carcinogenic activities (Okoh *et al*., 2010).

Essential oils, despite their wide uses and fragrances, constitute effective alternatives to synthetic compounds produced by chemical industries without showing the side effects (Faixova & Faix, 2008).

It is very difficult to attribute the antioxidant effect of a total essential oil to one or a few active principles because an essential oil always contains a mixture of different chemical compounds. In addition to the major compounds, minor molecules might make significant contributions to the oil activity. Therefore, the antioxidant property of the oils might be the combined activities of the various major and minor components of the oils.

Conclusion

This study has revealed significant antimicrobial, particularly antibacterial, activity of the investigated essential oil. The examined oil exhibited high RSC, which was found to be in correlation to the content of mainly monoterpene ketones and aldehydes. These results indicate that essential oils could serve not only as flavoring agents but also as safe antioxidant and antiseptic supplements in preventing deterioration of food, beverage and pharmaceutical products.

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