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**Research Article**

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# Chemical composition and antioxidant activity of essential oils and solvent extracts of *Foeniculum vulgare* Mill. from Morocco

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**ABSTRACT**

*The aim of this work was to characterize the essential oils and solvent extracts of Foeniculum vulgare Mill. from Morocco in terms of chemical composition and antioxidative activity. Analysis of the essential oil of F. vulgare was carried out using gas chromatography (GC) and gas chromatography-mass spectral (GC-MS). The main components were limonene (20.8%) and β-pinene (17.8%), followed by myrcene (15.0%) and fenchone (12.5%). The antioxidant activities of this aromatic plant were evaluated using a DPPH test system to suggest it as a new potential source of natural antioxidants. The amounts of phenolic and flavonoid compounds of solvent extracts (diethyl ether and ethyl acetate) were determined spectrometrically. Furthermore, The DPPH scavenging activity of extracts increased in the order essential oil < diethyl ether extract < ethyl acetate extract < ascorbic acid. Finally, a relationship was observed between the antioxidant activity potential and total phenolic and flavonoid levels of the extract.*

**Keywords**: *Foeniculum vulgare* Mill., Essential oil, Solvent extract, Antioxidant activity, Flavonoid content

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## INTRODUCTION

Antioxidants are widely used as food additives to provide protection against oxidative degradation of foods by free radicals [1]. Furthermore, many synthetic antioxidant components (BHA and BHT) have shown toxic and/or mutagenic effects; therefore, plant antioxidants are suggested as an interesting alternative. Several substances from aromatic and medicinal plants have been shown to contain antioxidants like flavonoid compounds. Compounds are the main agents that can donate hydrogen to free radicals and thus break the chain reaction of lipid oxidation at the first initiation step [2]. This high potential of phenolic constituents to scavenge radicals may be explained by their phenolic hydroxyl groups [3]. Numerous studies exhibited a strong relationship between total phenolic content and antioxidant activity in fruits, vegetables, and medicinal plants [4,5]. Polyphenolic components are also known for their ability to prevent fatty acids from oxidative decay [6].

Fennel (*Foeniculum vulgare* Mill.) belonging to the family Apiaceae (Umbelliferae) is native to the Mediterranean areas [7]. *F. vulgare* is a perennial herb (up to 40 cm high) with feathery leaves (up to 40 cm long) and yellow flowers (umbels 5–15 cm wide). The fruit is a dry and grooved seed from 4–10 mm long (Blamey, M. & Grey-Wilson, C, 1989).

*F. vulgare* is recognized as an aromatic plant growing wild in eastern Morocco. For instance, fennel fruits are widely used as culinary spices [9] and preparation of various dishes like soups, sauces, pastries, confectioneries, pickles, meat dishes, and alcoholic beverages, due to its characteristic anise odor [10, 11]. *F. vulgare* is also used in folk

medicine as a stimulant, diuretic, carminative and sedative [12, 13]. Widely used for its medicinal properties, it is nowadays cultivated for industrial uses such as cosmetic and pharmaceutical products [14, 15]. *F. vulgare* has shown antimicrobial [16], antioxidant, and anticholinesterase activity in various *in vivo* and *in vitro* experiments [17].

Phytochemical analysis of *F. vulgare* showed the presence of terpenes, alkaloids, flavonoids, tannins, and saponins [18]. The essential oil composition has been previously reported [19, 20]; the two major components are *trans*-anethol and fenchone [16, 21-23]. The characteristic odor of fennel has been attributed at the volatile compounds trans-anisole, estragole, fenchone and oct-1-en-3-ol [24], *F. vulgare* Essential Oil is alos known as green corrosion inhibitor of carbon steel in hydrochloric acid solution [25].

Solvent extracts of *F. vulgare* exhibited high amounts of hydroxylcinnamic acid derivatives and flavonoid components with high antioxidant activities such as 3-caffeoylquinic acid, 4-caffeoylquinic acid, 1,5-O-dicaffeoylquinicacid, rosmarinic acid, eriodictyol-7-O-rutinoside, quercetin-3-O-galactoside, kaempferol-3-Orutinoside and kaempferol-3-O-glucoside [26].

The aim of this work is to evaluate the antioxidative properties of the essential oils and solvent extracts of *F. vulgare* from Morocco. Additionally, the essential oil composition, the total phenolic and flavonoid contents of diethyl ether and ethyl acetate extracts have been determined.

## EXPERIMENTAL SECTION

### 2.1. Plant material

The aerial parts of *F. vulgare* were harvested in october 2009 (full bloom) from Al Hoceima, Morocco. Voucher specimens were deposited in the herbarium of Mohamed 1st University, Oujda, Morocco.

### 2.2. Essential oil isolation

The air-dried leaves of *F. vulgare* were submitted for 4 h to hydrodistillation using a Clevenger type-apparatus according to the method recommended in the European Pharmacopoeia [27]. The essential oils were dried over anhydrous sodium sulphate and then stored in sealed glass vials at 4 to 5°C prior to analysis.

### 2.3. GC and GC–MS analysis

Analysis was carried out using a Perkin-Elmer Autosystem XL GC apparatus (Waltham, MA, USA) and a Perkin-Elmer turbo mass detector (quadrupole) coupled to a Perkin–Elmer Autosystem XL equipped with a dual flame ionization detection (FID) system and the fused-silica capillary columns (60 m × 0.22 mm I.D., film thickness 0.25 ml) Rtx-1 (polydimethylsiloxane) and Rtx-wax (polyethyleneglycol). In previous studies [28,29], the complementarity of these two analytical techniques (GC-FID and GC-MS) with two chromatographic columns (apolar and polar) have been demonstrated for the identification and quantification of volatile components in complex mixture. The oven temperature was programmed from 60 to 230°C at 2°C/min and then held isothermally at 230°C for 35 min. Injector and FID temperatures were maintained at 280°C and MS source temperature at 150°C. Samples were injected in the split mode (1/50) using helium as a carrier gas (1 ml/min) and 0.2 µl injection volume of pure oil. Retention indices (RI) of compounds were determined relative to the retention times of a series of n-alkanes (C5–C30) (Restek, Lisses, France) with linear interpolation using the Van den Dool and Kratz equation and software from Perkin–Elmer. Electron ionization mass spectra (energy ionization: 70 eV) were acquired over the mass range 35 to 350 Da. Identification of individual components was based on: (i) comparison of calculated RI, on polar and apolar columns, with those of authentic compounds or literature data [30] ; and (ii) computer matching with commercial mass spectral libraries and comparison of mass spectra with those of our own library of authentic compounds or literature data [30,31] .

### 2.4. Preparation of the extracts

Boiling water extracts (100 ml) of plant samples obtained under reflux conditions (hydrodistillation process) were extracted three times (3 × 20 ml) with organic solvents (diethyl ether and ethyl acetate). Water extract residues were then extracted by boiling acidified water (2 N HCl) prior to liquid–liquid extraction. The diethyl ether and ethyl acetate extracts were filtered and concentrated under vacuum to obtain two extracts in yields of 0.36 % (w/w). The organic solvent extracts were dried over anhydrous sodium sulfate and then stored in sealed glass vials at 4 to 5°C prior to analysis. Each extraction was performed in triplicate.

### 2.5. Determination of total phenolic contents

Total phenolic contents of the extracts were determined by using Folin–Ciocalteu reagent according to the method previously reported by Slinkard and Singleton [32], using caffeic acid as a standard, and as modified by Li et al.

[33]. 200 µl of the diluted solution extract was mixed with 1 ml of Folin–Ciocalteu (diluted in distillated water) and the volumetric flask was vigorously shaken. After 4 min, 800 µl of $Na_2CO_3$ (75 mg/ml) solution was added and the mixture was allowed to stand for 45 min at room temperature. At the end of the incubation, the absorbance was measured at 760 nm. The same procedure was also applied to the standard solutions of caffeic acid, and a standard curve was obtained. The concentrations of phenolic compounds expressed as µg caffeic acid equivalent per mg of extract were calculated according to the standard caffeic acid graph. All experiments were carried out in triplicate, and caffeic acid equivalent values were reported as X (average) ± SD (standard deviation) of triplicates.

**2.6. Determination of total flavonoids contents**

Total flavonoid contents were determined using the Dowd method as adapted by Arvouet-Grand et al. [34]: 1 ml of 2% aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of extracts (200 µg). The absorption at 430 nm was measured after 10 min against a blank sample consisting of 1 ml methanol without $AlCl_3$. The concentrations of flavonoid compounds expressed as µg quercetin equivalent per mg of extract were calculated according to the standard quercetin graph. All experiments were carried out in triplicate, and quercetin equivalent values were reported as X ± SD of triplicates.

**2.7. Antioxidant activity**

The free radical-scavenging activities of solvent extracts were measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as described by Hatano et al. [35]; antioxidants react with the stable free radical DPPH (deep violet color) and convert it to 1,1-diphenyl-2-picrylhydrazine with discoloration. Various concentrations (0.1 ml) of the oil (1.5–24 mg/ml), the diethyl ether extract (40 to 400 mg/l) and ethyl acetate extract (28 to 160 mg/l) in ethanol and water were added to 3.9 ml of a DPPH radical solution in ethanol (the final concentration of DPPH was 0.05 mM). The mixture was strongly shaken and left to stand at room temperature for 30 min in the dark. The absorbance was measured at 517 nm against a blank. The radical-scavenging activity was expressed as percentage of inhibition (I%) according to the following formula [36]:

$$I(\%)= 100^* (A_{control} - A_{sample})/A_{control}$$

Where $A_{control}$ is the absorbance of the control reaction and $A_{sample}$ is the absorbance of the test compound. The sample concentration providing 50% inhibition (IC50) was calculated from the graph of inhibition percentage against sample concentration. Tests were carried out in triplicate. Ascorbic acid was used as a positive control.

## RESULTS AND DISCUSSION

**3.1. Essential oil composition**

Analysis of the essential oil of *F. vulgare* from Morocco was carried out using gas chromatography (GC) and gas chromatography-mass spectral (GC-MS). The oil was dominated by monoterpene hydrocarbons (68.4%) followed by oxygenated monoterpenes (28.2%). 21 constituents were reported amounting to 96.6% of the total oil (**Table 1**). Among them, limonene (20.8%), β-pinene (17.8%), myrcene (15.0%) and fenchone (12.5%) were identified as major components. Essential oil of Fennel from Morocco was characterized by the lack of *trans*-anethol in comparison with those of other geographic origins previously reported. Indeed, *F. vulgare* oils from Serbia [37], India [38], China [39] Turkish [9, 15] exhibited high contents of trans-anethol and fenchone.

**3.2. Total phenolic and flavonoid contents of solvent extracts**

The concentrations of total phenolic and flavonoid components in the plant extracts were determined spectrometrically and calculated as caffeic acid and quercetin equivalent values, respectively (**Table 2**). The total phenolic contents of the diethyl ether and ethyl acetate extracts of *F. vulgare* were 70 ± 4.2 µg/mg and 169 ± 5 µg/mg, respectively. The results showed that the ethyl acetate extract has higher total phenol amount than the diethyl ether extract. Similarly, the ethyl acetate extract was found to be richer in flavonoids (23 ± 3.1µg/mg) than the diethyl ether extract (14 ± 2.1 µg/mg).

**Table 1. GC and GC-MS analysis of essential oil composition from *F. vulgare***

| N°[a] | Components | RIl[b] | Ria[c] | RIp[d] | % [e] |
|---|---|---|---|---|---|
| **1** | α-Thujene | 932 | 922 | 1012 | 0.1 |
| **2** | α-Pinene | 936 | 930 | 1012 | 8.0 |
| **3** | Sabinene | 973 | 965 | 1109 | 1.0 |
| **4** | β-Pinene | 978 | 972 | 1099 | 17.8 |
| **5** | Myrcene | 987 | 981 | 1150 | 15.0 |
| **6** | α□□□□□□□□□□□□ | 1002 | 1000 | 1150 | 0.3 |
| **7** | 3-Carene | 1010 | 1006 | 1132 | 0.7 |
| **8** | p-Cymene | 1015 | 1013 | 1250 | 1.5 |
| **9** | Limonene | 1025 | 1022 | 1193 | 20.8 |
| **10** | γ□□□□□□□□□□ | 1051 | 1048 | 1226 | 0.8 |
| **11** | Fenchone | 1069 | 1067 | 1386 | 12.5 |
| **12** | Terpinolene | 1082 | 1078 | 1262 | 2.4 |
| **13** | Linalool | 1086 | 1083 | 1522 | 0.3 |
| **14** | Camphor | 1123 | 1115 | 1494 | 0.1 |
| **15** | *trans*-Pinocarveol | 1126 | 1122 | 1633 | 0.2 |
| **16** | Terpinen-4-ol | 1164 | 1160 | 1573 | 0.4 |
| **17** | α□□□□□□□□□□ | 1176 | 1167 | 1674 | 0.3 |
| **18** | Piperitenone | 1318 | 1308 | 1880 | 1.0 |
| **19** | Piperitenone oxide | 1335 | 1331 | 1910 | 12.5 |
| **20** | Geranyl acetate | 1362 | 1361 | 1732 | 0.2 |
| **21** | Nepetalactone | | 1374 | 1955 | 0.7 |
| **Total identified** | | | | | **96.6** |
| Monoterpene hydrocarbons | | | | | 68.4 |
| Oxygenated monoterpenes | | | | | 28.2 |

[a] *The numbering refers to elution order on apolar column (Rtx-1)*
[b] *RIl = retention indices on the apolar column of literature [30, 43]*
[c] *RIa = retention indices on the apolar column (Rtx-1)*
[d] *RIp = retention indices on the polar column (Rtx-Wax)*
[e] *Relative percentages of components based on GC peak areas on the apolar column (Rtx-1)*

**Table 2. Total phenol and flavonoid contents of solvent extracts from *F. vulgare***

| | Solvent extracts | Total polyphenol content (µg CA/mg extract) | Total flavonoid content (µg quercetin/mg extract) |
|---|---|---|---|
| *Foeniculum vulgare* | Diethyl ether | 70 ± 4.2 | 14 ± 2.1 |
| | Ethyle acetate | 169 ± 5.0 | 23 ± 3.1 |

### 3.3. Antioxidant properties

Free radical-scavenging capacity of the essential oil and solvent extracts were measured by DPPH method (**Table 3**). The antiradical activity of essential oil was weak (IC50: 900 µg/ml) in comparison with ascorbic acid (IC50: 0.97 µg/ml). Conversely, both extracts of *F. vulgare* exhibited potential antioxidant activity; the ethyl acetate and the diethyl ether extract scavenged 50% DPPH free radical at the concentration of 1.5 and 6.2 µg/ml. Thus, the DPPH scavenging effect increased in the order of essential oil < diethyl ether extract < ethyl acetate extract < ascorbic acid.

**Table 3. DPPH radical-scavenging of essential oils and solvent extracts (diethyl ether and ethyl acetate) from *F. vulgare***

| Sample | Antioxidant activities | | | | | | |
|---|---|---|---|---|---|---|---|
| Essential oil | Essential oil concentration (µg/ml) | 150 | 300 | 600 | 900 | 1200 | |
| | Scavenging effect on DPPH (%) | 16±2.4 | 30±0.7 | 43±3.6 | 50±2.1 | 57±1.8 | |
| | DPPH $IC_{50}$ (µg/ml) | | | | | | 900 |
| Diethyl ether | Extract concentration (µg/ml) | 1.0 | 1.5 | 2.5 | 3.5 | 10 | |
| | Scavenging effect on DPPH (%) | 6±2 | 14±1.7 | 25±3.5 | 35±4.3 | 72±5.3 | |
| | DPPH $IC_{50}$ (µg/ml) | | | | | | 6.2 |
| Ethyl acetate | Extract concentration (µg/ml) | 0.7 | 1.0 | 1.5 | 2.0 | 4.0 | |
| | Scavenging effect on DPPH (%) | 33±3.4 | 40±3.6 | 50±2.8 | 59±1.2 | 83±4.9 | |
| | DPPH $IC_{50}$ (µg/ml) | | | | | | 1.5 |
| Ascorbic acid | Concentrations (µg/ml) | 0.2 | 0.35 | 0.5 | 1.0 | 2.0 | |
| | Scavenging effect on DPPH (%) | 21±0.7 | 26±0.4 | 34±2.5 | 54±3.5 | 82±4.1 | |
| | DPPH $IC_{50}$ (µg/ml) | | | | | | 0,97 |

*Values expressed are means of three parallel measurements*

As shown in Table 3, free radical scavenging activity also increased with increasing concentration of solvent extracts. Indeed, the results showed that the ethyl acetate and diethyl ether extracts exhibited the strongest activities (83% and 72 %, respectively) at a concentration of 4 µg/ml and 10 µg/ml, respectively. At these high concentrations, the antioxidant activity of extracts was close to the scavenging effect of ascorbic acid (82% at 2.0 µg/ml).

It could be inferred from our results that there was a positive correlation between polyphenolic content and antioxidant activity. Indeed, the greater antioxidant activity of the ethyl acetate extract in comparison with diethyl ether extract could be attributed to the higher content of phenolic and flavonoids compounds ((**Table 2**). These results may be due to hydroxyl groups existing in the chemical structure of phenolic compounds from *F. vulgare* extracts that can provide the necessary component as a radical scavenger [40-42].

## CONCLUSION

From these results, it appeared that the ethyl acetate extracts of aerial parts of *F. vulgare* exhibited high antioxidant properties according to the amount of total polyphenolic compounds. Thus, *F. vulgare* extract can be used as easily accessible source of natural antioxidants for food applications as food supplement or preservative additives.

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