INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 4, No. 2, p. 148-153, 2014

RESEARCH PAPER OPEN ACCESS

Investigation of antioxidant and antifungal activity of fennel essence

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Key words: Essential oil, antioxidant, fennel, antifungal.

http://dx.doi.org/10.12692/ijb/4.2.148-153 Article published on January 18, 2014

Abstract

Essentiol oils and herbal extracts from medicinal plants have attracted a great deal of interesting applications in fresh and processed food preservation, pharmaceuticals, alternative medicine and natural-based therapies. In the present study, phytochemical composition, antioxidant and antifungal properties of fennel essential oil have been evaluated.Antifungal properties were evaluated by paper diffusion method. Antioxidant activity was evaluated through DPPH and Frap assay. The chemical analysis of the essential oil by Gas chromatography/ mass spectroohotometer (GC/MS) showed that Anisole,p-allyl (31/34%) was the major compound. Minimum inhibitory concentration of the essential oil were 2/8 mg/ml. This essential oil was able to reduce the stable free radical DPPH with an IC50 of 186/68 mg/ml and reduction Fe3+ (FRAP) of 400/528 µmol Fe II/ L. These results indicate that this essential oil has a high potential of antioxidant and antifungal properties . Therefore, it can be suggested to combine this essential oil with other agents for the preservation of foods against pathogenic and Toxigenic microorganisms.

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Introduction

Today, a wide range of additives with different objectives in various food preparations are used.

One of the most important food additives, chemical preservatives are antimicrobial, which played a decisive role in safety, longer shelf life food and reduce waste. The other concerns arising from the use of synthetic compounds in terms of health and safety issues, as well as restrictions on permitted use, food consumers is encouraged to use natural products in food (Teixeira *et al.,* 2004).

Essential oils and extracts from medicinal plants with antimicrobial compounds, anticancer and antioxidant (free radical-induced removal factor) as a new drug compounds are important (Bozin *et al.,* 2006; Hussain *et al.,* 2008).

Antimicrobial activity of essential oils actually is assigned to Terpenoids small group and phenolic compounds (thymol, carvacrol, eugenol). This essential oil is among substances permitted for use in food are safe, their use usually makes sense restrictions.Therefore, to determine the lowest concentration inhibiting growth of pathogenic bacteria that no affect the sensory quality of food is necessary. MIC is the lowest concentration of antimicrobial agent that has inhibitory effects on the growth of a particular microorganism, This means that the microorganism is present but cannot reproduce.

Reduce the number of microorganisms in the circumstances, but not essential because lethal effects caused by microorganisms reach the dying phase And the other does not multiply the number decreases. It should be noted that the use of plant essential oils in food storage does not create slightest problem in terms of health for the consumer (Aussalah *et al.,* 2007; Siger *et al.,* 2007). Many researchers antioxidant activity of essential oils evaluated by DPPH test and the results have been obtained. For example, essential oil Tulbaghia violacea showed the antioxidant properties of weak and essential oils of Eucalyptus grandis showed the antioxidant properties of strong (Molar, 2001; Thirupathi *et al.,* 2011). Kolisic *et al.* (2004) Antioxidant activity of oregano and its constituents were examined by DPPH test. Antioxidant activity of essential oils Less than alpha-tocopherol and ascorbic acid.Investigation showed the highest antioxidant activity of oxygenated compounds, Phenolic compounds including thymol and carvacrol and then are full oil and hydrocarbons. One of the valuable medicinal plants is fennel with scientific name of Foeniculum vuglar .Mill. It is of the family Apiaceae, herbaceous and perennial. Its variety of Vulgare (Bitter fennel oil) is mainly planted in Russia, Hungary, Germany, France, Italy, India, Japan, Argentina, and America and its variety of dulce (Sweet fennel oil) is planted in France, Italy and South Europe. Essence of fennel is formed from more than thirty terpene or terpenoid compounds. The most important of these compounds are *anthol*, *phencole*, *estragole*, and *methyl kavicole* (which are used as flavor-giving, antimicrobial, antioxidant ingredients (Salehi Surmaq, 2009).

Given the importance of medicinal plants, especially native plants in our study, we applied the important characteristics and components of fennel essential oil (anti-fungal and anti-oxidant) to examine, Thereby allowing the use of a readily available and affordable source provided, Prevent product loss and damage and ultimately lead to improved health and safety of the food is removed.

Materials and methods

Providing and preparing the samples

The seed of the sweet fennel plant was provided from rural cooperative Association for Women of Khorashad, Birjand. Its preparation, cleaning and grinding, was performed. Reagents DPPH, TPTZ, Methanol, Hexane, Chloridric acid, Acetic Acid, Three-water sodium acetate, and six-water ferric chloride with analytical degree were purchased from Sigma and Marak companies.

Essence making

To chemical analysis, evaluation, antifungal and antioxidant activity of fennel seeds, essential oil was

prepared. oil produced by steam distillation method was performed (Salehi Surmaq, 2009; Samsamshariat, 1991).

One hundred grams of crushed fennel seeds with electric grinder, and taking into machine oil spills, and after adding seven hundred ml of distilled water, turn on the device to be connected to two hours during distillation. After making essential oils, essential oil glass was refrigerated until use.

Analysis of constituents of essential oils using gas chromatography mass

Identification of essential oil compounds using a gas chromatograph GC-MASS (Model QP2010 PLUS), equipped with a column length of 30 m and a diameter of 0.25 mm and 0.25 mm thick layer of stationary phase, the ionization energy of 71 eV and an injection chamber temperature of 250 ° C. was performed. Identification of essential oil compounds using retention index and mass spectrum of each of the essential components and their comparison with reference spectra were carried out.

Evaluation of antifungal activity of essential oils

Bacterial strains of Aspergillus niger (classified as Aspergillus brasiliensis) PTCC 5011 (ATCC 16404) from the regional center of fungi and bacteria spawned the industry of Iran was prepared. This strain is the main cause of fungal infection in baked goods, it is necessary to control its growth in bakery products.Determination of minimum inhibitory concentrations of fennel seed essential oil was performed using disk diffusion method (Clin Pathol, 1975).

Evaluation of antioxidant activity

Measurement of free radical free radical scavenging activity (DPPH)[1] by fennel essence. A solution of 0.006% DPPH free radical in methanol was prepared. Then, 1ml was transferred to the test tubes containing 1 ml of methanol solution of fennel essence at different concentrations depending on its inhibitory potency. The tubes, then, vortexes for 1 h, kept in a dark place, and then their absorption at 512 nm wavelength was read against the control (the absorption of control must be about 0.700). The percentage of free radical scavenging was calculated using equation 1 (Siger, 2007).

$$\%A = \frac{A_C - A_S}{A_C} \times 100$$

Equation (1)

Where, A% is the percentage of DPPH free radical inhibition, A_C is the absorption of control and A_S is the absorption of sample. The diagram of the percentage of free radical inhibition against antioxidant concentration was drawn, the proper cure was fitted and then the concentration at which the antioxidant was able to inhibit 50 percent of free radicals was calculated as IC50[2].

Measurement of Ferric reducing activity of plasma (FRAP) by fennel essence

All of the solutions were prepared following the method of Benzi and Strin (1996).A solution containing 100 mg of sample in 10 ml of hexane was prepared of which 30 µl was mixed with 900 µl of FRAP solution and 90 µl of distilled water in a test tube. The tube was vortexed, placed under Ban mary conditions until reached 37°C, then its absorption was read at 595 nm wavelength against control. Fe II content was calculated using equation 2 (Benzie and Strain, 1996).

(2) Y = 1782x – 9.211

Where,

Y= µmol Fe II/ L

X= the read absorption at 595 nm wavelength

Results and discussion

Fennel essential oil constituents

Results of this study showed that the essential oil of anise essential oil, making 134/8 percent based on the dry weight of the sample.The major constituents of fennel essential oil constituents with time and percent inhibition for each compound are shown in Table 1.

[1] *2,2-Diphenyl-1-picrylhydrazyl*

[2] *Inhibitory concentration with 50%*

Table 1. Major components of anise essential oil by gas chromatography and mass.

Retention time (min)	relative amounts of the essential components (percent)	Combination	row
6/34	3/74%	D-limonene	1
7/1	7/5%	D- Fenchone	2
10/74	31/34%	Anisole, P alyl	3
11/7	26/48%	Trans-anethole	4

Most of the essential oil of fennel seed Anisole, P ALyl (31/34 percent), trans-anethole (26/48 percent), D-Fenchone (7/5 per cent), D-limonene (3/74 percent) of the account. The results of the present study was to investigate the chemical composition of the essential oil of fennel is somewhat consistent with other studies conducted in this regard. In most of these studies, compounds such as anethole, Fenchone, estragole and Methyl chavicol main component of anise oil and limonene in the present study, Fenchone, Anisole and Anatole major compounds in the essential oils (Table 1).

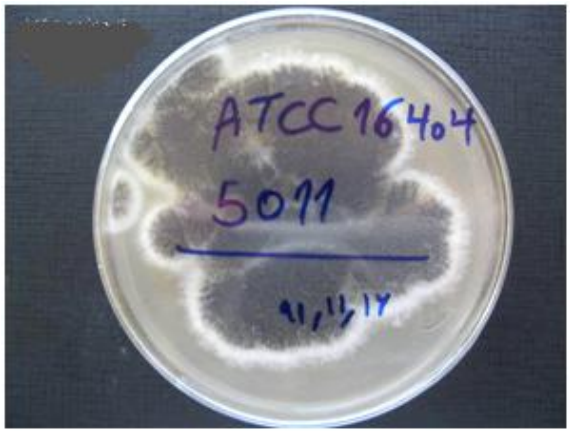

Fig. 1. Initial fungi to control the purity after 48 h at 27° C.

Antifungal activity of the essential oil of fennel

Antifungal effect of fennel essential oil was evaluated using minimum inhibitory concentration values were determined in the range 2/8 mg/ml. Figure 1 Initial fungi to control the purity after 48 h at 27 ° C is shown. Figure 2 Effect of yeast extract and potassium sorbate on growth inhibition after 24 and 48 h at 27 ° C is shown.

Fig. 2. Effect of yeast extract and potassium sorbate on growth inhibition after 24 and 48 h at 27° C.

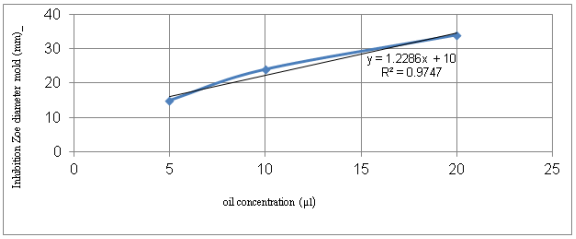

Fig. 3. The effect of different concentrations of essential oils to prevent the growth of mold *Aspergillus niger*.

As can be seen in the form of fennel essential oil antifungal properties stronger than potassium sorbate as a preservative in the food industry used to be there. So we can conclude that this plant can be used in food industry as a preservative (especially in baking products) is capable of. Figure 3 Effect of different concentrations of essential oils to prevent the growth of mold, Aspergillus niger is shown. According to the contract, the diameter of mold growth in different concentrations of essential oil is greater than 12 mm, this means fennel essential oil on mold growth is inhibitory. The regression equation obtained from the correlation coefficient is high.

Antioxidant activity of the essential oil of fennel

Ability to inhibit free radicals and iron reception ш order by DPPH test and evaluation Frap. In this study DPPH free radical scavenging fennel ninth mg ml of the FRAP rate, 528/400 micromoles per liter of iron was observed. In this study fennel DPPH radical scavenging 1/9 mg ml of the FRAP rate, 400/528 micromoles per liter of iron was observed. Many researchers antioxidant activity of essential oils evaluated by DPPH test and the different results are obtained. Investigation showed the highest antioxidant activity of oxygenated compounds, phenolic compounds, including thymol and carvacrol and then extract the hydrocarbons is complete. Souri *et al.* (2004) The amount of DPPH caraway essential oils to 5/76 micrograms per milliliter was the case in our study the rate of 1/9 microg ml (IC50 = 186/68) which demonstrates superior antioxidant capacity this study compared the antioxidant essence is Souri.

Conclusions

Due to the use of native plants for food and medicinal fennel and time away from our introduction to the practical application of this study could be due to the chemical composition of the essential oil of anise, anti-bacterial properties and is suitable antioxidant capacity, Thereby the advantage of providing a source of readily available and affordable, as well as product loss and damage is prevented. Finally, steps should be taken to promote public health and food safety.

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