International J. Seed Spices 8(1), January 2018:60-64

Analysis of fennel (*Foeniculum vulgare*) essential oil extracted from green leaves, seeds and dry straw

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Abstract

Seed spices, an important group of horticultural crops, are defined as vegetable products or mixture thereof, free from extraneous matter, primarily used for flavoring, seasoning and imparting aroma in foods. Value added form of spices has become the thrust area with tremendous potential. The global market is increasingly shifting from commodity to value added products. India exports raw seed spices as well as their value added items to around 90 countries. Main source of essential oil from seed spices are mature seeds of particular spice. In most of the seed spices after harvesting of economic product i.e. seeds considerable crop residues remain unutilized which contains significant amount of essential oil and need to be extracted for substantiating the farm income. Present analysis suggested that essential oil obtained from dry straw with no additional presence of undesirable compound make this suitable for blending with seed essential oil or to use alone. Thus seed spices residues may be utilized for substantiating the farm income.

Key words : Crop residues, essential oil, oil quality, seed spices, steam distillation.

Introduction

Fennel (*Foeniculum vulgare* Mill) belongs to the family *Apiaceae,* is an annual, biennial, and perennial herbaceous plant. The economic and useful plant part is seed. However, fennel leaves and stalks also possess fragrance and sweetness hence, can be eaten as a vegetable. Seed essential oil content varies as per time of harvest and climate. Fennel seeds are however the main source of fennel essential oil but study of a plant as a source of flavoring compounds requires the analysis of seeds as well as other parts of the plant. Accordingly, some authors have reported comparison between fruit oil and herb oil from sweet fennel (Embong *et al.*, 1977); components of oil produced from stems, leaves, flowers and the whole plant (Baldrich *et al.*, 1986); the volatile components of leaves, stems and fruits (Miura *et al.*, 1986) and the chemical composition of the essential oils obtained from various parts of the bitter Turkish fennel plant (Akgül & Bayrak, 1988). Essential oil and its constituents in available germplasm of fennel studies by Saxena *et al.,* (2016) reported 1.0 to 3.3% with maximum lines showing 2-3%. They detected eleven major compounds including α-pinene, camphene, β-pinene, myrcene, cymene, γ-terpinene, 4-allyl anisole/methyl chavicol or estragol, anethole, geranyl acetate and P-anisaldehyde in fennel essential oil. Due to its pleasant fragrance the essential oil is being used in food products such as bread, cheese, pickles, confectionary items and in beverages (Zoubiri *et al.*, 2014). It is also an ingredient of cosmetics and pharmaceutical products (Telci *et al.*, 2009). Fennel infusions are used as decoction for nursing babies to prevent flatulence and colic spasms (Perry *et al.*, 2011; Bruyas-Bertholon *et al.*, 2012). Apart from this fennel seeds are being used as antispasmodic, diuretic, hepatoprotective, anti-inflammatory, analgesic, secretomotor, secretolytic, galactagogue, eye lotion, and antioxidant remedy in Europe and Mediterranean areas (Agarwal 2017; Agarwal *et al.*, 2018; Lucinewton *et al.*, 2005). It is also used in the treatment of kidney stones, menopausal problems, nausea and obesity (Zahid *et al.*, 2009; Ehsanipour *et al.*, 2012).

Fennel plant grows up to two metres in height with considerable green biomass. Seed bearing umbles are harvested periodically as it mature and still green in colour. When crop is grown for seed purpose complete plant harvested when seeds turn yellow or light brown with 20% moisture. Seeds are separated by traditional means and somewhere using mechanical thresher. In any case in the process of manual and mechanical threshing a large amount of crop biomass with some broken seeds are left as residues. These residues contain some amount of essential oil. Present study was conducted with a view to use these crop residues for extracting essential oil and compare the quality of obtained essential oil with that of seeds and green herbs essential oil.

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Material and methods

Plant material

Fennel (*Foeniculum vulgare* Mill) variety AF-1 grown at ICAR-NRCSS farm was used for taking different plant parts. Mature green leaves were taken for essential oil extraction when plants attain full vegetative growth. Seeds were taken for essential oil extraction after harvesting of crop at its full maturity. Fennel straw with or without broken seeds were used for extraction of essential oil.

Extraction of essential oil from seeds

Thirty gram cleaned fennel seeds were used for essential oil extraction by hydro-distillation using a Clevenger apparatus (Clevenger 1928) for 3 h. After decanting and drying of the oil over anhydrous sodium sulphate the corresponding mild yellowish coloured oil were recovered and calculated in terms of percentage (V/W).

Extraction of essential oil from green herbs and crop residues

Steam distillation unit consisting of a boiling chamber with electric heaters connected with biomass containing chamber, condenser and receiver, is used for "dry steam" distillation of green herbs and crop residues. Biomass holding chamber of distillation unit is filled with thirty kilogram each of green herbs and dry crop residues. Steam is produced in the boiling by heating water. This steam travels into the biomass chamber where essential oils and water-soluble plant compounds are removed into the vapor stream. The vapor stream travels through the still head, condenses in the water-cooled condenser, and collects in the receiver, where the essential oil layer phase separates and collected in clean bottles.

Gas Chomatography-Mass Spectroscopy based analysis of oil constituents

One microlitres of essential oil extracted from fennel plant parts was injected to a HP 5 MS column (Agilent, USA, 30m×0.250mm film thickness 0.25 μm) using auto sampler (Agilent, 7693). The analysis was carried out under the following conditions: oven temperature was programmed at 50°C for 3 min followed by raising at 10°C/min to 180°C and 45 C/min to 280°C, injection port temperature was kept 250°C; detector temperature 250ºC; carrier gas: helium; flow rate 1 ml/min, split ratio was 10:1. Authentic standards of major constituents of fennel essential oil were procured from Sigma- Aldrich (USA). These standards were run alone and in combination to get retention time of each constituent. Retention indices of all the constituents were determined by chemstation software (Agilent technologies, USA). The volatile constituents were identified by a comparison of their retention indices and their identification was confirmed by computer matching of their mass spectral fragmentation patterns of compounds in the NIST-MS library and published mass spectra.

Results and discussion

Seed essential oil constituents

Table 1. showed essential oil constitments of fennel seeds, green herbs, fennel straw with broken seeds and fennel straw. A total of ten major constituents were detected in essential oil obtained from different plant parts and crop residues. This includes α-pinene, camphene, β-pinene, myrcene, cymene, γ-terpinene, 4-allyl anisole/ methyl chavicol, gerniol, anethole + estragol and geranyl acetate. The seeds of fennel contains 2% essential oil in which 4-allyl-anisole (53.69%) and anethole (44.30%) were the major constituents (Fig. 1). Similar composition of seed essential oil has been reported in our earlier publication (Saxena *et al.*, 2016). A group of terpenoids consisting of α- pinene, camphene, β-pinene, myrcene, cymene and γ-terpinene accounted for small portion of fennel seed essential oil. It is well documented that genetic constitution and environmental conditions influence the yield and composition of volatile oil produced by medicinal plants (Ramezani *et al.*, 2009; Omidbaigi 2007). Unlike other yield attributing characters that are quantitatively inherited and highly affected by environment, essential oil composition depends upon internal and external factors affecting the plant such as genetic structures and ecological conditions (Dubey *et al.*, 2017). Agricultural practices also have critical effects on yield and oil composition in the essential oil bearing plants (Telci *et al.*, 2009).

Fennel herb has sweet taste and a much stronger aniseed flavour due to presence of anethole more as compared to 4-allyl-anisole present in seeds. The leaves taste wonderful in salads, slaws, flavored butters, and dressings. The yellow flowers that appear late in the season are also edible and add beauty and flavor as toppings for soups and salads. They don't hold up well to long cooking, but are best used fresh. Though, fennel green herb contains a little 0.01% essential oil its major constituent is anethole (75.28%) and 4-allyl-anisole (15.8%) (Fig. 2). Significant amount of gamma terpinene (6.17%) is also present in green leaves. Stefanini *et al.*, (2006) while analyzing essential oil constituents from different organs of fennel (*Foeniculum vulgare*) found 46.11% trans anethole and 41.34% limonine as major constituents in fennel leaves and stem together. They also recovered fenchyl acetate in significant proportion (4.25%).

Table 1. Essential oil constituents of essential oil extracted from different plant parts of fennel.

Constituents	RT	Mature seeds	Dry straw	Green leaves
Essential oil percentage		2.0	0.22	0.01
α-pinene	5.27	0.17	0.03	0.10
Camphene	5.50	0.03	0	0
beta pinene	5.94	0.02	0.02	0.04
Myrecene	6.09	0.09	0.02	0.1
Cymene+cymol	6.64	0.01	0.26	0.51
gamma terpinene	6.72	1.62	0.66	6.17
4-allyl-anisole	9.30	53.69	16.86	15.80
Gerniol	9.95	0.05	0.008	1.93
Anethole+estragol	10.56	44.30	82.12	75.28
Geranyl acetate	11.84	0.007	0.01	0.081

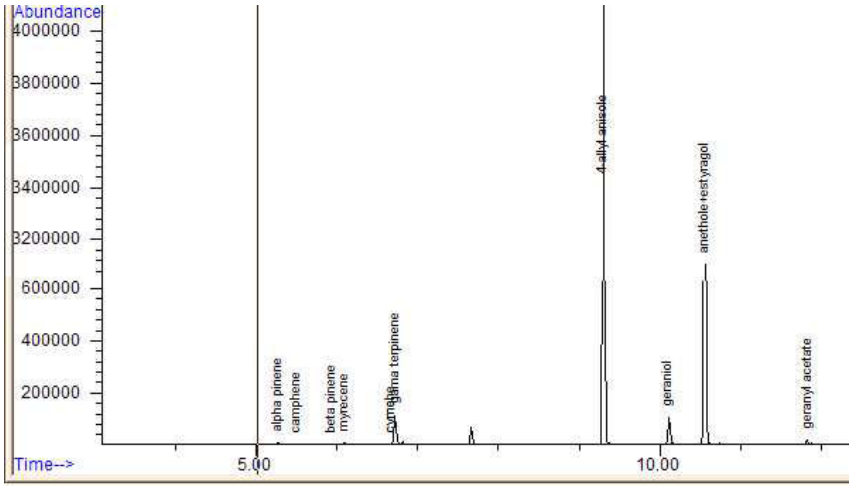

Fig 1: Chromatogram of fennel seeds essential oil

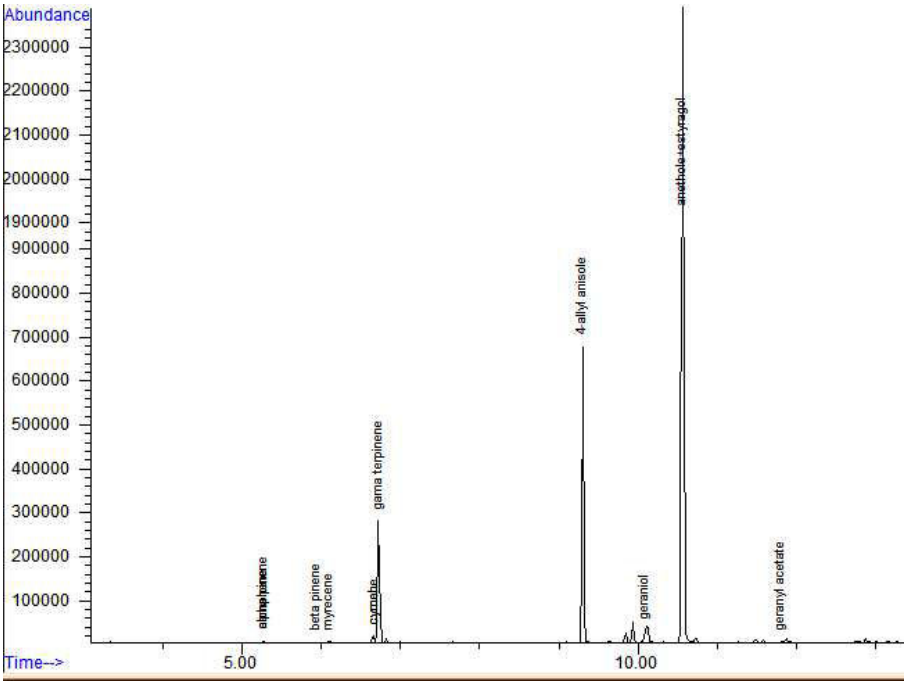

Fig. 2. Chromatogram of essential oil extracted from green leaves

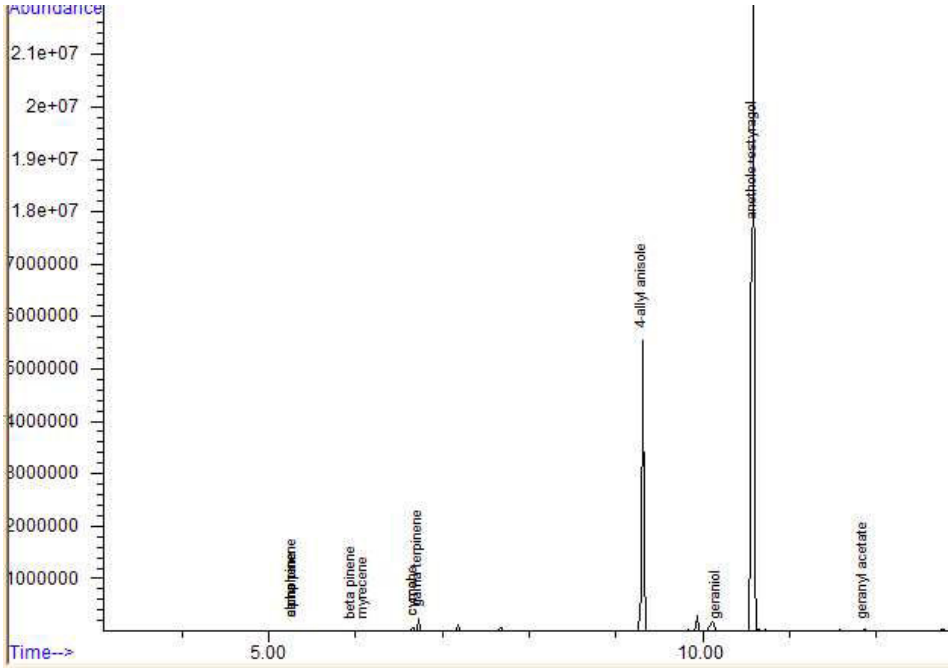

Fig. 3. Chromatogram of essential oil extracted from dry fennel straw

Dry fennel straw constitutes significant biomass after threshing of seeds. This straw gives a typical fennel flavour indicated presence of some flavouring compounds in it. This essential oil has been extracted using steam distillation technology and constitutes 0.22% by weight. Further, GC-MS analysis of extracted essential oil showed 82.12% anethole+estragol and 16.86% 4-allyl-anisole as major constituents. Gamma terpinene was found to reduce from 6.17% in green herb to 0.66% in dry fennel straw (Fig. 3).

Present analysis support the utilization of crop waste for meaningful purposes. Essential oil obtained from dry straw with no additional presence of undesirable compound make this also suitable for blending with seed essential oil or to use alone. Fennel essential oil is known to be a potent and powerful aid for digestive health. Its strong licorice flavor and aroma is gained from the steam distillation from the crushed seeds of the plant. In addition to digestive support, it's also popular with hormonal balancing. After extraction of oil the straw can be used to make compost or FYM. Dry fennel leaves can also be used as salad and sauces seasoning.

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Received : October 2017; Revised : November 2017; Accepted : December 2017.