

The effect of different extraction methods (steam distillation and supercritical fluid extraction) on essential oil production of aromatic tagetes and sweet basil.

Abstract

Tagetes minuta L. (Aromatic Tagetes) is an erect and stout summer annual herb. *Ocimum basilicum* L. (Sweet Basil) is considered a summer aromatic plant. There are several methods for extraction of essential oils i.e. (HD) hydro-distillation, (SD) steam distillation, (SFE) supercritical fluid extraction, and organic solvents. It is now considered the method of extracting essential oil with steam distillation is the commercial method used in many countries that produce essential oils. SFE method consider is the latest extracting essential oils method but the method spread is limited due to the high cost of its establishment. The present study is aimed to compare the two methods SD and SFE to extract the essential oil from sweet basil and aromatic tagetes plants, and its yield on the resulting essential oil yield. SFE method increases oil yield and improves oil quality. SFE method gave a high percentage of the following major's components for sweet basil oil (linalool (50.22%) and E- Citral (10.44%)) and aromatic tagetes oil (β -Ocimene (27.49%) and cis-Tagetone (9.93%)) compared to the steam distillation method.

Key words: *Tagetes minuta* L., *Ocimum basilicum* L., SD, SFE, GC-MS, Essential oil.

Introduction

Natural items, particularly volatile oils that are fragrant fluids isolated from grown aromatic plants, the most of crude materials for flavor, scents and pharmaceuticals. Flavoring and fragrance fixings are the foremost various single bunch of universal added substances utilized by the nourishment and cosmetic care businesses. (Karale, *et. al.*, 2011).

Volatile oils are composite blends of unstable compounds most regularly display at low concentrations in aromatic plants. A few diverse extraction commercial methods are broadly utilized for the extraction of essential oils such as steam distillation and solvent extraction method (Alexandros and koidis, 2016). These methods are characterized by downsides such as low extraction proficiency and selectivity, utilize of expansive sums of solvents, and long extraction times. In numerous cases, the quality of the essential oils gotten by among the previously mentioned methods (steam distillation and solvents), can be affected by hydrolyzation or oxidation than can take put due to long extraction time (more than 3h) and extracting temperature is high, energy consumption is big, and/or Consuming a large amount of water (Ronald Watson, 2018). Due to these confinements, effective methods for the extraction of volatile oils have been created. which can ordinarily overcome these issues. Considered SFE

(Supercritical fluid extraction) is novel method that are presently recognized as effective extraction method and can altogether diminish extraction times, improve of yields, and volatile oils quality. Although these method are predominantly exploited on the laboratory scale, despite this, the spread of this method has been limited on the commercial scale due to high expensive of its components.

Supercritical Fluid Extraction (SFE) method started with the beginning of the eighties and is a very expensive method with very complicated tools and equipment. The carbon dioxide is pressurized to a semi-liquid and half-gas state, and the aromatic molecules of the plant are extracted, and the pressure is changed to evaporate the gas, and the rest is the essential oil. Essential oils extracted by this method become “CO₂ essential oils. SFE is an original, clean and naturally attractive innovation with a particular interest in extracting volatile oils from aromatic plants and herbs. Supercritical CO₂ is specific method, there is no waste treatment associated with toxic soluble substances, and the extraction time is simple. Assisted supercritical fluids extraction are often considered to be of superior quality compared to those supplied by steam distillation, liquid solid extraction, or steam distillation. SFE method is characterized by the extraction of essential oil without waxes and fats that are usually present with the essential oil extracted by steam distillation or solvent method (Miguel, *et. al.*, 2017). On other hand from the perspective of industrial production of essential oil, supercritical CO₂ extraction is an efficient extraction method. Compared with other methods, CO₂ is low cost, easy to obtain, non-toxic and tasteless, and the extracted essential oil does not contain impurities. Supercritical CO₂ fluid has a strong attraction and a broad view of the application of environmentally friendly and highly safe essential oils to industrial production (Hua, *et.al.* 2021).

The steam distillation method is the simplest and the cost is low. Currently, 85% of the essential oils on the market are extracted by distillation. The method is to put dried aromatic plant raw materials for into a rectifier, and send steam from below to evaporate the essential oil of the plant. The water vapor containing the essential oil is collected and cooled by the conduit, and then condensed into a liquid, which is separated by the difference in specific gravity and density of the water and the essential oil. Distillation is often used to extract plants that are flowers, leaves, stems, and roots. During the cooling process, the circulating water can be used multiple times. In the remaining water, more or less some essential oils are dissolved inside. This is called soy water, which is pure dew (Erich, 1982). On the other hand, the steam distillation method is considered the most common at the present time, despite the high quality of the oil produced by the SFE method.

Aromatic Tagetes (*Tagetes minuta* L.); family, Asteraceae, is an erect and stout summer annual herb, widely cultivated as an ornamental aromatic plant. This plant's leaves are slightly glossy, green and pinnately dissected into 4–6 pairs of pinnae. In folk medicine, the crude plant preparations are used to have been used in

popular as anthelmintic, diuretic, antispasmodic and to treat stomach and intestinal diseases. (Chamorro, *et. al.*, 2008). The previous studies showed that the major components in *Aromatic tagetes minuta* oil are (Z)- β -ocimene, α -terpineol, (Z)-tagetone, dihydrotagetone, (E)-ocimenone and (Z)-ocimenone. (Moghaddam *et. al.*, 2007).

Sweet Basil (*Ocimum basilicum L.*) which is a summer annual; family, Lamiaceae. This family includes the most important medicinal aromatic plants. Basil is known with its highly aromatic leaves utilized either fresh or dried for culinary. In full blooming, the leaves and flowering tops of the plant are perceived as antispasmodic, carminative, galactagogue, stomachic and galactagogue in traditional medicine (Sajjadi, 2006). There are many major components of basil such as Linalool, Borneol, Terpinol, Cineole, Geraniol... etc., (Musa and Chalchat, 2002).

The study aims to compare the two methods Steam Distillation (SD) and Supercritical Fluid Extraction (SFE) to extract the essential oil from Sweet Basil and Aromatic Tagetes plants.

Material and Methods

Plant Material

Sweet Basil (*Ocimum basilicum L.*) and Aromatic Tagetes (*Aromatic tagetes minuta L.*) were collected from the Experimental Farm (Alqanater Alkhiria) of Medicinal and Aromatic Plants Res. Dept., ARC, Egypt, during the full blooming stage in June 2020 for basil (First cut) and August 2020 for aromatic tagetes plants.

Supercritical Fluid Extraction (SFE)

Use a Laboratory Unit a Single Pump Extraction ISCO SYSTEM (SFX 220). Leaves and flower tops of basil and aromatic tagetes plants were washed with water to remove any impurities and then dried at 45°C for 24 h in order to remove water content. Using screen analysis, the average particle size of 14 mm was obtained. Approximately 40 g of dried basil and aromatic tagetes were introduced into the 30 cm³ stainless steel fixed bed cell of the extraction vessel. The solvent used was 99.9% carbon dioxide (Air Products) with a flow rate of 1.38×10⁻⁴ kg s⁻¹ through the extraction vessel. The setup was initially purged with CO₂ for 2 min to remove any moisture or impurities. Then, the extraction cell was mounted on the apparatus where the extraction cell was submerged inside the water bath. The water bath was adjusted at the required operating temperature using an electrical heater. The extractor, and a temperature controlled metering valve used to perform the supercritical extraction. The flow rate of CO₂ is maintained at specific value for specific period of time. The range of temperature that tested is

between 40 and up to 60 °C, and the range of pressure is 100 and up to 300 bar. 3 gm sample of the ground herb has been taken each time (Cassel *et. al.*, 2010).

Steam Distillation (SD)

Used a laboratory unit for steam distillation (Apparatus, NC-13223). Steam distillation was carried out by passing steam from 1 liter boiling flask into a 2-liter round-bottomed flask containing 200 gm of the dried plant material (Leaves and flower tops of basil or aromatic tagetes plants) for 90 min and collecting the condensate (water and oil) in a erlenmeyer flask (500 ml). The condensate product (essential oil and water) is placed in a separating funnel and with the density difference between them, the essential oil is separated. Finally, sodium sulfate was added to the essential oil to remove moisture (Erich, 1982).

Physical and Chemical Properties Records:

- 1- Essential oil percentage according to British Pharmacopoeia (1980).
- 2- Refractive index and density.
- 3- Chemical composition of Essential oil by GC-MS.

Gas Chromatography-Mass Spectrometry Analysis (GC-MS)

The analysis of the *Ocimum basilicum L.* and *Tagetes minuta* essential oils extract was carried out by GC-MS system (Agilent Technologies) was equipped with gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, and Cairo, Egypt. Samples were diluted with hexane (1:19, v/v). The GC was equipped with HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 μ m film thickness). Analyses were carried out using helium as the carrier gas at a flow rate of 1.0 ml/min at a split ratio of 1:10, injection volume of 1 μ l and the following temperature program: 40 °C for 1 min; rising at 4 °C /min to 150 °C and held for 6 min; rising at 4 °C/min to 210 °C and held for 1 min. The injector and detector were held at 280 °C and 220 °C, respectively. Mass spectra were obtained by electron ionization (EI) at 70 eV; using a spectral range of m/z 50-550 and solvent delay 5 min. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

Measurement of the Refractive Index and Density

Essential oils were subjected to tests of index of refraction and density according to British Pharmacopoeia (1980) using an Abbe refractometer (Atago, Tokyo, Japan), while density was determined by using density bottle (Blaubrand, Germany).

Statistical Design and Analysis

A complete randomized design (CRD) was used according to Duncan. Means of the two methods were compared by L.S.D (Senedecor and Cochran, 1974).

Results and Discussion

The results in Table 1 and 2 indicate that the Supercritical Fluid Extraction (SFE) method gave a higher percentage of sweet basil oil (0.192%) as well as aromatic tagetes oil (0.387 %) compared to the oil extraction method by steam distillation. While the results showed that both extraction methods (Steam distillation (SD) or Supercritical Fluid Extraction (SFE)) have no significant differences on the physical properties (refractive index, and density) for both sweet basil and aromatic tagetes oils.

The results in Tables 3 and 4 showed that the percentage of some major components of both basil and aromatic tagetes essential oils were higher in the SFE method compared to the steam distillation method. It was found in Table 3 that the Supercritical Fluid Extraction (SFE) method gave a high percentage of the following major's components: linalool (50.22%), Terpinen-4-ol (6.91%) and E-Citral (10.44%) in sweet basil oil compared to Steam Distillation (SD) method. The same trend for the results obtained for sweet basil oil is found in the obtained results shown in Table 4, where the Supercritical Fluid Extraction (SFE) method gave a significant high percentage of some major components of aromatic tagetes essential oil such as β - Pinene (3.42%), β -Ocimene (27.49%), allo-Tagetone (7.41%) cis-Tagetone (9.93%) and Linalool (0.33%) compared to the steam distillation method. These results confirm that the SFE method increases oil yield and improves oil quality. This is reflected in the increase in the economic profits of producers (Miguel, *et.al.*, 2017). Supercritical Fluid Extraction (SFE) method appears to be an excellent method for extracting the essential oils of aromatic tagetes and sweet basil as it results in good yield, good recovery of essential oil constituents, is less labor-intensive, and is simpler and faster than steam distillation (Hua, *et.al.* 2021).

Conclusion

The Supercritical Fluid Extraction (SFE) method is considered one of the modern methods of extracting essential oils. It gives pure essential oils free of waxes and fats, in which the concentration of the main active substances increases, which makes the oil produced with a very high purity and is called absolute oil, which is reflected in the high price. But the Steam Distillation method is still the commercial method prevalent in most of the countries producing essential oils, as it is the least expensive way to set up their factories, opposite of the Supercritical Fluid Extraction (SFE) method, which is still expensive to set up its factories.

Table (1): Essential oil percentage and Physical Properties (Refractive index and Density) of Basil oil obtained from two extraction methods (SFE and SD).

Oil % Physical Properties	SD	SFE
Oil %	0.181 b	0.192 a
Refractive index	1.473 a	1.473 a
Density	0.8650 a	0.8651 a

Means with similar alphabetical letters are not significant different according to least significant difference test at 0.05 level of probability. (SD) Steam Distillation (SFE) Supercritical Fluid Extraction.

Table (2): Essential oil percentage and Physical Properties (Refractive index and Density) of aromatic tagetes oil obtained from two extraction methods (SFE and SD).

Oil % Physical Properties	SD	SFE
Oil %	0.319 b	0.387 a
Refractive index	1.447 a	1.447 a
Density	0.8543 a	0.8545 a

Means with similar alphabetical letters are not significant different according to least significant difference test at 0.05 level of probability. (SD) Steam Distillation (SFE) Supercritical Fluid Extraction.

Table (3): Chemical composition of Sweet basil oil obtained from two extract methods (SFE and SD).

Compounds	RT	SD	SFE
α-phylandrene	7.964	0.24a	0.26a
α- Pinene	8.124	1.18a	1.21a
β - Pinene	9.503	0.50a	0.50a
o - Cymene	11.248	0.56b	0.59a
Eucalyptol	11.431	4.31a	4.29a
cis-Verbenol	13.581	0.46a	0.39b
Linalool	14.155	49.62b	50.22a
Camphor	15.414	0.40a	0.36a
Terpinen-4-ol	16.610	6.86b	6.91a
α-Trepineol	17.232	0.38a	0.41a
Estragole	17.897	0.26a	0.23a
Z- Citral	18.847	7.83a	7.86a
E- Citral	18.860	10.35b	10.44a
Nerol	20.138	0.55b	0.59a
α-Copaene	22.984	0.62a	0.63a
Caryophyllene	24.335	1.65a	1.66a
Cis - α-Bergamtene	24.855	3.65a	3.68a
Humulene	25.370	0.49a	0.45b
Muurolene	27.184	0.55a	0.56a
β- Copaene	27.459	0.18a	0.19a
α-Bisabolene	28.037	1.45a	1.44a
Caryophyllene oxide	29.244	0.84a	0.85a
Cadinol acetate	31.264	0.74a	0.76a
Total identified compounds%	-	93.67	94.48
Total unidentified compounds%	-	6.33	5.52
	0.458		

Means with similar alphabetical letters are not significant different according to least significant difference test at 0.05 level of probability. (RT) Retention time (SD) Steam Distillation (SFE) Supercritical Fluid Extraction.

Table (4): Chemical composition of aromatic tagetes oil obtained from two extraction methods (SFE and SD).

Compounds	RT	Steam Distillation	SFE
α - Pinene	6.850	1.41a	1.40a
Sabinene	6.990	0.28a	0.28a
β - Pinene	7.213	3.38b	3.42a
p - Cymene	7.902	0.53a	0.55a
β -Ocimene	8.248	27.30b	27.49a
allo-Ocimene	8.381	2.31a	2.30a
α -phellandrene	9.176	0.30a	0.29a
Limonene	9.525	3.67a	3.66a
allo-Tagetone	10.419	7.20b	7.41a
cis-Tagetone	11.604	9.81b	9.93a
Carvacrol	12.905	1.41a	1.42a
Borneol	13.822	7.62a	7.65a
Linalool	14.338	0.25b	0.33a
β -Caryophyllene	15.357	1.34a	1.33a
Dihydrocarvone	16.291	3.78a	3.77a
Estragole	17.619	1.13a	1.02b
α -Humulene	18.980	0.17a	0.15a
L-Camphor	20.149	1.90a	1.88a
α -Terpinol	22.058	2.11a	2.10a
Verbenone	25.894	6.73a	6.70a
(-) – Bornyl acetate	27.607	1.56a	1.55a
Limonene oxide	28.991	0.71a	0.70a
Carvone	30.753	4.17a	4.16a
Anethole	31.899	6.67a	6.64a
Caryophyllene oxide	32.966	0.41a	0.40a
β -Cadinene	34.057	0.60a	0.62a
Total identified compounds%	-	96.75	97.15
Total unidentified compounds%	-	3.25	2.85
	0.458		

Means with similar alphabetical letters are not significant different according to least significant difference test at 0.05 level of probability. (RT) Retention time (SD) Steam Distillation (SFE) Supercritical Fluid Extraction.

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