Kastamonu Uni., Orman Fakültesi Dergisi, 2021, 21(2): 165-175 Research Article
Kastamonu Univ., Journal of Forestry Faculty
Doi:10.17475/kastorman.1000463

Chemical Compounds and Biological Activity of Turkish *Santolina chamaecyparissus* L. Essential Oil by Microwave Assisted Distillation

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Received Date: 08.05.2020 Accepted Date: 06.05.2021

Abstract

Aim of study: The essential oil of *S. chamaecyparissus* (SEO) was isolated by microwave assisted distillation system to analyze the antioxidant and antimicrobial activities.

Area of study: S. chamaecyparissus were collected from Osmaniye province of Turkey in April 2018.

Material and method: The chemical constituents and groups (by GC/MS and FTIR spectroscopy, respectively), antioxidant (by DPPH technique) and antimicrobial activities (by agar disc diffusion method) of SEO were investigated.

Main results: Twenty aromatic compounds were determined and artemisia ketone (39.83%), camphor (17.65%), β-phellandrene (8.03%) and β-bisabolene (7.32%) were the most abundant. IC_{50} values which were the indicators of antioxidant behavior of SEO, butylated hydroxytoluene (BHT) and gallic acid recorded as 88.301, 0.212 and 0.021 g/L respectively. The antimicrobial activity results showed that all tested microorganisms (*Escherichia coli*, *Streptococcus mutans*, *Bacillus cereus*, *Bacillus subtilis*, *Candida albicans*) were highly inhibited.

Highlights: SEO could be a good source of monoterpenes especially artemisia ketone. Its herbaceous fragrance may provide a new usage area in men's perfumery.

Keywords: *Santolina chamaecyparissus*, Essential Oil, Antioxidant and Antimicrobial Activity

Mikrodalga Destekli Destilasyon ile Elde Edilen Türk *Santolina chamaecyparissus* L. Uçucu Yağının Kimyasal Bileşikleri ve Biyolojik Aktivitesi

Öz

Çalışmanın amacı: S. chamaecyparissus uçucu yağı (SUY), antioksidan ve antimikrobiyal aktivitelerini belirlemek amacıyla izole edilmiştir.

Çalışma alanı: S. chamaecyparissus Türkiye'nin Osmaniye ilinden, Nisan 2018'de toplanmıştır.

Materyal ve yöntem: SUY'nin kimyasal bileşenleri ve grupları (sırasıyla gaz kromatografisi/kütle spektrometresi (GC/MS) ve Fourier dönüşümlü kızılötesi spektroskopisi (FTIR) yoluyla), antioksidan aktivitesi (DPPH yöntemiyle) ve antimikrobiyal aktivitesi (agar disk difüzyon tekniğiyle) araştırılmıştır.

Temel sonuçlar: Yirmi aromatik bileşen tespit edilmiş olup, artemisia keton (%39.83), kamphor (%17.65), β-phellandren (%8.03) ve β-bisabolen (%7.32) uçucu yağda miktarı en fazla olanlardır. Antioksidan davranışın bir ölçüsü olan IC_{50} değeri yağda, bütil hidroksitoluende (BHT) ve gallik asitte sırasıyla 88.301, 0.212 ve 0.021 g/L olarak hesaplanmıştır. Antimikrobiyal bulguları da, tüm test edilen mikroorganizmaların (*Escherichia coli*, *Streptococcus mutans*, *Bacillus cereus*, *Bacillus subtilis*, *Candida albicans*) SUY'da yüksek oranda inhibe olduğunu göstermiştir.

Araştırma vurguları: SUY, özellikle artemisia keton olmak üzere, monoterpenlerin iyi bir kaynağı olabilir. Yağın otsu kokusu, erkek parfümlerinde bu yağın yeni bir kullanım alanını açığa çıkartabilir.

Anahtar Kelimeler: *Santolina chamaecyparissus*, Esansiyel Yağ, Antioksidan ve Antimikrobiyal Aktivite

Citation (Atıf): Sufer, O., Ceylan, A., Onbasli, D., Celik Yuvali, G., & Bozok, F. (2021). Chemical Compounds and Biological Activity of Turkish *Santolina chamaecyparissus* L. Essential Oil by Microwave Assisted Distillation. *Kastamonu University Journal of Forestry Faculty*, 21 (2), 165-175.

Introduction

Santolina chamaecyparissus which belongs to the family Asteraceae is a strongly aromatic shrub with yellow inflorescences that widely distributed throughout the Mediterranean region (Niu et al., 2019). This species is called as cotton lavender, santolin, lavantin and garden cypress (Derbesy et al., 1989; Brunke et al., 1992; Pons & Caňavate, 2000). Besides *S. chamaecyparissus*, *S. viridis* and *S. pectinata* are the most common in growing areas (Labed et al., 2017).

It is known that plants have been utilizing in folk medicine for many years (Calvo et al., 2013). *S. chamaecyparissus* and its essential oil (SEO) are also used for various purposes, because of their anticandidal (Suresh et al., 1997), antioxidant (López et al., 2008), antimicrobial (Khubeiz & Mansour, 2016) and antifungal (López et al., 2008; Khubeiz & Mansour, 2016; Salah-Fatnassi et al., 2017) properties. The great antibacterial potential of related plant comes from artemisia ketone and dihydroaromadendrene which can be effective against *Listeria innocua* and *Aeromonas hydrophila* (Ruiz-Navajas et al., 2012; Niu et al., 2019). Furthermore, camphor and cubenol contained in *S. chamaecyparissus* were reported as important for decreasing the total amount of *Klebsiella pneumonia* and *Candida albicans* (Djeddi et al., 2012; Niu et al., 2019). Until today, volatile components (Niu et al., 2019), antioxidant capacity, phenolic content (Boudoukha et al., 2019), anti-inflammatory and xanthine oxidase inhibition activities (Djarmouni et al., 2018) and hepatoprotective activity (Messaoudi et al., 2018) of *S. chamaecyparissus* have been studied.

EO components of *S. chamaecyparissus* were investigated by several researchers by GC-MS (Villar et al., 1986; Derbesy et al., 1989; Vernin, 1991; Pérez-Alonso & Velasco-Negueruela, 1992; Demirci et al., 2000; Garg et al., 2001; Salah-Fatnassi et al., 2017, Nikolić & Radulović, 2018). But, most of them have focused the volatile compounds of SEO. However, active components can be impacted by climatic and environmental conditions (Niu et al., 2019) as well as distillation technique. Microwaves enhance the rate of distillation, reduce the processing time and are able to be combined with classical procedures easily. Besides microwave assisted distillation, supercritical fluid extraction method has started to use for recovering the EO of *S. chamaecyparissus* (Grosso et al., 2009). Although there have been studies about the EO of *S. chamaecyparissus*, it is required to develop a rapid and green (without using an organic solvent) distillation method. Up to now, hydrodistillation has been used apart from supercritical fluid extraction and both microwave assisted distillation and green technology for obtaining SEO are still missing. Best of our knowledge, functional groups in aforementioned EO by fourier-transform infrared (FTIR) spectroscopy have not been determined yet. In the present comprehensive study, the analysis of volatile constituents, antioxidant and antimicrobial activities of the EO of Turkish *S. chamaecyparissus* by microwave assisted distillation technique with no solvent were revealed as well as FTIR. Therefore, a good comparison with literature was enabled to observe the effects of differences in localities of samples and unit operations applied to obtain EO.

Material and Methods

Plant Sampling and EO Isolation

Fresh aerial and well-developed parts of *S. chamaecyparissus* were collected by cutting with scissors manually from Osmaniye province (37º02’28” N, 36º13’14” E, 155 m, voucher no FBozok00418) of Turkey in April 2018 and brought to the laboratory immediately to be powdered with a grinder (Loyka LKD 100, Turkey). About 2 kg of *S. chamaecyparissus* were obtained from the related area, many harvests were made within one week as needed. Homogenous sampling was done and nearly 300 grams of specimen was filled into a 1000 ml bottom flask by using a Clevenger apparatus containing a microwave oven (Arçelik, MD 574, Turkey) (Süfer & Bozok, 2020). Process continued during 30 minutes at 340 W until SEO flow stopped, and SEO was stored at -18°C in an amber glass vial until further analysis. Distillation process was performed at least five times. The yield of SEO on wet basis was determined from the Eq. (1);

$$\text{Yield (\%)} = \frac{Volume\ of\ EO\ (ml)}{Sample\ weight\ (g)} \times 100 \qquad (1)$$

Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

The analysis of volatile compounds in SEO was performed by a gas chromatograph (Agilent 7000 Series Triple Quad) with a capillary column (HP-5MS, 30 m length × 90.25 mm diameter × 90.25 µm film thickness). The inner surface of column contained 5% phenylmethylpolysiloxane and the device also equipped with a flame ionization detector (FID) (Thermo Scientific Focus). Scanning was carried out from m/z 50 to 600 in 70 eV. Initial temperature of the system was 50°C and reached to 240°C at a rate of 3°C/min. The flow rate of helium was 1 ml/min and split ratio was 1:20. The temperature of injector was 250°C. The retention indices (RI) which were calculated with the aid of a homologous series of n-alkanes (C_8-C_{20}), retention times (RT) and current mass spectra (Wiley and NIST libraries, Kovats retention index) were useful for determining the EO constituents. The chromatographic profile of SEO was depicted in Figure 1.

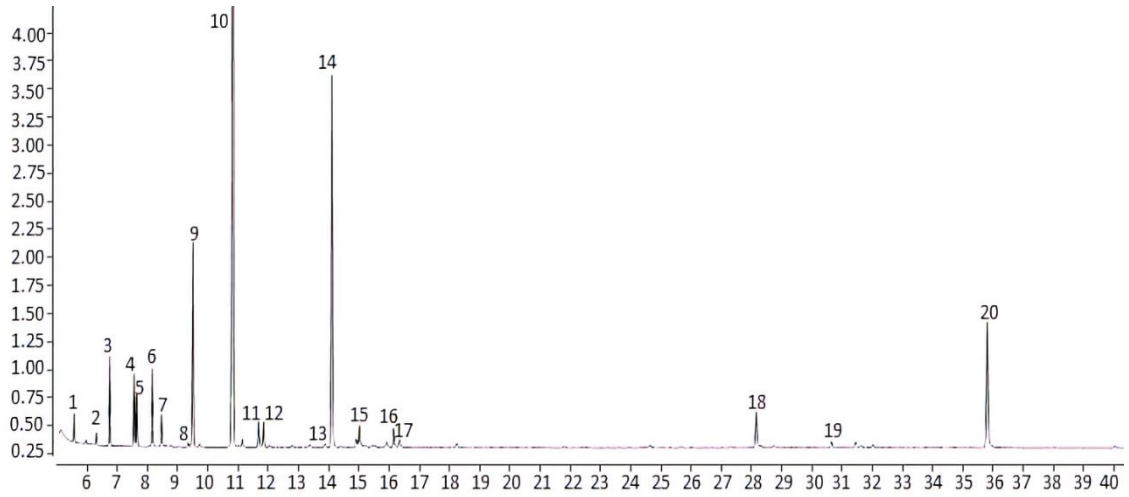

Figure 1. Chromatographic profile of SEO (1: Santolina triene, 2: α-Pinene, 3: Camphene, 4: Sabinene, 5: β-Pinene, 6: β-Myrcene, 7: Yomogi alcohol, 8: o-Cymene, 9: β-Phellandrene, 10: Artemisia ketone, 11: Artemisia alcohol, 12: Terpinolene, 13: Isopinocarveol, 14: Camphor, 15: Borneol, 16: Myrcenone, 17: Myrtenal, 18: Germacrene D, 19: (E)-α-Bisabolene, 20: β-Bisabolene)

Antioxidant Activity

The antioxidant potential of SEO was estimated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method modified from Viuda-Martos et al. (2010). Firstly, the specific methanolic concentrations of EO (10, 20, 40, 50, 100 g/L) and both butylated hydroxytoluene (BHT) (0.075, 0.150, 0.225, 0.300 g/L) and gallic acid (0.006, 0.012, 0.025, 0.050 g/L), which were selected as reference synthetic antioxidants were prepared and 100 µl sample reacted with 2 ml DPPH solution (0.025 g/L). After the incubation in dark place for 30 minutes, the absorbances of mixtures were read at 517 nm by a spectrophotometer (Shimadzu, UV 1800, Japan). The inhibition percentage of tested samples was specified from following formula (Eq. 2);

$$\text{Inhibition (\%)} = \frac{A_{DPPH} - A_{SAMPLE}}{A_{DPPH}} \times 100 \qquad (2)$$

where A_{SAMPLE} and A_{DPPH} were the absorbances of sample and DPPH solution, respectively. The concentration required to scavenge 50% of DPPH called as IC_{50} and it was determined from the graph which was plotted by the data of the inhibition percentages against concentrations. The analysis was performed in triplicate.

Antimicrobial Assays

The agar disc diffusion method was used to determine of antimicrobial activity of SEO (NCCLS, 2003). The following gram-negative bacteria were used: *Escherichia coli* (ATCC 35218), as well as the gram-positive bacteria: *Streptococcus mutans* (ATCC, 25175), *Bacillus cereus* (ATCC 11778), *Bacillus subtilis* (ATCC, 6633) and the fungus: *Candida albicans* (ATCC, 10231).

Bacterial species (*E. coli, S. mutans, B. cereus, B. subtilis*) were cultured overnight in Müller Hinton (MH) medium, while fungal strain (*C. albicans)* was grown on Sabouraud agar (SDA). The inocula prepared from 24-48 h broth cultures, and suspensions were adjusted to the turbidity of a 0.5 McFarland standard. Aliquots of 100 µL (suspension containing 10^6-10^7 CFU) were spread over the surface of growth medium. Filter paper discs (Whatman no.1, about 6 mm in diameter) were impregnated with 30 µL of the samples and placed on agar surfaces. The absolute methanol, absolute ethanol, water and ethyl acetate without SEO were used as a control. Standard antibiotics (ampicillin (AMP), gentamicin (CN), penicillin (P), nystatin (NYS)) were used in order to control the sensitivity of the tested microorganisms. Inoculated petri dishes were kept at 4ºC for 1 h and were incubated at 37ºC for 18-24 h for bacteria and at 28-30°C for 36-48 h for fungal strains. The diameters of the zones of inhibition were measured in mm after 24-48 h time periods. All assays were replicated three times.

SEO dissolved in 5% dimethyl sulfoxide was diluted to the highest concentration (20480 µg/mL) and then, serial two-fold dilutions were made in concentrations ranging from 20 to 5120 µg/mL in MH broth. The last DMSO concentration was 5% (v/v) and this solution was used as a negative control (MIC3). The evaluation of MICs was done using the agar dilution methods with slight modifications described by the National Committee for Clinical Laboratory Standards (NCCLS, 1990). The strain was cultured overnight in Mueller Hinton Broth and Sabouraud dextrose agar, the cultures adjusted to inoculum density of 8 log CFU/mL and used to 96-well plates including serial dilutions of EO (5120-20 µg/mL) on medium. Positive (containing the bacterial culture) and negative (broth without the EO) controls were performed for every test. On the basis of the previous studies, the inoculated microplate was incubated at 37ºC for 18-24 h for bacteria and at 28-30°C for 36-48 h for fungal strains (Barros et al., 2007; Zore et al., 2011; Tran et al., 2020). The MIC was defined as the lowest concentration of the SEO that showed no growth. The MBC (minimum bactericidal concentration) was determined by subculturing 100 µL from each showing no turbidity tube onto agar plates. All experiments were replicated three times.

FTIR Spectral Test

FTIR spectra of SEO was studied using a FTIR spectrometer (Perkin Elmer spectrum 65, USA) and obtained in the wavenumber range of 4000-600 cm^{-1}. Average curves from 16 scan were recorded and a drop of SEO (nearly 2 µL) which was put into the sample cell of equipment was enough to conduct the test. Also, an attenuated total reflectance (ATR) sampling accessory was equipped with device.

Results and Discussion

Chemical Composition of SEO

The yield of SEO obtained by microwave assisted distillation was calculated as 0.60% (v/v) and its colour was greenish yellow. The EO contained twenty aromatic compounds (Figure 1) and these were demonstrated in the order of elution with their percentage areas (Table 1). Twenty constituents represented the 93.14% of the total SEO. The prevalent volatiles were artemisia ketone (39.83%), camphor (17.65%), β-phellandrene (8.03%) and β-bisabolene (7.32%). The SEO was composed of 61.17% oxygenated monoterpenes, 21.33% monoterpene hydrocarbons and 10.62% sesquiterpene hydrocarbons.

Table 1. EO compounds of *S. chamaecyparissus* collected from Osmaniye, Turkey

No	RT	RI[a]	RI[b]	Compounds	Area (%)
1	5.56	906	906	Santolina Triene	0.93
2	6.30	917	932	α-Pinene	0.43
3	6.74	933	946	Camphene	3.04
4	7.54	935	969	Sabinene	2.53
5	7.64	938	974	β-Pinene	1.83
6	8.15	953	988	β-Myrcene	2.87
7	8.46	999	999	Yomogi Alcohol	1.25
8	9.33	1021	1022	o-Cymene	0.18
9	9.49	1026	1025	β-Phellandrene	8.03
10	10.83	1061	1056	Artemisia Ketone	39.83
11	11.67	1080	1080	Artemisia Alcohol	1.10
12	11.83	1087	1086	Terpinolene	1.06
13	13.87	1136	1135	Isopinocarveol	0.19
14	14.10	1140	1141	Camphor	17.65
15	15.01	1146	1147	Borneol	1.15
16	16.14	1152	1161	Myrcenone	0.92
17	16.34	1176	1195	Myrtenal	0.43
18	28.15	1483	1484	Germacrene D	2.04
19	30.63	1504	1506	(E)-α-Bisabolene	0.36
20	35.80	1506	1505	β-Bisabolene	7.32
				Oxygenated Monoterpenes	61.17
				Monoterpene Hydrocarbons	21.33
				Sesquiterpene Hydrocarbons	10.62
				Total	93.12

RT: Retention time, RI[a]: Retention indices in the present study, RI[b]: Retention indices in the literature

Table 2 summarized some previous researches focused on the EO content of *S. chamaecyparissus*. Artemisia ketone was dominant in Turkish (Demirci et al., 2000) and Indian (Garg et al., 2001) specimens and these findings were compatible with our research. The free radical scavenging capacity of related aromatic constituent was stronger than other monoterpenes (Berechet et al., 2017), the antimicrobial activity of that was known (Billia et al., 2014) and it could be a part of fragrance and perfumery (Haider et al., 2012; Liu et al., 2021). The amount of artemisia ketone in EO is able to be variable due to geography and harvest time (Liu et al., 2021). In Spanish (Villar et al., 1986) and Algerian (Djeddi et al., 2012) samples, camphor was the superior. Its antimicrobial and anticarcinogenic activity enables camphor to be used in cosmetic products and medicine formulations (Saeidnia et al., 2011). On the other hand, the differences in locations caused significant variations in major compounds of EOs. For instance, α-amorphene was dominant in Syria (Khubeiz & Mansour, 2016), however it was not accepted as an important volatile in other EOs as well as in current research.

Table 2. Comparison of the main constituents of SEO collected from several locations at different times

Country	Season	Major Compounds (%)	Reference
Syria	June 2016	α-Amorphene (12.11) β-Phellandrene (10.63) β-Myrcene (7.42) Nootkatone (6.97)	Khubeiz and Mansour, (2016)
Tunisia	Flowering Stage	1.8-Cineole (12.94) β-Eudesmol (10.49) Terpinen-4-ol (6.97)	Salah-Fatnassi et al., (2017)
Algeria	June 2005	Camphor (31.1) Cubenol (17.0) p-Cymene (8.3) Sabinene (4.0)	Djeddi et al., (2012)

Table 2 *(Continued)*

Country	Season	Major Compounds (%)	Reference
Turkey	February 1997	Artemisia ketone (38.1) Camphor (11.7) β-Phellandrene (9.2) α-Bisabolol (6.6) Mycrene (4.3)	Demirci et al., (2000)
India	July 1999	Artemisia ketone (31.8) 1.8-Cineole (15.6) Mycrene (14.2) Germacrene-D (8.8)	Garg et al., (2001)
Spain	June 1983	Camphor (25.19) Allo-aromadendrene (19.04) 1.8-Cineole (9.99)	Villar et al., (1986)

Antioxidant Activity

The inhibition percentages at definite concentrations and IC_{50} values of SEO, BHT and gallic acid were shown in Table 3. EO and reference samples did not possess the same antioxidant capability, because the sample: solvent ratio of solutions were different. The radical scavenging activities of both natural and synthetics were concentration-dependent. The IC_{50} value of SEO was the highest (88.301 g/L), followed by BHT (0.212 g/L) and gallic acid (0.021 g/L). The results stated that gallic acid exhibited the strongest DPPH scavenging capacity compared to other synthetic antioxidant, BHT and SEO sample, while the antioxidant potential of BHT was greater than SEO. The weak antioxidant behavior in an SEO could be attributed to sesquiterpene and monoterpene hydrocarbon contents (Ruberto & Baratta, 2000). In this paper, the 31.95% of SEO comprised of aforementioned organic groups, hence the inability for donating hydrogen with respect to BHT and gallic acid may be due to both monoterpenes and sesquiterpenes.

The EO of *S. chamaecyparissus* depicted lower DPPH scavenging activity than Moroccan *Cladanthus mixtus* EO (0.342 mg/ml) (Elouaddari et al., 2020), the EOs of *Citrus sinensis* (63.43 mg/ml), *C. grandis* (33.01 mg/ml), *C. aurantifolia* (7.11 mg/ml) peels (Chi et al., 2019) and *Johreniopsis stricticaulis* EO (18 mg/ml) (Feyzi et al., 2019). This obvious low IC_{50} value of SEO might be directly related to distillation process and microwaves can have harmful effects on bioactive compounds of SEO, because of rapid heating process. The percent DPPH inhibition of fresh sage EO by microwave distillation were reported lower than conventional hydro and steam distillations by Boutebouhart et al. (2019). Similarly, a study conducted by Albi et al. (1997) stated that, microwave mechanism caused more loss of polyphenols than convection in olive oil. Polyphenol content is almost correlated to antioxidant behavior; therefore extraction procedure of EO is a key factor to get highly active natural material depending on the structure of substances.

Table 3. Antioxidant activity of SEO and synthetic antioxidants

Sample	DPPH Inhibition (%)					IC_{50} (g/L)
SEO	10 g/L	20 g/L	40 g/L	50 g/L	100 g/L	
	14.264 ± 0.002	19.074 ± 3.509	28.478 ± 6.446	36.771 ± 1.431	60.133 ± 5.478	88.301
BHT	0.075 g/L	0.150 g/L	0.225 g/L	0.300 g/L		
	27.135 ± 0.001	40.323 ± 0.001	61.470 ± 2.363	75.341 ± 14.711		0.212
Gallic Acid	0.006 g/L	0.012 g/L	0.025 g/L	0.050 g/L		
	24.204 ± 0.012	39.212 ± 0.003	58.767 ± 0.010	92.420 ± 0.001		0.021

SEO: *S. chamaecyparissus* essantial oil, BHT: Butylated hydroxytoluene, DPPH: 2,2-Diphenyl-1-picrylhydrazyl, IC_{50}: Inhibitory concentration required to recover 50% of DPPH solution

Antimicrobial Activity

The *in vitro* antibacterial activity of EO of *S. chamaecyparissus* was qualitatively assessed by the presence or absence of inhibition zones. According to the results given in Table 4, SEO showed antibacterial effect against *E. coli*, *B. subtilis*, *B. cereus*, *S. mutans*, with their respective diameter zones of inhibition of 11.0, 14.1, 13.5, 10.4 mm and showed antifungal effect against *C. albicans*, having diameter zones of inhibition which was equal to 15.2 mm. On the other hand, regarding the findings of the agar diffusion method, *B. subtilis* (ATCC 6633) was the most susceptible microorganism which was strongly inhibited by SEO. The present study also revealed that the EO of *S. chamaecyparissus* showed a similar inhibitory type of activity to that of standard antibiotics.

The variability in the concentration of the main components present in SEO led us to evaluate the antimicrobial activity. As shown in Table 4, the MIC and MBC values for EO of *S. chamaecyparissus* were found in the range of 0.5–2.0 mg/ml and 1.0–2 mg/ml, respectively. Among tested bacterial species, the MIC value of EO was the least (0.5 µL/mL) for *S. mutans* and *B. cereus*.

Table 4. *In vitro* antibacterial activity of SEO

Microorganism	Growth Inhibition Zone Diameter (mm)[a]							
	GN	P	AMP	NYS	(-)C	SEO[b]	MIC	MBC[c] MFC[d]
E. coli	14.2	NA	19.5	NA	0.0	11.0	1/8 2mg/ml	1/8[c] 2mg/ml
B. subtilis	18.3	NA	NA	NA	0.0	14.1	1/16 1mg/ml	1/8[c] 2mg/ml
S. mutans	18.8	35.2	36.1	NA	0.0	10.4	1/32 0.5mg/ml	1/16[c] 1mg/ml
B. cereus	19.9	7.2	9.8	NA	0.0	13.5	1/32 0.5mg/ml	1/16[c] 1mg/ml
C. albicans	NA	NA	NA	17.6	0.0	15.2	1/16 1mg/ml	1/16d 1mg/ml

a: Inhibition zones including the diameter of the paper disc (6 mm), b: 20 µl of essential oil/disc, c: MBC (minimum bactericidal concentrations), d: MFC (minimum fungicidal concentration), SEO : *S. chamaecyparissus* essential oil, GN: Gentamicin, P: Penicillin, AMP: Ampicillin, NYS: Nystadin, (-)C: Negative control, NA: No activity.

The EO of *S. chamaecyparissus* and its constituents were reported with their antimicrobial effects against several bacterial and fungal species such as *B. subtilis*, *Streptococcus pyogenes*, *Micrococcus luteus*, *Salmonella typhimurium*, *Klebsellia pneumonia*, *Proteus vulgaris*, and *Vibrio parahaemolyticus* (Khubeiz & Mansour, 2016), *E. coli*, *Pseudomonas, aeruginosa*, *Proteus mirabilis*, *Citrobacter freundii*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Trichophyton rubrum*, *Microsporum canis*, *Epidermophyton floccosum*, *Candida albicans*, *Scytalidium dimidiatum*, *Scopulariopsis brevicaulis*, *Aspergillus fumigatus* (Salah-Fatnassi et al., 2017). Our data have perfectly matched with previous studies and small differences in the inhibitory effect of SEO on some microorganisms may arise due to changes in the amount of SEO components and extraction technique.

FTIR Fingerprints

FTIR spectroscopy has been utilized for getting reliable information about the identification of biochemical molecules (Sivakesava & Irudayaraj, 2001; Tulukcu et al., 2019). Figure 2 indicated that the components of SEO intensified in the waveband of 3439.70-668.06 cm^{-1}. Alcohols, hydrocarbons, esters, terpenes, carboxylic acid, ethers, aromatics and inorganics (sulphates) were the functional groups detected in SEO (Table 5) and these matchings were done with the aid of studies conducted by several researchers (Baba et al., 2012; Sheny et al., 2012; Oliveira et al., 2016; Chan & Wang, 2018; Taraj et al., 2019; Yuan et al., 2019). The FTIR results were also

supported by GC-MS analyses. For instance, characteristics bands at 1681.94 and 1620.98 cm^{-1} pertained to bicyclogermacrene (derived from germacrene) and pinenes (α and β) (Yuan et al., 2019). In addition, the constituent specified at 3439.70 cm^{-1} was remarked as phenols (Vanitha et al., 2019) which may be contributed the antioxidant activity of SEO.

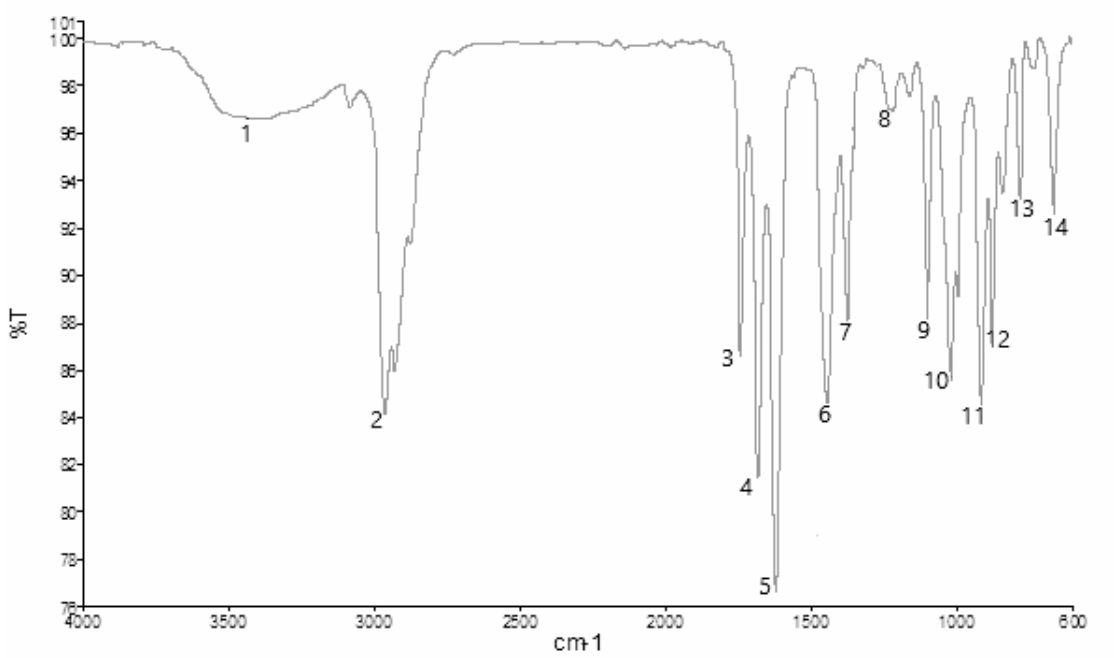

Figure 2. FTIR spectra of SEO (1: 3439.70, 2: 2965.46, 3: 1742.20, 4: 1681.94, 5: 1620.98, 6: 1446.52, 7: 1377.50, 8: 1222.21, 9: 1101.16, 10: 1021.73, 11: 915.69, 12: 877.29, 13: 784.74, 14: 668.06 cm^{-1})

Table 5. Chemical view of FTIR data

Peak No	Vibrational Motion	Wave Number (cm^{-1})	Functional Group	Reference
1	O–H Stretch	3439.70	Alcohols	Yuan et al., (2019)
2	C–H Stretch	2965.46	Unsaturated Hydrocarbon	Chan and Wang, (2018)
3	C=O Stretch	1742.20	Esters	Yuan et al., (2019)
4	C=C Stretch	1681.94	Terpenes	Taraj et al., (2019)
5	C=C Stretch	1620.98	Terpenes	Taraj et al., (2019)
6	C–O–H Bend	1446.52	Carboxylic Acid	Sheny et al., (2012)
7	C–O Stretch	1377.50	Carboxylic Acid	Sheny et al., (2012)
8	C–O Stretch	1222.21	Phenyl Alkyl Ethers	Chan and Wang, (2018), Baba et al., (2012)
9	C–O–C Stretch	1101.16	Ethers	Baba et al., (2012)
10	C–N Stretch (Alkyl)	1021.73	Amines	Baba et al., (2012)
11	C–H Bend	915.69	Aromatics	Chan and Wang, (2018)
12	C-C Stretch C-H Stretch	877.29	Aromatics	Oliveira et al., (2016)
13	C–H Bend (Mono)	784.74	Aromatics	Baba et al., (2012)
14	C–S Bend	668.06	Inorganics (Sulphates)	Chan and Wang, (2018)

Conclusions

The present work gives new information about microwave assisted distillation of SEO without using an organic solvent. Based on the above observations, the EO of *S. chamaecyparissus* could be a good source of monoterpenes especially artemisia ketone. Its herbaceous fragrance may provide a new usage area in especially men's perfumery. Also, antioxidant and antimicrobial properties

will benefit to related SEO in pharmaceutical industry. Optimization researches in microwave system are recommended to recover more bioactives which can affect the amount of antioxidant molecules and accordingly to enhance the ability of scavenging free radicals. For this goal, a gradual microwave program or lower process powers than 340 W might be useful in obtaining heat sensible polyphenols as well and/or decreasing the degradation rate of valuable constituents.

Ethics Committee Approval

N/A

Peer-review

Externally peer-reviewed.

Author Contributions

Conceptualization: Ö.S., F.B.; Investigation: Ö.S., A.C., F.B.; Material and Methodology: Ö.S., A.C., D.O, G.Y.Ç, F.B.; Supervision: Ö.S., F.B.; Visualization: Ö.S., A.C., F.B.; Writing-Original Draft: Ö.S., A.C., D.O, G.Y.Ç, F.B. Writing-review & Editing: Ö.S., A.C., F.B.; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest

The authors have no conflicts of interest to declare.

Funding

The authors declared that this study has received no financial support.

References

Albi, T., Lanzón, A., Guinda, A., León, M. & Pérez-Camino, M.C. (1997). Microwave and conventional heating effects on thermoxidative degradation of edible fats, *Journal of Agricultural and Food Chemistry*, 45(10), 3795-3798.

Baba, G., Lawal, A.O. & Shariff, H.B. (2012). Mosquito repellent activity and phytochemical characterization of essential oils from *Striga hermonthica*, *Hyptis spicigera* and *Ocimum basilicum* leaf extracts, *British Journal of Pharmacological Toxicology*, 3(2), 43-48.

Barros, L., Calhelha, R.C., Vaz, J.A., Ferreira, I. C., Baptista, P. & Estevinho, L.M. (2007). Antimicrobial activity and bioactive compounds of Portuguese wild edible mushrooms methanolic extracts, *European Food Research and Technology,* 225(2), 151-156.

Berechet, M.D., Manaila, E., Stelescu, M.D. & Craciun, G. (2017). The composition of essential oils obtained from *Achillea millefolium* and *Matricaria chamomilla* L. originary from Romania, *Revista de Chimie*, 68(12), 2787-2795.

Bilia, A.R., Santomauro, F., Sacco, C., Bergonzi, M. C. & Donato, R. (2014). Essential oil of *Artemisia annua* L.: an extraordinary component with numerous antimicrobial properties, *Evidence-Based Complementary and Alternative Medicine*, Article ID: 159819, 1-7.

Boudoukha, C., Elmastas, M. & Aksit, H. (2019). Antioxidant capacity and phenolic content of *Santolina chamaecyparissus* L. methanol extract. *International Journal of Green Pharmacy*, 13(3), 260-267.

Boutebouhart, H., Didaoui, L., Tata, S. & Sabaou, N. (2019). Effect of extraction and drying method on chemical composition and evaluation of antioxidant and antimicrobial activities of essential oils from *Salvia officinalis* L., *Journal of Essential Oil Bearing Plants*, 22(3), 717-727.

Brunke, E.J., Hammerschmidt, F.J. & Schmaus, G. (1992). The essential oil of *Santolina chamaecyparissus* L., *Dragoco Reports*, 39, 3-31.

Calvo, M.I., Akerrata, S. & Cavero, R.Y. (2013). The pharmacological validation of medicinal plants used for digestive problems in Navarra Spain, *European Journal of Integrative Medicine*, 5, 537-546.

Chan, W.P. & Wang, J. (2018). Formation of synthetic sludge as a representative tool for thermochemical conversion modelling and performance analysis of sewage sludge–Based on a TG-FTIR study, *Journal of Analytical and Applied Pyrolysis*, 133, 97-106.

Chi, P.T.L., Hung, P.V., Thanh, H. L. & Phi, N.T.L. (2019). Valorization of citrus leaves: chemical composition, antioxidant and antibacterial activities of essential oils, *Waste and Biomass Valorization*, 11, 4849-4857.

Demirci, B., Özek, T. & Başer, K.H.C. (2000). Chemical composition of *Santolina chamaecyparissus* L. essential oil, *Journal of Essential Oil Research*, 12, 625-627.

Derbesy, M., Touche, J. & Zokd, A. (1989). The essential oil of *Santolina chamaecyparissus* L., *Journal of Essential Oil Research*, 1, 269-275.

Djarmouni M., Baghiani A., Adjadj M. & Arrar L. (2018). Anti-inflammatory and xanthine

oxidase inhibition activities of *Santolina chamaecyparissus* extracts, *Annual Research & Review in Biology*, 22(6), 1-7.

Djeddi, S., Djebile, K., Hadjbourega, G., Achour, Z., Argyropoulou, C. & Skaltsa, H. (2012). *In vitro* antimicrobial properties and chemical composition of *Santolina chamaecyparissus* essential oil from Algeria, *Natural Product Communications*, 7(7), 937-940.

Elouaddari, A., Elamrani, A., Moutia, M., Oubrim, N., Habti, N. & Jamaleddine, J. (2020). Chemical composition and evaluation of antioxidant, antimicrobial and cytotoxic activities of Moroccan *Cladanthus mixtus* essential oil and extracts, *Journal of Essential Oil Bearing Plants*, 22(6), 1450-1466.

Feyzi, P., Kasaian, J., Haghbin, A., Nazari, A. & Hassani, H. (2019). Chemical composition and biological profile of *Johreniopsis stricticaulis* aerial parts essential oil, *Journal of Essential Oil Bearing Plants*, 22(5), 1386-1393.

Garg, S.N., Gupta, D., Mehta, V.K. & Kumar, S. (2001). Volatile constituents of the essential oil of *Santolina chamaecyparissus* L. from the southern hills of India, *Journal of Essential Oil Research*, 13, 234-235.

Grosso, C., Figueiredo, A.C., Burillo, J., Mainar, A.M. & Urieta, J.S. (2009). Supercritical fluid extraction of the volatile oil from *Santolina chamaecyparissus*, *Journal of Seperation Science*, 32, 3215-3222.

Haider, S.Z., Andola, H.C. & Mohan, M. (2012). Constituents of *Artemisia gmelinii* Weber ex Stechm. from Uttarakh and Himalaya: a source of artemisia ketone, *Indian Journal of Pharmaceutical Science*, 74(3), 265-267.

Khubeiz, M.J. & Mansour, G. (2016). *In vitro* antifungal, antimicrobial properties and chemical composition of *Santolina chamaecyparissus* essential oil in Syria, *International Journal of Toxicological and Pharmacological Research*, 8(5), 372-378.

Labed, F., Masullo, M., Cerulli, A., Benayache, F., Benayache, S. & Piacente, S. (2017). Chemical constituents of the aerial parts of *Santolina chamaecyparissus* and evaluation of their antioxidant activity, *Natural Product Communications*, 12(10), 1605-1608.

Liu, H., Guo, S., Lu, L., Li, D., Liang, J., Huang, Z., Zhou, Y-M., Zhang, W. J. & Du, S. (2021). Essential oil from *Artemisia annua* aerial parts: Composition and repellent activity against two storage pests, *Natural Product Research*, 35(5), 822-825.

López, V., Akerreta, S., Casanova, E., García-Mina, J., Cavero, R. & Calvo, M. (2008). Screening of Spanish medicinal plants for antioxidant and antifungal activities, *Pharmaceutical Biology*, 46(9), 602-609.

Messaoudi, D., Bouriche, H. & Demirtas, I. (2018). Phytochemical analysis and hepatoprotective activity of Algerian *Santolina chamaecyparissus* L. extracts, *Annual Research & Review in Biology*, 25(2), 1-12.

NCCLS – *National Committee for Clinical Laboratory Standards* (1990). Methods for dilution antimicrobial susceptibility tests for bacateria that grow aerobically - second edition; Approved Standard. NCCLS Document M7-A2. Villanova, Pa.: NCCLS.

NCCLS – *National Committee for Clinical Laboratory Standards* (2003). Performance standards for antimicrobial susceptibility testing: Eleventh informational supplement. Document M100-S11. National Committee for Clinical Laboratory Standard, Wayne, PA, USA.

Nikolić, M.G. & Radulović, N.S. (2018). Chemical composition of the essential oil from the aboveground parts of *Santolina chamaecyparissus* L. from Greece: NMR determination of the exocyclic double bond geometry of the major spiroketal-enol ether polyynic constituent, *Facta Universitatis, Series Physics, Chemistry and Technology*, 16(1), 130.

Niu, L., Qin, Q., Wang, L., Gai, Q., Jiao, J., Zhao, C. & Fu, Y. (2019). Chemical profiling of volatile components of micropropagated *Santolina chamaecyparissus* L., *Industrial Crops and Products*, 137, 162-170.

Oliveira, R.N., Mancini, M.C., Cabral, F., Oliveira, S.D., Passos, T.M. & Quilty, B. (2016). FTIR analysis and quantification of phenols and flavonoids of five commercially available plants extracts used in wound healing, *Matéria (Rio de Janeiro),* 11743, 767-779.

Pérez-Alonso, M.J. & Velasco-Negueruela, A. (1992). Essential oil components of *Santolina chamaecyparissus* L., *Flavour and Fragrance Journal*, 7, 37-41.

Pons, R.M.G. & Caňavate, J.L.R. (2000). *Santolina chamaecyparissus*: Especie mediterranea con potenciales aplicaciones terapeuticas en procesos inflamatorios y transtornos digestivos. *Revista de Fitoterapia*, 1, 27-34.

Ruberto, G. & Baratta, M.T. (2000). Antioxidant activity of selected essential oil components in two lipid model systems, *Food Chemistry*, 69, 167-174.

Ruiz-Navajas, Y., Viuda-Martos, M., Sendra, E., Perez-Alvarez, J.A. & Fernandez-Lopez, J. (2012). Chemical characterization and

antibacterial activity of *Thymus moroderi* and *Thymus piperella* essential oils, two *Thymus* endemic species from southeast of Spain, *Food Control*, 27, 294-299.

Saeidnia, S., Gohari, A.R., Mokhber-Dezfuli, N. & Kiuchi, F. (2011). A review on phytochemistry and medicinal properties of the genus *Achillea*, *Daru*, 19, 173-186.

Salah-Fatnassi, K.B.H., Hassayoun, F., Cheraif, I., Khan, S., Jannet, H.B., Hammami, M., Aouni, M. & Harzallah-Skhiri, F. (2017). Chemical composition, antibacterial and antifungal activities of flowerhead and root essential oils of *Santolina chamaecyparissus* L. growing wild in Tunisia, *Saudi Journal of Biological Science*, 24, 875-882.

Sheny, D.S., Mathew, J. & Philip, D. (2012). Synthesis characterization and catalytic action of hexagonal gold nanoparticles using essential oils extracted from *Anacardium occidentale*, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 97, 306-310.

Sivakesava, S. & Irudayaraj, J. (2001). Prediction of inverted cane sugar adulteration of honey by fourier transform infrared spectroscopy, *Journal of Food Science*, 66, 972-978.

Suresh, B., Sriram, S., Dhanaraj, S.A., Elango, K. & Chinnaswamy, K. (1997). Anticandidal activity of *Santolina chamaecyparissus* volatile oil, *Journal of Ethnopharmacology*, 55, 151-159.

Süfer, Ö. & Bozok, F. (2020). Characterization of essential oil from *Matricaria sevanensis* by microwave-assisted distillation, *Journal of Thermal Analysis and Calorimetry*, 140(1), 253-261.

Taraj, K., Malollari, I., Ciko, L., Llupa, J., Ylli, A., Ylli, F. & Andoni, A. (2019). Water distillation extraction of essential oil from *Sideritis raeseri* Herb, *Environmental Processes*, 6, 1051-1058.

Tran, H.N., Graham, L. & Adukwu, E.C. (2020). In vitro antifungal activity of *Cinnamomum zeylanicum* bark and leaf essential oils against *Candida albicans* and *Candida auris*, *Applied Microbiology and Biotechnology*, 104(20), 8911-8924.

Tulukcu, E., Cebi, N. & Sagdic, O. (2019). Chemical fingerprinting of seeds of some Salvia species in Turkey by using GC-MS and FTIR, *Foods*, 8(4), 1-12.

Vanitha, A., Kalimuthu, K., Chinnadurai, V. & Nisha, K.M.J. (2019). Phytochemical screening, FTIR and GC-MS analysis of aqueous extract of *Caralluma bicolor*–An endangered plant, *Asian Journal of Pharmaceutical Science*, 5(6), 1122-1130.

Villar, A., Giner, R.M. & Rios, J.L. (1986). Chemical composition of *Santolina chamaecyparissus* ssp. squarrosa essential oil, *Journal of Natural Products*, 49(6), 1143-1144.

Viuda-Martos, M., Navajas, Y.R., Zapata, E.S., Fernández-López, J. & Pérez-Álvarez, J.A. (2010). Antioxidant activity of essential oils of five spice plants widely used in a Mediterranean diet, *Flavour and Fragrance Journal*, 25(1), 13-19.

Vernin, G. (1991). Volatile constituents of the essential oil of *Santolina chamaecyparissus* L., *Journal of Essential Oil Research*, 3(1), 49-53.

Yuan, C., Wang, Y., Liu, Y. & Cui, B. (2019). Physicochemical characterization and antibacterial activity assessment of lavender essential oil encapsulated in hydroxypropyl-beta-cyclodextrin, *Industrial Crops and Products*, 130, 104-110.

Zore, G.B., Thakre, A.D., Rathod, V. & Karuppayil, S.M. (2011). Evaluation of anti-Candida potential of *geranium* oil constituents against clinical isolates of *Candida albicans* differentially sensitive to fluconazole: inhibition of growth, dimorphism and sensitization, *Mycoses*, 54(4), 99-109.