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Chemical Compositions and Antibacterial Activity of the Essential Oils From Some Plant Species

Bazı bitki türlerinden elde edilen uçucu yağların kimyasal kompozisyonu ve antibakteriyal aktivitesi

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Anahtar Sözcükler:

Uçucu yağlar, kimyasal kompozisyon, antibakteriyal aktivite

ÖZET

Bu çalışmada, kimyon (*Cuminum cyminum*), defne (*Laurus nobilis*), kekik (*Origanum onites* spp.), biberiye (*Rosmarinus officinalis*), anason (*Pimpinella anisum*) ve karanfil (*Syzygium aromaticum*) bitkilerinden elde edilen uçucu yağların kimyasal kompozisyonları GC/MS ile belirlenmiş ve *Salmonella typhimurium* CCM 5445, *Staphylococcus aureus* (MRSA) RSKK 95047, *Staphylococcus aureus* ATCC 6538P, *Escherichia coli* ATCC 29998, *Escherichia coli* O157:H7 RSKK 232 bakterileri üzerine iki farklı yöntem (disk difüzyon ve agar dilusyon) ile antimikrobiyal aktiviteleri araştırılmıştır. Araştırmadan elde edilen bulgulara göre kekik uçucu yağı (0.0625-0.125 mg/ml) en yüksek antimikrobiyal etkiyi göstermiş, bunu sırasıyla kimyon (0.0625-2.0 mg/ml) ve karanfil (0.25-1.0mg/ml) izlemiştir. Defne, biberiye ve anason uçucu yağları ise test bakterileri üzerinde antibakteriyal aktivite göstermemiş veya çok az aktivite göstermiştir.

Key Words:

Essential oils, chemical composition, antibacterial activity

ABSTRACT

In this study, the chemical composition of essential oils from cumin (*Cuminum cyminum*), laurel (*Laurus nobilis*), oregano (*Oreganum onites* spp.), rosemary (*Rosmarinus officinalis*), anise (*Pimpinella anisum*) and clove (*Syzygium aromaticum*) were determined by GC/MS, and their antibacterial activities were tested on *Salmonella typhimurium* CCM 5445, *Staphylococcus aureus* (MRSA) RSKK 95047, *Staphylococcus aureus* ATCC 6538P, *Escherichia coli* ATCC 29998 and *Escherichia coli* O157:H7 RSKK 232 by two different methods (disc diffusion and agar dilution). According to results, oregano essential oil showed the highest inhibition (0.0625-0.125mg/ml) effect followed by cumin (0.0625-2.0mg/ml) and clove (0.25-1.0mg/ml) respectively. The laurel, rosemary and anise essential oils did not show antibacterial activity or little activity on tested bacteria.

INTRODUCTION

Spices and herbs have been used for a wide variety of purposes for thousand of years to enhance the flavor and aroma of foods in all over the world. Recently, essential (volatile) oils or extracts from medicinal and aromatic plants are widely used in medicine, phyto- and aromatherapy, pharmaceutical industry, food industry as flavoring or preservation and cosmetics as fragrances.

The antimicrobial properties of spices and their products (extract or essential oil) have been known for a long time, and a number of studies on their antimicrobial effects have been reported (Zaika, 1988; Deans & Svoboda, 1990; Hsieh et al., 2001; Nanasombat and Lohasupthawee, 2005; Ertürk, 2006). Essential oils were obtained from plants or from parts by distillation methods, usually steam or hydrodistillation. Most essential oils consist of variable mixtures of terpenoids (monoterpenes [C10], sesquiterpenes [C15] and diterpenes [C20]), low molecular weight aliphatic hydrocarbons, alcohols, aldehydes, acyclic esters or lactones and exceptionally nitrogen- and sulphur-containing compounds, coumarins and homologues of phenylpropanoids. The antimicrobial activity of essential oils is assigned to terpenes and phenolic compounds. However, mechanism of antimicrobial action of essential oil constituents was unclearly characterized. Membrane disruption by the lipophilic constituents may be involved in this mechanism. Many researchers noted that phenolic components of oils sensitize the phospholipid bilayer of the cell membrane, causing an increase of permeability and leakage of vital intracellular constitutes or impairment of bacterial enzyme system and cell respiration (Singh et al., 2002; Moreira et al., 2005).

In animal nutrition, antibiotics have been used as antimicrobial agent, growth promoters and feed additives. The ban on the use of antibiotics as feed additives has accelerated and led to investigations of alternative feed additives in animal nutrition. Herbs, spices and products derived thereof, are mainly essential oils as phytogenic feed additives of the alternatives have gained attention in recent years (Lee et al., 2004).

Currently, consumers tend to be suspicious of chemical additives due to their carcinogenic and teratogenic attributes as well as residual toxicity. Thus, natural antimicrobials receive increasing attention by food scientists and animal breeders in view of safer food products.

Turkish flora includes more than 9000 plants species. About 3000 are endemic and 1000 are used as medicine and spice in Turkey (Özgüven et al., 2005). The main herbs and spices produced and exported following laurel (bay) leaves, thyme & oregano, anise seeds, cumin seeds, sage and are namely fennel seeds and juniper berries, red pepper, linden flowers, rosemary, mahaleb, sumac, and mint (Gürel Çakıroğlu, 2010). Turkey is one of the leading supplier countries of thyme & oregano and cumin seeds have the means to dominate the world trade. It has been started to cultivation of some medicine and aromatic plants such as cumin, thyme & oregano, anise, clove and rosemary with international standards.

This study explores the chemical composition and antibacterial activity of 6 essential oils from spices of medicinal and aromatic plants. In purpose, the antimicrobial activities of essential oils against Gram-positive and Gram-negative bacteria were determined using both disc diffusion and agar dilution methods.

MATERIALS and METHODS

The essential oils of cumin (*C. cyminum),* laurel (*L. nobilis),* oregano (*O. onites* ssp.), rosemary (*R. officinalis),* anise (*P. anisum)* and clove *(S. aromaticum)* were screened for chemical composition by GC/MS and antibacterial activity using disc diffusion and agar dilution methods against five bacteria.

Materials

Essential oils

Essential oils used in the study were identified in Table 1. Cumin and clove essential oils were obtained from Özdrog Company (Hatay, Turkey), laurel and anise essential oils from İnan Agriculture Company (Antalya, Turkey), oregano essential oil from Türer Agriculture Company (İzmir, Turkey), rosemary essential oil from Set Agriculture Company (Aydın, Turkey). The essential oils were obtained by hydrodistillation from plants.

Table 1. Essential oils screened for chemical composition and antibacterial activity

Common name	Family	Botanical name	Plant part	Collection site
Cumin	Umbelliferae	*Cuminum cyminum*	Fruits (Seeds)	Sultandağı-Akşehir
Laurel	Lauraceae	*Laurus nobilis*	Leaves	Hatay
Oregano	Labiatae	*Origanum onites spp.*	Leaves	İzmir
Rosemary	Labiatae	*Rosmarinus officinalis*	Leaves	Mersin
Anise	Umbelliferae	*Pimpinella anisum*	Fruits (Seeds)	Burdur
Clove	Myrtaceae	*Syzygium aromaticum*	Flower bud	Madagascar

Microorganisms

S. typhimurium CCM 5445, *S. aureus* (MRSA) RSKK 95047, *S. aureus* ATCC 6538P, *E. coli* ATCC 29998 and *E. coli* O157:H7 RSKK 232 were used as test organisms to determine antibacterial activity. Bacterial strains were cultured overnight at 37°C in Mueller Hinton agar (MHA, Oxoid).

Methods

GC–MS analysis of essential oils

The essential oils were subsequently analyzed and their individual components identified by GC/MS (HP 6890GC/5973 MSD) system [Enjection temperature: 250 °C; enjection split:1/100; colummm:DB-17 30m, 0.25 µm, 0.32 mm (agilent); oven program: initial temp., 70 °C, rate 8 °C/min, final temp:200 °C, enjection vol., 1 µl.].

Determination of antibacterial activity of essential oils

Disc diffusion method

Disc diffusion method was employed for the preliminary antibacterial evaluation of the essential oils according to NCCLS (2000a). Bacterial strains were cultured overnight at 37°C in MHA and then test strains were suspended in physiological saline solution [0.85 % (w/v) sodium chloride] with reference to the Mc Farland standard to give a final density of 10^8 cfu/ml. A 0.1ml portion of this suspension was spread onto the MHA plates. Sterile filter paper discs (6mm in diameter) were impregnated with 3 µl of the oil and placed on the inoculated plates (mg values of each essential oil are given in Table 3). The diameters of the inhibition zones were measured in millimetres after incubation at 37°C for 24 h. Gentamycin was used as control compound (10µg/disc).

Agar dilution method

Agar dilution susceptibility assay was performed according to NCCLS (2000b), for the determination of minimum inhibition concentration (MIC) of the essential oils. Bacterial strains were cultured to activation for 24h at 37°C in Mueller Hinton agar (MHA). Test media were prepared containing two fold serial dilutions of essential oils ranging from 0.0039 to 2mg/ml. The suspension of the test microorganisms were adjusted using the 0.5 McFarland standard and was then further diluted in physiological saline to achieve 10^6 cfu/ml. Finally, 1-2 µl of suspension of the test organism was spotted onto agar media. Petri dishes were incubated at 37°C for 24 h. The same test was performed using gentamycin as a reference control compound (0.05-12µg/ml). The MIC is defined as the lowest concentration of the essential oil at which the microorganism does not demonstrate visible growth.

RESULTS and DISCUSSION

Chemical composition of the essential oils

The identified combinations in essential oils (cumin, laurel, oregano, rosemary, anise and clove) restrictive index (RI) and quantitative percentage of the compounds are presented in Table 2.

The cuminaldyde (33.57%), safranal (17.90%), p-cymene (12.72%), γ-terpinene (8.23%) and β-pinene (5.76%) constituted the main fraction of cumin. Compared to our results, Beis et al. (2000) found less cuminaldehyde (27.60%) and p-cymene (5.51%), higher γ-terpinene (17.25%) and β-pinene 10.22%), and no safranal were determined in cumin essential oil from *C. cyminum* cultivated in Eskişehir. Interestingly, it should also mentioned that ρ-mentha-1,3 dien-7-al (15.18%) and ρ-mentha-1,4-dien-7-al (9.48%) were determined as major components by Beis et al. (2000), but cumin essential oil used in the study did not contain these components. A number of studies on *C. cyminum* of different origin have been performed. Jirovetz et al. (2005) found similar amounts of cuminaldyde (36.0%), higher β-pinene (19.3%) and γ-terpinene (15.3%) and less p-cymene (1.4%) in *C. cyminum* oil of fresh seeds from Bulgaria. Other investigations on seeds from China (Li and Zi-Tao, 2004) and Iran (Oroojalian et al., 2010), almost have similar results (cuminaldehyde 36.32% and 30.2%; safranal 10.87% and 9.4%; p-cymene 9.85% and 14.1%, γ-terpinene 11.14% and 12.8%, β-pinene 7.75% and 6.4%, respectively).

In *L. nobilis* essential oil, 25 compounds were identified (Table 2), representig 94.26% of total oils. The main component was 1,8-cineole (53.74%), and also contains α-terpinenly acetate (12.02%) and smaller levels of sabinene (7.28%) and α-pinene (5.49%). Çelikel & Kavas (2008) noted that major components of laurel essential oil from plant cultured in İzmir (Turkey) were 1,8-cineole (56.7%), α-terpineol (7.0%), sabinene (6.8%), α-pinene (4.9%), β-pinene (3.6%), and smaller amounts were found terpinen-4-ol, ρ-cymene, trans pinocarveol and γ-terpinene. In another study, 1,8-cineole, sabinene and α-terpinenly acetate were major components of leaves of *L. nobilis* L. collected from natural growing sides of Hatay in Turkey (Sangun et al., 2007). In Turkey, *L. nobilis* L. grows in Mediterranean, Marmara and Aegean regions, and Turkey is the major producer of high quality laurel (bay) leaves in the world. As a result, there is an impression in the world expressed by the phrase "True laurel (bay) leaves come from Turkey". Laurel (Bay) leaves are one of the traditional and stable export items of Turkey (Sangun et al., 2007).

Table 2. Chemical composition of essential oils of cumin (*C. cyminum*), laurel (*L. nobilis*), oregano (*O. onites* spp.), rosemary (*R. officinalis*) anise (*P. anisum*) and clove (*S. aromaticum*) (%)

Component	RI[a]	Cumin	Laurel	Oregano	Rosemary	Anise	Clove
α-Thujene	921.0	-	-	0.26	-	-	-
α-Pinene	929.0	0.75	5.49	0.22	24.50	-	-
Camphene	968.0	-	0.25	-	5.57	-	-
β-Pinene	1010	5.76	3.55	-	1.44	-	-
Sabinene	1020	0.78	7.28	0.31	-	-	-
Verbenone	1028	-	-	-	1.90	-	-
Myrcene	1054	0.37	0.40	0.39	-	-	-
δ-3-Carene	1057	-	-	-	4.10	-	-
α-Phellandrene	1077	1.74	0.13	-	0.36	-	-
α-Terpinene	1098	0.14	0.30	0.65	0.67	-	-
Limonene	1117	0.61	1.11	-	6.40	-	-
1,8-Cineole	1132	-	53.74	-	12.86	-	-
γ-Terpinene	1165	8.23	0.54	2.10	0.58	-	-
p-Cymene	1200	12.72	2.39	4.35	3.21	-	-
Terpinolene	1211	-	0.12	-	0.69	-	-
Filifolone	1375	-	-	-	0.26	-	-
Sabinene Hydrate	1387	-	0.25	-	-	-	-
α-copaene	1426	-	-	-	0.58	-	0.09
(+)-Camphor	1458	-	-	-	14.24	-	-
Linalool	1461	-	0.67	2.85	-	0.03	-
Cis sabinen hydrate	1470	-	0.29	-	-	-	-
Pinocamphon (Cis/Trans)	1486	-	-	-	1.34	-	-
Bornylester	1510	-	-	-	1.46	-	-
β-Elemene	1518	0.11	0.19	-	-	-	-
Terpinene-4-ol	1526	0.48	2.83	1.28	0.57	-	-
β-Caryophyllene	1531	0.33	0.34	1.01	2.87	-	6.98
(+)-Aromadendren	1540	-	-	0.13	-	-	-
γ-Terpineol	1590	-	0.47	-	-	-	-
Estragole	1595	-	-	-	-	0.57	-
α-humulene	1600	-	-	-	0.72	-	0.69
α-Terpineol	1613	0.57	-	0.39	4.69	-	-
α-Terpinenly acetate	1617	-	12.02	-	-	-	-
Bicyclogermacrene	1618	3.19	-	-	-	-	-
(+)-Borneol	1620	-	-	1.30	4.69	-	-
γ-Himachalene	1624	-	-	-	-	0.76	-
Berbenone	1641	-	-	-	2.73	-	-
β-Bisabolene	1642	-	-	1.89	-	0.05	-
Anethole	1649		-	-	-	0.34	-
(+)-Carvon	1661	0.35	-	0.14	-	-	-
δ-Cadinene	1676	-	-	-	0.39	-	-
α-Amorphone	1682	-	-	-	0.34	-	-
β-Bisabolene	1685	-	0.11	-	-	-	-
Myrtenol	1705	-	0.44	-	-	-	-
Cuminaldhyde	1707	33.57	-	-	-	-	-
Safranal	1720	17.90	-	-	-	-	-
Trans-anethole	1724	-	-	-	-	95.40	-
Caryophyllene oxide	1903	-	-	-	-	-	0.39
Eugenol-methylether	1907	0.18	0.90	-	-	-	-
Caratol	1925	1.11	-	-	-	-	-
Anisaldehyde	1929	-	-	-	-	0.85	-
Cuminic alcohol	1991	0.78	-	-	-	-	-
Eugenol	2054	-	0.25	-	-	-	77.85
Thymol	2057	0.32	-	7.33	-	-	-
Carvacrol	2087	1.40	0.20	74.01	0.40	0.22	0.86
Eugenly Acetate	2134	-	-	-	-	-	11.79
Chavicol	2208	-	-	-	-	-	0.31
Total identified		91.39	94.26	98.61	97.56	98.22	98.96

[a]Retention indices relative to n-alkane series on the DB-5 column

The two major components of essential oil obtained from *O. onites* were determined as 74.01% carvacrol and 7.33% thymol followed by ρ-cymene (4.35%), linalool (2.85%) and γ-terpinene (2.10%). Turkish oregano (*O. onites*) growing in Southeastern Anatolia of Turkey, and it is the most exported origanum species (Yaldız et al., 2005) and is commonly known as thyme, 'İzmir kekiği', 'Bilyalı kekik' or 'White thyme'. Many of the studies reported that main components of essential oil from *O. onites* are carvarol, thymol, ρ-cymene, γ-terpinene, borneol, linanool and α-terpinene (Başer et al., 1993; Ceylan et al., 2003; Demirci et al., 2004; Toncer et al., 2009).

The most abundant components in rosemary essential oil were α-pinene (24.50%), (+) camphor (14.24%) and 1,8- cineole (12.86%) and *R. officinalis* is widely found in the lands of Aegean and Mediterranean regions of Turkey. The compositions of essential oils, representing different locations and the time intervals, were studied by Yeşil-Çeliktaş et al. (2007). These researchers identified 1,8 cineole, camphor, verbenone and α-pinene as the major components of *R.officinalis* from Turkey. Moghtader & Afzali (2009) reported that 41 components were identified in the essential oil of rosemary obtained from Kerman region of İran, and the main constituents are α-pinene (15.52%), camphor (11.66%), verbenone (11.10%) and 1,8-cineole (10.63%). In a further study (Bozin et al., 2007), limonene (21.70%), α-pinene (13.50%), camphor (21.60%), linalool (10.80%) were identified as the main constituents in rosemary essential oil from leaves of cultivated plants of rosemary collected in Serbia, and borneol (6.20%), camphene (3.90%), sabinene (2.0%), 1,8-cineole (2.1%) and α-terpineol (1.9%) were also detected in a considerable amount.

The relative content of the main compositions in anise was *trans*-anethole (95.40%), and remaining chemical compounds (carvacrol, linalool, anisaldehyde, γ-himachalene, estragole, anethole and β-bisabolene) were in trace amounts. *P. anisum* is primarily cultivated for its fruits, commercially called seed, which are used extensively in an alcoholic beverage (rakı) in Turkey. The anise essential oil is valuable in perfumery, medicine and food (as flavoring) industries. In earlier study conducted with Turkish anise, the major compounds were determined as *trans*-anethole, methyl chavicaol and anisaldehyde (Bayram, 1992). In previous study, the major component of the anise essential oil was determined *trans*-anethole, which ranged from 78.63%-95.21%, in twenty-nine essential oil from anise seeds collected from different locations in Turkey (Arslan et al., 2004).

GC/MS analysis of clove essential oil (from *S. aromaticum* L. of Madagascar origin) identified nine phytochemicals as constituents; eugenol was the major compound (77.85%) followed by eugenly acetate (11.79%) and β-caryophyllene (6.98%). In clove essential oil from *S. aromaticum* L. cultivated in Turkey, higher eugenol (87.0%), less eugenyl acetate (8.01%) and β-caryophyllene (6.98%) as main components were determined (Alma et al., 2007). In another study used clove essential oil from *S. aromaticum* L. of Chinese origin (Fu et al. 2007), it is noted that the relative components of clove essential oil were eugenol (68.52%), β-caryophyllene (19.00%), 2-methoxy-4-[2-propenyl] phenol acetate (10.15%) and α-caryophyllene (1.85%).

The differences in chemical compositions of essential oils varies slightly across different collection periods, harvest-time, plant part, numerous species, extraction- method, varieties, storage conditions and geopraphical regions (Baydar et al., 2004; Toncer et al., 2009).

Antibacterial activity

The inhibition zones measured in millimetre by disc diffusion method for preliminary antibacterial evaluation of the essential oils are presented in Table 3.

Table 3. Antibacterial activity of essential oils

Test organism	Inhibition zone (mm)						
	Cumin 2.4 mg	Laurel 3.3 mg	Oregano 2.4mg	Rosemary 2.7mg	Anise 3 mg	Clove 5mg	A* 10µg
S. typhimurium CCM 5445	0	0	25	0	0	12	13
S. aureus (MRSA) RSKK 95047	8	0	40	0	0	21	26
S. aureus ATCC 6538P	0	0	41	8	0	12	25
E. coli ATCC 29998	0	0	28	0	0	14	13
E. coli O157:H7 RSKK 232	11	10	52	11	9	25	20

*Gentamycin

Table 4. Minimum inhibitory concentration (MIC) of essential oils (mg/ml)

Test organism	Cumin	Laurel	Oregano	Rosemary	Anise	Clove	A*
	mg/ml						µg/ml
S. typhimurium CCM 5445	≥2.0	>2.0	≥0.125	≥2.0	>2.0	≥1.0	≥0.48
*S. aureus (*MRSA) RSKK 95047	≥0.0625	>2.0	≥0.125	≥2.0	>2.0	≥0.5	-
S. aureus ATCC 6538P	≥0.125	≥2.0	≥0.125	≥2.0	>2.0	≥0.5	≥0.48
E. coli ATCC 29998	≥2.0	≥2.0	≥0.125	≥2.0	>2.0	≥1.0	≥3.9
E. coli O157:H7 RSKK 232	≥0.125	≥2.0	≥0.0625	≥2.0	≥2.0	≥0.25	-

*Gentamycin

The most susceptible strain was *E. coli*, while the most effective essential oils were oregano and clove on all tested bacteria.

The MIC results of essential oils of cumin, laurel, oregano, rosemary, anise and clove against tested bacteria are shown in Table 4. In general, MIC values confirmed the results obtained with the disc diffusion. The essential oils exerted varying levels of antibacterial effects against tested bacteria.

The laurel and anise possessed little or no antibacterial activity on the tested bacteria while rosemary did not show any activity. The essential oil of anise was effective on *S. typhimurium*, MRSA, *S. aureus* and *E. coli* at high concentration. Similar, essential oil of laurel was effective on S. *typhimurium* and S. *aureus* at high concentration.

The oregano essential oil was effective against all the bacterial strains tested. The MIC values of oregano were ≥0.0625mg/ml for *E. coli* O157:H7 and ≥0.125mg/ml for *S. typhimurium*, MRSA, *S. aureus* and *E. coli* respectively. The chemical composition of the most effective essential oil of oregano consists of carvacrol (74.01%) as main compounds. Antimicrobial properties of carvacrol and thymol were reported in other studies. Dorman and Deans (2000) demonstrated the individual antimicrobial effect of carvacrol on *E. coli*. Helander et al. (1998) also reported that carvacrol, thymol and *trans*-cinnamaldehyde inhibited *E. coli* O157:H7 and *S. typhimurium* at 1-3 mM. The membrane disturbing effect of the phenols is known and phenolic character of the carvacrol might be related to its inhibitory effect (Keweloh et al., 1990). Accordingly, the high carvacrol content of the oregano essential oil makes it strong inhibitor. Similarly, the inhibitory effect of the oregano essential oil (carvacrol content 68.23%) was demonstrated a set of food borne pathogens as *E. coli* O157;H7, *Listeria monocytogenes* and *S. typhimurium* (Dadalıoglu and Akdemir-Evrendilek, 2004). In our study, oregano is the most effective against *E. coli* O157:H7 with MIC value of ≥0.0625mg/ml, followed cumin and clove with MIC values of ≥0.125 and ≥0.25 mg/ml. Among the six different essential oils, the essential oil of rosemary which contains the highest level (24%) of α-pinene did not show any antibacterial effect of tested concentration in our study. This result may be related to the test concentration. In other studies, it has been reported that rosemary essential oil has moderate (Gachkar et al., 2007) or weak (Lopez et al., 2005) antibacterial activity against *E. coli* and *S. aureus*.

The other promising result was obtained from cumin essential oil which contains cuminaldhyde (33.57%) as major compound. While cumin essential oil have no effect on *E. coli* and *S. typhimurium*, significant effect was determined as ≥0.0625 mg/ml for MRSA and ≥0.125 mg/ml on *E.coli* O157:H7 and *S. aureus* respectively. Nanasombat and Lohasupthawee (2005) who reported the cumin essential oil was potent inhibitor of bacterial growth, showing the lowest MIC of 4.2 µl/ml to *S. typhimurium* (DMST 0562, non-DT104) and *E. coli* (DMST 4212). Researchers have used higher concentration (1.5 fold) of cumin essential oils compared with this study. Result most probably is varying due to the tested concentration of essential oils.

GC-MS analysis of 26 different essential oils of anise was reported that the trans anethol content was ranging from 78.63% to 95.21% (Arslan et al., 2004). The results show that the content of the anise essential oil was 95.40% trans anethol that only active on *E. coli* O157:H7.

The clove essential oil, which contains eugenol (77.85%) as major compound, inhibited all bacterial strains tested in the study. Results showed that the MIC value of essential oil of clove was determined as ≥0.25mg/ml for *E.coli* O157:H7 and ≥0.5 mg/ml for MRSA and *S. aureus* and ≥1mg/ml for *E.coli* and *S. typhimurium* respectively. The antibacterial effect of cloves may be explained by the action of eugenol as many investigators have reported (Ağaoğlu et al., 2007). Dorman & Deans (2000) also showed the antimicrobial effect of eugenol individually on *E. coli* and *S. aureus*. The ten different plant essential oils were tested to detect their antimicrobial effects on *E. coli* O157:H7 and the most effective result obtained with clove (Moreira et al., 2005). In our study, oregano is the most effective aganist *E. coli* O157:H7 with MIC value of ≥0.0625mg/ml, followed by cumin and clove with MIC values of ≥0.125 and ≥0.25mg/ml, respectively. Nanasombat and Lohasupthawee (2005) clove extract and essential oil had the inhibitory effect on the growth all bacterial strains tested (20 serotypes *Salmonella* and 5 species of other enterobacteria) among all crude ethanolic extracts and essential oils of 14 spices. Results showed that the MIC value of essential oil of clove was determined as ≥0.25mg/ml for *E.coli* O157:H7 and ≥0.5 mg/ml for MRSA and *S. aureus* and ≥1mg/ml for *E.coli* and *S. typhimurium* respectively.

The results of the present study indicated that oregano exhibited the strongest antibacterial activity (especially to *E.coli* O157:H7), followed by cumin and clove respectively. The results confirm the potential use of oregano, cumin and clove essential oils represent promising natural antimicrobial agents in food industry and animal nutrition against common foodborne and animal pathogens. The antibacterial activity of mixtures of the oregano, cumin and clove should be tested, and the underlying modes of action remain to be explained in future investigations. Also, *in vivo* studies are necessary to carry out which might yield significant information as to whether these essential oils in individual or combinations can be used as replacement to antibiotics in animal nutrition.

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