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CHEMICAL COMPONENTS, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF GARLIC, CUMIN AND PARSLEY VOLATILE OILS

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ABSTRACT: **In this study, the chemical components of garlic, cumin and parsley volatile oils as well as the antioxidant and antimicrobial activities of these volatile oils and their mixtures were determined. Results indicated that garlic, parsley and cumin volatile oils contained eleven, thirteen and fourteen components which represented 96.47, 97.79 and 95.41%, respectively. Diallyl disulfide, diallyl trisulfide and allyl methyl trisulfide were the most abundant chemical compounds in garlic volatile oil, while myristicin was the major component of parsley volatile oil followed by α-pinene and γ-terpinene. However, cumin aldehyde, γ-terpinene, β-pinene and cuminic alcohol were the most predominant chemical compounds in cumin volatile oil. The highest antioxidant and antimicrobial activities were recorded for garlic and cumin volatile oils mixture (GC) followed by garlic and parsley volatile oils mixture (GP), while the lowest antioxidant and antimicrobial activities were found for parsley volatile oil. Finally, GC and GP volatile oils mixtures could be used in food industry as alternatives to synthetic antioxidants and antimicrobial substances.**

Key word: **Chemical component, antioxidant, antimicrobial, volatile oils, cumin, garlic, parsley,**

INTRODUCTION

Lipid oxidation and microbial alteration are the main reasons for the deterioration of quality, safety of food and shelf life of food. Lipid oxidation in foods generates end-products which may be harmful to human health. Compounds such as malondialdehyde and cholesterol oxidation products are reported to have cytotoxic and genotoxic potential and have been linked to the promotion of atherosclerosis, cardiovascular disease, and cancer (Kanner, 2007). Also, the presence of pathogens in food might be responsible for serious diseases leading to death (Mnayer *et al.,* 2014). Traditional preservation methods such as cold storage and freezing do not completely inhibit microbial and chemical reactions in food. Application of antioxidants and antimicrobial agents in food help to reduce lipid oxidation and microbial growth. Due to modern trends consumers adopt towards the consumption of minimally processed foodstuffs containing no chemical preservatives, lightly preserved food products with natural additives have become popular (Khalafalla *et al.,* 2015).

Spices and herbs were used as green materials, plant extracts; essential oils (EOs) and powders for enhancing the storage life of foods by preventing rancidity through their antioxidant activity and inhibiting microorganisms as well as foodborne pathogenic bacteria through their bactericidal activity (Singh *et al.,* 2005). Essential oils (EOs) contain a mixture of compounds, (terpenes, alcohols, acetones, phenols, acids, aldehydes, and esters) which are mainly used as food flavorings or functional components in pharmaceuticals (Corbo *et al.,* 2009)

Parsley (*Petroselinum crispum* Mill.) Fuss (Apiaceae), is a widely cultivated and used herb. Myristicin, β-phellandrene, 1,3,8-*p*-menthatriene, α- pinene and β-myrcene were the major compounds in leaf parsley volatile oil (Nemeń et *al.*, 2018). Also, cumin (*Cuminum cyminum*), belonging to the family Umbelliferae, is generally used as a spice for foods in the form of powder or volatile oil for flavoring different food preparations (Kafie *et al.,* 2002). Cumin aldehyde, γ-terpinene, β-pinene, *p*-cymene, α -terpinene and 2-caren-10-al were the main components of cumin volatile oil (Vieira *et al.*, 2019 and Ali and Jumma, 2019). Moreover, Garlic (*Allium sativum*) is one of the oldest known vegetables, and it is extensively used as a food flavoring. Garlic has been used as a medicinal plant for a variety of ailments including respiratory disorders, diabetes, asthma, rheumatism, headache, bites, intestinal worms and tumors (Corzo-Martínez *et al.,* 2007). Diallyl trisulfide, diallyl disulfide, allyl methyl trisulfide, allyl methyl disulfide and diallyl sulfide were the main components of garlic volatile oil (Satyal *et al.*, 2017 and Süfer and Bozok, 2019). Therefore, the objective of this study was to determine the chemical components of garlic, cumin and parsley volatile oils as well as to evaluate the antioxidant and antimicrobial activities of each volatile oil and their mixtures.

MATERIALS AND METHODS

Materials:

Spices volatile oils

Three different spices volatile oils (garlic, parsley and cumin) were purchased from Kato Flavors & Fragrance Company at Shooting Club Street, Mohandessn, Giza, Egypt.

Chemicals

The 2, 2-dipheny-l-1picrylhydrazyl (DPPH) and methanol were obtained from Sigma -Aldrich Corp, Cairo, Egypt.

Microbial cultures

Four bacterial strains representing gram- negative (*Escherichia coli* and *Salmonella typhimurium*), gram positive bacteria (*Staphylococcus aureus* and *Listeria monocytogenes*), in addition two yeast strains (*Candida albicans* and *Saccharomyces cerevisiae*), and two mold strains (*Aspergillus niger,* and *Aspergillus flavus*) were obtained from Department of Chemistry of Natural and Microbial product, National Research Center, Cairo, Egypt. These microorganisms were checked for their purity and identity and finally recultivated to obtain active cultures.

Methods:

Preparation of volatile oils mixtures

Two volatile oils mixtures (GC and GP) were prepared by mixing equal volume (1: 1) from garlic and cumin volatile oils to obtain mixture (GC) and also equal volume from garlic and parsley to obtain mixture (GP).

Separation and identification of volatile oils components by gas chromatographic (GC)

The essential oils were analyzed and performed using Gas Chromatography analysis instrument (Hewlett-Packard 5890 A series II) equipped with flame ionization detector (FID) and a carbowax fused silica column (50 m length, 0.25 mm width, film thickness 0.32 μm). The oven temperature was programmed from

60ºC to 230ºC at the rate of 3ºC/min, Nitrogen (1 ml/min) was used as carrier gas; split ratio was 1: 100; the temperature of injection port and detector were 250ºC and 280ºC, respectively. Percentages of peak area were calculated with Hewlett Packard 3396 integrator (Abd El-Qader, 2004).

Antioxidant activity of volatile oils and their mixtures:

Antioxidant activity of volatile oils (garlic, cumin and parsley) and their mixtures (GC and GP) was determined using the 2,2-dipheny-l-1picrylhydrazyl (DPPH) radical scavenging method according to the procedure described by Sreejayan and Rao (1996).

Antimicrobial activity of volatile oils and their mixtures:

The effect of different volatile oils (garlic, cumin and parsley) and their mixtures (GC and GP) on bacteria, yeasts and mold growth was determined using the disc diffusion method according to Karimi *et al* (2014), by measuring the diameter of inhibition zone (mm).

RESULTS AND DISCUSSION

Chemical components of garlic volatile oil:

From the results in Table (1) it could be indicated that there were eleven components fractionated and identified from garlic volatile oil by GC technique. The identified components represented 96.47% from the garlic volatile oil. Diallyl disulfide, diallyl trisulfide and allyl methyl trisulfide were the most abundant chemical compounds in garlic volatile oil which represented 70.38% of the total identified chemical compounds. Diallyl disulfide (28.46% of the total chemical compounds) was the major component of garlic volatile oil followed by diallyl trisulfide (25.54%). However, allyl methyl trisulfide, (16.38%) was the lowest one among the most abundant chemical compounds in garlic volatile oil. These results are in agreement with those obtained by Romeilah *et al.* (2010) reported that diallyl disulfide (25.2%), allyl methyl trisulfide (23.8%) and diallyl trisulfide (21.1%) were the major compounds in Egyptian garlic essential oil.

Furthermore, diallyl sulfide (7.74%), allyl methyl sulfide (6.60%), dimethyl disulfide (4.57%) and diallyl tetrasulfide (2.51%) were present in moderate amount of garlic volatile oil which represented 21.42% of the identified chemical compounds. Relatively similar diallyl sulfide percentage (7.20%) was reported by Douiri *et al*. (2013).

Also, 2-vinyl-1-1.3-dithiane (1.89%), dimethyl trisulfide (1.43%) and dimethyl disulfide (1.05%) were present in small amount of garlic volatile oil which represented (4.37%) of the total chemical compounds. Moreover, garlic volatile oil also contained allyl propyl trisulfide in trace amount which represented (0.30 %) of the total chemical compounds. These results are on line with Satyal *et al.* (2017) who found that garlic volatile oils which obtained by different distillation methods contained 1.5 to 2.0% diallyl tetrasulfide, 1.8 to 2.50% 2-vinyl-1.3-dithiane, 1.30 to 2.90% dimethyl trisulfide, 0.4 to 1.40% dimethyl disulfide and 0.2 to 0.3% allyl propyl trisulfide.

Generally, the differences in results of chemical composition may be due to various factors like genetic variation, geographical location, climatic conditions and pretreatments (drying etc.) as well as extraction techniques (Süfer and Bozok, 2019).

Table (1): Chemical components of garlic volatile oil fractionated by GC technique.

Compounds	RT (min)	RRT	Area %
Di methyl disulfide	2.458	0.132	1.05
Di allyl tetrasulfide	3.375	0.182	2.51
Di methyl disulfide	3.942	0.212	4.57
Di allyl sulfide	7.060	0.380	7.74
Allyl methyl sulfide	10.934	0.589	6.60
2-vinyl-1-1.3 dithain	14.491	0.781	1.89
Di methyl trisulfide	17.664	0.952	1.43
Di allyl diasulfide	18.556	1.00	28.46
Allyl methyl trisulfide	22.961	1.211	16.38
Di allyl trisulfide	30.052	1.619	25.54
Allyl propyl trisulfide	32.471	1.749	0.30
Total known			96.47
Total unknown			3.53

The retention time of diallyl diasulfide, 18.556min, was taken as standard retention time, its relative retention time is equal one.
RT: retention time. RRT: relative retention time.

Chemical components of parsley volatile oil:

The fractionated and identified chemical components of parsley volatile oil were presented in Table (2). From these data it could be noticed that, thirteen compounds were identified from parsley volatile oil. The identified components represented (97.79%) from the parsley volatile oil. Myristicin, α-pinene and γ-Terpinene were the most abundant chemical compounds in parsley volatile oil which represented 47.38% of the total identified chemical compounds. Myristicin (22.08%) was the major component of parsley volatile oil followed by α-pinene (13.25%). However, γ-Terpinene (12.05%) was the lowest one among the most permanent chemical compounds in parsley volatile oil. Similar results were obtained by Al-Saqqa *et al.* (2018) revealed that the main constituents of the parsley seeds essential oil were myristicin (34.18%) and α- pinene (16.14%).

Also, parsley volatile oil contained β-phellanderene (8.21%), camphene (7.51%), α-phellandrene (6.75%), *p*-cymene (5.79%), meristic acid (5.87%), myrcene (4.64%), apiole (3.91%), terpinolene (3.43%), limonene (3.17) and *β*-pinene (1.19%). Sabry *et al.* (2013) who found that the main constituents essential oil of five parsley cultivars grown in Egypt were myristicin (8.96 to 62.35 %), bisabolene (0.17 to 31.02 %), carotol (0.25 to 13.12%), β-phellandrene (3.24 to 13. 69 %), 1,3,8-p-menthatriene (3.87 to 15.95 %), α-terpinolene (0.25 to 5.52 %), apiole (0.14 to 6.80 %). Also, Nemeń et *al.* (2018) reported that main components of the essential oils obtained from parsley leaves were 1,3,8-menthatriene (22.8-50.9%), myristicin (12.8-36.8%), β-phellandrene (14.1-29.0%), and β-myrcene (1.4-12.7%).

Table (2): Chemical components of parsley volatile oil fractioned by GC technique.

Compounds	RT (min)	RRT	Area %
α-pinene	4.763	0.116	13.25
Camphene	6.235	0.152	7.51
limonene	7.340	0.179	3.17
γ-Terpinene	8.473	0.206	12.05
β-pinene	10.469	0.255	1.13
α-phellandrene	14.912	0.363	6.75
β-phellanderene	16.602	0.406	8.21
Terpinolene	17.387	0.424	3.43
Myrcene	18.745	0.457	4.64
p-cymene	20.882	0.509	5.79
Myristicin	41.033	1.00	22.08
Apiole	47.027	1.146	3.91
Meristic acid	53.682	1.308	5.87
Total known			97.79
Total unknown			2.21

The retention time of myristicin, 41.033 min, was taken as standard retention time, its relative retention time is equal one.
RT: retention time. RRT: relative retention time.

Chemical components of cumin volatile oil:

From the results presented in Table (3), it could be noticed that fourteen volatile components were fractionated and identified from cumin volatile oil. The identified components represented (95.41%) from the cumin volatile oil. Cumin aldehyde, γ-terpinene, β-pinene and cuminic alcohol were the most abundant chemical compounds in cumin volatile oil which represented 81.54% of the total identified chemical compounds. Cumin aldehyde (35.78% of the total chemical compounds) was the highest chemical compound of cumin volatile oil. However, cuminic alcohol (10.08% of the total chemical compounds) was the lowest one among the most abundant chemical compounds in cumin volatile oil. These results are in agreement with Vieira *et al.* (2019) who found that the chemical main components of cumin were cumin aldehyde (32.66%), γ-terpinene (19.87%) and β-pinene (15.22%). Also, Moawad *et al.* (2015) reported that cumin aldehyde considered the major compound followed by gamma terpinene.

Also, *p*-cymene (5.29%), p-meth-3-en-7-ol (3.25%), *α*-phellandrene (1.41%), *β*-myrecen (1.17%) and *α*-pinene (1.13%) were found in moderate amount of cumin volatile oil which represented 12.25% of the total identified chemical compounds. These results are on line with those obtained by Beis *et al.* (2000) who reported that cumin essential oil contained 5.25% p-cymene, 2.91% p-meth-3-en-7-ol and 1.60% α-phellandrene, but higher than Ali and Jumma (2019) who mentioned that cumin essential oil contained 0.19% α-phellandrene, 0.72% *β*-myrecen and 0.42% *α*-pinene.

Table (3): Chemical components of cumin volatile oil fractioned by GC technique.

Compounds	RT (min)	RRT	Area %
α-pinene	3.577	0.416	1.13
Linalool	3.880	0.159	0.08
β-pinene	4.324	0.177	14.91
α-phellandrene	4.839	0.198	1.41
Carene	5.414	0.221	0.09
β-myrecen	5.587	0.228	1.17
γ-Terpinene	6.369	0.260	20.77
p-cymene	6.802	0.278	5.29
D- limonene	10.109	0.413	0.82
Cuminic alcohol	12.179	0.498	10.08
p-meth-3-en-7-ol	13.814	0.565	3.25
Cumin aldehyde	24.461	1.00	35.78
Cumic acid	32.779	1.340	0.52
Carotol	34.361	1.405	0.11
Total known			95.41
Total unknown			4.59

The retention time of cumin aldehyde, 24.461min, was taken as standard retention time, its relative retention time is equal one.

RT: retention time. RRT: relative retention time.

Moreover, cumin volatile oil contained D- limonene (0.82%), cumic acid (0.52%), carotol (0.11%), careen (0.09%) and linalool (0.08%) in trace amounts which represented 1.62 % of the total chemical compounds. Similar results were obtained by Ali and Jumma (2019) reported that cumin volatile oil contained trace amounts of D- limonene, cumic acid, carotol, careen and linalool.

Antioxidant activity of volatile oils and their mixtures:

Antioxidant activity of different volatile oils (garlic, cumin and parsley) and their mixtures (GC and GC) were presented in Table (4). Antioxidant activity of different volatile oils and their mixtures was significantly ($p \leq 0.05$) affected by the type of volatile oils. The highest ($p \leq 0.05$) antioxidant activity (93.63 %) was recorded for GC mixture followed by GP mixture (88.90%). On the other hand, the lowest ($p \leq 0.05$) antioxidant activity (79.56%) was observed for parsley volatile oil. Antioxidant activity of volatile oils mixtures was higher than that of garlic, cumin and parsley volatile oils individually. This is might be due to volatile oils mixing have led to synergistic or potentiating effect (Burt, 2004).

Also, from the same table, it could be noticed that, garlic volatile oil had higher ($p \leq 0.05$) antioxidant activity (86.97%) compared to cumin volatile oil (82.43%) and parsley volatile oil (79.56%). These results are on line with those obtained by Khan *et al*. (2017).

Table (4): Antioxidant activity of volatile oils and their mixtures

Volatile oils	DPPH free radical scavenging (%)
Garlic	$86.97^{c}\pm0.32$
Cumin	$82.48^{d}\pm0.37$
Parsley	$79.56^{e}\pm0.51$
Garlic + cumin	$93.63^{a}\pm0.43$
Garlic + parsley	$88.90^{b}\pm0.49$
LSD at 0.05%	0.57

Means in the same column with different letters are significantly different ($p \leq 0.05$)

The antioxidant activity of garlic essential oil might be due to allicin derivative products such as diallyl disulfide and diallyl trisulfide found in garlic essential oil which have good antioxidant activities (Mnayer *et al.,* 2014). Also, the antioxidant activity of cumin essential oil might be due to cumin aldehyde, γ-Terpinene and *p*-cymene which have antioxidant activity (Bag and Chattopadhyay, 2015). However, the antioxidant activity of parsley essential oil might be due to apiol which is described as the major contributor to the antioxidant activity of oil followed by myristicin which exhibited moderate activity (Zhang *et al.*, 2006).

Antimicrobial activity of volatile oils and their mixture:

The antimicrobial activity of volatile oils and their mixtures was affected by the type of volatile oils and microbial strains as shown in Table (5). From these results it could be noticed that the highest antimicrobial activity was recorded for garlic and cumin volatile oils mixture (GC), with inhibition zones ranged from 35.0 to 55.0 mm followed by garlic and parsley volatile oils mixture (GP) with inhibition zones ranged from 34.0 to 51.0 mm. The inhibitory activity of volatile oils mixtures was higher than that of each volatile oil individually. This is might be attributed to the synergistic effect of volatile oils (Bassolé and Juliani, 2012).

Also, data cleared that garlic volatile oil had higher inhibition zones for all tested microbial strains compared to cumin and parsley volatile oil. The antimicrobial activity of garlic volatile oil might be attributed to its rich in sulfide compounds such as diallyl disulfide (28.46%), diallyl trisulfide (25.54%), allyl methyl trisulfide (16.38%), diallyl sulfide (7.74%), allyl methyl sulfide (6.6%), dimethyl disulfide (4.57%), diallyl tetrasulfide (2.51) and dimethyl disulfide (1.05%) (Corzo-Martinez *et al.*, 2007). These results were in accordance with Babu *et al.* (2011) who reported that garlic volatile oil had antibacterial activity against gram-negative and gram-positive bacteria.

The efficiency of cumin volatile oil against all microbial strains is related to its components such as cumin aldehyde (35.78 %) and other chemical compounds such as γ-terpinene (20.77%), *β*-pinene (14.91%) and cuminic alcohol (10.08%) and *p*-cymene (5.79%) (Johri, 2011). Moreover, the antimicrobial effect of parsley might be attributed to its chemical composition such as myristicin (22.08 %) and other chemical compounds such as α-pinene, γ-terpinene, β-phellanderene, camphene, α-phellandrene and *p*-cymene (Vokk *et al.,* 2011). These results are in agreement with those obtained by Karimi *et al.* (2014) who reported that the essential oils from seed and leaves of parsley have anti-bacterial effect.

Table (5): Diameter of inhibition zones (mm) of volatile oils and their mixtures against some selected microorganisms.

Microorganisms	Diameter of inhibition zones (mm) of different volatile oils				
	Garlic	Cumin	Parsley	GC	GP
<u>Gram negative bacteria</u>					
Escherichia coli	37.0	34.0	32.0	40.0	38.0
Salmonella typhimurium	32.0	30.0	28.0	35.0	34.0
<u>Gram positive bacteria</u>					
Staphylococcus aureus	40.0	37.0	35.0	45.0	42.0
Listeria monocytogenes	37.0	35.0	31.0	41.0	38.0
<u>Yeasts</u>					
Saccharomyces cerevisiae	47.0	45.0	41.0	52.0	49.0
Candida albicans	42.0	41.0	38.0	47.0	43.0
<u>Molds</u>					
Aspergillus niger	50.0	47.0	43.0	55.0	51.0
Aspergillus flavus	45.0	42.0	40.0	47.0	44.0

GC: Mixture of garlic and cumin volatile oils (1:1, v: v)

GP : Mixture of garlic and parsley volatile oils (1:1, v: v)

Moreover, the antibacterial effects of different volatile oils and their mixtures were more pronounced on gram positive bacteria than gram negative bacteria. These results are in agreement with Abd El-Qader, (2014) who showed that gram negative bacteria had slightly higher resistance to essential oils than gram positive bacteria. This might be due to the variation in cell wall structures. More specifically, gram negative bacteria have an outer membrane that is composed of high density lipopolysaccharides that serves as a barrier to many environmental substances including antibiotics (Palombo and Semple, 2001).

Also, from the same results, it could be noticed that all mold strains were more sensitive to all tested volatile oils and their mixtures than bacteria and yeast strains. These resulted were similar to those obtained by Badei *et al*. (2002) and Abd El-Qader (2014). The highest sensitive mold strain was *Aspergillus niger* with inhibition zones ranged from 43.0 to 55.0 mm. While, the highest sensitive yeast strain was *Saccharomyces cerevisiae* inhibition zones ranged from 41.0 to 52.0 mm.

Finally, volatile oils and their mixtures could be arranged in descending order according to their antimicrobial potency as follows: garlic and cumin mixture (GC) > garlic and parsley mixture (GC) > garlic > cumin > parsley volatile oil. Also, microbial strains types could be arranged descendingly according to their resistance to volatile oils as follows: Gram negative bacteria > Gram positive bacteria > yeasts > molds.

Conclusion

From the above results, it could be concluded that GC and GP volatile oils mixtures had higher antioxidant and antimicrobial activities than each volatile oil individually. The GC and GP volatile oils mixtures could be used in food industry as alternatives to synthetic antioxidants and antimicrobial substances.

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المكونات الكيميائية و الأنشطة المضادة للأكسدة و الميكروبات للزيوت الطيارة للثوم و الكمون و البقدونس

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الملخص العربى

فى هذه الدراسة تم تقدير المكونات الكيميائية للزيوت الطيارة للثوم و الكمون و البقدونس وكذلك الأنشطة المضادة للأكسدة و الميكروبات لهذه الزيوت الطيارة ومخاليطها. أوضحت النتائج أن الزيوت الطيارة للثوم و الكمون و البقدونس احتوت على عدد 11 , 13 , 14 مركب و كانت تمثل96.74 و و97.79 و 95.41٪ على التوالى. وكانت مركبات الداي أليل داي سالفيد , الداي اليل تراي سالفيد وأليل الميثيل تراي سالفيد أكثر المركبات الكيميائية توافرا في زيت الثوم الطيار، بينما كان الميريستسين هو المكون الرئيسي لزيت البقدونس الطيارمتبوعًا بـ ألفا بينين و جاما تربينين .كذلك فإن ألكيومن الدهيد، جاما تربينين ، بيتا بينين والكيومن الكحول كانت هي المركبات الكيميائية الأكثر شيوعًا في زيت الكمون الطيار. كما أشارت النتائج أن أعلى أنشطة مضادة للأكسدة و مضادة للميكروبات تم تسجيلها لمخلوط الزيوت الطيارة للثوم و الكمون يليه مخلوط الزيوت الطيارة للثوم و البقدونس بينما أقل أنشطة مضادة للأكسدة و الميكروبات وجدت لزيت البقدونس الطيار وأخيرا ، يمكن استخدام مخلوط الزيوت الطيارة للثوم و الكمون ومخلوط الزيوت الطيارة للثوم والبقدونس في تصنيع الأغذية كبدائل لمضادات الأكسدة ومضادات الميكروبات المخلقة كيميائيا.

الكلمات الدالة: المركبات الكيميائية ، النشاط المضاد للأكسدة ، النشاط المضاد للميكروبات ، الزيوت الطيارة ، الكمون ، الثوم ، البقدونس

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