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Antibacterial activity of two *Phlomis* essential oils against food pathogens

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Abstract

Phlomis species from the Lamiaceae family are widely distributed in Turkey. In this study, the essential oils of *Phlomis russeliana* (Sims.) Bentham and *Phlomis grandiflora* H.S. Thompson var. *grandiflora* collected from North and Southern parts of Turkey, were obtained by hydrodistillation of the aerial parts. The essential oils were subsequently analysed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC/MS). The major constituents of *P. russeliana* essential oil were identified as sesquiterpenes β-caryophyllene (23%), germacrene-D (15%), and caryophyllene oxide (8%). Analysis of *P. grandiflora* var. *grandiflora* oil also showed oxygenated sesquiterpenes such as β-eudesmol (42%) and α-eudesmol (16%) as major constituents.

Furthermore, essential oils were tested *in vitro* against common food borne bacteria such as *Aeromonas hydrophila*, *Bacillus cereus*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Yersinia enterocolitica*, and the anaerobic pathogen *Clostridium perfringens* using the micro-broth dilution assay. When compared with antimicrobial standards weak to moderate (125 to >1000 μg/ml) minimum inhibitory concentrations (MIC) were observed. The results show that *Phlomis* essential oils might be an alternative to conventional antimicrobials in various foods.

Keywords: *Phlomis* sp.; Essential oil; Antibacterial activity

1. Introduction

Plant extracts and essential oils constitute a natural source of antimicrobial mixtures or pure compounds for centuries. Essential oils and purified components are used as natural antimicrobials in food systems, as well as to prevent the growth of food borne bacteria and molds resulting in extension of the shelf life of processed foods (Burt, 2004; Cowan, 1999; Kalemba & Kunicka, 2003). Fruits, vegetables, grains and food constituents can be contaminated by various microorganisms and their hazardous toxic metabolites. Enterotoxins produced by *Escherichia coli*, *Staphylococcus pyogenes, Salmonella, Yersinia* and *Clostridium* species are responsible for toxicity in the intestinal tract causing vomiting, diarrhea, etc. Moreover, microorganisms are also associated with food spoilage causing economical loss (D'Mello, 2003; Harris, 1988; Rocourt, Moy, Vierk, & Schlundt, 2003; Smith-Palmer, Stewart, & Fyfe, 1998). Research into more effective antimicrobial food agents in particular natural antimicrobials such as essential oils received attention in the last decade (Burt, 2004; Cowan, 1999; Draughon, 2004).

Lamiaceae is an important economic plant source of essential oils and the genus *Phlomis* L. has more than 100 species distributed in Euro-Asia and North Africa (Azizian & Moore, 1982). It is recently documented that the 52 taxa including 6 varieties, 12 natural hybrids and 34 endemic

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taxa are growing in Turkey (Demirci, Baser, & Dadandi, 2006).

Both *Phlomis russeliana* and *Phlomis grandiflora* var. *grandiflora* species used in this study are endemic among the Turkish Flora and are characterized by yellow bilabiate corolla (Dadandi, 2002; Huber-Morath, 1982). Baytop (1999) reported that Turkish *Phlomis* species are used as herbal teas (Dağçayı), as tonic, carminative, appetizer and stimulants in the folk medicine and recognized by local names as "Ballıkotu, Calba, Çalba or Şalba". Some medicinal usages of *P. grandiflora* as treatment for stomach disorders were documented by Gurbuz, Ustun, Yesilada, Sezik, and Kutsal (2003) and Ozcelik (1987).

Phytochemical investigations of *Phlomis* species were the subject of several studies and consequently, iridoid and phenylpropanoid glycosides were isolated and reported from the aerial parts of *P. grandiflora* var. *fimbrilligera* and *P. grandiflora* var. *grandiflora* (Ersoz, Saracoglu, Harput, Calis, & Donmez, 2002; Takeda et al., 1999). Phytochemical analyses of *P. russeliana* revealed the presence of various phenylethanoid glycosides (Kirmizibekmez et al., 2005).

To the best of our knowledge this is the first report on the detailed analysis of essential oils in *P. russeliana* and identification of its sesquiterpenes. The essential oil composition of various *P. grandiflora* var. *grandiflora* were recently reported by Celik, Gokturk, Flamini, Cioni, and Morelli (2005).

In this present study, it was aimed to investigate the application and usage of essential oils, in particular *Phlomis* sp. essential oils, against food borne pathogenic bacteria. A selection of common Gram (+) and Gram (−) pathogens such as *Aeromonas hydrophila*, *Bacillus cereus*, *E. coli* O157:H7, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Yersinia enterocolitica* and *Clostridium perfringens* were screened. The *in vitro* micro-broth dilution assay was used to test the growth inhibitory activity of *Phlomis* essential oils and compare the minimum inhibitory concentrations (MIC) versus commercial antimicrobial standards.

2. Materials and methods

2.1. General

All chemicals, solvents, media, and standards were purchased from Sigma/Aldrich (Taufkirchen, Germany) and were reagent grade (purity >99%), if not indicated otherwise.

2.2. Plant material

Aerial parts (leaves, flowers and stems) of the flowering plants were collected from North and Southern regions of Turkey by the authors. Voucher specimens were deposited at the Herbarium of Erciyes University, Faculty of Science and Letters. Detailed information on the plant materials used are given in Table 1.

Table 1
Information on the plant material and essential oil yields

Phlomis sp.	Collection site	Altitude (m)	Collection period	Oil yield[a] (%)	MYD[b]
P. russeliana	Bolu: Abant lake	1220	12.8.1999	0.10	1718
P. grandiflora var. *grandiflora*	Antalya: Termessos antique city	800	05.7.2003	0.19	1681

[a] Essential oil yields are given on moisture free basis.
[b] MYD: Herbarium code of the collector.

2.3. Isolation of the essential oils

Plant materials were dried in the shade at room temperature and were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus. The percentage (%) yields were calculated on dry weight basis as given in Table 1.

2.4. Gas chromatography (GC) and gas chromatography–mass spectrometry (GC/MS)

Phlomis essential oils were analysed by GC using a Hewlett-Packard 6890 (Sem Ltd., Istanbul, Turkey) system and an HP Innowax FSC column (60 m × 0.25 mm ∅, with 0.25 μm film thickness) was used with nitrogen at 1 ml/min. Initial oven temperature was 60 °C for 10 min, and increased at 4 °C/min to 220 °C, then remained constant at 220 °C for 10 min and increased at 1 °C/min to 240 °C. Injector temperature was set at 250 °C. Percentage composition of the individual components were obtained from electronic integration using flame ionization detection (FID) at 250 °C. *n*-Alkanes were used as reference points in the calculation of relative retention indices (RRI).

GC/MS analysis was performed with a Hewlett-Packard GCD (Sem Ltd., Istanbul, Turkey), system and Innowax FSC column (60 m × 0.25 mm, 0.25 μm film thickness) was used with helium. GC oven temperature conditions were as described above, split flow was adjusted at 50 ml/min, the injector temperature was at 250 °C. Mass spectra were recorded at 70 eV. Mass range was from *m*/*z* 35 to 425.

2.5. Identification of components

Identification of the essential oil components were carried out by comparison of individual relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley and MassFinder 2.1) (Joulain, König, & Hochmuth, 2001; McLafferty & Stauffer, 1989) and in-house "Baser Library of Essential Oil Constituents" libraries made up

Table 2
The essential oil composition of *Phlomis russeliana* (Pr) and *P. grandiflora* var. *grandiflora* (Pg)

RRI	Compound	Pr	Pg	Identification method
1280	*p*-Cymene	0.1	0.1	a,b
1400	Tetradecane	0.1	nd	a,b
1452	1-Octen-3-ol	nd	0.1	b
1466	α-Cubebene	0.4	nd	b
1497	α-Copaene	0.4	0.1	b
1499	α-Campholene aldehyde	nd	0.1	b
1506	Decanal	nd	0.1	a,b
1528	α-Bourbonene	0.1	tr	b
1535	β-Bourbonene	1.3	0.2	b
1549	β-Cubebene	0.2	nd	b
1553	Linalool	0.3	1.8	a,b
1568	*trans*-α-Bergometene	nd	0.1	b
1577	α-Cedrene	nd	1.5	b
1589	Isocaryophyllene	1.0	nd	a,b
1589	β-Ylangene	0.7	nd	b
1594	*trans*-β-Bergamotene	nd	0.4	b
1612	β-Caryophyllene	22.6	0.6	a,b
1613	β-Cedrene	nd	0.4	b
1627	(*Z,E*)-2,5-Epoxy-6,8-megastigmadiene	nd	0.1	b
1638	β-Cyclocitral	nd	0.1	b
1650	γ-Elemene	0.5	nd	b
1654	(*E,E*)-2,5-Epoxy-6,8-megastigmadiene	nd	0.4	b
1659	γ-Gurjunene	0.2	nd	b
1668	(*Z*)-β-Farnesene	0.1	nd	b
1674	Muurola-4,11-diene	nd	0.9	b
1683	*trans*-Verbenol	nd	0.8	a,b
1687	α-Humulene	1.5	0.1	a,b
1704	γ-Muurolene	0.5	nd	b
1706	α-Terpineol	tr	0.6	a,b
1715	Geranyl formate	nd	0.1	b
1718	2-Dodecane	0.1	nd	b
1722	Dodecanal	0.1	nd	b
1725	Verbenone	nd	0.2	b
1726	Germacrene-D	15.1	nd	a,b
1740	α-Muurolene	0.4	nd	b
1742	β-Selinene	nd	0.8	b
1743	α-Cadinene	0.1	nd	b
1744	α-Selinene	nd	0.2	b
1747	Sesquicineole	nd	0.1	b
1755	Bicyclogermacrene	0.5	nd	b
1773	δ-Cadinene	0.6	nd	b
1776	γ-Cadinene	0.2	nd	b
1786	*ar*-Curcumene	nd	2.8	b
1800	Octadecane	0.1	nd	a,b
1808	Nerol	nd	0.2	a,b
1815	2-Tridecanone	0.2	nd	b
1827	(*E,E*)-2,4-Decadienal	0.1	nd	b
1830	β-Damascone	nd	0.1	b
1838	(*E*)-β-Damascenone	0.1	nd	b
1845	*trans*-Carveol	nd	0.1	a,b
1854	Germacrene-B	0.3	nd	b
1857	Geraniol	nd	0.4	a,b
1868	(*E*)-Geranyl acetone	0.1	0.1	b
1878	2-Tetradecanone	0.2	nd	b
1900	*epi*-Cubebol	0.1	nd	b
1916	α-Agarofuran	nd	0.4	b
1933	Tetradecanal	0.1	nd	b
1957	Cubebol	0.4	nd	b
1958	(*E*)-β-Ionone	nd	0.1	b
1984	γ-Calacorene	0.1	nd	b
2001	Isocaryophyllene oxide	1.0	0.4	a,b
2008	Caryophyllene oxide	8.1	3.2	a,b
2036	2-Pentadecanone	0.2	nd	b
2037	Salvial-4(14)-en-1-one	0.1	nd	b
2050	(*E*)-Nerolidol	0.3	0.2	a,b
2057	Ledol	0.3	nd	b
2071	Humulene epoxide-II	0.5	0.2	b
2096	Elemol	nd	0.4	b
2100	Heneicosane	0.4	nd	b
2104	Viridiflorol	1.2	nd	b
2127	10-*epi*-γ-Eudesmol	nd	0.9	b
2131	Hexahydrofarnesyl acetone	3.9	0.4	b
2144	Rosifoliol	nd	0.3	b
2144	Spathulenol	0.5	nd	b
2148	(*Z*)-3-Hexen-1-yl benzoate	nd	0.1	b
2157	5-*epi*-7-*epi*-α-Eudesmol	nd	0.2	b
2170	β-Bisabolol	nd	0.2	b
2179	Nor-Copaonone	0.2	nd	b
2179	3,4-Dimethyl-5-pentylidene-2(5H)-furanone	nd	0.2	b
2185	γ-Eudesmol	nd	0.8	b
2187	T-Cadinol	0.2	nd	b
2200	Docosane	0.2	nd	a,b
2204	Eremoligenol	nd	0.3	b
2209	T-Muurolol	0.6	nd	b
2210	Hinesol	nd	0.3	b
2214	*ar*-Turmerol	0.1	2.7	b
2219	δ-Cadinol	0.3	nd	b
2250	α-Eudesmol	0.4	16.1	b
2255	α-Cadinol	0.5	nd	b
2257	β-Eudesmol	1.0	42.0	b
2273	Selin-11-en-4α-ol	0.4	nd	b
2300	Tricosane	4.1	nd	a,b
2300	γ-Undecalactone	nd	0.8	b
2316	Caryophylladienol I	0.5	nd	b
2324	Caryophylladienol II	0.8	nd	b
2384	Farnesyl acetone	0.3	nd	b
2389	Caryophyllenol I	0.3	nd	b
2392	Caryophyllenol II	1.4	nd	b
2400	Tetracosane	0.4	nd	a,b
2500	Pentacosane	1.3	nd	a,b
2600	Hexacosane	0.4	nd	a,b
2607	1-Octadecanol	0.1	nd	b
2622	Phytol	0.6	nd	b
2700	Heptacosane	1.0	nd	a,b
2800	Octacosane	0.1	nd	a,b
2822	Pentadecanoic acid	nd	0.1	a,b
2900	Nonacosane	1.3	nd	a,b
2931	Hexadecanoic acid	1.4	0.5	a,b
	Monoterpene hydrocarbons	0.1	0.1	
	Oxygenated monoterpenes	0.3	4.2	
	Sesquiterpene hydrocarbons	48.8	8.3	
	Oxygenated sesquiterpenes	17.5	68.7	
	Others	16.6	3.1	
	Total	83.3	84.4	

RRI: relative retention indices calculated against *n*-alkanes on the HP Innowax column.
% calculated from flame ionization detector (FID) data.
nd: not detected.
a, identification based on retention times of genuine compounds on the HP Innowax column.
b, tentatively identified on the basis of computer matching of mass spectra of peaks from the Wiley and MassFinder libraries (see references).

by genuine compounds and components of known oils, as well as MS literature data (Jennings & Shibamoto, 1980; Joulain & König, 1998) were also used for the identification (Table 2).

2.6. Antibacterial assay

2.6.1. Microorganisms

All microorganisms were recovered from long term storage at −85 °C in 15% glycerol. Bacteria were refreshed in Nutrient Broth (Merck, Darmstadt, Germany) at 35–37 °C, and afterwards inoculated on Nutrient Agar (Merck, Darmstadt, Germany) plates for checking the microbial purity. *C. perfringens* was inoculated and grown on Tripticase Soy Agar (TSA, Fluka, Buchs, Switzerland) under anaerobic conditions. Strain numbers and sources of the acquired microorganisms are listed in Table 3.

2.6.2. Antibacterial activity using the microdilution assay

The minimal inhibition concentration (MIC) values were evaluated according to published procedures (Guven, Celik, & Uysal, 2005; Iscan, Demirci, Kirimer, Kürkcüoglu, & Baser, 2002; Koneman, Allen, Janda, Schreckenberger, & Winn, 1997). Stock solutions of essential oil and the antimicrobial standards were prepared in dimethyl sulfoxide (DMSO, Carlo-Erba, Milan, Italy). Dilution series using sterile distilled water were prepared from 2000 μg/ml to 1.95 μg/ml in test tubes which were later transferred into 96-well microtiter plates as 100 μl aliquots. Bacterial suspensions were incubated in double strength Nutrient Broth (Merck, Darmstadt, Germany) overnight at 37 °C and then standardized to 10^8 CFU/ml (McFarland No: 0.5). Freshly prepared bacterial suspensions were pipetted into each well in an equal volume. The last-well row containing sterile distilled water and the medium served as positive growth control. After incubation at 37 °C for 18–24 h the first well without turbidity was assigned as minimum inhibitory concentration (MIC) in μg/ml. *Clostridium* was incubated under anaerobic conditions at 37 °C and 5% CO_2, separately in an CO_2-incubator (Sanyo, Japan), using double strength Tripticase Soy Broth (TSB, Fluka, Buchs, Switzerland). Chloramphenicol, ascorbic acid, and citric acid were used as antibacterial standards against all pathogens. Experiments were repeated at three different times and results were expressed as the average of three values (Table 3).

3. Results and discussion

3.1. Determination of essential oil components

Essential oils were obtained by hydrodistillation from air dried aerial parts of *P. russeliana* and *P. grandiflora* var. *grandiflora*, respectively. Oils were subsequently analysed by GC and GC/MS and the individual identified components with their relative percentages are given in Table 2.

In the oil (Pr) of the *P. russeliana* 84 components were characterized representing 83.3% of the total oil, with β-caryophyllene (22.6%), germacrene-D (15.1%), and caryophyllene oxide (8.1%) as the main constituents. The essential oil was dominated mainly by sesquiterpene hydrocarbons (48.8%). Oxygenated sesquiterpenes (17.5%) and hydrocarbons such as alkanes and fatty acids were classified as "others" and shared a sum of 16.6%, as seen at the end of Table 2. *P. russeliana* essential oil was rather poor in monoterpene hydrocarbons (0.1%) and oxygenated monoterpenes (0.3%). α-Phellandrene, dodecane and hexadecane were detected as trace (corresponding to less than 0.1%) compounds in the essential oil of *P. russeliana*.

A total of 77 compounds were characterized in *P. grandiflora* var. *grandiflora* essential oil (Pg), representing 84.4% of the total oil. This oil which was characterized by a relatively high content of β-eudesmol (42.0%) and α-eudesmol (16.1%) was dominated by oxygenated sesquiterpenes (68.7%). Myrcene, *o*-mentha-1(7),5,8-triene, (*Z*)-β-ocimene, (*E*)-β-ocimene, *trans*-linalool oxide (furanoid type), *trans*-sabinene hydrate, *cis*-linalool oxide (furanoid type),

Table 3
Antibacterial results in MICs (μg/ml) for the test materials and standards

Pathogen	Strain source	Pr	Pg	Ca	Aa	Cp
Escherichia coli, G (−)	O157:H7[a]	1000	1000	1000	1000	250
Aeromonas hydrophila, G (−)	[b]	250	500	500	500	125
Listeria monocytogenes, G (+)	[b]	500	250	500	1000	125
Salmonella typhimurium, G (−)	NRLL B-4420[c]	1000	250	250	1000	15.6
Staphylococcus aureus, G (+)	NRRL B- 767[c]	1000	500	250	1000	7.8
Bacillus cereus, G (+)	NRLL B-3711[c]	250	250	500	1000	15.6
Yersinia enterocolitica, G (−)	O:9[d]	500	125	500	1000	7.8
Pseudomonas aeruginosa, G (−)	NRRL B-23[c]	500	500	500	500	62.5
Clostridium perfringens, G (+)	NRRL B-3526[c]	125	125	250	>1000	3.9

G (−): Gram negative, G (+): Gram positive, Pr: *P. russeliana* essential oil, Pg: *P. grandiflora* var. *grandiflora* essential oil, Ca: citric acid, Aa: ascorbic acid, Cp: chloramphenicol.

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α-ylangene, pentadecane, *cis*-sabinene hydrate, octanol, sesquisabinene, *trans*-pinocarveol and geranial were identified as trace compounds in the oil of *P. grandiflora* var. *grandiflora*. A recent study by Celik et al. (2005) on the essential oil compositions of various *Phlomis* species inlculding *P. grandiflora* var. *grandiflora* demonstrated that germacrene-D, β-caryophyllene, bicyclogermacrene, α-humulene and limonene were main constituents.

Furthermore, components like α-pinene, β-pinene, δ-2-carene, limonene, 1,8-cineole, γ-terpinene, terpinolene, tridecane and nonane were identified in trace amounts, in both *P. russeliana* and *P. grandiflora* var. *grandiflora* essential oils in this study.

3.2. Antibacterial activity of essential oils

Common pathogenic Gram (+) and (−) bacteria associated with food poisoning and spoilage are listed in Table 3, were challenged with *Phlomis* essential oils (Pr and Pg). The objective was to evaluate the antibacterial properties of the *Phlomis* essential oils in comparison with common antibacterial and preservative agents. Using a modified micro-broth dilution assay (Guven et al., 2005; Iscan et al., 2002; Koneman et al., 1997) minimum inhibitory concentrations for each essential oil were compared with chloramphenicol, ascorbic and citric acid, respectively. Essential oils from *Phlomis* showed MICs from weak to a moderate range (125–>1000 μg/ml).

The essential oils of *P. russelina* (Pr) and *P. grandiflora* var. *grandiflora* (Pg) exhibited notable antibacterial activity against all the bacteria species tested except *E. coli* O157:H7 (Table 3). The anaerobe *C. perfringens* was the most susceptible organism at low concentration (125 μg/ml) to both essential oils. *Y. enterocolitica* was sensitive to the essential oil Pg at 125 μg/ml.

Previous antimicrobial activity studies on various *Phlomis* species from different localities showed inhibitory activity against a wide spectrum of microorganisms such as human, animal and plant pathogens, including food poisoning bacteria and several fungi (Aligiannis, Kalpoutzakis, Kyriakopoulou, Mitaku, & Chinou, 2004; Couladis, Tanimanidis, Tzakou, Chinou, & Harvala, 2000; Ristic et al., 2000; Tsitsimi, Loukis, & Verykokidou, 2000). *Phlomis* extracts (Digrak, Ilcim, Alma, & Sen, 1999; Ristic et al., 2000), essential oils, and pure compounds (Aligiannis et al., 2004; Calis et al., 2005; Kyriakopoulo, Magiatis, Skaltounis, Aligiannis, & Harvala, 2001), were tested in these studies. However, to the best of our knowledge this is the first study reporting on the antibacterial activity of *P. russeliana* and *P. grandiflora* var. *grandiflora* essential oils.

The selection of pathogenic bacteria used in this evaluation was based on their frequent occurrence and pathology associated by food contamination or origin as listed in Table 3. In this present study only a weak inhibitory effect was observed against *E. coli* O157:H7 with a MIC value of 1000 μg/ml by all of the tested essential oils.

Another important cause of food borne disease is *C. perfringens*, and the literature on the inhibitory effects of chemical antimicrobials on the growth of *C. perfringens* is limited. Buffered sodium citrate and buffered sodium citrate supplemented with sodium diacetate (Thippareddi, Juneja, Phebus, Marsden, & Kastner, 2003), sodium and potassium lactates (Aran, 2001) were used for inhibition of *C. perfringens* growth during chilling of meat products. *C. perfringens* was inhibited by both essential oils relatively good with a MIC of 125 μg/ml when compared to the tested standard controls.

Yersinia enterocolitica, a pathogen also from food origin was inhibited at the level as *C. perfringens*.

Overall, both essential oils displayed good and selective inhibitory activity against *C. perfringens*, whereas, only *P. grandiflora* var. *grandiflora* essential oil showed strong antibacterial activity against *Y. enterocolitica* with a MIC value of 125 μg/ml.

Preservatives are designed to prevent food decay and the growth of food borne pathogens, and to increase the storage shelf lives of foods. Currently, many food additives i.e., benzoic acid and sorbic acid are used in the food industry. Although these synthetic preservatives are effective, they can be detrimental to human health and consequently an increasing number of consumers choose food products which are preservative-free or contain only trace amounts (Kim, Hwang, & Shin, 2005). The exploration of naturally occurring antimicrobials for food preservation receives increasing attention due to consumer awareness of natural food products and a growing concern of microbial resistance towards conventional preservatives (Cowan, 1999; Draughon, 2004).

Essential oils are fairly complex mixtures, which are well known to possess varying *in vitro* and *in vivo* antimicrobial effects. They generally show selective toxicity towards various pathogens and are relatively safe both to animals and humans. In complex mixtures, synergism of individual components is also expected so that microorganisms hardly can develop resistance towards essential oils. Their mode of action works on various bacterial sites inside and outside the cell (Burt, 2004; Cowan, 1999). Various studies on different foods and food environments showed significant antimicrobial effects of essential oils both *in vitro* and *in vivo*. The effective dose *in vitro*, however, needs to be enhanced when applied to food systems to achieve the same effect (Burt, 2004). It has also been noticed that, essential oils were found more active against Gram (+) bacteria when compared to Gram (−) (Burt, 2004; Cowan, 1999; Kalemba & Kunicka, 2003), which partially support our findings.

Limitations of employing essential oils and components as food antimicrobials include changes in sensory attributes such as aroma and flavor, furthermore, solubility difficulties associated with lipophilic nature. However, recent encapsulation techniques using various surfactants seem to overcome such problems (Gaysinsky, Davidson, Bruce, & Weiss, 2005). Appropriate essential oil selections and

usage according to the type of food can also overcome undesirable organoleptic characters while preventing the growth of microorganisms.

In conclusion, there is an immense need for food borne disease investigation and effective prevention. Essential oils and their active components, possessing antioxidant activity besides several biological activities, can be employed instead of costly antibiotics in the effective control of food borne pathogens. *Phlomis* essential oils evaluated in this study showed varying inhibitory activity on all tested food pathogens. It is worthwhile also to test other fractions for their antimicrobial and biological activity potential.

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