

Investigating the Phytochemical, Antibacterial and Antifungal Effects of *Thymus Vulgaris* and *Cuminum Cyminum* Essential Oils

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Abstract

Background and Objective: Phytochemical and antimicrobial properties of *Thymus vulgaris* and *Cuminum Cyminum* essential oils (Eos) against foodborne pathogens and *Candida* species in vitro were assessed.

Methods: The EOs were extracted using a Clevenger apparatus. Analysis of the EO constituents was performed using gas chromatography-mass spectrophotometry. The antibacterial activity of EOs against *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella typhimurium* were evaluated in agar culture medium. The minimum inhibitory concentration (MIC) of these EOs against fungal strains of *Candida albicans*, *C. tropicalis*, *C. parapsilosis* and *C. dubliniensis* was measured.

Results: Thymol (64.45%) and cuminaldehyde (29.02%) were the main components of the *Thymus vulgaris* and *Cuminum Cyminum* EOs, respectively. The largest inhibition zone diameter in the *Thymus vulgaris* and *Cuminum Cyminum* EOs in the agar disk diffusion method was related to *B.cereus* with 30 and 21 mm diameter, respectively. The largest growth inhibition zone diameter by the *Thymus vulgaris* EO in the well diffusion method was 21 mm and against *B.cereus*. The MIC of *Thymus vulgaris* EO in the microdilution method was 0.09% against all the four *Candida* strains. The MIC of *Cuminum Cyminum* EO against strains of *C. albicans* and *C. tropicalis* was 0.39%, while it was found as 0.19% against *C. parapsilosis* and *C. dubliniensis*.

Conclusion: In this study, *Cuminum Cyminum* and *Thymus vulgaris* Eos show suitable inhibitory effects against the growth of pathogenic bacteria and *Candida* species. This study has raised the possibility of using these EOs as an alternative chemical preservative in the foods.

Keywords: essential oil, *Cuminum cyminum*, *Thymus vulgaris*, antibacterial, antifungal.

INTRODUCTION

Inappropriate use of antibiotics such as non-compliance of patients with required dosage or overuse leads to the development of bacterial resistance to antibiotics (1). Essential oils are volatile oily liquids with antimicrobial properties. Numerous studies have been performed on their use in controlling the growth of foodborne pathogenic bacteria and spoilage bacteria (2,3). Since essential oils are used in many types of foods to create exquisite tastes, the simultaneous presence of their antimicrobial properties may encourage their further usage (4). *Thymus* is a main genus of the Lamiaceae family which includes about 215 species of perennial herbaceous plants and small shrubs. Overall, 14 species of this genus grow in different parts of Iran (5). It is also used as a medicinal herb for strengthening the nervous system, treatment of depression and insomnia, and has antimicrobial, anti-parasitic and anti-fungal properties. When boiled, it is also used as an anti-flatulence, antispasmodic, antitussive and to control common cold and improve digestion (6). *Thymus vulgaris* is a native plant of Iran and its vegetative tissue contains antimicrobial active substance. Thymol and Carvacrol are the main components of essential oils of this family. This plant also contains Tannin, Flavonoids, Saponin and bitter substances (7,8,9). *Cuminum cyminum* is an annual herbaceous plant, delicate and aromatic member of the Apiaceae family (10). This plant is native to the Middle East, especially South East of Iran and its wild form grows in Kerman province. Sabinene, flavonoids, polysaccharides, coumarin, cuminaldehyde, pinene and terpinene are some of the key and major compounds of this plant (11). *Cuminum cyminum* is used in treatment of various diseases as an anticonvulsant, anti-epileptic, Stomach-strengthening, diuretic, anti-flatulence, anti-dyspepsia and sweating stimuli, as well as being useful for diabetics (12). The aim of this study was to determine the chemical composition and evaluate the antimicrobial activity of essential oils of *Thymus vulgaris* and *Cuminum cyminum* against foodborne pathogens using disc assay and agar well diffusion assay. Moreover, microdilution method was used to assess the antifungal efficacy of the essential oils and determine their minimum inhibitory concentration (MIC) against the *Candida* species, as the most

common cause of fungal infections in humans, especially in immunocompromised patients.

MATERIAL AND METHODS

In order to extract the essential oils, *Thymus vulgaris* and dried seed of *Cuminum cyminum* were purchased. The scientific name of the plants was confirmed by the herbarium center of faculty of Pharmacy, Tabriz University, Iran. The plants were fully grinded (aerial parts of *Thymus vulgaris* and *Cuminum cyminum* seed), and their essential oil were obtained by 3 hour water distillation and extraction using a Clevenger apparatus. Dewatering was done using dried sodium sulfate and the obtained oil was kept in dark glass containers at refrigerator temperature (13). The prepared samples were first injected into a gas chromatograph and the optimum temperature of column for the separation of essential oils' constituents was obtained. Also, the percentage of constituents for each essential oil sample was calculated. The essential oil was injected to the gas chromatograph, attached to a mass spectrograph and the mass spectra of the compounds were achieved. Identification of essential oil compounds was performed using evaluation of mass spectrum for each essential oil components and their comparison with the reference spectra. In this study, a Agilent 6890 gas chromatograph with capillary column of 30m length, an inner diameter of 0.25 mm and 0.25 μm thick inner layer of HP-5MS type. The column temperature program started as 70 $^{\circ}\text{C}$ for 2 minutes, then the temperature was increased to 220 $^{\circ}\text{C}$ with rate of 15 $^{\circ}\text{C}$ per minute and then it was increased to 300 $^{\circ}\text{C}$ for 2 minutes. Injection chamber temperature was 290 $^{\circ}\text{C}$ and helium was used as the carrier gas with a flow rate of 0.8 ml/min. Agilent 5973 mass spectrometer with ionization energy of 70 eV was used and electron ionization source detector temperatures was 220 $^{\circ}\text{C}$ (14). The antimicrobial activity of *Cuminum cyminum* and *Thymus vulgaris* essential oils was evaluated against two Gram-positive bacteria (*Bacillus cereus* ATCC 11178 and *Listeria monocytogenes* ATCC 19118) and two Gram-negative bacteria (*Escherichia coli* ATCC 43894 and *Salmonella typhimurium* ATCC 13311). Also, the activity of essential oils against fungal strains of *Candida albicans* ATCC 10231, *C. tropicalis* ATCC 750, *C. parapsilosis* ATCC 22019 and *C. dubliniensis*

CD 36 was determined. The Selection of bacterial species was based on their importance in food contamination and food poisoning among humans. Moreover, *Candida* was used in this study due to its importance as the most common cause of fungal infection in humans, and particularly immunocompromised patients. Bacterial suspension of each bacteria was obtained from the Department of Microbiology, Faculty of Veterinary Medicine, University of Tabriz and then prepared separately (0.5 McFarland equivalent). In the disk diffusion method, 100 μ l of the second 24-hour bacterial culture (containing 10^8 CFU/ml) were cultured in nutrient agar. The essential oils were dissolved in 10% dimethyl sulfoxide (DMSO) solution, at the highest concentrations used (400 μ l/ml). Four serial dilutions of the essential oils were prepared in the range of 25 to 400 μ l and then 35 μ l of the prepared serial dilutions were added to 6 mm sterile paper discs, which were transferred to plates after drying. Plates were incubated for 24 hours and the inhibition zone diameter was measured. All the experiments were done in triplicate (15). Next, 100 μ l of the refreshed bacterial cultures containing 10^8 CFU/ml were cultured on nutrient agar using sterile swabs. Then, wells with a diameter of 6 mm were created using a sterile tool on the agar surface. Afterwards, 40 μ l of each concentration of the essential oils were inoculated in the related well. The plates were incubated for 24 hours at 37 °C. Finally, bacteria growth inhibition zone diameter was measured. These experiments were not performed in triplicate (16, 17). Fungi were cultured in the Potato (potato extract) dextrose agar (Merck, Germany) containing chloramphenicol and incubated for 5 days at 28 °C. Broth microdilution and serial dilutions in 96-well plates were used to investigate the antifungal effects of *Thymus vulgaris* and *Cuminum cyminum* essential oils according to the recommended method of CLSI (M27-A2). A fungal suspension was prepared in 5ml of 0.85% sterile physiological saline, containing Tween 80 (1%) of the fungus spores grown on Potato dextrose agar, so that the resulted suspension had 78 to 90% dispersion at wavelength of 520 nm (equivalent to 1×10^6 - 5×10^6 CFU/ml). The resulting suspension was first diluted 1/100 and then 1/20 to be used for the experiment (equivalent to 0.5×10^3 - $2.5 \times$

10^3 CFU/ml yeast cells). An initial concentration of the essential oils was prepared as 100 μ l of essential oils in one ml of 10% DMSO, then dissolved in the RPMI1640 medium and became sterile using a filter. About 100 μ l of the twice concentration (2X) of RPMI1640 medium containing L-glutamine in 0.165 M MOPS buffer (pH=7) were added to each well of the 96-well plate. This double concentration was achieved by adding the following compounds to the optimum concentration (1X). Then, 100 μ l of the prepared solution was added to the first well of each row. Later, 100 μ l of the first well was transferred to the next well until the last well of the plate. Finally, 100 μ l of the fungal suspension containing 0.5×10^3 - 2.5×10^3 CFU/ml yeast were added to all wells of the microplate. The microplate was incubated for 48 hours at 37 °C. After this period, the resulting turbidity was evaluated. The concentration of essential oils that reduced the fungal growth to 80% was considered as the MIC for fungi. All the experiments were performed in triplicate (18).

RESULTS

As seen in Table 1, in the essential oil of *Thymus vulgaris*, thymol (64.45%), gamma-terpinene (9.22%) and p-cymene (6.18%) were found as the main compounds. While, cuminaldehyde (29.02%), alpha-terpinene (20.7%), gamma-terpinene (12.94%), gamma-terpinene 3L (8.9%) and p-cymene (8.55%) were identified as the major compounds in the essential oil of *Cuminum cyminum*. Table 3 shows the inhibitory effects of the essential oil of *Thymus vulgaris* and *Cuminum cyminum* at different concentrations in the disk diffusion method. Accordingly, the maximum diameter of the inhibition zone of *Thymus vulgaris* essential oil was measured against *B. cereus* (30 mm). The maximum size of the inhibition zone of *Cuminum cyminum* in this method was also recorded against *B. cereus* (21mm). Table 2 shows the inhibition zone diameter of different concentrations of the two essential oils using the agar well diffusion method. The largest inhibition zone of *Thymus vulgaris* was recorded against *B. cereus* (21mm). The MIC of *Thymus vulgaris* was lower compared to *Cuminum cyminum*, which indicates the high antifungal activity of *Thymus vulgaris* essential oil.

Table 1- The main components of *Cuminum cyminum* and *Thymus vulgaris*

Compounds	<i>Cuminum cyminum</i>	<i>Thymus vulgaris</i>
Thymol		64.45
Gamma-terpinene	12.94	9.22
P-cymene	8.55	6.18
Carvacrol	-	2.82
Beta-caryophyllene	-	2.14
Borneol	-	2.04
Linalool	-	1.28
Cuminaldehyde	29.02	-
Alpha-terpinene	20.7	-
Gamma-terpinene 3 L	8.9	-
cis-dihydrocarvone	4.45	-
Beta-pinene	3.3	-
Myrcene	1.1	-
1,8-cineol	0.84	-

Table 2-The growth inhibition zone diameter (mm) of the essential oil of *Thymus vulgaris* and *Cuminum cyminum* at different concentrations, using agar disk and well diffusion methods

Essential oil of <i>Cuminum cyminum</i> (µg/ml)								Essential oil of <i>Thymus vulgaris</i> (µg/ml)								Method		
25	50		100		200		25	50		100		200		400				
well	Disk	well	Disk	well	Disk	well	Disk	well	Disk	well	Disk	well	Disk	well	Disk			
*	8	-	9	13	10	14	11	-	14	12	16	15	20	18	21	*	23	<i>S. typhimurium</i>
*	10	*	11	*	12	*	13		18	*	20	*	21	*	22	*	24	<i>L. monocytogenes</i>
*	-	10	8	12	8	13	9	-	10	11	16	15	18	17	19	*	21	<i>E. coli</i>
*	8	*	9	*	14	*	21	13	10	15	12	17	26	21	28	*	30	<i>B. cereus</i>

No inhibition zone : -

Not done : *

Table 3-The MIC of essential oils of *Thymus vulgaris* and *Cuminum cyminum* against *Candida* species using the broth microdilution method

Essential oil of <i>Cuminum cyminum</i> (%)	Essential oil of <i>Thymus</i> <i>vulgaris</i> (%)	<i>Candida</i> species
0.39	0.09	<i>albicans</i>
0.39	0.09	<i>tropicalis</i>
0.19	0.09	<i>parapsilosis</i>
0.19	0.09	<i>dublinsiensis</i>

DISCUSSION

In this study, chemical composition and antimicrobial effect of essential oils of *Thymus vulgaris* and *Cuminum cyminum* were evaluated. Cuminaldehyde (29.02%) and alpha-terpinene (20.7%) were the major components of the *Cuminum cyminum* essential oil. Results of evaluating the chemical composition of *Cuminum cyminum* seed in the present study are somewhat consistent with other studies. In most studies, compounds such as cuminaldehyde, alpha-terpinene, beta-pinene, gamma-terpinene, cymene and P-menthane were found as the major components of this essential oil. Cuminaldehyde (30.2%) and p-cymene (14.13%) were found as the main components of the essential oil of *Cuminum cyminum* in the study of Aroojalian et al., which is largely consistent with the present study (19). In Attar-Rahman et al. study, 20 compounds were identified the essential oil of *Cuminum cyminum* where cuminaldehyde, p-cymene, beta-pinene and gamma-terpinene were found as the main compounds (20). This essential oil is composed of pinene, alpha-terpineol, apigenin and flavonoids, which have antimicrobial properties in addition to a particular aroma. These compounds often exert their antimicrobial effect by creating pores in the cell membrane of Gram-positive bacteria and destructing the outer membrane of Gram-negative bacteria (21). Iacobellis et al. showed that the presence of high levels of cuminaldehyde (16.1%) in this essential oil can have antibacterial activity against certain Gram-negative and Gram-positive bacteria (22). Thymol (64.45%) was found as the major constituent of *Thymus vulgaris* essential oil in this study, which is largely consistent with the results of other similar studies (23, 24). In these investigations, thymol and p-cymene were the major compounds in the essential oil of *Thymus vulgaris*. In Moghtader study to assess the antifungal activity of *Thymus vulgaris* essential oil, thymol and p-cymene were found as the major compounds. The antifungal activity of this essential oil in high concentrations was more than Streptomycin sulfate. It is reported that thymol has high fungicidal activity and its antimicrobial activity is due to its phenolic structure. Also, the inhibitory effect of thymol was almost thrice the effect of *Thymus vulgaris* essential oil (25). The constituents of certain species

of plants may differ compared to the same species in different regional conditions. This could be due to differences in harvesting season, oil extraction time, geographical areas and even different parts of plants (21). In this study, essential oil of *Thymus vulgaris* and *Cuminum cyminum* had the greatest impact against *B. cereus* in the disk and well diffusion methods. *B. cereus* and *L. monocytogenes* growth inhibition zone diameter was larger compared with the Gram-negative bacteria that were studied. In general, Gram-positive bacteria show greater sensitivity to plant extracts and essential oils in comparison with Gram-negative bacteria (26). The increase in the antimicrobial activity of the essential oils by increasing their concentration was evident. In study of Daneshmadi et al., the antibacterial activity of *Cuminum cyminum* essential oil against several bacterial strains was assessed. Standard strains of *B. cereus*, *B. subtilis*, *S. aureus*, *E. coli* and *Shigella flexneri* showed the greatest sensitivity to the essential oil's constituents, but the sensitivity of *Enterococcus faecalis*, *S. typhimurium* and *Pseudomonas aeruginosa* strains was not significant (27). In another study, the antimicrobial effect of *Cuminum cyminum* seed against *E. coli* and *S. typhimurium* was demonstrated (28). Results of Mahmoudi et al. study showed the relatively good antibacterial activity of *Cuminum cyminum* essential oil, with the highest activity observed against *S. aureus*. However, *S. typhimurium* and *E. coli* were the most resistant bacteria against this essential oil (29). As demonstrated by the results of the present study, the antibacterial effect of essential oils used against Gram-positive bacteria is more than Gram-negative bacteria. Ranjbarian et al. studied the antibacterial effects of four plant extracts including *Cuminum cyminum*, on *Helicobacter pylori* using disk diffusion method and reported the inhibitory effect of cumin against this bacterium (30). In a study conducted by Yano et al., the antimicrobial effect of *Cuminum cyminum* on growth of *Vibrio parahemolyticus* was assessed and its inhibitory effect at concentration of 0.02% was confirmed (31). Derakhshan et al. also investigated the antimicrobial effect of this essential oil on growth of *Klebsiella pneumoniae* and reported its inhibitory effect at concentration of 0.03% (32). In a study, the

antimicrobial activity of *Cuminum cyminum* essential oil against foodborne pathogens such as *L. monocytogenes* and *E. coli* are well-demonstrated (33). In study of Pajohi et al., the main constituents of this essential oil and its antibacterial effect at different concentrations against *B. cereus* were investigated in food models kept at different temperatures. The main compounds were benzaldehyde (27.18%) and phenyl-propanol (17.5%), respectively. Storage temperature had a significant effect on the growth of bacteria in the samples. So that the temperature of 10 °C significantly reduced the growth of bacteria compared to 25 °C. The high concentration of the essential oil significantly inhibited the growth of bacteria in the samples (34). Nowadays, *Thymus vulgaris* is considered as a plant with high antimicrobial effects. The antibacterial, antifungal and antiviral activities of this plant have been demonstrated. High antimicrobial activity of *Thymus vulgaris* essential oil and its major constituents such as thymol and carvacrol, against *S. aureus*, *S. epidermidis*, *E. faecalis*, *B. cereus*, *V. cholerae*, *E. coli*, *S. typhimurium* and many other microorganisms have been reported (25). The results of Haghighi et al. study on essential oils of *Thymus vulgaris*, parsley, *Cuminum cyminum* and black cumin on *C. albicans* showed that, the leaves of *Thymus vulgaris* have the highest inhibitory effect on this fungus. Carvacrol is among the main constituents of *Thymus vulgaris*, and the antifungal properties of this plant can be attributed to this compound (35).

Sokovic et al. studied the antifungal activity of essential oil of *Thymus vulgaris* on Dermatophytosis in animal models and reported its positive effect (9). Kon et al. study on the antibacterial effect of this essential oil also showed its good growth inhibitory effect against *E. coli* and *S. aureus* (33). Kačániová et al. study also showed the high antibacterial activity of this essential oil against *E. coli* and *B. cereus* (36). The present study showed that the MIC of essential oil of *Thymus vulgaris* is lower than the essential oil of *Cuminum cyminum*, which indicates higher antifungal activity of *Thymus vulgaris*. Kalembe et al. attributed the high antimicrobial activity of plants to their high levels of phenolic compounds. Accordingly, the high activity of *Thymus vulgaris* which contains high levels of phenolic compounds is justified (37).

CONCLUSION

Essential oils of *Cuminum cyminum* and *Thymus vulgaris* have antibacterial and antifungal properties with different effects against various species. This study has raised the possibility of using these essential oils as suitable antimicrobial compounds and alternatives for chemical preservatives in the food industry.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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