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Original Article

Antibacterial activity of *ajwain* essential oil against some zoonotic bacteria

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Summary

Harmful pathogens such as viruses, bacteria, parasites, and fungi can cause different types of diseases in people and animals, ranging from mild to severe illness and even death. Due to the increasing frequency of antimicrobial resistance among germs causing zoonotic diseases, more studies have focused on the usage of natural agents against them. This study aimed to evaluate the antibacterial effects of *ajwain* essential oil on some of the most common zoonotic bacteria. Antibacterial activity of *ajwain* essential oil was screened against *Bacillus anthracis*, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* Typhimurium, *Pseudomonas aeruginosa,* and *Klebsiella pneumonia* using disc diffusion method and broth microdilution assays. *Ajwain* essential oil exhibited antimicrobial activity against all the tested bacteria with minimal inhibitory concentrations (MIC) at a range of 2.5 to 10 µg/ml for Gram-positives and 40 to 80 µg/ml for Gram-negatives and minimum biocidal concentration (MBC) at a range of 10-40 µg/ml for Gram-positives and 80 µg/ml for Gram-positives. However, *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Bacillus anthracis* were the most susceptible to this essential oil, respectively. The results suggest that the activity of *ajwian* essential oil can be mainly attributed to the presence of phenol, benzene methyl, γ-terpinene, and thymol which appears to possess similar activities against all the tested bacteria. In conclusion, this material could be served as an important natural alternative to prevent zoonotic bacterial growth.
Keywords: Antimicrobial activity, Ajwain, Essential oil, Zoonotic bacteria

Introduction

According to the World Health Organization (WHO), zoonoses are diseases or infections that are naturally transmissible from vertebrate animals to humans and vice-versa (Rahman et al., 2020). Approximately 61% of all known human pathogens, such as viruses, bacteria, fungi, and parasites are mentioned as zoonotic germs, with 73% of emerging and re-emerging infections being considered as zoonoses (Jones et al., 2008). Each year, 2.5 billion cases related to zoonotic infections are recorded from all over the world which resulting in 2.7 million deaths (Asante et al., 2019). Bacterial zoonotic diseases can be transferred from animals to humans in many different ways, including animal bites and scratches, vectors, contaminated animal food products, improper food processing, and insufficient cooking. Also, farmers, livestock keepers, and animal health workers are at high risk of exposure to certain zoonotic bacteria and they may catch them. They can also become carriers of these pathogens and spread them in the society (Cantas and Suer, 2014). In 2017, the WHO presented a global priority pathogens list and categorized them as critical, high, and medium antibiotic-resistant bacteria that urgently need applying of new treatment strategies. In this regard, the majority of the WHO list was Gram-negative bacterial pathogens, including *Enterobacteriaceae* and *Pseudomonas aeruginosa*. Because of their remarkable structure, Gram-negative bacteria are more resistant than Gram-positive bacteria and thus causing important morbidity and mortality worldwide (Breijyeh et al., 2020). Also, among the listed pathogens, Gram-positive bacteria which can cause significant diseases are mentioned as a major problem and a health care concern, particularly multidrug-resistant (MDR) bacteria, including vancomycin-resistant *Enterococcus faecium* (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), and β-lactamase-resistant *Streptococcus pneumonia* (Jubeh et al., 2020). Although natural resistance of *Bacillus anthracis* to different antibiotics has been reported (Athamna et al., 2004), a long-term antibiotic therapy, as would be used for anthrax, may also result in antimicrobial resistance in *Bacillus anthracis* by the selection of resistant mutants.. Finally, since the isolation of the first multidrug-resistant *L. monocytogenes* strain in France, several strains isolated from food and environmental and clinical samples have exhibited resistance to some antibiotics (Şanlıbaba et al., 2018).

Antimicrobial resistance (AMR) and its relationship to human and animal morbidity are significant challenges facing modern medicine. The occurrence of AMR among bacterial pathogens is an evolutionary process that has several reasons including the improper use and overuse of therapeutic agents, prescribing antibiotics where bacterial species are not the causative agent of infection, using antibiotics as growth promoters in aquaculture, and promoting the faster growth of livestock in agriculture (Meade et al., 2017). The side effects of chemical and synthetic antimicrobial agents and the widespread prevalence of resistance to antibiotics among many bacteria have led to the search for green solutions like medicinal plants (Mahmoudi et al., 2013a). Ajwain is one of the members of the Apiaceae plants family. It is a native of Egypt but grows in Iraq, Iran, Afghanistan, Pakistan, and India (Boskabady et al., 2014). Ajwain seeds contain brown oil named *ajwain* which contains fiber, minerals, vitamins, and anti-oxidants. However, the main component of the oil is a phenolic compound named thymol (Sharifi mood et al., 2014). Thymol is one of the major antibacterial among the herbal essence components (Inouye et al., 2001). It has been shown that Ajwain essence can be effective against many bacteria, including Pseudomonas species, *E. coli*, *Bacillus subtilis*, *S. aureus*, *Klebsiella*, Proteus, and Shigella (Kumar et al., 2012; Sagdic et al., 2003; Usha et al., 2012). The purpose of this study was to investigate the chemical composition and antibacterial activity of *ajwain* essential oil on some common zoonotic bacteria.

Materials and methods

Bacterial strains

Eight pathogenic bacteria, four gram positive including *Bacillus cereus ATCC 1247*, *Staphylococcus aureus ATCC 25923*, *Streptococcus pneumoniae ATCC 49615*, and *Listeria monocytogenes ATCC 7644* and four gram negative including *Salmonella* Typhimurium *ATCC 14028*, *Escherichia coli ATCC 8739*, *Pseudomonas aeruginosa ATCC 9027*, and *Klebsiella pneumoniae ATCC 10031* were selected for the current study. The pure cultures of these bacteria were obtained from the Microbiology Laboratory of the Faculty of Veterinary Medicine, University of Tabriz (Tabriz, Iran). They were all subcultured on nutrient broth media (Sigma Aldrich, USA).

Plant material

The seeds of ajwain were bought from The Spice Market in Tabriz city which is located in the East Azerbaijan province of Iran.

Extraction and isolation of ajwain essential oil

The essential oil was extracted by hydrodistillation of 100 g of dried seeds using Clevenger-type apparatus for 3 h. As a collector solvent, 10 ml of diethyl ether was used. After evaporation of the solvent, the essential oil was dried using anhydrous sodium sulfate and then stored at -20ºC (Mahmoudi et al., 2013b).

GC-MS analysis

The chemical compositions of *ajwain* essential oil were determined by gas chromatography-mass spectrometry (GC-MS; Agilent 6890 gas chromatography equipped with Agilent 5973 mas selective detector, USA). The chromatograph had HP-5MS capillary column (30 × 0.2 mm ID × 0.2 µm film thickness) and data were acquired under the following conditions: initial temperature 70 ºC holding for 2 min, then increasing the temperature from 70 to 220ºC at a rate of 4ºC. The injector temperature was 290ºC, and helium was used as carrier gas and the split ratio was 0.8 ml^{-1} min with the final temperature of 300ºC (holding for 2 min; Mahmoudi et al., 2013b).

Antibacterial activity

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the essence were determined by broth micro-dilution susceptibility method. For microdilution method, a stock solution of essential oil was prepared in dimethyl sulfoxide (DMSO; oil to DMSO ratio 1:3). Then, serial two-fold dilutions were made in a concentrations range 80-0/31µg/ml (Goudarzi et al., 2011; Vitali et al., 2016). The bacterial strains were cultured for 12 h in nutrient broth media. Then, the turbidity of suspensions was adjusted to 0.5 McFarland standards. The 96 well plates were used in this study. 95 µl of nutrient broth and 5 µl of 0.5 McFarland adjusted microbial suspension were added to the plate. The 100 µl of the essential oil with the above concentrations was added. The last well of the plate contained 195 µl of nutrient broth medium and 5 µl of bacterial suspension without essential oil. This well was the negative control. Contents of each well were mixed on a plate shaker at 300 rpm for 20 s, then incubated for 24 h at 37ºC. Microbial growth was confirmed by absorbance at 600 nm applying microplate reader (Biotech Instrument, Highland Park, Vermont, USA) (Galvao et al., 2012; Gharajalar and Hassanzadeh, 2017). Ciprofloxacin (5 µg disc; from Oxoid) was used as reference antimicrobial against bacteria. For MBC determination, 10 µl of each sample from clear wells was cultivated on a nutrient agar plate at 37ºC for 24 h. The MIC and MBC were detected as the lowest concentrations of the *ajwain* essential oil which inhibited the growth and killed of the isolates (Mahmoudi et al., 2013b).

Disc diffusion test

The petri dishes with 150 mm sterile Mueller Hinton agar were prepared. After culturing the bacteria in BHI broth for 24 h, the turbidity of bacterial suspension was adjusted to 0.5 McFarland standard and cultured on the Mueller Hinton agar plates. Sterile discs were saturated with 15 µl of sterile essential oil dissolved in 10% DMSO and then placed on the plates. Finally, the inoculated plates were incubated anaerobically at 37ºC for 18-20 h (Mahmoudi et al., 2013b; Majdi et al., 2017). The zone of inhibition diameters (mm) around the disks were measured and interpreted by referring to the performance standard for antimicrobial susceptibility testing, as described by the Clinical and Laboratory Standards Institute (CLSI) guidelines (2015).

Statistical analysis

Descriptive statistics, such as the percentage of main ingredients of ajwain essence was done using the statistical Package, SPSS, version 15.0.

Results

GC- MS analysis

Table 1 shows the results of GC-MS analysis of the *ajwain* essential oil. The compounds are listed in the order of their retention time. A total number of 31 compounds were detected in the oil, representing 94.48% of the total oil. The main ingredients were as follow: phenol (42.26%), benzene methyl (23.11%), γ-terpinene (19.69%), and thymol (7.75%).

Antibacterial activity

The MIC and MBC values of the essence for the isolates are presented in Table 2. The analysis of *ajwain* essential oil showed that this essence had more antibacterial activity against Gram-positive bacteria than Gram-negatives. The results of this research showed that the essential oil worked well against Gram-positive and Gram-negative bacteria studied with MIC at a range of 2.5 to 10 µg/ml for Gram-positives and 40 to 80 µg/ml for Gram-negatives and MBC at a range of 10-40 µg/ml for Gram-positives and 80 µg/ml for Gram-negatives. The MIC value of the *ajwain* essential oil was obtained the lowest against *Streptococcus pneumoniae* (2.5 µg/ml), which was followed by *Staphylococcus aureus* (10 µg/ml), and *Bacillus anthracis* (10 µg/ml). Also, the MIC of the essential oil towards *Listeria monocytogenes* was 20 µg/ml. Among the Gram negative species, the MIC values against *E. coli*, *Salmonella* Typhimurium, and *Klebsiella pneumonia* were 80 µg/ml which were double than the one obtained for the *Pseudomonas aeruginosa* (40 µg/ml).

Similarly, the MBC values of the *ajwain* essential oil against the tested bacteria were also determined, which were found to be the highest against *E. coli*, *Salmonella* Typhimurium, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* (80 µg/ml). While the lower MBC values of the *ajwain* essential oil were obtained against *Streptococcus pneumoniae* (10 µg/ml) and *Staphylococcus aureus* (20 µg/ml) in comparison with *Bacillus anthracis* (40 µg/ml) and *Listeria monocytogenes* (40 µg/ml). In the microdilution tests, ciprofloxacin was found to act at the higher concentrations (Table 2).

Table 1. Phytochemical composition of *ajwain* essential oil

No.	Phytochemicals	RT*	%
1	Thymol	5.68	% 7. 75
2	Benzene, methyl	6.72	% 23.11
3	gamma.-Terpinene	7.31	% 19.69
4	CIS-SABINENE HYDRATE	7.73	% 0.17
5	Ethanone	8.81	% 0.18
6	1-HYDROXYLINALOOL	8.84	% 0.24
7	Dodecane	9.42	% 0.55
8	Ethanone	10.26	% 1.67
9	Phenol	12.14	% 42.26
10	Ether, 3-butenyl pentyl	12.78	% 0.19
11	5-Isopropenyl-2-methylcyclopent-1-enecarboxaldehyde	12.90	% 0.10
12	Tetradecane	13.49	% 0.25
13	trans-Caryophyllene	14.01	% 0.16
14	2(3H)-Furanone	14.63	% 0.12
15	gamma.-himachalene	14.87	% 0.23
16	spathulenol	16.26	% 0.10
17	Caryophyllene oxide	16.35	% 0.36
18	Dillapiole	16.77	% 0.19
19	(1R*,5R*,8S*)-1,8-Dimethylbicyclo[3.3.0]oct-3-en-2...	19.75	% 0.12
20	Spongia-13(16),14-dien-19-al	20.80	% 0.16
21	Thymyl acetate	20.95	% 0.31
22	3,7-dimethoxy-11a-methylpterocar...	21.04	% 0.07
23	3,4-Diethylphenol	21.52	% 0.09
24	Heneicosane	21.74	% 0.06
25	Totarol-7-one	22.14	% 0.07
26	2-Methoxy-6-(3',5'-dimethoxyphen...	22.36	% 0.07
27	Hexadecane, 2,6,10,14-tetramethyl-	22.65	% 0.15
28	Heneicosane	23.67	% 0.23
29	Tetracosane (CAS)	24.84	% 0.24
30	Pentacosane	26.06	% 0.19
31	Octacosane (CAS)	27.41	% 0.12

*RT= Retention time (min).

Disc diffusion assay

The results of the disc diffusion assay demonstrated that the largest growth inhibition zone diameter belonged to *Bacillus anthracis* (250 mm), followed by *Streptococcus pneumoniae* (230mm), and *Staphylococcus* aureus (218 mm). On the other hand, the lowest growth inhibition zone was for *Salmonella* Typhimurium (150 mm), *E. coli* (160 mm), and *Klebsiella pneumonia* (180mm). Of note, the ajwain oil antibacterial activity was comparable to that of the reference antibiotic ciprofloxacin, in the disc diffusion test (Table 2).

Table 2. Antibacterial activity of *ajwain* essential oil against tested pathogens

Bacteria	Inhibition Diameter(mm) Ajwain essence (15µl)	Inhibition Diameter(mm) Ciprofloxacin	MIC Ajwain essence	MIC Ciprofloxacin	MBC Ajwain essence	MBC Ciprofloxacin
E. coli	160	145	80	80	80	80
Salmonella Typhimurium	150	145	80	80	80	80
Listeria monocytogenes	210	200	20	40	40	40
Klebsiella pneumonia	180	160	80	80	80	80
Bacillus anthracis	250	236	10	40	40	80
Staphylococcus aureus	218	200	10	40	20	80
Streptococcus pneumoniae	230	215	2.5	5	10	20
Pseudomonas aeroginosa	200	200	40	40	80	80

Discussion

In this study, the antibacterial properties of *ajwain* essential oil were screened against some zoonotic bacterial pathogens and the values of MIC, MBC, and inhibition diameter of the essence were determined.

Medicinal plant includes plants having some bioactive compounds that could be precursors for the synthesis of therapeutic drugs. They are popular for their low toxicity, pharmacological activities, and economic viability (Manzo et al., 2017). Essential oils, secondary metabolites formed by aromatic and medicinal plants, are significant for their antimicrobial properties against a wide range of microbial pathogens (Panday et al., 2017). *Adjwan* essential oil has components like thymol, p-cymene, and γ-terpinene with some potential antibacterial activities (Mahboubi et al., 2011). The main components of the Iranian and African *ajwain* essential oil are carracrol, γ-terpinene, and p-cymene. However, the major component of south Indian plant oil is thymol (Mahboubi et al., 2011). Mahboubi *et al*. (2011) evaluated chemical composition and antimicrobial activity of *ajwain* essential oil against different kinds of microorganisms in Kashan (Iran). According to their results, the dominant components of this essence were thymol, γ-terpinene, and o-cymene. In addition, thymol, γ-terpinene, and p-cymene were reported by Akbarnia *et al*. (2005) as the main components of ajwain oil chemical. Also, Haghiroalsadat *et al*. (2011) studied the active ingredients and anti-oxidant effects of *ajwain* seeds harvested in Yazd (Iran). The results indicated that thymol and γ-terpinene were the most significant components of the essence. Here, the essential oil was mainly composed of phenol (42.26%), benzene methyl (23.11%), γ-terpinene (19.69%), and thymol (7.75%). As can be seen, γ-terpinene, and thymol were identified among the most common compounds in ajwain oil, confirming the other reports. However, different chemical profile for *ajwain* oil especially thymol, may be related to the geographic origin, genetic variability, and harvesting time of the samples (Vitali et al., 2016). In some studies, the antibacterial properties of essential oils such as *ajwain* against bacteria were screened (Mahboubi et al., 2011; Sharifi mood et al., 2014; Mobaiyen et al., 2015; Gharajalar and Hassanzadeh, 2017). In one study, *ajwain* essential oil was used for controlling the *Listeria monocytogenes* activity in the fish model system (Rabiey et al., 2014), or in another one, the inhibitory effect of this oil on *Staphylococcus*, *E. coli,* and *Klebsiella* were studied. They found that *ajwain* essential oil has antibacterial effects against human pathogens which are in accordance with our results (Sharifi mood et al., 2014). Also, MehriArdestani *et al*. (2020) evaluated the antimicrobial activity of *ajwain* essential oil against vaginal pathogens which could inhibit vaginal pathogens' growth. In another study, the antimicrobial activity of *ajwain* essential oil was evaluated by disc diffusion method against methicillin-resistant *Staphylococcus aureus* (MRSA), other extended-spectrum beta-lactamases (ESBLs) producing, as well as Gram-negative and Gram-positive bacteria. This study confirmed that the essence of *Ajwain* had in vitro antibacterial activity against Gram-negative and Gram-positive pathogens, which is comparable with those of our study (Mobaiyen et al., 2015). Antimicrobial activity of *ajwain* essential oil is apparently attributable to high phenolic compounds such as thymol, which can damage the membrane integrity through changes in pH hemostasis as well as equilibrium of inorganic ions. Also, non-oxygenated monoterpene hydrocarbons such as γ-terpinene and p-cymene appear to produce antagonistic effects against more tolerant micro-organisms. It is found that the whole essential oil has a greater antimicrobial activity than the mixed major component. Thus, the minor components of *ajwain* essential oil play a critical role for the activity of oil (Mahboubi et al., 2011).

Conclusion

Here, we used *ajwain* essential oil for controlling some zoonotic pathogens. Our results showed that *ajwain* essential oil may act as a potent inhibitor of the growth of bacteria in an in vitro, which can spread between animals and people. Thus, this essential oil can be considered as a promising medicine for the management of these important pathogens.

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Conflict of interest statement

There is no conflict of interest.

Ethical approval

Not applicable

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