International Food Research Journal 25(3): 908-912 (June 2018)
Journal homepage: *http://www.ifrj.upm.edu.my*

Antimicrobial effect of ajwain seed ethanolic extract against food borne pathogenic bacteria

*Bhatt, V., Mahesh Kumar, M. and Periyar Selvam, S.

Department of Food Process Engineering, School of Bioengineering, SRM University, Kattankulathur Campus, Chennai- 603203 India

Article history

Received: 12 February 2017
Received in revised form: 21 March 2017
Accepted: 24 March 2017

Keywords

Antioxidant
Antibacterial
Pathogenic bacteria
Trachyspermum ammi

Abstract

Ajwain *(Trachyspermum ammi)* is a source of medicinally active compounds and has various pharmacological effects and therapeutic uses. The aim of this study was to evaluate the antimicrobial activities of ajwain seeds ethanolic extract against different pathogenic bacteria. Ethanolic extraction of ajwain seeds was carried out by supercritical fluid extraction method and GC-MS of the extract showed the presence of thymol as its major component (71.06%) along with o-Cymene (3.37%), γ-Terpinene (3.83%), 2-methyl-5-(1-methylethyl)-phenol (0.51%). The extract was analyzed for its ethanolic DPPH (2, 2-diphenyl-1-picrylhydrazyl) scavenging potential, showing increase in percent inhibition (50% to 83%) with increase in concentration (50 µg/mL to 250 µg/mL). The antimicrobial test results revealed that the pure ethanolic extract of ajwain seeds is significantly active against the food-borne pathogenic Gram positive and Gram negative bacterial strains (range of inhibition, 15-19 mm) and was compared with that of the broad-spectrum antibiotic, chloramphenicol (range of inhibition, 33-37 mm). Results concluded that ajwain seeds contain high amount of phenolic compound (mainly thymol) and have high antioxidant and antimicrobial activity and, hence, can be used as an excellent bio-preservative and for medicinal purpose, and can be useful for various perishable and fat rich food products by enhancing their shelf-life.

Introduction

Nature has been as a rich source of medicinal floras for a long period of time to maintain human health, having a tremendous sanative potential to heal many infectious maladies, without any side effects (Shelef, 1983). Apart from providing aroma and savor, spices have been recognized for their properties of preserving foods and medicinal values due to the presence of bioactive compounds (Papp *et al*., 2007). Resistance among pathogenic microbes against various antimicrobial drugs has been an increasingly important and most appalling problem, globally. Synthetic chemicals can be toxic in nature; hence, the plant extracts containing phytochemical, which have both antimicrobial and antioxidant properties, must be taken to control this problem (Agaoglu *et al.,* 2007). The antioxidant activity of several polyphenolic extracts and compounds derived from different plant parts, such as foliage, fruits, seeds, bark or skin, roots and oil-rich seeds has been extensively analyzed and documented (Naz *et al*., 2008; Li *et al*., 2012). It has been found that spices are rich in antioxidant activity as compared to fruits, cereals and nuts.

Ajwain *(Trachyspermum ammi)* is one the natural botanical sources, having several testified officinal properties. Being rich in constituents with promising bioactivity, Ajwain is an extremely reputable plant source (Bairwa *et al*., 2012). The essential oil is employed in the treatment of gastrointestinal ailments, lack of appetency and bronchial problems, at therapeutic level. Thymol being the major phenolic compound present in Ajwain has been reported to be a germicide, spasmolytic and antimycotic agent (Nagalakshmi *et al*. 2000). Thymol has antibacterial (Kumar *et al*., 2011), antifungal (Paul *et al*., 2011), and antioxidant (Moein *et al*., 2015) effects. Depending on the concentration used, such phenolic compounds can act as either bactericidal or bacteriostatic agents (Ashrafi Tamai *et al.*, 2013). The concentration of the polyphenols and carotenoids in ajwain leaves extracts has been found to influence the antioxidant activity (Raza *et al.*, 2015).

The foods containing such phytochemicals have proved their strong protective effects against many diseases and are increasingly been gaining importance (Orhan *et al.*, 2010; Phapale *et al*., 2010; Ozçelik *et al*., 2011; Guest *et al*., 2012). The main aim of this study was to evaluate the antimicrobial activity of the *Trachyspermum ammi* seeds ethanolic extract against some of the important food borne pathogenic bacteria

*Corresponding author.
Email: *vishalbiotech25@gmail.com*

as well as its antioxidative properties for its potential use as a natural food additive.

Materials and Methods

Sample preparation

Ajwain seeds were purchased from the local market of Chennai, India. The seeds were ground using electronic grinding machine, to get fine and uniform powder. The sample was stored at -20°C until further use.

Supercritical fluid extraction (SFE)

Extraction was carried out in a pilot-plant scale supercritical fluid extractor (Thar Technology), comprising a 2 L cylinder extraction cell and a separator (of 0.5 L capacity) with temperature and pressure control. Recirculation system was also included in the extraction device, where CO_2 was condensed and pumped up to the desired extraction pressure. For each extraction cycle, the extraction vessel was filled with 5 g of ground ajwain seed. Parameters were set as per Table 1. Each cycle was run for 60 minutes. Solvent was removed using rotary evaporator at 40°C. Pure extract was collected, weighed and stored at 4°C for future use. Percentage Yield was calculated on w/w basis (g extract/g sample load) (Mónica *et al*., 2011). Greater yield cycle was continued for further extract collection.

Qualitative analysis of the extract using GC-MS technique

The analysis of ajwain seed extract was carried out in Agilent 7890B GC System (Agilent Technologies) connected to 5977A mass spectrometer detector, comprising a split/splitless injector, G4513A auto injector, and data was analyzed using Mass Hunter software. The column used was an Agilent HP-5MS 5% Phenyl Methyl Silox -60ºC-325ºC capillary column (30 m × 250 µm i.d. × 0.25µm phase thickness).

Parameters were set as described by Villanueva *et al.* (2015), with some modifications. Oven temperature was programmed to 60ºC isothermal for 4 min then increased to 106ºC at 2.5ºC/min and from 106ºC to 130ºC at 1ºC/min and finally from 130ºC to 250ºC at 20ºC/min and this temperature was kept constant for 10 min. Sample injections (1 µL) were performed through auto-sampler in split mode (1:10). The chromatographic separation was carried out at constant pressure of 20.74 psi and Helium (99.996 mass %) was used as a carrier gas (He flow rate= 2mL/min.). Injector temperature was 250ºC, whereas, mass spectrometer ion source and interface temperatures were 230ºC and 280ºC, respectively. Mass spectrometer was used in total ion current (TIC) mode and sample scanning was done from 40 to 500 amu. Thymol was identified by comparison with the mass spectra from NIST (The National Institute of Standards and Technology) library.

Table 1. Extraction conditions and yield of ajwain seed SFE assay

Set	Pressure (bar)	Temperature (°C)	CO_2 flow rate (g/min)	Co-solvent flow rate (g/min)	Yield (%)
1	200	40	5	0.5	19.71
2	250	45	5	0.5	21.51

DPPH radical scavenging activity

The free radical scavenging activity of the sample was measured in vitro by DPPH assay (Sarker *et al*., 2006) with minor modifications. Ajwain seed ethanolic extract was taken and 0.1mM solution ethanolic DPPH solution (0.04g/L) was prepared. Different concentrations of working solutions (50, 100, 150, 200 and 250µg/mL of pure extract using absolute ethanol) were prepared and left in dark, at room temperature, for 30 minutes incubation. Absorbance was measured at 517 nm. Absolute ethanol without DPPH was used as Blank and ethanolic DPPH was used as Control. Butylated hydroxytoluene (BHT) was taken as reference.

Equation 1 was used to express the result as percentage of inhibition of the DPPH radical.

$$I\ (\%) = (A_{blank} - A_{sample}/A_{blank}) \times 100 \quad (1)$$

Where, A_{blank} is the absorbance of the control and A_{sample} is the absorbance of the test compound. The sample concentration showing 50% inhibition (IC_{50}) was calculated by plotting graph of inhibition percentage vs sample concentrations. IC_{50} value and its 95% confidence interval were calculated.

Antimicrobial property of the extract

The food borne pathogenic bacterial strains were obtained from MTCC, and were sub cultured every 30 days in nutrient agar medium at 30°C. The susceptibility of antibiotics was carried out using disc diffusion method on Muller-Hinton (MH) agar (Luangtongkum *et al*., 2007). MH agar plates were prepared and the strains of *S. aureus, Shigella, Salmonella* and *E.coli* were taken. On the other hand, antimicrobial discs (~9 mm) were prepared

Table 2. GC-MS result showing the presence of thymol and other phenolic compounds in the extract

Peak No.	Retention Time	Compound Name	Molecular Formula	Molecular Weight	Percentage of Total
1	7.986	o-Cymene	$C_{10}H_{14}$	134	3.368
2	9.469	γ-Terpinene	$C_{10}H_{16}$	136	3.826
3	21.817	Thymol	$C_{10}H_{14}O$	150	71.064
4	22.008	Phenol, 2-methyl-5-1-methylethyl	$C_{10}H_{14}O$	150	0.512

using the pure extract and 250 µg/mL ethanolic concentration. One untreated disc was used as control. Chloramphenicol (50 mg/mL) antibiotic disc was used as standard to compare the results. Wells were bored (~9 mm diameter) in the agar plates, the discs were placed gently and the plates were incubated at 37°C for 48 hours. Later, the diameter of the zone of inhibition was measured, which gave the extent of microbial inhibition.

Statistical analysis

Standard deviation was applied for determination of significant difference between mean values of zone of inhibition used to calculate antimicrobial property (Hernández-Carranza *et al*., 2013).

Results and Discussion

Extraction of thymol from ajwain seeds using SFE technique

The efficiency of supercritical fluid extraction was similar to that obtained by conventional liquid extraction, but the quality of the super critically extracted oil was higher, equivalent to a degummed liquid – extracted oil. In SFE, the stages like solvent distillation and oil refining can be omitted; hence, it is competitive with conventional liquid extraction (Gomez *et al.*, 1996).

SFE result showed that at 45^{O}C temperature and 250 bar pressure the yield was greater than at lower temperature and pressure, as shown in Table 1, even though the CO_2 flow rate and Co-solvent flow rate remained constant. This showed that with increase in temperature and pressure the percent yield of the extract increased. Hence, Set 2 cycle was continued for further extract collection. These results were in accordance with study that said both high temperature and pressure helped in increasing extraction efficiency (Langenfeld *et al*., 1993).

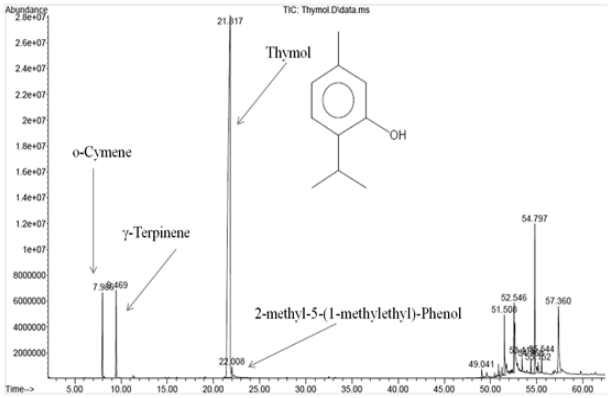

Figure 1. GC-MS result of ajwain seed ethanolic extract

Qualitative analysis of the extract using GC-MS technique

GC-MS result showed the presence of a mixture of components, mainly thymol together with a small amount of other volatile compounds. Four main components were identified, which represented 99.97% of the total oils: Thymol (71.06%), o-Cymene (3.37%), γ-Terpinene (3.826%) and 2-methyl-5-(1-methylethyl)-phenol (0.512%) were found as main phenolic components, as shown in Table 2.

Figure 1 shows the GC-MS result in the form of peaks. From the figure it can be observed that the highest peak is of thymol. Here the peaks depict the abundance of that particular compound in the sample at that period of time. For example, the presence of thymol appeared at 21.817 seconds. In the figure, the presence of other non-phenolic compounds can be observed. Some previous studies have already reported that thymol is the major component of this oil but its concentration varies due to time of plant growing, preparation process, cultivar differences and geographical location from which the plants were collected (Saei-Dehkordi *et al.*, 2010).

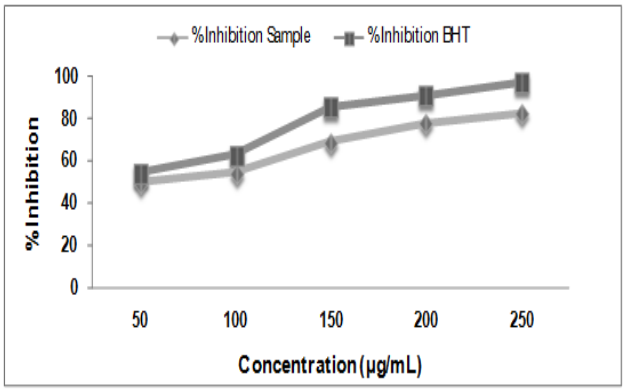

Figure 2. Comparative DPPH activity of the sample with BHT standard

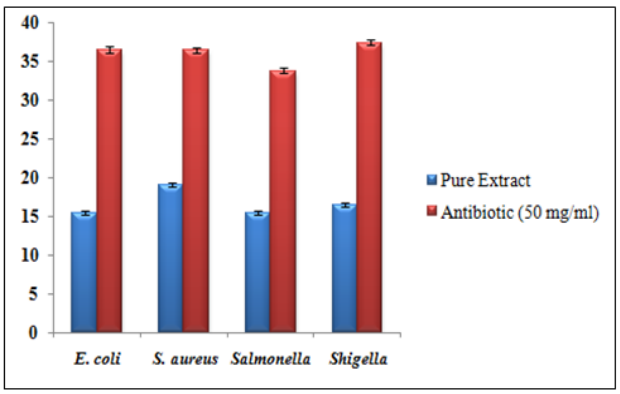

Figure 3. Graphical representation of zone of inhibition against different pathogenic bacteria and compared with chloramphenicol as standard antibiotic

Antioxidant property using DPPH radical scavenging activity

It was observed that with increase in concentration from 50 µg/mL to 250 µg/mL, the percentage inhibition increased from 50% to 83%. IC_{50} was calculated and its 95% confidence interval (CI) value came out to be between the lower endpoint 5.484 and the upper endpoint 17.53188. The graphical representation, in Figure 2, depicts the activity of the sample extract compared with BHT (Butylated hydroxytoluene) standard.

Antimicrobial property of the extract

Agar well diffusion method showed the antimicrobial activity of the pure ajwain seed extract with zone of inhibition of 15.33±0.35 mm, 19.02±0.34 mm, 16.29±0.32 mm and 16.46±0.13 mm against *Salmonella, S. aureus, E. coli* and *Shigella*, respectively, whereas, the control discs did not show any zone of inhibition. The diameter of zone of inhibition recorded includes the size of filter paper discs. This showed that the ajwain seed extract has antimicrobial properties against various pathogenic and food spoilage microbes as shown in few studies (Wadhwa *et al.*, 2010; Masih *et al.*, 2012; Hassanshahian *et al.*, 2014). In Figure 3, the graph shows the zone of inhibition of different concentrations against the pathogenic bacteria. Here the control and 250 µg/mL ethanolic concentration of the extract did not show any inhibitory activity against the microbes.

Conclusion

From this research work, it was concluded that ajwain seeds' ethanolic extract, obtained via supercritical fluid extraction method, is rich in phenolic compounds (mainly thymol) and it has high antioxidant and antimicrobial activity. DPPH radical scavenging activity showed that the extract has comparative antioxidant property as that of the BHT standard. The disc diffusion method showed the effectiveness of the extract against various Gram positive and Gram negative bacteria. Hence, this extract can be used as an excellent bio-preservative and for medicinal purpose. It can also enhance the shelf-life of various perishable and fat rich food products, even at room temperature.

Acknowledgment

The authors express their gratitude to the research facilities and the lab assistants of SRM University, Chennai, India.

References

Agaoglu, S., Dostbil, N. and Alemdar, S. 2007. Antimicrobial activity of some spices used in the meat industry. Bulletin of the Veterinary Institute in Pulawy 55:53-57.

Ashrafi Tamai, I., Zahraei Salehi, T., Khosravi, A., Sharifzadeh, A. and Balal, A. 2013. Chemical composition and anti-candida activity of *Trachyspermum ammi* essential oil on azoles resistant Candida albicans isolates from oral cavity of HIV+ patients. Journal of Medicinal Plants 12: 137-149.

Bairwa, R., Sodha, R. and Rajawat, B. 2012. Trachyspermum ammi. Pharmacognosy Reviews 6(11): 56–60.

Guest, J. and Grant, R. 2012. Effects of dietary derived antioxidants on the central nervous system. International Journal of Nutrition, Pharmacology, Neurological Diseases 2(3): 185–197.

Gomez M. A., Lopez P. C. and de la Ossa M. E. 1996. Recovery of grape seed oil by liquid and supercritical carbondioxide extraction: A comparision with conventional solvent extraction. The Chemical Engineering Journal and the Biochemical Engineering Journal 61(3): 227-231.

Hassanshahian M., Bayat Z., Saeidi S. and Shiri Y. 2014.

Antimicrobial activity of *Trachyspermum ammi* essential oil against human bacterial. International journal of Advanced Biological and Biomedical Research 2(1): 18-24.

Hernández-Carranza, P., López-Malo, A., and Jiménez-Munguía, M.-T. 2014. Microencapsulation Quality and Efficiency of *Lactobacillus casei* by Spray Drying Using Maltodextrin and Vegetable Extracts. Journal of Food Research, 3(1): 61-69.

Kumar, A., Mishra, R. K., Srivastava, S., Tiwari, A., Pandey, A., Shukla, A. C. and Dikshit, A. 2011. Role of phylogenetic analysis for anti-bacterial activity of essential oil of *Trachyspermum ammi* (L.) against water borne pathogens. Advances in Environmental Biology 5(6): 1271-1278.

Langenfeld J. J., Hawthrone S. B., Miller D. J. and Pawliszyn J. 1993. Effects of temperature and pressure on supercritical fluid extraction efficiencies of polycyclic aromatic hydrocarbons and polychlorinated biphenyls. Analytical Chemistry. 65: 338-344.

Li, T., Hu, W., Li, J., Zhang, X., Zhu, J. and Li, X. 2012. Coating effects of tea polyphenols and rosemary extract combined with chitosan on the storage quality of large yellow croaker. Food Control 25(1): 101–106.

Luangtongkum, T., Morishita, T. Y., El-Tayeb, A. B., Ison, A. J. and Zhang, Q. 2007. Comparison of antimicrobial susceptibility testing of *Campylobacter* spp. by the agar dilution and the agar disk diffusion methods. Journal of Clinical Microbiology 45: 590-594.

Masih U., Shrimali R. and Naqvi S. M. A. 2012. Antibacterial Activity of Acetone and Ethanol Extracts of cinnamon *(Cinnamomum zeylanicum)* and ajowan *(Trachyspermum ammi)* on four food spoilage bacteria. International Research Journal of Biological Sciences 1(4): 7-11.

Moein, M., Zomorodian, K., Pakshir, K., Yavari, F., Motamedi, M. and Zarshenas, M. 2015. *Trachyspermum ammi* (L.) Sprague: Chemical composition of essential oil and antimicrobial activities of respective fractions. Journal of Evidence-Based Complementary and Alternative Medicine 20(1): 50-56.

Mónica, R., García, R., Gonzalo, V., Guillermo, R. and Tiziana, F. 2011. Fractionation of thyme (*Thymus vulgaris* L.) by supercritical fluid extraction and chromatography. Journal of Supercritical Fluids 55(3): 949–954.

Nagalakshmi, G., Shankaracharya, N. B. and Puranaik, J. 2000. Studies on chemical and technological aspects of Ajwain *(Trachyspermum ammi)* syn *(Cerumcopticum hiren)* seeds. Journal of Food Science and Technology 37: 277–281.

Naz, S., Siddiqi, R. and Sayeed, S. 2008. Effect of flavonoids on the stability of corn oil. International Journal of Food Science and Technology 43(10): 1850–1854.

Orhan, D., Ozçelik, B., Ozgen, S. and Ergun, F. 2010. Antibacterial, antifungal, and antiviral activities of some flavonoids. Microbiological Research 165(6): 496–504.

Ozçelik, B., Kartal, M. and Orhan, I. 2011. Cytotoxicity, antiviral and antimicrobial activities of alkaloids, flavonoids, and phenolic acids. Pharmaceutical Biology 49(4): 396–402.

Papp, L. V., Lu, J., Holmgren, A. and Khanna, K. K. 2007. From Selenium to Selenoproteins: Synthesis, Identity, and Their Role In Human Health. Pakistan Journal Botany 9(7): 775-806

Paul, S., Dubey, R., Maheswari, D. and Kang, S. 2011. *Trachyspermum ammi* (L.) fruit essential oil influencing on membrane permeability and surface characteristics in inhibiting foodborne pathogens. Food Control 22(5): 725–731.

Phapale, R. and Thakur, S. M. 2010. Antioxidant activity and antimutagenic effect of phenolic compounds in *Feronia limonia* (L.) Swingle Fruit. International Journal of Pharmacy and Pharmaceutical Sciences 2(4): 68–73.

Raza, M., Shukla, A. K., Fatima, T. and Ali, S. 2015. Comparative study of antioxidant activity of polyphenols isolated from frozen and fresh leaves of *Trachyspermum ammi* (Ajwain). Journal of Pharmacognosy and Phytochemistry 3(6): 122-124.

Saei-Dehkordi, S., Tajik, H., Moradi, M. and Khalighi-Sigaroodi, F. 2010. Chemical composition of essential oils in *Zataria multiflora* Boiss from different parts of Iran and their radical scavenging and antimicrobial activity. Food and Chemical Toxicology 48(6): 1562–1567.

Sarker, S. D., Latif, Z. and Gray, A. I. 2006. Natural Products Isolation, p. 529. Totowa, New Jersey: Humana Press Inc.

Shelef, L. A. 1983. Antimicrobial effects of spices. Journal of Food Safety 6: 29-34.

Villanueva, B. D., Angelov, I., Vicente, G., Stateva, R. P., Rodriguez García-Risco, M., Reglero, G., Ibañez, E. and Fornari, T. 2105. Extraction of thymol from different varieties of thyme plants using green solvents. Journal of the Science of Food and Agriculture 95(14): 2901-2907.

Wadhwa S., Bairagi M., Bhatt G., Panday M. and Porwal A. 2010. Antimicrobial activity of essential oils of *Trachyspermum ammi*. International Journal of Pharmaceutical and Biological Archives 1(2): 131-133.