



Isolation of bioactive components of Carom: A Review

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Abstract

Essential oil is a complex mixture of volatile compounds and can be extracted from various parts of plants. It has been used for thousands of years for medicinal and health purposes. Because of their detoxifying, antibacterial, antiviral and calming properties, it is used as a natural, safe and cost-effective therapy for a number of health concerns. Ajwain seed is very valuable for its oil contents. It is a high value medicinally important seeds. Ajwain is most widely used for the treatment of many diseases in both animals and humans. Ajwain seeds are most commonly used as a household remedy for diarrhea, asthma, colic and dyspepsia and it also have antifungal, antibacterial, antihelminthic, antioxidant properties. The active components of ajwain essential oils are phenolic carvacrol and mainly thymol. Both are responsible for antitussive and antiseptic properties. The phenolic compound thymol is germicide, antifungal and antispasmodic agent. This review article is designed to compile extraction techniques and isolation techniques for the separation of bioactive components present in essential oils of ajwain. Furthermore, biological activities of isolated components are also discussed briefly.

Key words: Ajwain seed, Essential oils, Biological activities, Bioactive components, Thymol

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1. Introduction

Essential oils (EOs) are the fascinating natural plant products. Essential oils have various biological properties [1-2]. Essential oil can be defined as etheric oil or volatile oil, made of the mixture of those volatile components which are biologically synthesized by living organisms. Essential oils are more usually extracted from the plant material by water and steam and distillation [3]. Essential oils produced by the plant are used as remedies and for the cure of diseases [4]. Furthermore, it is also used in religious ceremonies because of their healing properties and their pleasant odors [1]. Essential oils (EOs) show antimicrobial action [5] against pathogenic bacteria but their less water solubility limits the application in foods. Essential oils can be extracted from different aromatic plants which includes species and herbs. Essential oils obtain from various specie has been used to produce natural preservatives, nourishing food products, increase the shelf life and for the reduction of pathogenic bacteria [6]. The chemical composition of essential oils depends on environmental conditions, harvesting period, type of extraction technique and geographic conditions [3-7]. There are different methods which are extensively used for the extraction of essential oils from the plant materials such as solvent extraction, hydro (HD) and steam distillation (SD)

etc. Among these approaches, HD is the most common method for the extraction of essential oils from the plant/medicinal herbs. However, these methods are thermally sensitive and vulnerable to chemical changes. Low extraction efficiency, damages of certain volatile compounds, presence of toxic solvent in the extract and degradation of unsaturated or ester compounds through thermal or hydrolytic effect can be met by using these extraction methods. These failings have led to the consideration of the usage of new green techniques for the extraction of essential oils from the plant materials, that use lesser amount of both solvent and energy, for example microwave, ultrasound, supercritical fluid techniques [8]. Various techniques are used to analyze the extracted essential oils such as gas chromatography (GC), high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) [2-9-11]. However GC-MS is considered to be a best analytical technique for the detection of components present in the extracted essential oils [12]. The biological activities of essential oils depend upon the composition, type and concentration of essential oils [7]. Almost 300 essential oils are most commonly traded on world market. Essential oils are most widely used in pharmaceutical, cosmetics, in food and household goods industries. About 20 percent essential

oils are used in food industries for flavor purposes and about 20 percent used in pharmaceutical industry and the remaining used in cleaning products, in skin, in hair products, in fragrance industry and in aromatherapy [13]. In recent times essential oils have become famous as aromatherapy agents. Various aromatic plants have been used for the protection of stored products from pest infection in certain countries [14]. However, inappropriate use of essential oils can give rise adverse effect to the human being such as nausea, headaches and skin irritation [15]. Essential oils obtained from herb or spices have number of applications in various industries as describe above. Among spices, essential oils extracted from the Ajwain (Carom) are most widely used for the treatment of many diseases in both animals and humans. Ajwain seeds are most commonly used as a household remedy for diarrhea, asthma, dyspepsia and it also have antifungal, antibacterial, antihelminthic, antioxidant properties. Ajwain seeds are used medicinally and also used as herbs and spices in the preparation of numerous edible dishes. Chemical composition of the Ajwain seed oil is responsible for its extensive applications in various fields. There are number of chemical constituents present in Ajwain seed oil and all these constituents have different activities. That is why, in the present article various isolation techniques are discussed for the isolation of various biologically active components from the Ajwain seed oil.

2. Essential oil of Ajwain

Spices have remarkable therapeutic value. It is used to increase the flavor as well as aroma [16]. There are some other names of ajwain in history such as omum, owa, Ethiopian cumin, carom and bishops weed. Carom seeds are belongs to the Apiaceae family. The botanical name of carom seeds is *Trachyspermum ammi*. Essential oils which is extracted from carom seeds have volatile nature and have physio-chemical and organoleptic properties [17]. *Trachyspermum ammi* is a native of Egypt and it is cultivated in Pakistan, India, Iran, Iraq, and Afghanistan. *T. ammi* is a medicinally important seed as the seeds of *T. ammi* contain excellent aphrodisiac properties and its roots are diuretic in nature. The *T. ammi* seeds consisted of 2-4.4 % brown colored oil which is known as ajwain oil [18] in which (40-50)% thymol is present. Thymol can easily be crystalized from the essential oils of ajwain and the remainder contains beta pinene, carvacrol, dipentene, beta terpinenes, and rho-cymene. Ajwain seeds are most commonly used as a household remedy for diarrhea, asthma, colic and dyspepsia and it also have antifungal, antibacterial, hypocholesterolemic, antihelminthic, antioxidant and bronchodilator effects [19].

3. Composition of ajwain oil

The principle active components of essential oil of *T. ammi* are phenols, carvacrol and mainly thymol. Both phenolic compounds carvacrol and thymol are responsible

for antitussive and antiseptic properties. Thymol has antiseptic activity and carvacrol contains antifungal properties. Ajwain is rich source of thymol, γ -terpinene and p-cymene and carvacrol. [20]. Major components which are present in the ajwain essential oil are α -pinene, α -thujene, β -myrcene, β -pinene, β -phellendrene, o cymene, γ -terpiene, limonene, 4-terpineol, dodecane, cis limonene oxide, β -fenchyl alcohol, tetra decane, thymol, ethylene methacrylate, heptadecane, diethyl phthalate [21].

4. Extraction Techniques

Various methods are used for the extraction of ajwain oil such as solvent extraction, microwave-assisted extraction, Hydrodistillation, steam distillation, soxhlet extraction and super critical CO₂ extraction. Yield of essential oils extracted from the Ajwain seeds was calculated by some researcher via hydrodistillation as it is most general and extensively used method. Extraction of ajwain oil through hydro distillation method took place in 3 h, by using Clevenger type apparatus. Hydrodistillation showed that ajwain seeds contained 2.99 % (w/w) EOs and Ajwain oil had 16 compounds, representing 98.51% of total oils. Soxhlet extraction method can also be used for the extraction of essential oils from the plant materials. It was found that the ajwain seed mass was dried and 13.33 g of this mass introduced in soxhlet apparatus with 400 mL methanol. This extraction took 24 hours with methanol. In this extraction thymol yield was calculated in the in ajwain seed and found that its amount varies from source to source, about 3.18% (w/w) thymol yield was recorded [22]. In microwave assisted extraction, the thymol was extracted in self-tuning single mode microwave cavity. Inside the microwave reactor, the pressure was kept from 0 to 300psi and the temperature was kept 25 to 250 degree centigrade. Thymol was extracted by the addition of 1g of dried ground seeds in methanol solvent and put it in a vessel and then the vessel was placed symmetrically in the microwave field under agitation condition (600 rpm). The maximum percentage of thymol extraction was 99% [22].

5. Isolation techniques

Biological components present in ajwain essential oils can be isolated by using different techniques such as high speed counter current chromatography (HSCC), fractionation by vacuum distillation and fractional distillation. HSCC is a recent separation technique in which two immiscible solvents are used in form of two layers for isolation of required components of essential oil where lower layer acts as mobile phase and upper layer works as stationary phase. Initially, stationary phase is filled in helical column and then mobile phase is pumped in the coiled column through inlet that is usually found on chromatographic column. When equilibrium is established, sample of essential oil is introduced which undergo fractionation and continuously detected by detector. It is most commonly used for purification or sanitization of

different functional compounds from customary classical Chinese aromatic plants and also various other usual natural products. Fractional distillation separates the chemical constituents on the basis of their boiling point. Boiling point of any liquid is the most significant physical property that shows important role in fractionation of essential oil, as various fractions are known to have different chemical constituents depend upon temperature ranges. However, fractions of essential oils are generally obtained at variable temperatures under controlled conditions and decreased pressure. Every fraction constitutes entirely different fragrances due to different chemical compounds. *Furthermore*, column chromatography was also used by some researcher in order to isolate the thymol from the extracted essential oils of ajwain. The essential oil of the ajwain was adsorbed on silica gel with 60-120 mesh size and then the column was eluted with hexane and the polarity of the column was increased by using dichloromethane. In this way thymol was isolated successively [23].

6. Biological activities of ajwain oil

A number of therapeutic and medicinal properties has been attributed to certain parts of this ajwain plant. Essential oils of Ajwain seeds consisted of 40-50 % thymol which is strong anti-spasmodic, fungicide and germicide. Thymol is used as an ingredient of mouthwashes, toothpastes, deodorants and in different pharmaceutical preparations. Ajwain essential oils shows many biological activities including scolicidal, antitermitic, antibacterial, antifungal, the cytotoxicity on tumour cells, induction of lymphocyte proliferation, digestive stimulant, hypolipidemic, antiplatelet-aggregatory. It is also used as febrifugal, antipyretic and in the treatment of typhoid fever. Essential oils due to their antimicrobial [24-25], antioxidant and therapeutic activity used in pharmaceutical and food [26]. *T. ammi* has much value due to its stimulant, carminative and antispasmodic effects which was indicated by different scientists [27]. The oil obtained from ajwain seeds is used in mouthwash because of its excellent antimicrobial properties and the smoke of heated ajwain seeds are used for the treatment of cough. Researcher estimated the in vitro antioxidant and antibacterial activity of essential oil of ajwain and the same in combination with essential trace elements iron (III), copper (II), and zinc (II). The addition of iron and copper salt to all the ajwain seed extract increase its antibacterial activity against antibiotic resistant bacteria as *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* [28].

7. Pharmacological activities

Ajwain oil at therapeutic level is used for the treatment of gastrointestinal ailments, bronchial problems and lack of appetite. In about 1923 and 1250 AD, it has been reported that *T. ammi* seeds were used for the removal of urinary calculi. *T. ammi* seeds were also used to release the pain of kidney stone and also used in different drug

formulations [27]. The seeds of ajwain revealed to possess stimulant, carminative, anesthetic, diuretic, nematocidal, antiviral, antihypertensive, antiulcer, bronchodilatory, antiplatelet, antitussive, hepatoprotective, antiplatelet, antihyperlipidemic and antiseptic effects. With these pharmacological activities, seeds of ajwain can be applied efficiently in clinical practice [29].

8. Biological activities of isolated bioactive components of Ajwain oil

Ajwain essential oils is a major source of biological active components mainly thymol, phenolic compounds, para-cymene, γ -terpinene, α - and β -pinene, carvacrol, α -terpinene, limonene, dillapiole and dipentene. Biological activities of these chemical components are described as follow.

8.1. Thymol

Essential oil of ajwain seeds consisted of 40-50 % thymol which is strong fungicide, anti-spasmodic and germicide. It is used as ingredient of toothpastes, deodorants, mouthwashes and various other pharmaceutical preparations. Essential oil obtained from ajwain are used in pharmaceutical and food industries due to their antimicrobial, antioxidant and therapeutic activities [26].

8.2. Phenolic monoterpene

Due to the presence of phenolic compounds ajwain oil can be used as natural antioxidant. Methanolic extract of *T. ammi* fruits along with mature bovine filarial *Setaria digitate* worms was investigated by some researchers. EOs of ajwain showed significant activity against the mature *S. digitata* by both MTT [(2, 5-diphenyltetrazolium bromide)-3-(4, 5-dimethylthiazol-2-yl)] and a worm motility reduction assay [30].

8.3. p-cymene

p-Cymene is a monoterpenes originate in over 100 plant species used for medicine and food purposes. It shows a variety of biological activities including antinociceptive, anticancer, antimicrobial, anxiolytic, antioxidant and anti-inflammatory effect. Medicinally, *p-cymene* is used to inhibit phlegm and coughs and being used in the production of fungicides and pesticides [31].

8.4. γ -Terpinene

γ -Terpinene (TH), a monoterpene hydrocarbon present in essential oils, retards the peroxidation of linoleic acid (LH). The peroxidation of TH has been shown to yield *p-cymene* as the only organic product in a chain reaction in which the chain carrier is the hydroperoxyl radical, HOO•. The peroxidation of LH is well-known to be a chain reaction in which the chains are carried by linoleylperoxyl radicals, LOO•, and the products are linoleyl hydroperoxides. The retardation of LH peroxidation by TH has been found to be due to rapid chain termination via a very fast cross-reaction between HOO• and LOO• radicals [32].

8.5. Carvacrol

Carvacrol has been categorized as an inhibitor of growth of diverse pathogens [33]. It is a natural phenolic compound that have great care for its high antimicrobial actions [34].

9. Conclusion

Ajwain seeds served as an important components of human diet and also used in the treatment of diseases in both humans and animals. Ajwain essential oils used as antibacterial, antimicrobial, antifungal and antioxidant agent. Bioactive components of ajwain oil also participates to its nutritious value. Furthermore, these components have various applications in pharmaceutical field. Hence the isolation of these components from the EOs of ajwain is crucial in order to make sure the complete separation as well as purity of the chemical constituents. From the above discussion, it is concluded that microwave assisted extraction method is better as compare to other methods which are discussed above for the extraction of thymol from the ajwain seeds. Thymol is a major component present in the essential oils of Ajwain and responsible for various biological activities. Major components responsible for the antimicrobial activity of ajwain essential oils are thymol and carvacrol. Thymol is a strong fungicide, germicide, anti-spasmodic and used in perfumery and toothpaste. Thymol and carvacrol both are considered to be either bacteriostatic agents or bactericidal depend upon the concentration.

References

- [1] K.H.C. Baser, G. Buchbauer. (2015). Handbook of essential oils: science, technology, and applications. CRC press: pp.
- [2] M.A. Hanif, M.Y. Al-Maskari, A. Al-Maskari, A. Al-Shukaili, A.Y. Al-Maskari, J.N. Al-Sabahi. (2011). Essential oil composition, antimicrobial and antioxidant activities of unexplored Omani basil. *Journal of Medicinal Plants Research*. 5(5): 751-757.
- [3] A.Y. Al-Maskri, M.A. Hanif, M.Y. Al-Maskari, A.S. Abraham, J.N. Al-sabahi, O. Al-Mantheri. (2011). Essential oil from *Ocimum basilicum* (Omani Basil): a desert crop. *Natural product communications*. 6(10): 1934578X1100601020.
- [4] I. Ahmad, M.A. Hanif, R. Nadeem, M.S. Jamil, M.S. Zafar. (2008). Nutritive evaluation of medicinal plants being used as condiments in South Asian Region. *JOURNAL OF THE CHEMICAL SOCIETY OF PAKISTAN*. 30(3): 400-405.
- [5] M.A. Hanif, H.N. Bhatti, M.S. Jamil, R.S. Anjum, A. Jamil, M.M. Khan. (2010). Antibacterial and antifungal activities of essential oils extracted from medicinal plants using CO₂ supercritical fluid extraction technology. *Asian journal of chemistry*. 22(10): 7787.
- [6] R. Lanciotti, A. Gianotti, F. Patrignani, N. Belletti, M. Guerzoni, F. Gardini. (2004). Use of natural aroma compounds to improve shelf-life and safety of minimally processed fruits. *Trends in food science & technology*. 15(3-4): 201-208.
- [7] H. Baydar, O. Sađdıç, G. Özkan, T. Karadođan. (2004). Antibacterial activity and composition of essential oils from *Origanum*, *Thymbra* and *Satureja* species with commercial importance in Turkey. *Food Control*. 15(3): 169-172.
- [8] M.E. Lucchesi, F. Chemat, J. Smadja. (2004). Solvent-free microwave extraction of essential oil from aromatic herbs: comparison with conventional hydro-distillation. *Journal of Chromatography A*. 1043(2): 323-327.
- [9] M.A. Hanif, S. Nisar, G.S. Khan, Z. Mushtaq, M. Zubair, *Essential Oils*. In *Essential Oil Research*, Springer: 2019; pp 3-17.
- [10] M.A. Hanif, A.Y. Al-Maskri, Z.M.H. Al-Mahruqi, J.N. Al-Sabahi, A. Al-Azkawi, M.Y. Al-Maskari. (2011). Analytical evaluation of three wild growing Omani medicinal plants. *Natural product communications*. 6(10): 1934578X1100601010.
- [11] I. Shahzadi, R. Nadeem, M.A. Hanif, S. Mumtaz, M.I. Jilani, S. Nisar. *Chemistry and biosynthesis pathways of plant oleoresins: Important drug sources*.
- [12] S. Kilani, J. Ledauphin, I. Bouhlel, M.B. Sghaier, J. Boubaker, I. Skandrani, R. Mosrati, K. Ghedira, D. Barillier, L. Chekir-Ghedira. (2008). Comparative study of *Cyperus rotundus* essential oil by a modified GC/MS analysis method. Evaluation of its antioxidant, cytotoxic, and apoptotic effects. *Chemistry & biodiversity*. 5(5): 729-742.
- [13] R. Tisserand, R. Young. (2013). *Essential oil safety: a guide for health care professionals*. pp.
- [14] S. Ramezani, M.J. Saharkhiz, F. Ramezani, M.H. Fotokian. (2008). Use of essential oils as bioherbicides. *Journal of Essential Oil Bearing Plants*. 11(3): 319-327.
- [15] S. Paul, R. Dubey, D. Maheswari, S.C. Kang. (2011). *Trachyspermum ammi* (L.) fruit essential oil influencing on membrane permeability and surface characteristics in inhibiting food-borne pathogens. *Food Control*. 22(5): 725-731.
- [16] A. Shukla, N. Yadav. (2018). ROLE OF INDIAN SPICES IN INDIAN HISTORY. *International Journal of Management Research and Reviews*. 8(11): 1.
- [17] S.S. Kadam, S.B. More, J.S. Waghmare. (2017). *Trachyspermum Ammi*: Natural pesticides. *Journal of Biopesticides*. 10(2): 90-98.
- [18] R. Bairwa, R. Sodha, B. Rajawat. (2012). *Trachyspermum ammi*. *Pharmacognosy reviews*. 6(11): 56.

- [19] M.A. Rajput, R.A. Khan, N. Qazi, Z. Feroz. (2012). Effect of methanol extract of ajwain (*Trachyspermum ammi* L) on blood coagulation in rats. *JLUMHS*. 11(02): 105.
- [20] S. Javed, A.A. Shahid, M.S. Haider, A. Umeera, R. Ahmad, S. Mushtaq. (2012). Nutritional, phytochemical potential and pharmacological evaluation of *Nigella Sativa* (Kalonji) and *Trachyspermum Ammi* (Ajwain). *Journal of Medicinal Plants Research*. 6(5): 768-775.
- [21] M. Mahboubi, N. Kazempour. (2011). Chemical composition and antimicrobial activity of *Satureja hortensis* and *Trachyspermum copticum* essential oil. *Iranian journal of microbiology*. 3(4): 194.
- [22] J. Gujar, S. Wagh, V. Gaikar. (2010). Experimental and modeling studies on microwave-assisted extraction of thymol from seeds of *Trachyspermum ammi* (TA). *Separation and Purification Technology*. 70(3): 257-264.
- [23] K. Dhaiwal, K.K. Chahal, D. Kataria, A. Kumar. (2017). Gas chromatography-mass spectrometry analysis and in vitro antioxidant potential of ajwain seed (*Trachyspermum ammi* L.) essential oil and its extracts. *Journal of Food Biochemistry*. 41(3): e12364.
- [24] E.M. Abdallah, A.E. Khalid. (2012). A preliminary evaluation of the antibacterial effects of *Commiphora molmol* and *Boswellia papyrifera* oleo-gum resins vapor. *International Journal of Chemical and Biochemical Sciences*. 1: 1-15.
- [25] A.A. Hamid, O.O. Aiyelaagbe. (2012). Pharmacological investigation of *Asystasia calyciana* for its antibacterial and antifungal properties. *International Journal of Chemical and Biochemical Sciences*. 1: 99-104.
- [26] Y. Rohamare, T. Nikam, K. Dhumal. (2013). Effect of foliar application of plant growth regulators on growth, yield and essential oil components of Ajwain (*Trachyspermum ammi* L.). *International Journal of Seed Spices*. 3(2): 34-41.
- [27] T. Kaur, R.K. Bijarnia, S.K. Singla, C. Tandon. (2009). In vivo efficacy of *Trachyspermum ammi* anticalcifying protein in urolithiatic rat model. *Journal of ethnopharmacology*. 126(3): 459-462.
- [28] M. Prashanth, H. Revanasiddappa, K. Rai, K. Raveesha, B. Jayalakshmi. (2012). Antioxidant and antibacterial activity of ajwain seed extract against antibiotic resistant bacteria and activity enhancement by the addition of metal salts. *Journal of Pharmacy Research*. 5(4): 1952-1956.
- [29] M.M. Zarshenas, M. Moein, S.M. Samani, P. Petramfar. (2013). An overview on ajwain (*Trachyspermum ammi*) pharmacological effects; modern and traditional. *Journal of natural Remedies*. 14(1): 98-105.
- [30] N. Mathew, S. Misra-Bhattacharya, V. Perumal, K. Muthuswamy. (2008). Antifilarial lead molecules isolated from *Trachyspermum ammi*. *Molecules*. 13(9): 2156-2168.
- [31] A. Marchese, C. Arciola, R. Barbieri, A. Silva, S. Nabavi, A. Tsetegho Sokeng, M. Izadi, N. Jafari, I. Suntar, M. Daglia. (2017). Update on monoterpenes as antimicrobial agents: A particular focus on p-cymene. *Materials*. 10(8): 947.
- [32] M.C. Foti, K. Ingold. (2003). Mechanism of inhibition of lipid peroxidation by γ -terpinene, an unusual and potentially useful hydrocarbon antioxidant. *Journal of agricultural and food chemistry*. 51(9): 2758-2765.
- [33] Z. Mazarei, H. Rafati. (2019). Nanoemulsification of *Satureja khuzestanica* essential oil and pure carvacrol; comparison of physicochemical properties and antimicrobial activity against food pathogens. *LWT*. 100: 328-334.
- [34] X. Sun, R.G. Cameron, J. Bai. (2019). Microencapsulation and antimicrobial activity of carvacrol in a pectin-alginate matrix. *Food Hydrocolloids*. 92: 69-73.