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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, anti-bacterial, anti-viral, and calming properties, it is used as a natural, safe, and cost-effective therapy for several health concerns. Ajwain seed is unbelievably valuable for its oil contents. It is a high valued and medicinally important seed. Ajwain is most widely used for the treatment of many diseases in both animals and humans. Ajwain seeds are mostly used as a household remedy for diarrhea, asthma, colic and dyspepsia and it also have anti-fungal, anti-bacterial, anti-helminthic and anti-oxidant properties. The active components of ajwain essential oils are phenolic carvacrol and mainly thymol. Both are responsible for anti-tussive and anti-septic properties. The phenolic compound thymol is germicide, anti-fungal and anti-spasmodic agent. This review article is designed to compile extraction techniques and isolation methods 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 [1-19]. Essential oils have various biological properties. 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 usually extracted from the plant material by water and steam through distillation [1-19]. Essential oils produced by the plants are used as remedies and for the cure of diseases. Furthermore, it is also used in religious ceremonies because of its healing properties and pleasant odors [1-19]. Essential oils (EOs) show anti-microbial action against pathogenic bacteria but their less water solubility limits the application in foods. Essential oils can be extracted from different aromatic plants which include species and herbs. Essential oils obtain from various specie has been used to produce natural preservatives, nourishing food products, to increase the shelf life and for the reduction of pathogenic bacteria [20]. The chemical composition of essential oils depends on environmental conditions, harvesting period, type of extraction technique and geographical conditions [1-19]. 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 [21]. 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). However GC-MS is considered to be a best analytical technique for the detection of components present in the extracted essential oils [22]. The biological activities of essential oils depend upon the composition, type and concentration of essential oils [23]. Almost 300 essential oils are most traded

in world market. Essential oils are most widely used in pharmaceutical, cosmetics, in food and household goods industries. According to some recent estimates, almost twenty percent essential oil is used in food and flavor industry and twenty percent in pharmaceutical industry. Rest of the percentage is being used by routine cleaning products, skincare articles, hair care products and aromatherapy lotions [24]. 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 [25]. However, inappropriate use of essential oils can give rise to adverse effects in human being including nausea, headaches and skin irritation [26]. Essential oils obtained from herb or spices have number of applications in various industries as described above. Among spices, essential oil extracted from the ajwain (Carom) is most widely used for the treatment of many diseases in both animals and humans. Ajwain seeds are mostly used as a household remedy for diarrhea; asthma, dyspepsia, and it also have anti-fungal, anti-bacterial, anti-helminthic and anti-oxidant properties. Ajwain seeds are used as a medicine in conventional and modern system of medicines and as herb in 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 review 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 [27]. There are some other names of ajwain in history such as omum, owa, Ethiopian cumin, carom and bishops weed. Carom seeds belongs to the Apiaceae family. The botanical name of carom seeds is *Tranchyspermum ammi*. Essential oils extracted from carom seeds possess highly volatile organic compounds having unique physiochemical characteristics and organoleptic properties [28]. *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. The seeds of *T. ammi* contain excellent aphrodisiac properties and its roots are diuretic in nature. *T. ammi* seeds consisted of 2-4.4% brown colored oil which is known as ajwain oil [29] containing 40-50% of thymol as a major component. Thymol can be 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 anti-fungal, anti-bacterial, hypocholesterolemic, anti-helminthic, anti-oxidant and bronchodilator effects [30].

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 anti-tussive and anti-septic properties. Thymol has anti-septic activity and carvacrol contains anti-fungal properties. Ajwain is rich source of thymol, γ-terpinene and p-cymene and carvacrol [31]. The components commonly 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 and diethyl phthalate [32].

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 CO_2 extraction. Extraction of ajwain oil through hydrodistillation method takes about 3 h, by using Clevenger type apparatus. The essential oil yield obtained from ajwain seeds is about 3%. Soxhlet extraction method can also be used for the extraction of essential oils from the plant materials. This extraction required almost 24 hours with methanol [33]. In microwave assisted extraction, the thymol was extracted in self-tuning single mode microwave cavity. Inside the microwave reactor, the pressure was programmed from 0 to 300 psi and the temperature was programmed 25 to 250˚C. Thymol was extracted by the addition of 1g of dried ground seeds in methanol solvent taken in a vessel and then the vessel was placed symmetrically in the microwave field under agitation condition (600rpm). The maximum percentage of thymol extraction was 99% [33].

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 used method 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 based on 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 depending 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 researchers 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 [34].

6. Biological activities of ajwain oil

Several therapeutic and medicinal properties have 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, anti-termitic, anti-bacterial, anti-fungal, the cytotoxicity on tumor cells, induction of lymphocyte proliferation, digestive stimulant, hypolipidemic and anti-platelet-aggregatory. It is also used as febrifugal, anti-pyretic and in the treatment of typhoid fever. Essential oils due to their anti-microbial [35-36], anti-oxidant and therapeutic activity, used in pharmaceutical and food [37]. *T. ammi* has much value due to its stimulant, carminative and anti-spasmodic effects which was indicated by different scientists [38]. The oil obtained from ajwain seeds is used in mouthwash because of its excellent anti-microbial properties and the smoke of heated ajwain seeds are used for the treatment of cough. *In vitro* anti-oxidant and anti-bacterial activity of essential oil of ajwain and the same in combination with essential trace elements iron (III), copper (II), and zinc (II) has been studied previously. The addition of iron and copper salt to all the ajwain seed extract increased, its anti-bacterial activity against anti-biotic resistant bacteria such as *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* [39].

7. Pharmacological activities

Ajwain oil at therapeutic level is used for the treatment of gastrointestinal ailments, bronchial problems, and lack of appetite. Since long times, *T. ammi* seeds are 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 [38]. The ajwain seeds are found to possess stimulant, carminative, anesthetic, diuretic, nematocidal, anti-viral, anti-hypertensive, anti-ulcer, bronchodilatory, anti-platelet, anti-tussive, hepatoprotective, anti-platelet, anti-hyperlipidemic and anti-septic effects. With these pharmacological activities, seeds of ajwain can be applied efficiently in clinical practice [40].

8. Biological activities of isolated bioactive components of ajwain oil

Ajwain essential oils is a major source of biologically 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 a 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 anti-microbial, anti-oxidant and therapeutic activities [37].

8.2. Phenolic monoterpene

Due to the presence of phenolic compounds, ajwain oil can be used as natural anti-oxidant. Methanolic extract of *T. ammi* fruits along with mature bovine filarial *Setaria digitate* worms was investigated previously. 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 [41].

8.3. p-cymene

p-Cymene is a monoterpene that is present in over 100 plant species and is used for medicine and food. It shows a variety of biological activities including anti-nociceptive, anti-cancer, anti-microbial, anxiolytic, anti-oxidant, and anti-inflammatory effects. Medicinally, p-cymene is used to inhibit phlegm, coughs and being used in the production of fungicides and pesticides [42].

8.4. γ-Terpinene

γ-Terpinene (TH), a monoterpene hydrocarbon present in essential oils, retards the per-oxidation of linoleic acid (LH). The per-oxidation 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 [43].

8.5. Carvacrol

Carvacrol has been categorized as an inhibitor of growth of diverse pathogens. It is a natural phenolic compound that have great care for its high anti-microbial actions [44].

9. Conclusions

Ajwain seeds serve as an important component of human diet and used in the treatment of diseases in both humans and animals. Ajwain essential oils used as anti-bacterial, anti-microbial, anti-fungal and anti-oxidant agent. Bioactive components of ajwain oil also participate to its nutritious value. Furthermore, these components have various applications in pharmaceutical field. Thymol is a major component present in the essential oils of ajwain and is responsible for the various biological activities. Major components responsible for the anti-microbial activity of Ajwain essential oils are thymol and carvacrol. Thymol is a strong fungicide, germicide and anti-spasmodic agent. It is abundantly used in perfumery and toothpastes. Thymol and carvacrol both are considered to be either bacteriostatic or bactericidal agents depending upon the concentration.

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