An extraction and utilization of essential oil from ajwain (*Trachyspermum ammi* L.) seed: a systematic review

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Abstract

Ajwain (*Trachyspermum ammi*) has been used as one of the most important spices due to its potential pharmacological and functional values for centuries in India. It is primarily used to treat flatulence, atonic dyspepsia, diarrhoea, abdominal tumours, stomach discomfort, piles, and bronchial difficulties. It is also used to treat amenorrhea, asthma and appetite deficiency. Ajwain possesses of significant antibacterial, antifungal, antimicrobial and antioxidant characteristics and activities as well. Essential oil derived from ajwain seed nowadays gained significant importance due to occurrence of thymol, γ-terpinene, and p-cymene as major terpenoids Moreover, aromatic essential oils contains the chemical compounds like aldehydes, phenols, sulphur, and nitrogen etc.The use of the essential oils (EO) as safe flavouring agent and medicinal goods is widespread. This review paper deals γ-with extensive survey on occurrence of essential oil, its chemistry, availability of functional components and different extraction methods including conventional as e.g. steam distillation, hydrodistillation, solvent extration etc. and non-conventional e.g. Microwave assisted extraction (MAE), Supercritical Fluid Extraction (SCFE) and Pulsed Electric Field (PEF) etc. based upon latest research finding.

Keywords: essential oil, ajwain seed, therapeutic value, extraction, utilization

1. Introduction

The ajwain or carom seed plant resembles parsley in appearance. The fruit pods are sometimes referred to as "ajwain seeds" or "bishop's weed seeds" due to their resemblance to seeds (Panda et al., 2020). Middle Eastern countries, possibly Egypt, where ajwain first appeared. Currently, Afghanistan, Iran, Egypt, and the Indian subcontinent are where it is mostly farmed and utilised (Pooja et al., 2023).Also, the biological classification of Ajwain is shown in Table 1.

Table 1. Biological classification of ajwain spice (Hejazian ., 2006).

Kingdom	**Plantae**
Subkingdom	Tracheobionota
Division	Magnoliophyta
Class	Magnoliosida
Subclass	Rosidae
Order	Apiales
Family	Apiaceae
Genus	Trachyspermum
Species	Ammi

It is occasionally a component in berbere, a blend of spices that is popular in Ethiopia and Eritrea. Because it also contains thymol, raw ajwain has a scent is like thyme but is more fragrant, less delicate in flavour, somewhat bitter, and pungent (Shahrajabian et al., 2021).Seeds of ajwain are oval shaped, yellowish-brown to greyish in colour with a wrinkled appearance (Gaba et al., 2019). Essential oil extraction is expressed diagrammatically in figure 1.

Figure 1. Process flow of essential oil extraction from ajwain

2. Essential oil characteristics

Essential oils are very complicated blends that can contain hundreds of different aromatic components and essential oils sometimes have the same low water solubility as oils (Mahian & Sani, 2016). Some of essential oil are recognised as safe chemicals by FDA in accordance with the Code of Federal Regulations, and some of them contain components that can be utilised as antibacterial additives (Mahian & Sani, 2016). Ajwain essential oil's bioactive compounds, thymol and carvacrol, have been considered safe by the European Commission and the US Food and Drug Administration (FDA) (Anwar et al., 2015). An analysis of the ajwain derived essential oil's cytotoxic effects on colon cancer cells and significant antioxidant capabilities has been recently published (Mazzara et al., 2021). Due to its hydrophobic nature, essential oil is only partially soluble in polar solvents like water. However, this mostly yellow-color solution is highly soluble in nonpolar solvents.(Adeyemi et al., 2022)

3. Classification of Essential oil Based on its aroma

EO can be classified according to the aroma/smell of the oil. This category of oils may be classified into earthy, floral, medicinal/camphorous, herbaceous, resinous oils and woody, citrus and spicy oils (Herman et al., 2019). Table 2 represents different varieties of essentials oils based upon plant resources.

Table 2. Essential oil type based on its aroma and It's source

Essential oil type	Plant source	References
Floral Oils	Chamomile	(Nogueira et al., 1998)
	Rose	
	Geranium	

	Neroli	(Downs et al., 2000) (Simpson et al., 1990)
	Lavender	
	Ylang-Ylang	
	Jasmine	
Earthy Oils	Vetiver	(Priestap et al., 1990) (Jirovetz et al., 2002)
	Angelica	
	Valerian	
	Patchouli	
Citrus Oils	Lemon	(Viuda-Martos et al., 2008)
	Tangerine	
	Bergamot	
	Orange	
	Grapefruit	
	Lime	
Spicy Oils	Ginger	(López-Cortés et al., 2013) (Maurya et al., 2013)
	Black Pepper	
	Nutmeg	
	Cumin	
	Cinnamon	
	Cloves	
	Ajwain	
Camphoraceous Oils	dried plum	(Weyerstahl et al., 1993)
	rosemary	
	Tea Tree	
	mugwort	
	borneol	
Woody Oils	Juniper Berry	(Xu et al., 2010) (Li-Chang-Zhu et al., 2006)
	Cedar wood	
	Sandalwood	
	Cypress	
	Cinnamon	
	Pine	
Herbaceous Oils	Marjoram	(López-Cortés et al., 2013) (Maurya et al., 2013)
	Coriander	
	Melissa	
	Clary Sage	
	Chamomile	
	Hyssop	
	Basil	
	Peppermint	

4. Essential oil chemistry

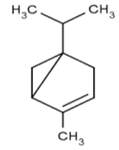
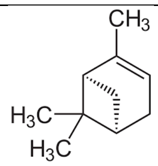
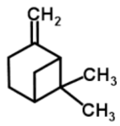
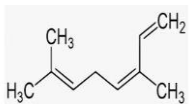
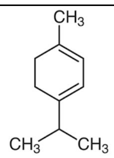
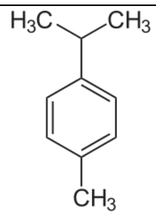
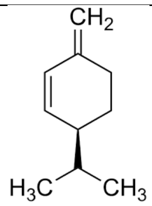
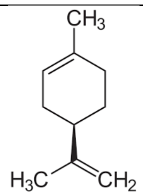
All organic compounds, including essential oils, are composed of hydrocarbon molecules and may be further categorised as phenols, aldehydes , terpenes, ketones, alcohols, and esters etc (Tabanca et al., 2007). Availability of various bioactive components will be represented by Table 3.

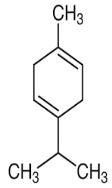
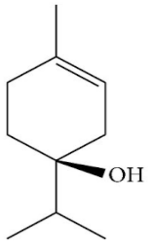
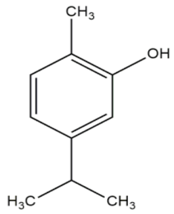
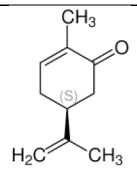
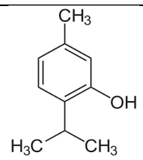
Generally, thymol percentage in ajwain seed essential oil is 40-50%.Ajwain essential oil is strong Nematicidal, fungicide, and Antioxidant (Chahal et al., 2017). Agilent Technologies model 7890A gas chromatograph linked to mass detector was used for the process of finding compound of essential oil (Zarshenas et al., 2014). Based on a comparison of the mass spectra of the components with those described in the literature as well as those from the Willey (nl7) and Adams libraries, the components were identified (Zarshenas et al., 2014).

Table 3. Detailed status of bioactive components in essential oil

Bioactive constituents	Chemistry	References
Terpenoids	The main components of the essential oils produced by the many plant species and flowers are terpenes and terpenoids	(Thimmappa et al., 2014)
Sesquiterpenes and Oxygenated Compounds	The essential oil isolated from the flowers of *E. klotzschiana* has a high concentration of hydrocarbon sesquiterpenes, mainly α-(E)-bergamotene, germacrene-D, and β-bisabolene.	(Carneiro et al., 2017)
Ketones	Generally defined by –C=O group. Ketonic groups are not usually frequent in most of essential oils. These are generally stable compounds and are not relevant as perfumes or taste ingredients. Essential oils contains the major ketonic derivatives e.g. jasmone, pinocamphone, pulegone, verbenone, camphor, Carvone, thujone, methyl nonyl ketone, menthone and fenchone	(Chaubey, 2019)
Monoterpene/ Monoterpenoid	These substances, which have a structure of 10 carbon atoms and at least one double bond, are present in almost all essential oils. Geraniol, pinene (found in pine trees), limonene (found in citrus fruits), terpineol (found in lilacs), linalool (found in lavender), or myrcene are a few examples of monoterpenes and monoterpenoids.	(Eberhard Breitmaier, 2007)
Esters	The essential oil produced from the flower of Jasminum officinale L. contains an ester group of benzyl acetate, linalyl acetate, and benzyl benzoate .High-ester group plants contains benzoin, bergamot, sweet birch, clary sage, helichrysum, jasmine, bergamot mint, sweet thyme etc.	(Sharmeen et al., 2021)

Table 4. Physicochemical properties of essential oil found in Ajwain seed (Zarshenas et al., 2014)

Compound	Chemical formula	Chemical structure	Molecular weight (g/mol)	Density at 25°C (g/cm^3)	Boiling point (°C)
Thujene	$C_{10}H_{16}$		136.234	0.92	151
α- pinene	$C_{10}H_{16}$		136.23	0.858	156.85
β -pinene	$C_{10}H_{16}$		136.23	0.872	165-167
Myrcene	$C_{10}H_{16}$		136.23	0.794	167
α- terpinene	$C_{10}H_{16}$		136.23	0.837	173-174
ρ-cymene	$C_{10}H_{14}$		134.21	0.857	177
β-phellandrene	$C_{10}H_{16}$		136.23	0.85	171
Limonene	$C_{10}H_{16}$		136.23	0.841	176

γ-terpinene	$C_{10}H_{16}$		136.23	0.853	183
Terpinene-4-ol	$C_{10}H_{18}O$		154.25	0.933	211-213
Carvacrol	$C_{10}H_{14}O$		150.22	0.9772	236.8
Carvone	$C_{10}H_{14}O$		150.22	0.96	231
Thymol	$C_{10}H_{14}O$		150.22	0.96	232

5. Methods of extracting essential oils

The essential oil extraction methodologies are determined by the properties and components required in the Ajwain seed. The process of extraction is the primary determinant of the quality of essential oils since improper extraction techniques may result in the degradation and alter the activity of phytochemicals found in aromatic oils. Two different kinds of extraction techniques are described in figure 2.

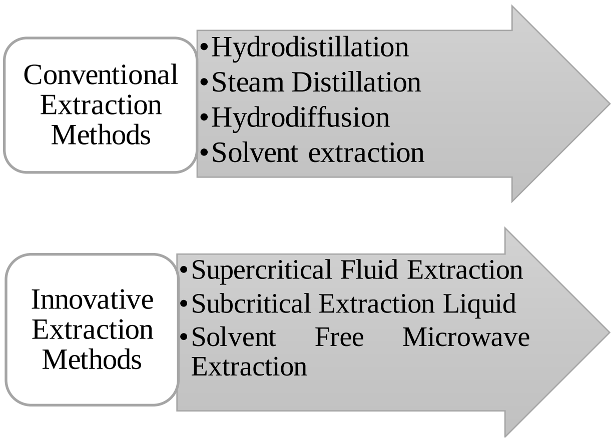
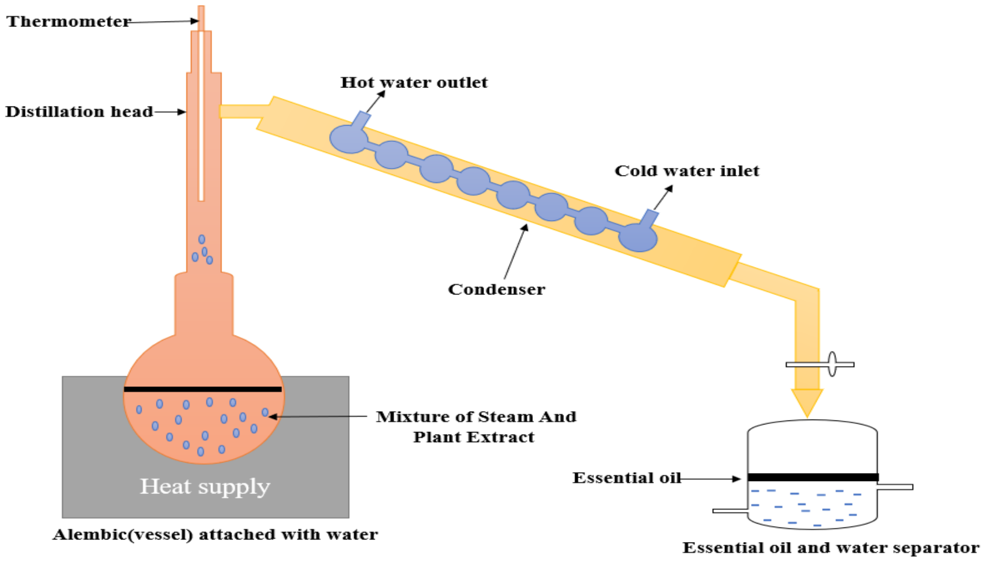


Figure 2. Classification of essential oil extraction method

5.1 Hydrodistillation

Avicenna invented the ancient and easiest technique of oil extrication, hydrodistillation, and was the first to produce extraction by using alembic. During the hydrodistillation process, plant materials are dissolved in water, and then extracts are boiled using a heating medium to extract essential oil. The clevenge apparatus consists of a heating origin, an alembic container, a condenser to transform vapour from the container to liquid, and a decanter to acquire the condensate and isolate essential oils from water. The hydrodistillation method is extensively utilised due to its affordable equipment cost and key



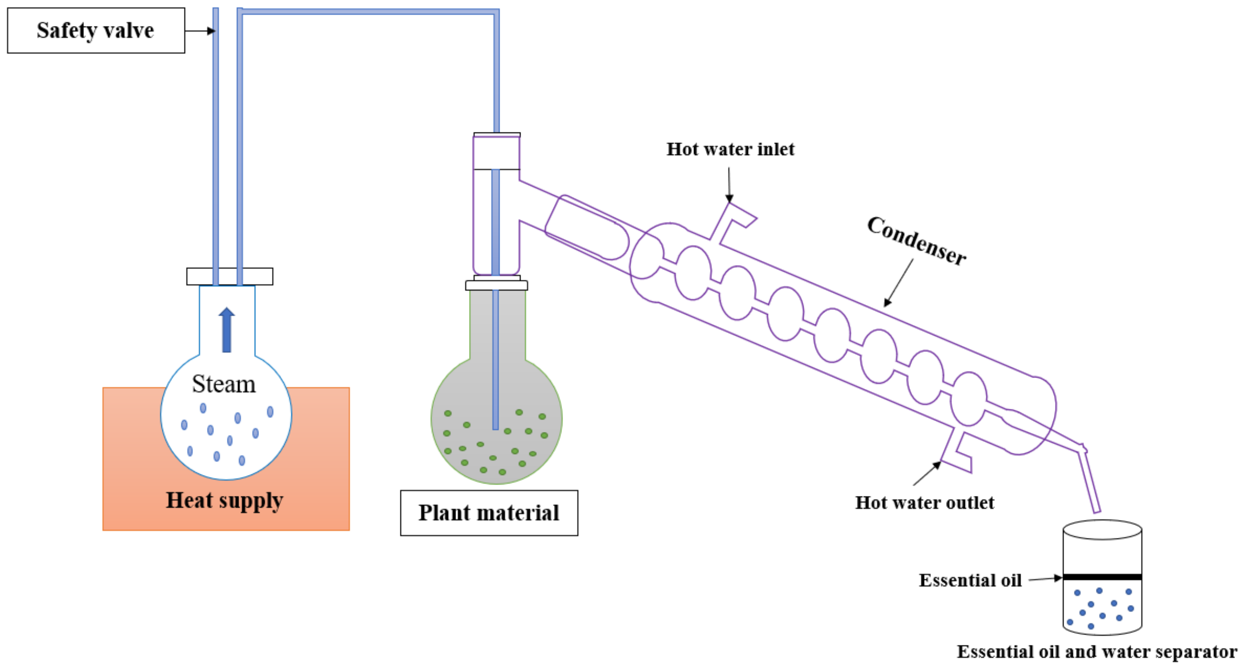
advantage: the water boiling temperature is less than 100^{o}C, resulting in less aromatic component loss.

Figure 3. Hydrodistillation process (Azmin et al., 2016)

Aziz et al., (2018) described that the overall hydrodistillation process has been modified by the use of new technology. We may increase extraction yield by varying the ratio of solvent and time, and temperature. (Samadi et al. 2017) find the high extraction yield of *Aquilaria malaccensis* malaccensis leaf oil with a soil to solvent ratio of 1:10 and a time of 3 hours at effective temperature. MAHD extraction process technology proposed by (Golmakani and Rezaei.,2008) shown advantages in energy transmission and separationtime (75 min in comparison to Hydrodistillation of 4hr)

5.2 Steam distillation

Steam distillation has traditionally been used to separate volatiles from aromatic plant components. Steam is injected through the plant material during the steam distillation process. The heated vapour combination is collected and condensed to create a liquid with two separate layers of oil and water (Irmak et al., 2008, Božović et al., 2017) create a novel 24-hour steam distillation procedure for extracting plant essential oils. Longer distillation



may result in higher yields, but it may also result in the build up of more contaminants.

Figure 4. Steam distillation process(Silori et al., 2019)

The basic benefit of the steam distillation method is governed by an organic solvent-free approach that can be used for large production lines with less expensive equipment. The drawback of this procedure is that it takes a long time and consumes a lot of energy.

5.3 Hydrodiffusion

Hydrodiffusion procedure is only used on dried plant samples that are susceptible to harm at boiling temperatures. Steam distillation method deals with the supply of steam through the bottom of the boiler, whereas hydrodiffusion technique involves steam supply from the top of the boiler. This procedure was carried out under vacuum condition, and the steam temperature may be decreased less than 100°C(Aziz et al., 2018). Addition of microwave technology was considered to be an effective way for the enhancement of steam diffusion technique (Aziz et al., 2018). Investigation was carried out to differentiate the performance between microwave hydrodiffusion and gravity (MHG) and hydrodistillation. Experimental work was done on the microwave hydro-diffusion and gravity (MHG) process for extracting essential oil from thyme (Calinescu and Mason.,2017). This system provides uniform microwave irradiation. The mechanism allows for low-pressure operation and the injection of additional water either before or during the extraction. Extraction efficiency was improved by the increase in occurrence of water for thyme by pulverising the thyme seed with distilled water. Microwave hydrodiffusion coupled with gravity technique operated under reduced pressure less than one atmosphere can enhance yields. Microwave Steam Diffusion (MSDF) was developed by (Wang et al., 2006) and provides a schematic explanation of these processes.

5.4 Solvent extraction

Soxhlet apparatus was utilized for determining milk fat in 1879 (William., 2007). Solvent extraction (SE) is a common approach for extracting bioactive essential oil from plant sources. The efficacy of solvent extraction methods is determined by the type of solvent employed and how it interacts with other plant or food ingredients. The sample is transferred in a thimble-holder and filled with condensed solvent from a distillation flask. A syphon aspirator is the excess of solvents that transports the extracted essential oil to the bulk liquid when it reaches the overflow level. This technique is continued until the completion of extraction phenomenan.The solvent extraction technique has the benefit of being a quick and simple procedure. There are various downsides, such as high solvent consumption, long treatment times, and sample thermal degradation (Suwal and Marciniak, 2019). EO was extracted from lemongrass using a soxhlet device (Suryawanshi et al., 2016). As a result of less exposure to heat and air than steam distillation, he also concludes that solvent extraction produced the highest yield.

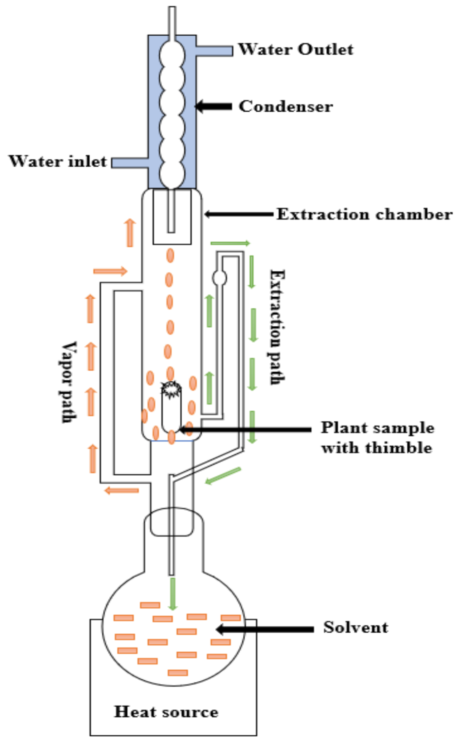


Figure 5. Diagram of Soxhlet apparatus (Dabbs et al., 2006)

5.5 Supercritical Fluid Extraction

The supercritical fluid state is principally determined by two variables: the fluid's critical pressure (Pc), and critical temperature, (Tc). CO_2 is utilised as a supercritical solvent for essential oil extraction because of its many outstanding characteristics, including its potential to reach critical points rapidly (Sardar et al.,2018) being chemically inert and toxic, being non-flammable, and being available in high purity at a cheap cost. By rapidly compressing and heating CO_2, a supercritical state may be created. After that, it goes through the raw plant material to extract volatile substances and plant extracts. Because of its high extraction yield of essential oils, the supercritical fluid extraction technology is frequently employed in industry. Extraction of thymol-rich essential oil with significant sensory characteristics The SFE-CO2 method is most effective at temperatures of 40 °C and pressures of 7 Mpa (Morsy., 2020). Because the equipment was excessively costly, small-scale manufacturing has not been used (Fekadu Egza, 2020).

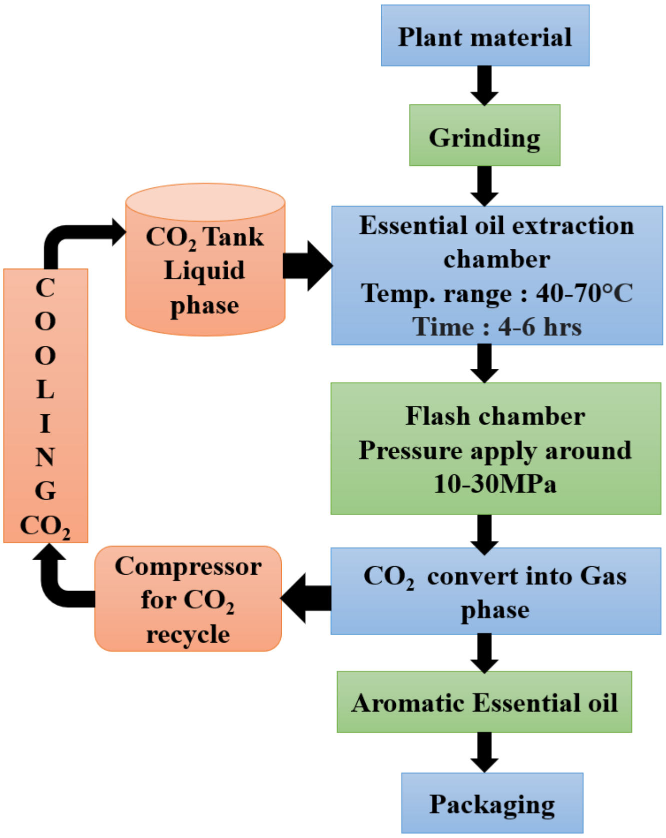


Figure 6. Flow diagram of SFE-CO_2 method (Pourmortazavi and Hajimirsadeghi, 2007)

5.6 Microwave Extraction without Solvent

Microwave assisted extraction (MAE), which was invented in the 1980s, is a procedure that includes heating the solvent in contact with the sample using electromagnetic radiation for molecular partition of interest from the surrounding medium to the solvent used. The yield of essential oils in MAE is affected by a number of factors, including solvent composition, solvent composition, plant material loading, time combination, and microwave power (Bagade & Patil, 2021).The extraction process differs from other traditional methods in that it happens as a result of changes in cellular structure generated by microwaves.MAE has benefits such as shorter extraction time, regulated temperature and other extraction conditions, less solvent, greater extraction rate, energy savings, and quality product (Suwal & Marciniak.,2019). Different non conventional essential oil extraction methodologies are suitably represented in table 5

Table 5. Advanced innovative extraction method

Method	Operating conditions	Findings	References
Enzyme-assisted extraction	Enzyme – cellulose and hemicellulose Temp. – 40°C	Extraction of essential oils from *Thymus capitatus* and *Rosmarinus officinalis* leaves. To enhance extraction yield, dried leaves are treated with cellulose and hemicellulose enzymes before conducting extraction.	(Hosni et al., 2020)
Microwave-Assisted Hydro-Distillation (MAHD) extraction	Microwave power -900 W	An innovative extraction approach for obtaining *Rosmarinus officinalis* L. essential oil from its leaves was carried out. The essential oil's quality was increased, and energy was saved throughout the MAHD extraction process.	(Moradi et al., 2018)
Microwave hydro diffusion gravity extraction	Microwave power – 800 W	Combinations of extraction techniques—microwave and hydrodiffusion gravity extraction to extract *Tunisian cumin* seed essential oil. This technique provides a larger extraction yield, a smaller environmental damage, and less energy consumption.	(Benmoussa et al., 2018)
Pressurized Liquid Extraction	Pressure 10.34 MPa Ethanol percentage in aqueous solution (50% water and 70% Ethanol)	Pressurized Liquid Extraction, as an innovative advanced green extraction process, was designed for the effective separation of polyphenols from laurel leaves	(Dobroslavi&Pedisi, 2022)

Pulsed Electric Field-Assisted Extraction(PEF)	Intensity of the field- 1and 2 kV/cm Number of pulses – 100 and 200 Duration of distillation 30 and 60 min	Essential oil yield of *Mentha spicata* enhanced after the employment of the PEF due to: the field intensity, the number of pulses, and the duration of distillation. The PEF treatment was designed to increase extraction yield for plants with lower essential oil content. So, generally, its use in industry.	(Miloudi et al., 2022)
Ultrasound assisted hydrodistillation	ultrasound time –35 min ultrasound power -300 W extraction time –60 min liquid-solid ratio -7	Ultrasound-assisted hydrodistillation approach for the extraction of essential oil from cinnamon barks was carried out. According to study, this method produces more bioactive compounds, has a greater yield, and consumes less time than the hydrodistillation method.	(Chen and Wang., 2020)
Subcritical water extraction(SCWE)	Sub critical fluid - water	Extraction process of coriander seeds (*Coriandrum sativum* L.) derived essential oil was carried out by using the subcritical water extraction technique. According to research, this approach does not give a greater extraction yield than the conventional method. The principal objective was to employ this process since the ultimate excerpt using the SCWE method was comparatively better and more beneficial due to the large presence of oxygenated components.	(Eikani et al., 2007)

Ultrasound-assisted supercritical CO2 extraction	dynamic extraction time -60 min Pressure -25 MPa Temperature - 50 °C Ultrasonic power -200W	Extraction of active compound cucurbitacin E (CuE) with biological activity from *Iberis amara* seeds was carried out by employing an ultrasound-assisted supercritical CO2 extraction technique. This is a hybrid technology with significant extraction that is feasible and eco-friendly.	(Liu et al., 2020)

The amount and composition of the bioactive components derived from ajwain essential oil are significantly influenced by the extraction conditions. The chosen extraction technique, solvent, temperature, duration, and other factors can have a big impact on how well bioactive chemicals are extracted as well as their profile. Here it is describe in table 6 of how various extraction procedures might affect the bioactive component.

Table 6. Impact of extraction conditions on bioactive constituents

Method	Condition	Extraction yield, %	Plant source	Bioactive component	References
Hydro distilled	Temperature below 100°C	2.8	Ajwain	Thymol (49.0%) γ-terpinene (30.8 %) p-cymene (15.7%) β-pinene (2.1%) myrcene (0.8%) limonene (0.7%)	(Chahal et al., 2017)
Solvent extraction	Solvent use as hexane	1.82	Ajwain	Thymol (49.33%) γ-terpinene (20.90 %) p-cymene (21.62%)	(Singh & Ahmad, 2017)
Supercritical fluid (CO_2) Extraction	Pressure: 30.4 MPa Temperature: 30°C	1-5.8	Ajwain	γ-terpinene (14.2%) p-cymene (23.1%) Thymol	(Chahal et al., 2017)

	Time:30 min			(62.0%)	
Microwave-Assisted Extraction	Microwave power: 1.37 (W/g) Time: 162.7 min	1.6-3.7	Ajwain	Thymol (66.4 %)	(Mazzara et al., 2021)
				γ-terpinene (12.5 %)	
				p-cymene (13.7%)	

6. Nutritional Value and Utilization

Ajwain seed are edible in nature. It contains fibre, carbohydrates , vitamins, minerals (such as calcium, phosphorus, and iron), and proteins (Bairwa et al., 2012). Ajwain refers to using the seeds in particular breads, including naans and parathas. When combined with lemon juice and black pepper and then dried, the seeds may also be used as a mouth refresher. Additionally, ajwain seeds contain an essential oil that, when added to culinary goods, imparts a familiar flavour. The yields of ajwain EO ranged between 1.6 and 3.7% (v/w). (Mazzara et al., 2021) and main fatty acid is petroselinic acid, oleic acid, and palmitic acids.(Zarshenas et al., 2014). According to studies (Javed et al., 2012), ajwain seeds have the following composition like moisture (11.5%), protein (20.23%), fibre (21.89%), fat (4.83%), carbohydrate (47.54%) and total ash (11.5%).

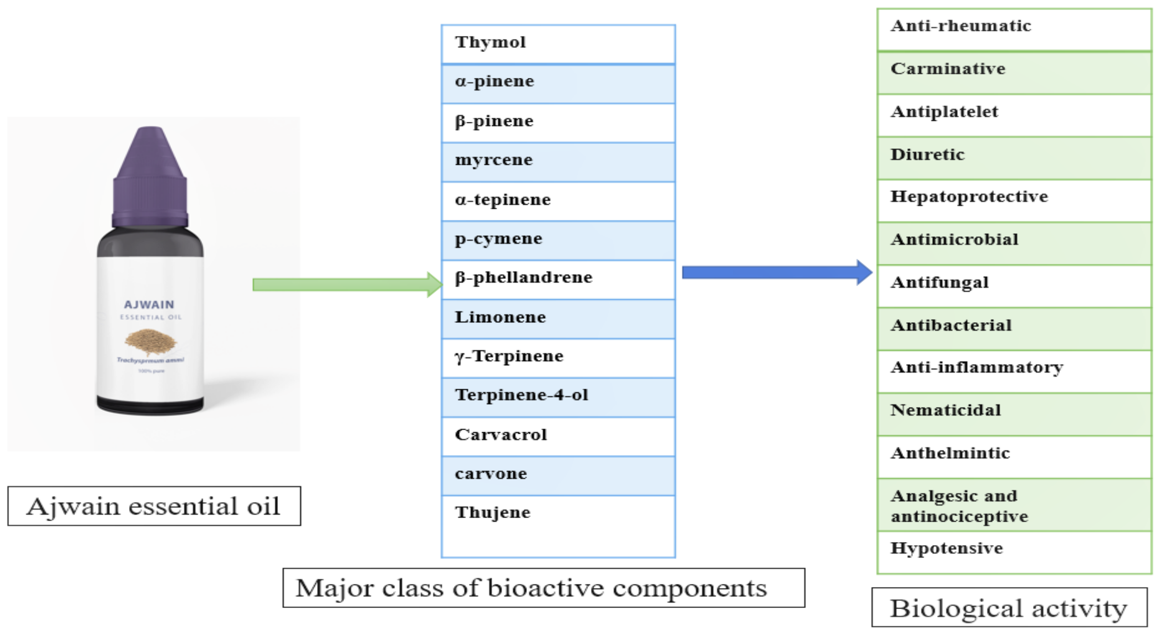


Figure 7. Bioactivity of Ajwain essential oil (Awais Hanif et al., 2021).

According to Polat and Kezban, the use of edible coatings consisting oregano and thyme EO's on fresh beef slices might have a potential for preventing pathogenic microorganisms and improved colour stability with acceptable sensory qualities(Herman et al., 2019).According to (Dagli et al., 2015), essential oils have the potential to be developed as preventive measure or therapeutic agents for a variety of dental disorders (Singh et al., 2022). Essential oils may increase the shelf stability of foods by reducing lipid oxidation (Oussalah et al., 2006) . As a function, including essential oils into biodegradable films may give antioxidant activity for the resulting films. By encapsulating essential oils in liposomes, polymeric particles, and solid lipid nanoparticles, several emerging technologies have produced new techniques to increase their stability (Sharma et al., 2020).

7. Therapeutic applications

According to (Sabar.,2017), nine days oral administration of ajwain seed in milk showed significant efficacy against patients suffering from urinary stone due to the deposition of pure calcium oxalate. The extract's hepatoprotection against paracetamol- and CCl_4-induced liver damage in rats further supports the traditional use of *Carum copticum* seeds as a hepatobiliary diseases preventative measure (Gilani et al., 2005). It has now been shown that ajwain may boost the production of bile acids, gastric acid, and digestive enzymes. It may also shorten the duration of meal transitory (Zarshenas et al., 2014a). Ajwain essential oil has also been shown to have fungitoxic properties against pathogenic fungi isolated from food materials such as *Asperigillus niger, Aspergillus flavus, Aspergillus oryzae, Aspergillus ochraceus, Fusarium monoliforme, Fusarium graminearum, Penicillium citrinum, Penicillium viridicatum, Penicillium madriti, and Curvularia lunata* (Sridhar et al., 2003). Experimental results showed that ajwain extract has antinociceptive effects on both early and late stages of pain (Hejazian.,2006). According to (Boskabady et al., 2003), the quantity of carvacrol in essential oil fractions may be the cause of the relaxing and bronchodilatory effects. An in vitro experiment using samples of human blood, ajwain seeds prevented platelet aggregation brought on by arachidonic acid, collagen, and the stress hormone adrenaline (Srivastava., 1988). According to the findings of (Kong et al., 2006), the dietary ajwain extract would be able to lessen the toxicity caused by hepatic free radical stress. For treating rheumatic, headache, joint, and neuralgic pain, *C. copticum* has been utilised in conventional medicine (Awais Hanif et al., 2021). According to (Dashti-Rahmatabadi et al., 2007) research, the ethanolic extract of *C. copticum* has analgesic effects equivalent to those of morphine (Hejazian.,2006). This effect is thought to be caused by the substance's parasympathomimetic properties via its downward pain-modulating trails (Awais Hanif et al., 2021). Therefore, health benifits of ajwain seed was well established by several experimental studies.

8. Future application

Reserch is going on continusly to explore the therapeutic potential of ajwain (*Trachyspermum ammi* L.*)* for prevantion of various diseases.Because of its technological efficiency and biosecurity, *T. Ammi* may soon be used as an immunotherapeutic agent, along with other infection control agents, to dominate cancer therapy efforts.without negative consequences (Awan et al., 2021).

9. Conclusion

After comprehensive review of chemistry, bioactivity (Awais Hanif et al., 2021), different extraction techniques (Božović et al., 2017 and Suwal & Marciniak et al., 2019). it has been established that ajwain is one of the most useful spice. Moreover, ajwain has a significant pharmacological potential due to its ant rheumatic, carminative, antiplatelet, diuretic, hepatoprotective, antimicrobial, antifungal and antibacterial properties (Awais Hanif et al., 2021). Higher antioxidant activity of ajwain essential oil is due to occurance of active constituants e.g. thymol, carvacrol, γ-terpinene, and p-cymene (Gandomi et al., 2013). There is a vast research scope in order to extract greater amount of essential oil from ajwain by means of combination of one conventional (Elyemni et al., 2019) and other novel or nonconventional (Belwal et al., 2020) technologies or multiple non conventional technologies in future.

Conflict of Interest
Authors declare none.
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