

GC-MS Analysis, Identification of Bioactive Compounds, and Their Bioactivity in *Foeniculum Vulgare* Mill Essential Oil

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ABSTRACT

Essential oils are also known to be used in different applications including cosmetic, pharmaceutical and food industries. Objective of the work: to extract the essential oil of *Foeniculum vulgare* by the hydro-distillation clevenger type method through hydro-distillation technique with 4ml oil per 100g fresh leaves/seeds of *Foeniculum vulgare* and to determine the antimicrobial activity of the essential oil.

GC-MS. Nine compounds were determined. The predominant one was 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester (83.10%). The antimicrobial activity of the essential oil was determined using agar well diffusion technique. The oil tested on four types of bacteria showed significant zones of inhibitions (20mm), *E. coli* (20mm and 16mm), and *S. aureus* (22mm and 15 mm): a strong indicative of the antibacterial potential of essential oil. *P. mirabilis* was however insensitive (0mm ZOI). The *Foeniculum vulgare* essential oil has significant antimicrobial activity, which is probably explained by its abundant phytochemical makeup and it can be regarded as suitable in its natural therapeutic use.

Keywords: *Foeniculum Vulgare*, Essential Oil, Hydro-Distillation, Gc-Ms Analysis, Bioactive Compounds, Antimicrobial Activity, Agar Well Diffusion, 1,2-Benzenedicarboxylic Acid, Phytochemicals, Antibacterial Potential

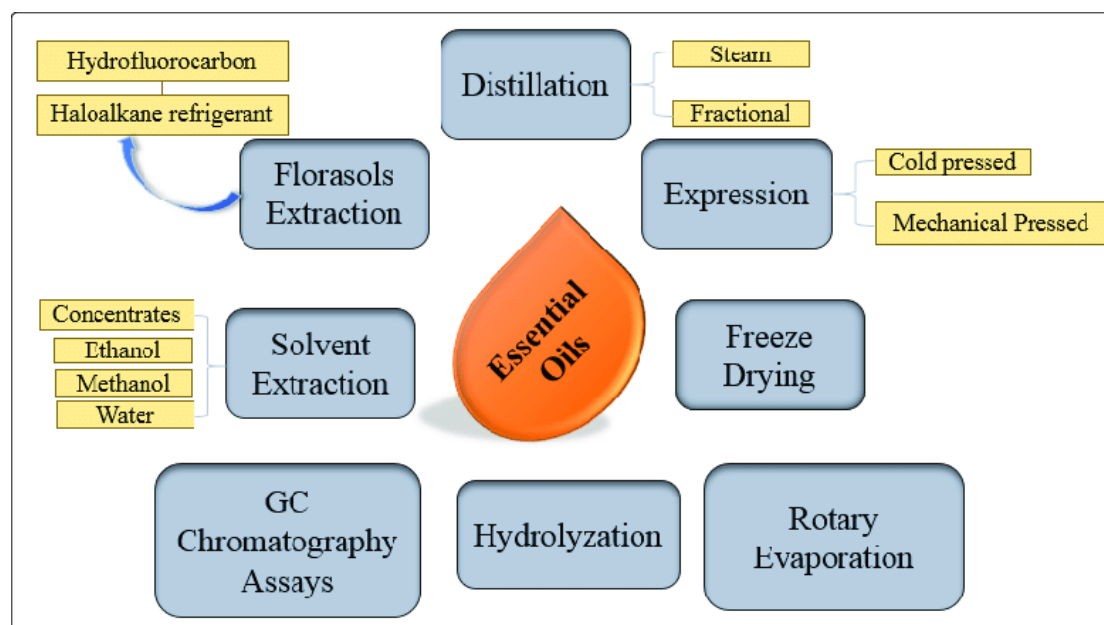
Introduction and Background

Natural products are chemical materials which are obtained through plants, animals, micro organisms or minerals and traditionally have been used nutritionally, as cosmetics and medicines. In most cases, these products are extracted or separated using separation processes and have varying chemical characteristics (Sarker and Nahar, 2012). The past years witnessed a large growth in the demand of natural products because of the growing popularity of sustainable, eco-friendly, and health-oriented alternatives in the pharmaceuticals, food technology, cosmetics, and biomaterials industries (Chopra and Dhingra, 2021). The traditional medical systems and the drug discovery modern domain are both based on natural products as

numerous modern medicines are natural in their origin (Atanasov et al., 2015).

The scale of natural products is that they selectively react with biological objects, which has been molded by evolution mechanisms (Li & Vederas, 2009). Biotic animals- especially plants, microorganisms and marine animals have always been an important source of bioactive compounds in medical chemistry. In the past, natural products played an important role in the treatment of infectious diseases, cancer and cardiovascular diseases by providing structural diversity and bioactivity in many cases unexplored by synthetic compounds (Newman, and Cragg, 2020). The key natural product sources are plants (source of alkaloids, flavonoids, and terpenoids), microorganisms (particular antibiotics, e.g. penicillin of *Penicillium* species), and marine animals, which have produced powerful bioactive molecules such as anticancer ones of sponges and algae.

Essential oils are a well-known group of natural products and are volatile, hydrophobic substances that are obtained using physical agents like steam or hydro-distillation and are mostly obtained using different aromatic plants. These oils are well extracted as flavoring agents, fragrances, and medicinal cures because of their antibacterial, antiviral, and antioxidant powers and their healing effects (Tongnuanchan and Benjakul, 2014). The essential oils are accumulated in their special plant structures like, oil glands and sacs, and are made up of sophisticated mixes of aromatic compounds. The use of essential oils has been very traditional in traditional medicinal systems in most countries and has continued to be used up to the present day especially in developing countries (K. Satish Kumar, 2010). The extraction and analysis methods have also advanced, which also boosts the knowledge on essential oils, confirming the contribution of natural products as a useful asset in contemporary pharmacology and healthcare.



Essential oil classification based on Aroma

Another way to categorize essential oils is by their scent or aroma. These oils can be classified as woody, earthy, minty, spicy, medicinal/camphorous, floral, citrus, herbaceous, and resinous

Citrus Oils: This group includes essential oils with a pronounced citrus flavor. Citrus oils are produced by a variety of plants, such as bergamot, citrus paradisi, citrus limon, citrus aurantiifolia, orange, and tangerine. (Fernández-López, J Pérez-Álvarez (2008)

Herbaceous oils: made from plants, which are generally considered to be the most beneficial herbs. These oils can be derived from a variety of plants, including peppermint, rosemary, hyssop, marjoram, basil, chamomile, Melissa, and clary sage (Arumugam G, M (2016).

Camphoraceous Oils: These essential oils have a specific therapeutic effect. These are also derived from tea tree, cajeput, and borneol. They have a background of dried plums and are earthy, Artemisia vulgais, and Salvia rosmarinus. with a fruity, prune. (Sudipta Kumar Mohanty (2015).

Flower Oils: This type of oils derived from plant parts or that include the essence of plants. Plants that generate these oils include geranium, jasminum, Lavandula Rosa, orange blossom oil, Matricaria, cananga odorata, and others. (López-Cortés I, DC Salazar-García, B Velázquez-Martí, DM Salazar (2013).

Woody Oils: Essential oils derived from plant stem or branches parts that have a woody scent. These oils are produced from cedrus, cinnamomum, cypress, juniper berries, pinus, sandalwood and so on. (Sun Yunjuan (2010)

Hot Oils: Oils made from spices or hot plants like nutmeg, cloves, Thymus, cardamom, anise, piper nigrum, cinnamon, coriander, cumin, and zingiber officinale.

Extraction of Essential oil

Essential oils are obtained through different methods which include:

Cold pressing

Solvent extraction

Steam distillation

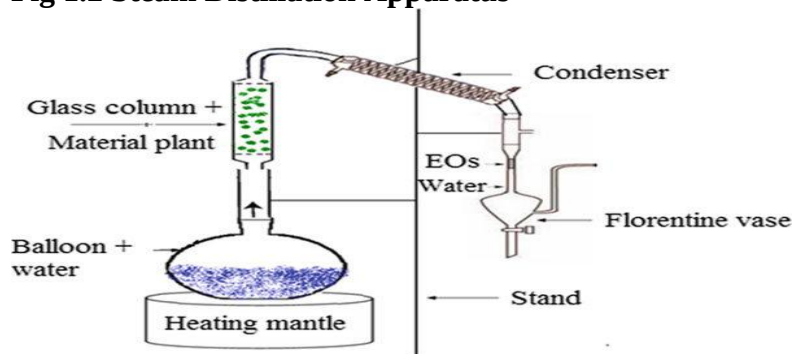
Hydro distillation

Steam Distillation for Essential oils Extraction

Steam extraction is the most common technique of essential oil production, with approximately 93 percent of the world output. The load with plant material is directed to a stream of steam that ruptures the cells and releases oil without any preliminary maceration. The volatile compounds that are carrying the steam get condensed and the oil and water are separated. It keeps the oil in place, does not produce hydrolysis or changes, and can extract high-boiling compounds at temperatures around 100°C

and a pressure modification is made on the more recalcitrant materials (M'SILA, 2021; Souiy et al., 2022; Raaman, 2006; Li et al., 2014).

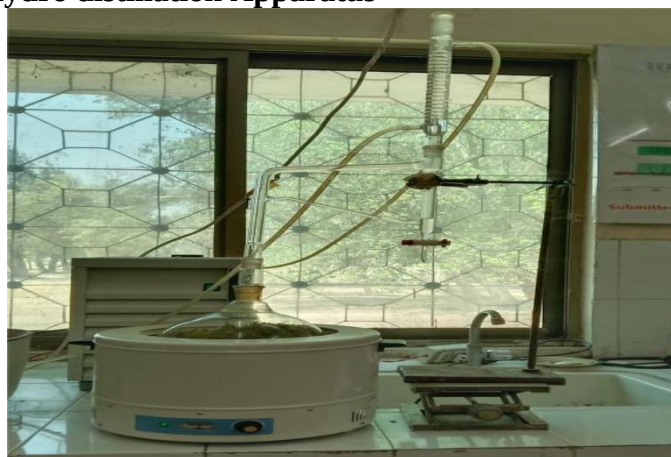
Fig 1.2 Steam Distillation Apparatus



The Hydro Distillation Method of Essential oil Extraction

The process of hydrodistillation (HD) represents one of the common procedures to extract essential oils, enabling to obtain water-insoluble compounds of a high boiling point. Water is added to plant material and boiled and steam condensed through the process of refrigeration to avoid excessive heat on oils. The technique allows distillation to take place below 100degC and has disadvantages, such as the scalding of plant matter, and burnt aromas. The boiling may hydrolyze the esters over time, which is a compromise of quality in oils, and rectification might be incurred to eliminate undesired compounds. The time of distillation depends on the type of plants, and perennial structures take more time (Rao & Sardeshpande, 2022).

Fig 1.3 Hydro distillation Apparatus



Bioactive compounds with significant antimicrobial and antioxidant effects are abundant in essential oils, and these types of substances are considered as Generally Recognized as Safe (GRAS) food additives by the US FDA, attracting interest in the food industry (Prieto et al., 2015; Crandall and O, 2015). They have been applied in the active packaging that achieves the reduction of microbial loading and prevents microbial growth in processing and storage (Yildirim, 2018). Other that are used not

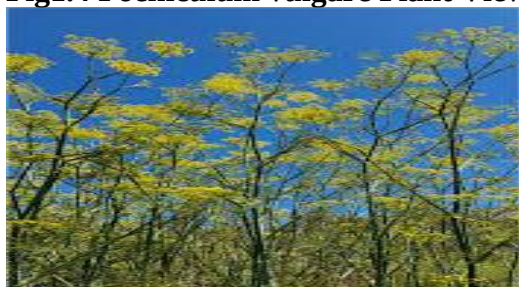
only in food but oils containing the essential oils have antibacterial and anti-inflammatory effect and could potentially help to prevent respiratory infections (Sharifi-Rad et al., 2017). Essential oils inhalation aromatherapy is another area that has been examined to respond to SARS-CoV-2 and associated effects of inflammation (Asif et al., 2020).

Foeniculum vulgare Mill

The first name, *Foeniculum foeniculatum*, was not a valid name, and was given *Foeniculum foeniculatum* by Karsten. By the international standards of nomenclature Philip Miller appears to be the first author to name it formally, in the 1768 eighth edition of *Gardeners Dictionary*: *Foeniculum vulgare*. It is therefore, right to call the plant *Foeniculum vulgare* Mill. (Hill, 1756).

Foeniculum vulgare (fennel) is a fragrant, therapeutic herb of family *Apiaceae* having aromatic roots, stems, leaves, and seeds which are used either in culinary or medicinal purpose (Badgujar et al., 2014). The primary substance phytochemicals in it are phenols, phenolic glycosides, and volatile organic compounds, such as trans-anethole, methyl chavicol, and fenchone (Rather et al., 2016). Fennel is also used in various parts of Europe, Asia, America and Africa to produce seeds and essential oils despite low production in certain countries such as Bangladesh (Kapoor et al., 2004; BBS, 2016). It is a temporary herb, and has the benefits of being nutritious, low-stress and memory-promoting, and it can be taken orally, as a raw or cooked herb, or as tea (Krishnamurthy, 2011; Koppula & Kumar, 2013). The increased attention to its therapeutic effect has encouraged the cultivation of it on a large scale and further studies on its metabolites and therapeutic uses (Barros and Carvalho, 2010)

Fig1.4 Foeniculum vulgare Plant View



Foeniculum vulgare (Plant)



Foeniculum vulgare (flowers)



Foeniculum vulgare (seeds)

Plant Botanical Description

One of the oldest seasonal shrubs is *Foeniculum vulgare*, which grows either wild or in plantations in Asia, North America, and Europe. Total strangers to us could be the ancient Egyptians, Romans, Indians and Chinese, but it is very common in southern Italy and the tender shoots are consumed as a vegetable, and aromatic seeds consumed in cooking. The emperor, Charlemagne encouraged its growing in Central Europe, and it is currently a significant ingredient in the Italian and French food (Krishnamurthy, 2011).

The plant is erect and porous becoming cylindrical, leaves bright green, and finely partitioned, and threadlike pieces or segments up to 14 cm in length. Small white flowers appear in umbels large and flat as well in July and August. Fruits are long, ribbed and have desirable oval and slenderly curved seeds about 3-5 mm long. The *F. vulgaris* is typically used in vegetable and herb gardens because of its meal and incense properties.

Fig 1.5 *Foeniculum vulgare* Mill



Taxonomic Classification

Classification

Domain:	Plantae
Part:	Tracheophyta
Subpart:	Spermatophytina
Group:	Magnoliopsida
Rank:	Apiales
Family:	Apiaceae
Genus:	Foeniculum

Variety:

vulgare

Herbal name:

Foeniculum vulgare Mill.

Foeniculum vulgare Mill Essential oil

The essential oil of *Foeniculum vulgare* is also famous because of its characteristic smell that is very popular to use as a flavoring agent in baked products, meat and fish products, ice cream, and alcoholic drinks. Anethole, fenchone, methyl chavicol, and α -phellandrene are the main components of fennel seed essential oil but their relative content is different depending on the phenological stage and geographical origin (Diaz-Maroto et al., 2006).

Essential oils are complex secondary metabolites and they are very important in protecting plants against bacteria, viruses, fungi and insect pests (Rehman et al., 2016). The chemical profile of *Foeniculum vulgare* is variable due to the aspect of the growth stage, harvesting season, climatic conditions, genetic variation, and the agricultural practices (Demirtas and Sahin, 2009). Difference in extraction technique and the maturity of the plant also influence the composition of the oil as the amount of essential oil tends to decline with the age of the fruits (Gross et al., 2009; Ilic et al., 2019). The trans-anethole is the most prominent one a concentration of up to 81.63 to 87.85 has been reported (Telci et al., 2009).

Chemical Composition and Nutritional Value of Fennel

Foeniculum vulgare is commonly grown by people to serve as their food (eat seeds and the fruit). The perfect specimen is a beautiful fruit; they are dry and sweet. Fennel is a common herb that is often dried to be used later and it is a valuable commodity (Garvin). Fennels contain the best sources of calcium, phosphorus, sodium, and potassium patients. The vitamin and dietary fiber contents in Fennels are the highest when compared to those required by human beings as per the data provided by USDA about the Mission cultivar. They contain a lot of other nutrients in lower concentrations (Barros L, Carvalho AM, Ferreira ICFR.(2010))

Literature Review

Pharmacological Activities of *Foeniculum vulgare*

Foeniculum vulgare (fennel) is a popular medicinal plant that is traditionally used in the treatment of digestive, metabolic, neurological, and inflammatory disorders. A number of its bioactivities have been confirmed by modern pharmacological research, most of which can be credited to the essential oil and phenolic compounds that are found in it.

Anti-diabetic Activity

The experimental research on streptozotocin-induced diabetic rats showed that the fennel essential oil can have significant amounts to reduce the blood glucose level and better the pathological effects of hyperglycemia. Fennel oil (35 mg/kg) as an oral agent was protective to the pancreatic functions and glucose metabolism, and this demonstrated that it could be effectively used in diabetes treatment (Abou N et al., 2011; El-Soud and El-Laithy, 2011).

Antibacterial and Antifungal Activity

Fennel is broad-spectrum antimicrobial in relation to foodborne and pathogenic microorganisms. Some of its compounds of activity include anethole, fenchone, linoleic acid, phenolic derivatives, which explain its antibacterial actions. The extracted fennel samples were demonstrated to be very powerful inhibitors of *E. coli*, *Staphylococcus aureus* and *Candida albicans*, but with significant minimum inhibitory concentrations (Favela-Hernandez et al., 2012; Cwikla et al., 2010). Its antifungal effect on *Aspergillus*, *Fusarium*, and *Sclerotinia* also demonstrates their preservative and therapeutic effects (Rahimi and Ardekani, 2013).

Antioxidant Activity

Fennel is an excellent source of the natural antioxidant because it contains high polyphenol and flavonoid. Depending on the experiment, solvent and essential oils demonstrate high free radical scavenging in DPPH, FRAP and H₂O₂. Research has indicated that the antioxidant activity can be equal or higher than that of α -tocopherol, which shows that it is able to mediate in oxidative stress-related diseases (Shahat et al., 2011; Scalbert, and Manach, 2005).

Neuroprotective and Anti-anxiety Activity

Fennel extracts antiquated anxiolytic and memory improving action in animals. These actions act by mediating changes in the GABAergic system and estrogen receptors. This is because prevention of acetylcholinesterase activity may have a future use in the neurodegenerative disorders like the Alzheimer disease (Koppula and Kumar, 2013; Joshi and Parle, 2006).

Gastroprotective, Hepatoprotective, and Cardiovascular Effects

Fennel essential oil has been demonstrated to possess protective effects in gastric ulcer, presumably owing to its antioxidant and anti-inflammatory effects. Hepatoprotective tests indicate the prevention of liver damage in the models by giving a decrease in ALT, AST, ALP, and lipid peroxidation products. Also, fennel has the effects of hypolipidemics, antihypertensives, and anti-atherogenics, which helps in supporting cardiovascular values (Al-Mofleh et al., 2013; Wang et al., 2012).

Anticancer Activity

Trans-anethole and its derivatives either suppress TNF- α and NF- κ B signaling, which is the cause of the anticancer activity of fennel. There is in vitro and in vivo evidence of apoptotic and cytotoxic activity against breast, liver, leukemia, and prostate cancer cells, which is mostly exerted via the anti-oxidant and anti-inflammatory mechanism (Mohamad et al., 2011; Smolarz et al., 2008).

Phytochemical Composition of *Foeniculum vulgare*

Fennel seeds contain a lot of carbohydrates, proteins, fats, minerals, and dietary. Content of essential oils varies between 4-6 percent and is made of trans-anethole (50-80 percent), fenchone, estragole, and limonene. Fennel also contains phenolic acids, flavonoids, coumarins and tannins including quercetin and derives of kaempferols that play an important role in terms of its pharmacological characteristics (Rahimi and

Ardekani, 2013; Faudale and Viladomat, 2018).

Traditional and Medicinal Uses

Traditionally, fennel was used in the Chinese, Indian, Middle East, and European traditional medicine in the treatment of digestive disorders, respiratory diseases, colic, lactation, and hormonal dysregulation. Its intense conventional clinical application makes it clear that scientific research on its bioactive compounds and medicinal application remains feasible (Badgujar and Patel, 2014).

Essential Oil Isolation and Significance

The chemical compound of fennel essential oil consists of aromatic terpenes and phenylpropanoid that have antimicrobial, antioxidant, and anti-inflammatory activity. Traditional techniques like steam and hydro-distillation are common but other modern techniques like the micro-wave-assisted hydrodistillation are better as it improves the yield of oils, as well as, conserving thermolabile compounds. Pharmaceutical applications and industrial applications require proper isolation (Chen et al., 2018).

Literature supports that *Foeniculum vulgare* especially its essential oil is endowed with important pharmacological effects like antioxidant, antimicrobial, antidiabetic, neuroprotective, hepatoprotective, and anticancer activity. These properties are mainly associated with its complicated phytochemical content and this makes it worthy of additional studies on its therapeutic and industrial use.

Research Methodology:

Plant Collection

Fennel plant were collected from village (shah alam , Tehsil Daraban kalan)

Plant Material Identification

Plant material identification through her-barium department

Place of Work

I conducted my research in the Marine Natural Products Laboratory at the institute of chemical science Gomal university D.I.Khan

Equipment

All tools and equipment applied during the research.

Heating mantle	Cleavenger
Round bottom flask	Condenser
Chiller	Reciver
Dropper	Glass container(vial)
Distillation flask	

solvents used in this method

N-Hexane (C₆H₁₄)

Ethanol

Distilled water

Methanol

Ways and process

Hydro distillation

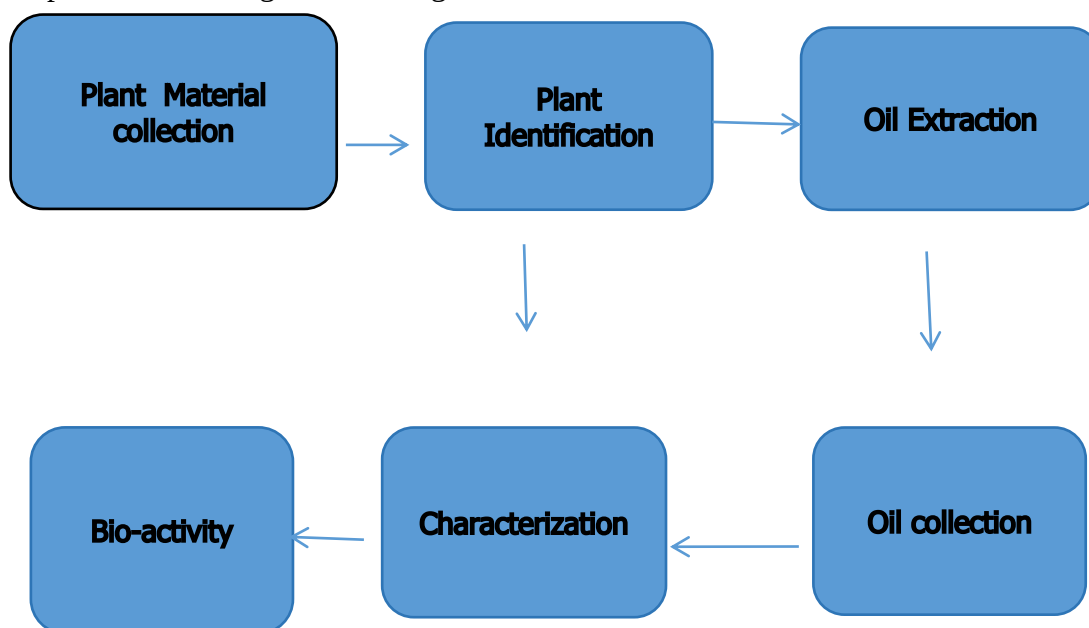
GCMS

FTIR

Agar well diffusion

Extraction of oil

Foeniculum vulgare Mill specimen preparation To obtain the essential oil, the plants were chopped into very small pieces and placed into the distillation flask; the typical equipment used in the process includes the following: clevenger, distillation vessel, receiver, and condenser of heat. Sufficient water was also available to help extract the improved process. A heating mantle or a water bath was used to heat the distillation flask. The volatile compounds of the plant matter were transported by the steam generated. When steam generated the water in a flask was boiled due to the amount of heat that was applied to the volatile compounds within the plant materials, the steam that had condensed was then collected in a receptacle. The essential oils were easy to isolate since they were very light in comparison to water and a funnel was used to separate the water and the essential oil. Essential oil was used to transfer it to an airtight and dark container. To ensure the quality of the oils over time it was necessary to protect the oils against heat, light and air.



Extraction Method

Clevenger Apparatus Hydrodistillation

The clevenger apparatus hydro-distillation is a method to extract essential oils from plant materials.

Heating

Place the round bottom flask on a heating mantle or water bath.

Gradually heat the water to boiling, ensuring controlled and even heating

Distillation

Steam passes through the plant material as the water boil & carrying volatile essential oils.

The vapour rises and passes through the condenser

Condensation

The condenser cools the vapor, turning it into liquid form.

The liquid (mixture of H₂O and essential oil) flows into the graduated arm of the clevenger apparatus.

Oil Separation

Essential oil separates from water due to difference in density.

The oil either floats on top or sinks (depending on the oil type) in the graduated arm

Excess water from the graduated arm is recycled back into the boiling flask to ensure continuous distillation

Result and Discussion

Result of Antimicrobial Activity

After applying *Foeniculum vulgare* oil to chosen microbial strains for 24 hours, the outcome was zone inhibition. The zone demonstrated oil action against the tested microorganisms. The zone diameter was measured in millimeters and depended on the concentration of essential oil used. for 100% oil content.

Table 4.1 Antimicrobial activity of *Foeniculum vulgare* oil

Sr.No	Bacteria	ZOI R1 mm	ZOI R2 mm
1	S.mutans	20	20
2	E.coli	20	16
3	S.aureus	22	15
4	P.mirabilis	0	0

Figure 4.1 Graph of Antimicrobial activities

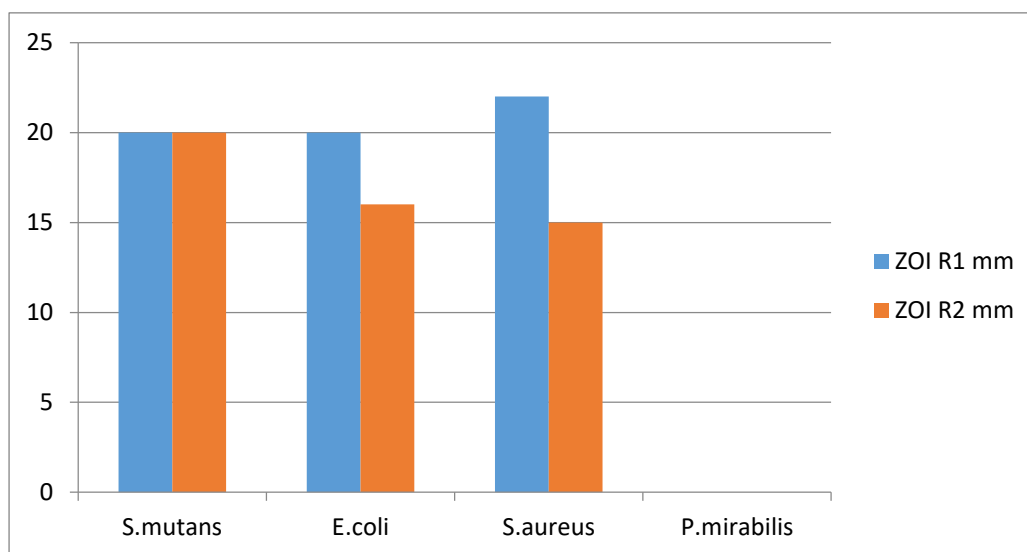
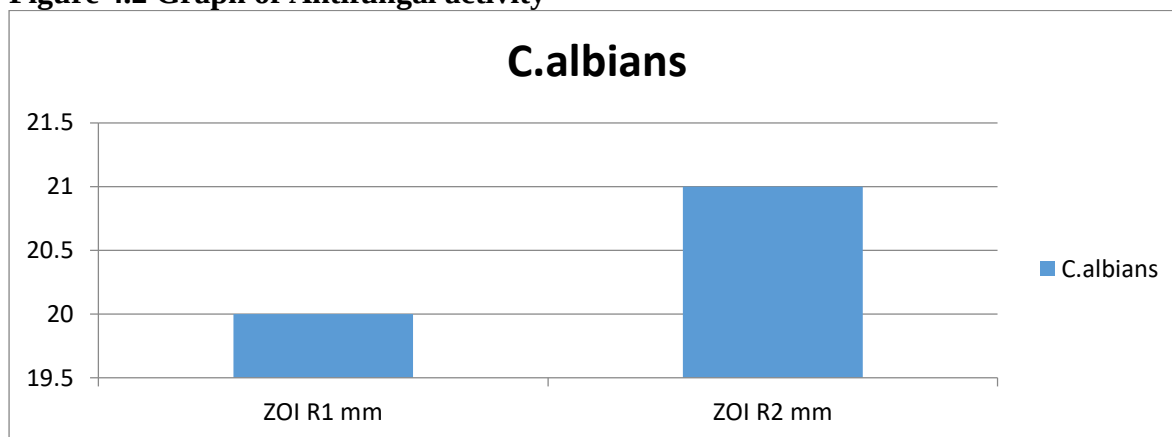


Table 4.2 Antifungal Activity of Foeniculum vulgare Mill

Sr.No	Fungi	ZOI R1 mm	ZOI R2 mm
1	C.albians	20	21

Figure 4.2 Graph of Antifungal activity



FTIR Spectra of Foeniculum vulgare

FTIR spectrum of the existence of hydroxyl (OH) aliphatic (C-H) and potential alkene (C=C) functional group . Hydroxyl functional group indicated by the presence of broad O-H stretch (3341cm) and intense C-H stretching (at 2924 cm) indicative of the presence of alkanes. Its peak at (1642cm) can be associated with C=C bonds e.g alkene or aromatic structures. The peaks within the fingerprint division (under 1500 cm) prove the occurrence of numerous functional groups, i.e., of the ether(s), ester or complex hydrocarbons.

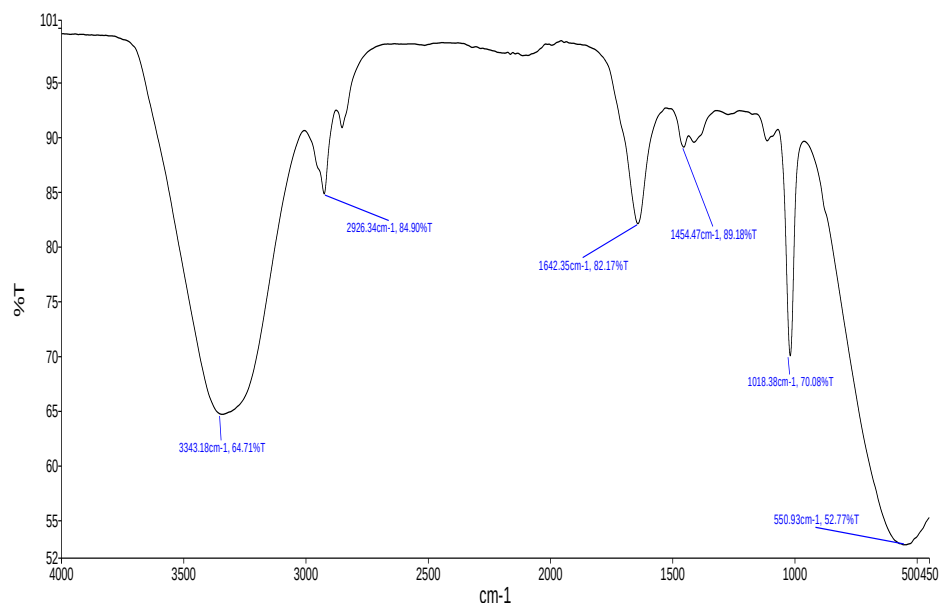


Figure 4.3 : FTIR spectra of Foeniculum vulgare

FTIR spectrum peak values

Wavenumber (cm)	Transmittance (T%)	Possible Functional Group
3341.16 cm	14.71 % T	O-H stretching (alcohols, carboxylic acids)
2924.36 cm	84.96% T	C-H stretching (Alkanes)
1642.36 cm	82.73% T	C=C stretching (Alkenes,Aromatics)
1454.72 cm	89.85% T	CH2 bending (Alkanes)
1018.38 cm	76.28% T	C-O stretching (Esters,Ethers)
550.63 cm	12.75% T	Possibly metal-oxygen bond

GCMS

The chemical composition of Foeniculum vulgare essential oil was examined using GCMS. During the analysis, nine different compounds were found in the essential oil. The results indicate that GCMS effectively determined the chemical makeup of Foeniculum vulgare essential oil. Among the identified components, stigmasterol was present at 0.64%, and Di-ethyl phthalate at 5.32%. Other detected compounds included:

1,2-Benzenedicarboxylic acid, bis (2-methyl propyl) ester – 0.45%

1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester – 0.72%

Gamma-sitosterol – 0.80%

Gamma-sitosterol (another form) – 0.18%

1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester – 83.10% (the major component)

4,4a,5,6,6a,6b,7,8,8a,9,10,11,14b compound – 6.89%

1,2-cyclohexanedicarboxylic acid, bis (2-ethylhexanyl) ester – 2.08%

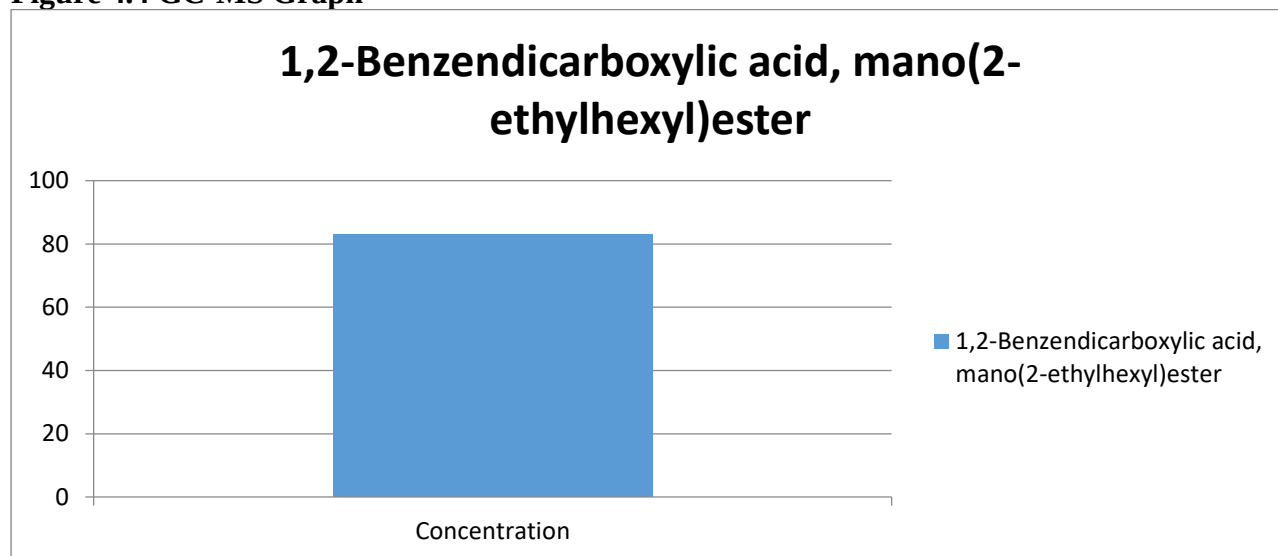
Table 4.4 Foeniculum vulgar Mill essential oil Qualitative GCMS Result

S.No	Name of the Compounds	R.Time	Area	Conc. %
1	Stigmasterol	26.531	184703	0.64
2	Diethyl phthalate	27.168	1533530	5.32
3	1,2-Benzenedicarboxylic acid,bis (2-methylpropyl)ester	30.430	129082	0.45
4	1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester	31.455	206505	0.72
5	.gamma-sitosterol	40.682	230280	0.80
6	.gamma-sitosterol	41.223	50972	0.18
7	1,2-Benzenedicarboxylic acid ,mono(2-ethylhexyl)ester	41.967	23942649	83.10
8	4,4,6a,6b,8a,11,11,14b-octamethyl-1,4,4a,5,6a,6b,7,8,8a,9,10,11	43.310	1985751	6.89
9	1,2-cyclohexanedicarboxylic acid ,bis (2-ethylhexyl)ester	43.686	599119	2.08

Table 4.5 Major compound

S.No	Major compound	R.Time	Area	Conc %
1	1,2-Benzenedicarboxylic acid ,mono(2-ethylhexyl)ester	41.967	23942649	83.10

Figure 4.4 GC-MS Graph



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Table 4.6 Minor Compounds

S.No	Minor Compound	R.Time	Area	Conc %
1	Diethyl phthalate	27.168	1533530	5.32
2	4,4,6a,6b,8a,11,11,14b-octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11	43.310	1985751	6.89
3	1,2-cyclohexanedicarboxylic acid ,bis (2-ethylhexyl)ester	43.686	599119	2.08

Figure 4.5 GC-MS Minor Graph

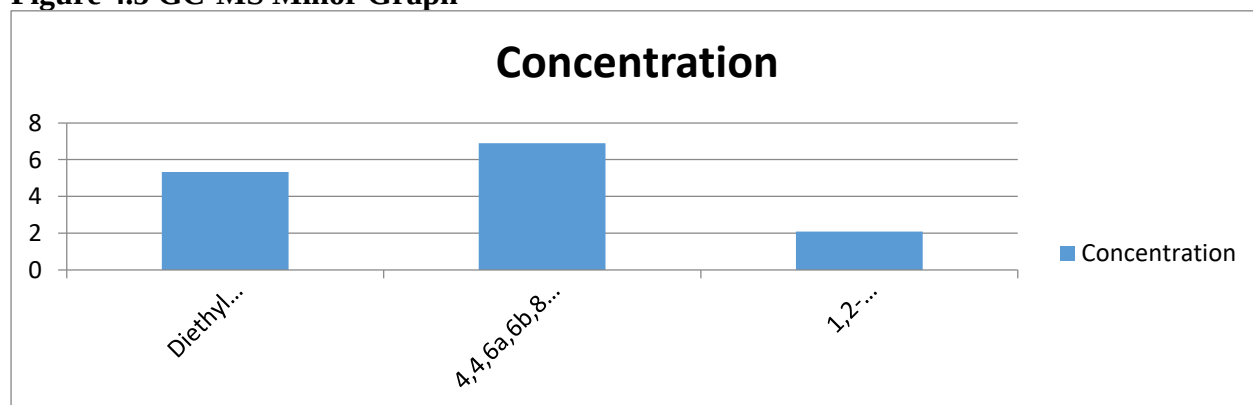
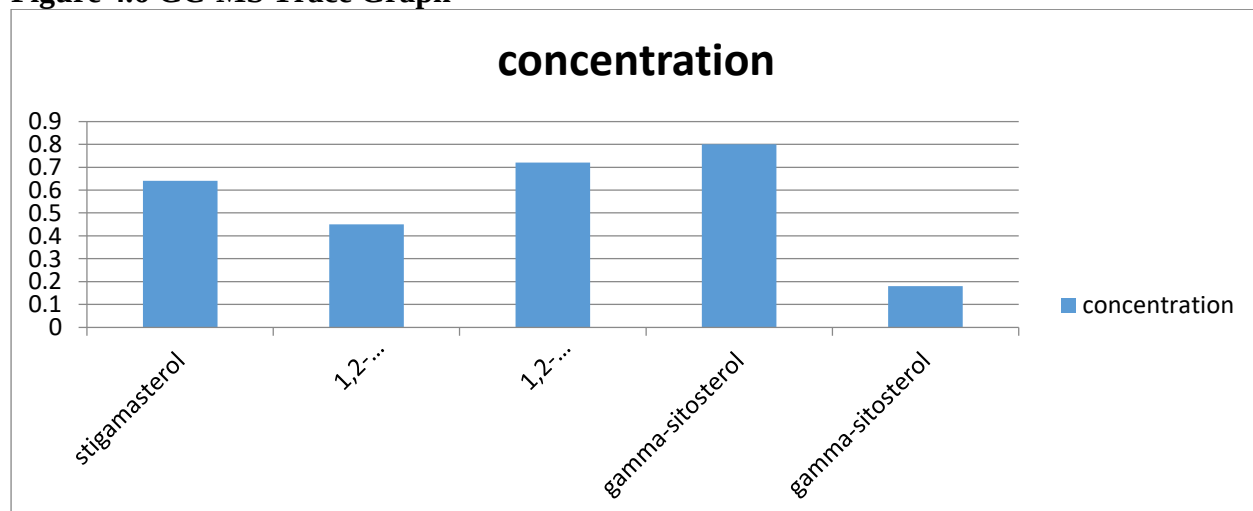


Table 4.7 Trace Compounds

S.No	Trace Compounds	R.Time	Area	Conc %
1	Stigmasterol	26.531	184703	0.64
2	1,2-Benzenedicarboxylic acid ,bis (2-methylpropyl)ester	30.430	129082	0.45
3	1,2-Benzenedicarboxylic acid ,butyl 8-methylnonyl ester	31.455	206505	0.72
4	gamma-sitosterol	40.682	230280	0.80
5	gamma-sitosterol	41.223	50972	0.18

Figure 4.6 GC-MS Trace Graph



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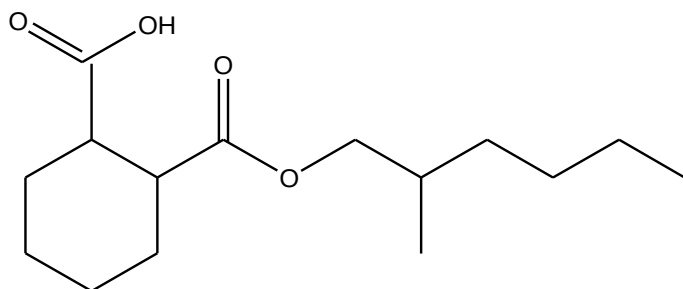
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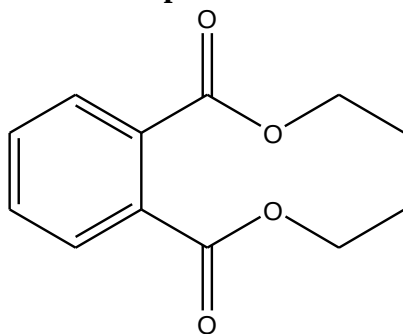
4.4 Structure of Major and Minor compounds

a) Major compound

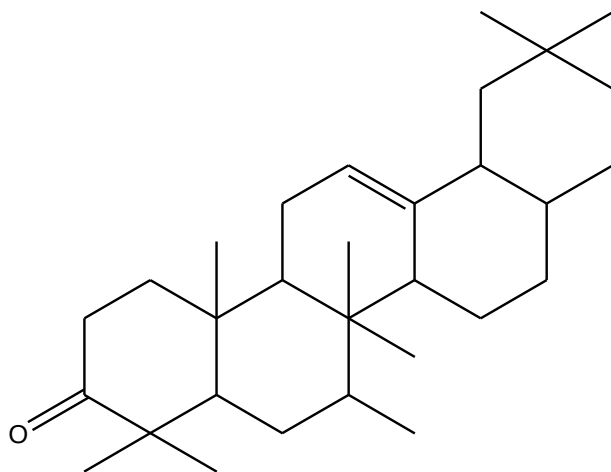


1,2-Benzendicarboxylic acid ,mono(2-ethylexyl)ester

Minor Compounds



Diethyl phthalate



4,4,6a,6b,8a,11,11,14b,-octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11

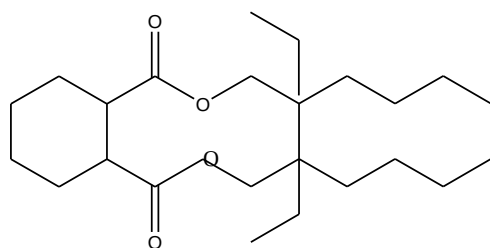
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1,2,cyclo hexanedi carboxylic acid ,bis (2-ethylhexyl) ester

Conclusion

In the current research, the extraction of the essential oil of *Foeniculum vulgare* was achieved by using the hydro-distillation technique to extract the oil. By analyzing through the gas chromatography-mass spectrometry (GC-MS), nine chemical constituents were identified indicating that 12-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester was the dominant compound found among the other chemical constituents as it occupies 83.10 percent of total oil composition.

Evaluation of the antimicrobial action against four bacterial strains coded *Streptococcus mutans*, *Escherichia coli*, *Staphylococcus aureus*, and *Proteus mirabilis* using the agar well diffusion technique was done on the extracted essential oil. It had high antibacterial effects on *E. coli* and *S. aureus* with inhibition zones of 20 mm and 22 mm, respectively. The moderate inhibition was detected in the case of *Streptococcus mutans* and no action was seen in the case of *Proteus mirabilis*.

In general, the findings suggest that the *Foeniculum vulgare* essential oil has substantial antibacterial potential, which might be explained by the fact that this product contains a substantial amount of phytochemicals. These results justify its potential use as a natural antimicrobial agent in both pharmaceutical preparation, and as a natural preservative agent in food and medical care products. More research is also suggested to assess its action mechanism, toxicity, and efficacy with a wider variety of microbial pathogens.

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