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"Extraction, Evaluation and Pharmacological Activities of Fennel Seeds: A Comprehensive Review"

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Abstract: Fennel is a popular fragrant and medicinal plant. Flavonoids, glycosides, and other phytoconstituents found in fennel are utilized as remedies for many illnesses. Fennel contains phenolic chemicals that are beneficial to human health. From this plant, bioactive substances like trans-anethole, estragole, fenchone and bioactive compounds kaempferol, quercetin, rosmarinic acid have been isolated from this plant and several associate with prospective human body mechanisms. The aim of the study is to discuss pharmacological activities including antibacterial, antidiabetic, anticancer, antioxidant, and other activities. The objective of this review to focusing on the nutritional value, botanical studies, phytochemicals and some major pharmacological actions of fennel to reveal the medicinal potential and future investigation aspects & may be utilized in the creation of many drugs

Keywords: Fennel extract, identification test, pharmacological activities

Introduction:

Nature has offered a vast array of treatments to treat all human maladies. In the past, man relied almost exclusively on plants as his only source of chemistry. There is currently a substantial body of research about the healing abilities of various plants. All plant phyla, including Thallophyta, Bryophyta, Pteridophyta, and Spermatophyte—of which a conservative estimate places the total number of known species at about 3,35,000—contain species that yield important official and unofficial medical products. As long as humans have existed, herbal remedies have a long history. The work contains more than 800 equations and 700 unique medications. Acacia, castor oil, and fennel are among the drugs listed, and there are also obvious allusions to iron oxide, salt chloride, sodium carbonate, and sulphur. The majority of the medicinally effective compounds discovered in the nineteenth and twentieth centuries were utilised as crude extracts. Many medicinal herbs have been used in China from 5000 B.C. Pen-t-sao, which was composed by the emperor Shen Ning sometime

around 3000 B.C., is the oldest herbal that is currently understood. For every day of the year, there are 365 drugs in it. [1]

Due to its commercial significance and significant pharmaceutical industry applications, fennel (Foeniculum vulgare Mill), one of the world's most significant medicinal herbs, is among the oldest known spice plants.

Scientific classification of fennel[19]

Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Clade	Eudicots
Clade	significant medicinal herbs, is among the oldest known spice plants.
Order	Apiales
Family	Apiaceae
Genus	Foeniculum

F. vulgare is the species. A perennial herb is called foeniculum vulgare. Marathon is the Greek word for fennel. It has an upright, glucose-green stem that can reach a height of up to 2.5 metres (8 feet) and is hollow. The leaves are finely divided, with the final segments being filiform (threadlike) and measuring 0.5 mm in length. The leaves can grow up to 40 centimetres (16 inches) in length. Its leaves are narrower than dill's but generally similar. Flowering occurs in terminal compound umbels that range in size from 5 to 17.5 (2 to 7 in) wide, with each umbel section having 20 to 50 small yellow flowers on short pedicels. Dry schizocarps that are 4-10 mm long, half that width, or less, and grooved make up the fruit. Since the fruit's seed is affixed to the pericarp, the entire fruit is frequently referred to as a seed by accident.Despite being grown in many regions of the world on dry soils near rivers or coasts, fennel is still thought of as a native plant of the Mediterranean region (Rather et al., 2016). The best growing conditions for fennel are in well-drained, calcareous soils with enough of sunshine (Bhattacharya, 2016).India, Egypt, Turkey, Syria, and Iran are significant fennel producers. India is known to have produced roughly 150,000 tonnes of fennel in 2018 (NHB, 2019), despite the fact that real production data for several other nations are not yet available. [2]

Fennel, a member of the Apiaceae family, is utilised for a variety of traditional therapeutic uses. According to reports, foeniculum vulgare has a moisture content of 6.3%, 9.5% protein, 10% fat, 13.4% minerals, 18.5% fibre, and 42.3% carbs. Foeniculum vulgare contains calcium, potassium, sodium, iron, phosphorus, thiamine, riboflavin, niacin, and vitamin C amongst other minerals and vitamins. Compilated information on their a lot antimicrobial, antiviral, anti-inflammatory, anti-mutagenic, antinociceptive, antipyretic, antithrombotic,

antitumor, hepatoprotective, hypoglycemic, hypolipidemic, and memory-enhancing pharmacological properties in vitro and in vivo indicates their effectiveness. [3]

A well-known Mediterranean fragrant plant called fennel (Foeniculum vulgare) is employed in traditional medicine. The fennel fruit also exhibits diuretic and analgesic properties in addition to antioxidant properties. The distinctive aroma and flavour are provided by the essential oils, which are primarily concentrated in the mericarps . According to Siahi et al. (2009), phytoestrogen is an active biological compound that functions in a manner similar to oestrogen. Additionally, it boosts libido and milk production while alleviating menopausal symptoms in women . In fact, due to the plant's sedative, estrogenic, analgesic, and anti-inflammatory effects, infusions and essential oils made from its fruits and aerial parts are both part of the herbalist's arsenal.

In addition to its antimicrobial activity due to its potential essential oil constituents and several pharmacological benefits through its bioactive constituents, which are very significant for human health , preliminary phytochemical screening confirmed the presence of flavonoids, tannins, saponins, steroids, glycosides, and terpenoids. [4] Many previous articles had extensively studied the pleiotropic activities of fennel using different solvents, and all of them recommended methanol, ethanol, or hydro-alcoholic extracts so, the current study aimed to assess the ability of ethyl extract (dissolved in 60% ethyl alcohol) of fennel seeds to inhibit the growth of a wide range of indicator microorganisms including Gram-positive bacteria, Gram-negative bacteria, yeasts, and filamentous fungi. Antioxidant, anticancer, and antiviral activities would also be included in the report. As a preliminary method for characterising an object, GC-MS would anticipate its chemical contents.

Organoleptic characteristic of Fennel:

Colour	Green to yellowish-brown
Odour	Sweet aromatic
Taste	Strongly aromatic
Size	5-10 x 2-4mm
Shape	Straight or slightly curved
Condition	Dried Cremo carp
Surface	glabrous

Microscopic characteristic of Fennel:

1.	Pericarp: [a] Epicarp	A layer of quadrangular to polygonal cells, with smooth cuticle
	[b]Mesocarp	Reticular, lignified parenchyma surrounding the vascular bundles
	[c]Vascular bundles	Five in number, bicollateral present below ridge (primary ridge)
	[d]Vittae	Schizogenous oil cells, 4 on dorsal side, 2on commissural surface/ventral surface. About 250 micron in maximum width, the walls are brown.
	[e]Endocarp	Consist of narrow elongated cells having a parquetry arrangement
2.	Seed: [a]Testa	Single layered yellowish brown in colour
	[b]Endosperm	Thick walled, polygonal, cellulosic parenchyma containing oil globules, aleurone, grains and rosette crystal of calcium oxalate
	[c]Raphe	A single ridge of vascular strands appears in the middle of commissural surface
	[d]Carpophore	With very thick walled sclerenchyma in 2 strands

Functional and therapeutic properties of fennel:

For thousands of years, fennel has been used in traditional medicine to treat a number of diseases and has a long history of use by humans. Fennel was thought to have calming characteristics in the fifth century, and from the ninth through the fourteenth centuries, it was credited with a wide range of medicinal benefits.[20] Fennel seed, according to the Romans, might enhance eyesight. The English thought that the herb may help with digestion and provide comfort from a bloated stomach. Since the 18th century, fennel has been used therapeutically, and several research has been conducted. The various plant components are now utilized to treat a variety of illnesses, including digestive system discomfort. Additionally, it is highly effective in the treatment of kidney stones, bronchitis, diabetes, and persistent cough.[21] Meals with cream use fennel seeds as an ingredient. The plant is used to treat kidney and bladder disorders because of its diuretic properties. It is also

used to stop vomiting and ease nausea. The herbs are useful for treating chronic fever as well as obstructions in the hepatic, gastrointestinal, respiratory, and urinary tracts. In addition, they are utilized to treat conditions relating to the eyes, such as cataracts, and the stomach, such as persistent diarrhea, endocrine, reproductive, and respiratory systems. In addition to that, it is also utilized in the treatment of breastfeeding women as a galactagogue agent.

Pharmacological activities of fennel:

1. Antibacterial activity: [5]

According to Mohsenzadeh (2007), E. coli 0157:H7, Listeria monocytogenes, and S. aureus, as well as Dadalioglu and Evrendilek (2004) and Cantore et al. (2004),[6] the essential oil isolated from the fruits of F. vulgare displayed antibacterial efficacy against these foodborne pathogens. Both aqueous and organic extracts of F. vulgare exhibit antibacterial activity against particular bacterial strains, according to Kaur and Arora (2008). Additionally, it has been asserted that the essential oil from the seeds of F. vulgare has antibacterial activity against a few human pathogenic pathogens. It has been shown that ethanol and water extracts of F. vulgare are effective against Campylobacter jejuni and Helicobacter pylori [7]According to a different study, F. vulgare essential oil may be able to treat Acinetobacter baumannii infections that are resistant to numerous medications. The potent antibacterial component of the F. vulgare stem was revealed to be a phenyl propanoid derivative known as Dillapional. Other chemical elements of F. vulgare have also been recognised as active antibacterial elements. Another substance, named Scopoletin, a coumarin derivative, has been found in the F. vulgare plant, according to Kwon et al. (2002).

2. Antifungal activity:

The essential oil of fennel is said to have antifungal properties. Reports claim that the antimycobacterial and anticandidal effects of fennel essential oil and seed extracts. [8]A number of F. vulgare bark extracts have been proven in studies by Pai et al. (2010) to have antifungal effects against Candida albicans. The essential oil of F. vulgare has also been demonstrated to impede the mycelial growth and germination of Sclerotinia sclerotiorum, suggesting that it may be used as a natural substitute for synthetic fungicides to treat phytopathogenic fungi. [9]

3. Antioxidant activity:

The different antioxidant capabilities of fennel species from different Mediterranean countries have been identified. Wild fennel has more radical scavenging action than both edible and therapeutic types, according to studies by Faudale et al. (2008). Malondialdehyde levels were discovered to be lower in the F. vulgare fruit methanol extract group than in the control group, indicating that the fruit's methanolic extract may have antioxidant properties. Compared to butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), it has been found that F. vulgare's essential oil and acetone extracts exhibit a significant amount of antioxidant activity. The inhibitory action of oil and acetone extracts in the linoleic acid system was investigated by monitoring peroxide accumulation in the emulsion throughout incubation using the ferric thiocyanate technique. Antioxidant activity has been demonstrated for the isolated compounds cis-miyabenol C, trans-

miyabenol C, sinapyl glucoside, and syringing 4-O-glucoside as well as the fruit extract from F. vulgare. In the n-BuOH extract of the F. vulgare fruit, a negligible level of lipid peroxidation activity was seen, but a substantial quantity of activity was observed at the higher quantities that were investigated. The antioxidant activity of isolated pure components from F. vulgare was higher than that of crude extracts, according to Marino et al. (2007).

The blooming aerial parts of bitter fennel were left behind after distillation for essential oils and contained isolated phenolic compounds with potent antiradical scavenging properties. The pharmacological impact of F. vulgare may now be better understood in light of this information. The isolated compounds were identified by Parejo et al. (2004) to be 3-caffeoylquinic acid, 4-caffeoylquinic acid, 1,5-O-dicaffeoylquinic acid, rosmarinic acid, eriodictyol-7-rutinoside, quercetin-3-O-galactoside, and kaempferol-3-O-rutinoside. In another study, antioxidant activity was discovered in both water and ethanol-based extracts of F. vulgare seed. Inhibition of peroxidation in the linoleic acid system by 100 g of water and ethanol extracts was 99.1% and 77.5%, respectively, in compared to the same quantity of -tocopherol (36.1%). Both extracts were reported to have strong reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, and metal chelating activities. The essential oils from the fruits of three organically grown Egyptian fennel cultivars, F. vulgare var. azoricum, F. vulgare var. dulce, and F. vulgare var. vulgare, have been discovered to have antioxidant activity. In comparison to essential oils from the vulgare cultivar, those from the azoricum and dulce cultivars had stronger antioxidant properties. [10]

4. Antithrombotic activity:

The essential oil of F. vulgare and its main ingredient, anethole, have been proven to have a safe antithrombotic activity because of its broad-spectrum antiplatelet activity, clot destabilising effect, and vasorelaxant action. Inhibition of arachidonic acid, collagen-ADP, and U46619-induced aggregation in guinea pig plasma was equally effective when anethole, the main component of fennel oil, was used. Anethole also prevented the thrombin-induced clot reaction at dosages comparable to fennel oil. When tested in vitro on rat aortas with or without endothelium, fennel oil and anethole both demonstrated similar NO-independent vasorelaxant effectiveness at antiplatelet levels that were confirmed to be free of deleterious consequences. Tognolini et al. (2007) found that anethole (100 mg/kg oral dose) and F. vulgare essential oil (also combined) substantially prevented ethanol-induced stomach ulcers in rats. features that reduce inflammation. [11]

The methanolic fruit extract of F. vulgare (200 mg/kg) has been demonstrated to have inhibitory effects against acute and subacute inflammatory diseases as well as type IV allergic reactions, according to Choi and Hwang (2004).

6. Oestrogenic activity:

F. vulgare has a long history of usage as an oestrogenic agent. It is said to increase libido, promote menstruation, make labour and delivery easier, increase milk production, and minimise symptoms of male climacteric. It is believed that anethole, which constitutes the majority of the oil, is the active oestrogenic component in fennel essential oil. According to several additional studies, the real pharmacologically active compounds are polymers of anethole such dianethole and photoanethole. [12]

7. Hepatoprotective activity:

Essential oil from fennel has been shown to have hepatoprotective properties. The levels of the blood enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and bilirubin were found to have decreased in a study that looked at how fennel essential oil affected the hepatotoxicity brought on by acute CCl4 injection. [13]

8. Antidiabetic activity:

The essential oil of F. vulgare has been demonstrated to exhibit hypoglycaemic activity in diabetic rats caused by streptozotocin. The serum glutathione peroxidase activity of diabetic rats receiving F. vulgare essential oil increased from 59.72 +(99.60 + 6.38 u/g Hb) to (2.78 u/g Hb), and their hyperglycemia decreased from (162.5 + 3.19 mg/dl) to (81.97 + 1.97 mg/dl). Inclusion in the market for diabetes drugs is now a possibility as a result [14]

9. Miscellaneous:

9.1 In vitro cytoprotection and antitumour activity:

By using the trypan blue exclusion assay to test the melanoma cell line B16F10 and the micronucleus assay to test normal human blood cells, it has been established that the methanolic extract of F. vulgare possesses anti-tumor activity. A 70% methanolic extract of F. vulgare was used to treat lymphocyte cultures, and it resulted in a considerable reduction in the number of micronuclei, which went from 0.018% with doxorubicin to 0.006%. A 70% methanolic extract of F. vulgare at a concentration of 200 g/ml, however, showed effective anti-tumor activity. This, according to Pradhan et al. (2008), demonstrates that F. vulgare may be exploited as a natural source of anticancer medications in addition to offering cytoprotection for normal cells.

9.2 Entomological activity:

When compared to the commercial repellent benzyl benzoate, direct contact application of F. vulgare fruit oil has been demonstrated to exhibit acaricidal effect against D. farinae and D. pteronyssinus. The physiologically active components of F. vulgare fruit oil have been discovered as P-anisaldehyde, (+)-fenchone, ()-fenchone, thymol, and estragol [15]

It has been established by skin and patch testing that the methanol extract of the fruit of F. vulgare has mosquito-repellent qualities against female Aedes aegypti mosquitoes. The physiologically active components of the fruits of Foeniculum were found to be (+)-Fenchone and (z)-9-Octadecanoic Acid .

9.3 Antihirustism activity:

There is evidence that the ethanolic extract of F. vulgare has antihirustism benefits. In a double-blind study, creams containing 1%, 2%, and placebo fennel extract were given to participants. Javidnia et al. (2003) assert that cream with 2% fennel is superior to cream with 1% fennel.[16]

9.4 Effect on uterine contraction:

Studies have been done to determine how fennel essential oil affects rat uterine contraction. Oxytocin and PGE2-induced contractions were significantly less strong when fennel essential oil was given at different doses (25 and 50 g/ml for oxytocin and 10 and 20 g/ml for PGE2, respectively). Fennel essential oil also reduced the frequency of contractions induced by PGE2, but not by oxytocin. An estimated LD50 of 1326 mg/kg was attained in female rats by employing the moving average method. The vital organs of the dead animals likewise didn't seem to have any obvious damage. [17]

9.5 Human liver cytochrome P450 3A4 inhibitory activity:

Thirteen chemicals isolated from fennel's methanolic extract have been found to have inhibitory effect against human liver cytochrome P450 3A4. Out of all of these medicines, 5-methoxypsoralen (5-MoP) displayed the strongest inhibition, with an IC50 value of 18.3 M and a mixed type of inhibition. The year 2007 [18]

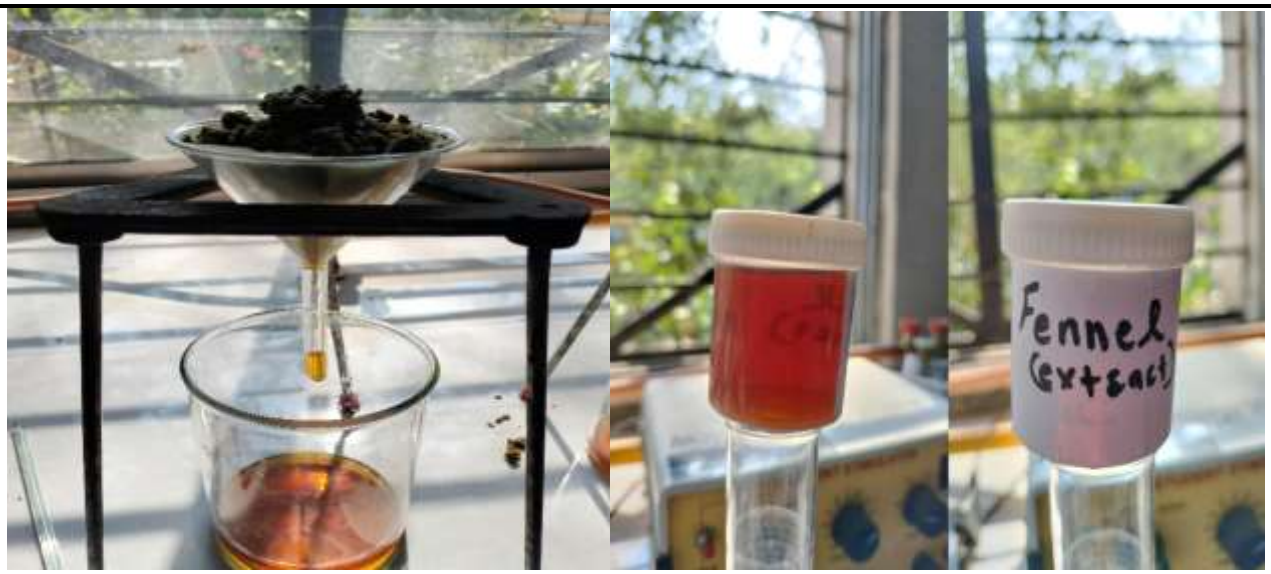
The safety of culinary and medicinal plants as well as the goods derived from them should be the subject of more scientific study. One of the main conditions for employing herbal medicines in medical diseases is the lack of risks such as mutagenicity, carcinogenicity, and teratogenicity. Generally speaking, these products ought to be as safe as feasible and have few negative consequences.

MATERIALS AND METHODS:

Plant matter: The fresh fennel seeds utilised in this investigation were acquired from a local Nagpur market.

Chemicals and Sample Acquisition: To prevent the loss of chemical components and to maintain the samples' quality, the samples were housed in a secure environment. In this investigation, n-hexane, methanol, and other chemicals and reagents were used. Every single one of the compounds and agents was either laboratory- or analytical-grade.

Using the maceration technique, volatile compounds from the seeds of F. vulgare were extracted. Using a weight-to-solvent ratio of 7:3, or water to methanol, a weight of sample (25g) is added to a total volume of 100 ml. Approximately 7 days are spent in the maceration process.



Extraction of bioactive Compounds: The bioactive compounds were extracted by using Maceration process. A weight amount of sample are placed (25g) in a solvent of total 100ml using a ratio 7:3 i.e. Water (70%) Methanol (30%). The maceration process is conducted for 7days.

Maceration process:

Plant material (crushed or cut small or moderately coarse powder)

(Fennel seeds)

↓

Placed in closed vessel

↓

Whole of the selected solvent added

↓

Allowed to stand for 7 days shaking occasionally

↓

Liquid strained off Solid residue (marc) pressed to recover as much as occluded solution

↓

Strained and expressed liquid mixed

↓

Clarified by subsidence or filtration

↓

Evaporation and concentration

Phytochemical Screening:

The fennel seeds were purchased from the local market in Nagpur. The methanolic extracts were subjected to chemical tests for the detection of different phytoconstituents.

Powdered plant material:

The powdered plant material of fennel is grayish-brown to grayish-yellow in color. It contains polyglonal secretory cells which are yellowish-brown in color and these cells are associated with the thin -walled transversely elongated cells, which are 2–9 μm wide. It also contains numerous fibre bundles. Endosperm fragments contain aleuronic grains along with very small microrosette crystals of calcium oxalate, and fibre bundles from the carpophores

Chemical Test:

1.Chemical test for cardiac glycoside:

(a) Baljet reaction: When picric acid (or sodium picrate) is added to the test solution, a stable orange colour result. With saponin, these effects are adverse, and with bufadienolides, they are adverse or considerably weaker.

(b) Raymond test: When the test solution is added to a hot methanolic alkali solution, a violet colour develops.

(c) Legal test: An appearance of pink to blood-red hue results from the treatment of the aqueous or alcoholic extract with pyridine (1 ml) and sodium nitroprusside (1 ml) solution.

(d) Killer-kiliani test: Dry some extract made by chloroform. Following that, incorporate 0.4 ml of glacial acetic acid and a minuscule amount of ferric chloride. Place a tiny test tube's sidewall with 0.5 ml of concentrated sulfuric acid in it, then transfer the contents there. In the presence of deoxy sugars, the acetic acid solution gradually turns bluish-green.

2. Chemical tests for coumarin glycosides

(a) fecl3test: Concentrate alcoholic extract of drug sample + few drops of alcoholic FeCl3 solution →appearance of dark green colour → turned to yellow after some time on addition of conc. HNO3 → indicates the presence of coumarins.

3. Chemical test for flavonoid glycosides

(a) Ammonia test: Ammonia vapour was inhaled after filter paper that had been dipped in a drug's alcoholic solution. emergence of a yellow spot on the filter paper. Magnesium turns and dilates when combined with alcohol as a medicine extract. After adding HCl, the creation of a red hue denotes the presence of flavonoids.

(b)Shinoda test: Because a carbonyl group is reduced in the Shinoda test, it is comparable to the Clemmensen reduction. However, a reductive-elimination takes place in this instance. The hydroxyl-like intermediate goes through elimination to produce the stable anthocyanidin due to the stability (extended conjugation) of the anthocyanidin product rather than the reduction proceeding all the way to the equivalent methylene molecule. Magnesium is utilised in place of the zinc mercury amalgam in the Shinoda test. The Shinoda test is used to determine whether flavones are present. Under the circumstances of the Shinoda reaction, they are converted to anthocyanidins if they are present in the test sample, as indicated. While the extended conjugation in the resulting anthocyanidin extends the colour further out into the red area of the visible spectrum, the conjugation in flavinoid compounds generates a yellow colour. This is a straightforward visual test for flavone presence because of the striking colour change.

Result:

Sr. No	Tests	Observations	inferences
1.	**Test For Cardiac Glycoside**		
a.	**Baljet test**	Stable orange colour	Test Complies
b.	**Raymond test**	A Voilet colour develops	Test Complies
c.	**Legal test**	Pink to blood-red colour appears	Test Complies
d.	**Killer-killani test**	The acetic acid solution slowly turns bluish-green	Test Complies
2.	**Tests For Coumarin Glycosides**		
a.	**fecl3test**	Appearance of dark green colour turned to yellow after some time on addition of conc. HNO3	Test Complies
3.	**Test For Flavonoid Glycosides**		
a.	**Ammonia Test**	Formation of red colour	Test Complies
b.	**Shinoda test**	Yellowish/ Reddish colour develops	Test Complies

Discussion & Conclusion:

This review discuss the chemical constituent, pharmacological and therapeutic effects of Foeniculum vulgare as promising herbal drug because of its safety and effectiveness. Studies that are presently accessible have shown that fennel extracts have a variety of pharmacological properties, including anti-allergic, analgesic, anti-inflammatory, antioxidant, antibacterial, anti-cancer, anti-stress, and cytotoxicity activities. The plant's numerous chemical components are what give it its therapeutic properties. Among the many components found in fennel plant essence, phenolic molecules are thought to be the most important and active ones. Fennel contains bioactive chemicals that are essential to preserving human health and are employed in the production of many different medications. remarkably popular and productive studies have been conducted on the antioxidant, antibacterial, and estrogenic effects of fennel in different animal models.

In traditional medicines the plant has been used as a treatment option against anxiety, arthritis, water retention, appetite suppressant, amenhorrea, angina etc. Traditional knowledge regarding the use of this plant is many but the scientific research available today to support this knowledge is limited. Here we have tried to compile all the available information from both traditional and published scientific literatures regarding the medicinal uses of Foeniculum vulgare. It will helpful for the future researchers to get the information. This will provide tremendous opportunities for planning and conduct research related to various aspects of this medicinal plant.

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