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REVISIONARY STUDY ON THE PHYTOCHEMICAL, PHYTOMEDICINAL AND OTHER ASPECTS OF *Foeniculum vulgare* MILL.

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ABSTRACT

Fennel, a multi traditional medicinal plant, is world-wide in distribution. Fruits and seeds are medicinally important parts and have various uses ranging from flavour to various dyspeptic complaints such as gastro-intestinal problems, flatulence, disturbance in female climacteric and increase libido. Essential oil is used in some respiratory complaints and in pediatric colic because of its antispasmodic effects. The chemistry of fennel is elaborated in details and large number of active and useful compounds are obtained and identified. These chemicals were then tested against range of ailments, for example, diuretic, analgesic, antipyretic, antimicrobial, cytotoxic activities in different animals and the positive effects were obtained. Successes were made in propagating the plants through tissue culture techniques using various nutrients and hormones. It has been established that the plant is diploid and no nuclear DNA polymorphism is found. The plant is sensitive to many pathogens which cause diseases to the plants. About 10% of the plants may be affected in commercial fields; however, the use of certain chemicals may reduce the chances of infection.

Key words: Fennel, *Foeniculum vulgare* Mill, Phytochemical, phytomedicinal.

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INTRODUCTION

Plant height of *Foeniculum vulgare* is about 0.4-2 m. Leaf blade is broadly triangular in outline about 4-30 × 5-40 cm, pinnatisect 4-5 and ultimate segments linear about 1-6 × ca. 0.1 mm and its lower petiole is 5-15 cm. Infloresence is umbel 5-9 cm with peduncles about

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2-25 cm and have 6-29(-40) rays which are unequal about 1.5-10 cm and umbellules have 14-39 flowers with pedicels about 2-10 mm which are unequal. The fruit is about 4-6(-10) × 1.5-2.2 (2.5) mm. It starts flowering from May to June and fruiting from July to September.

It is cultivated and adventive about 200-2600 m all over the China (It is indigenous to the region of Mediterranean but it is cultivated and adventive throughout the world). The fruit, leaves and stem are usually used in Chinese traditional medicine as dietary herb "xiao hui xiang" to help in digestion (Hui xiang, 2005).

Source

Fennel is apparently native to coast of Mediterranean and spreading towards east however it is cultivated in Wurtemberg, Galicia, Russia, Saxony, Japan, India and in south of France for medicinal use. Moreover, *F. vulgare* is well known to the ancients like other aromatic umbelliferous fruits and during the middle ages it was greatly used in Europe. The fruits of Russia, Romania, Galicia and Saxon are ideal for medicinal use because it was revealed that the fruits of these areas contain most essential oil and fenchone is present in Romania fruits (Greenish, 1999).

Description of the fruit

The fruits of *F. vulgare* have many commercial varieties and their size are greatly variable and also the appearance. The best fruit may be observed as Saxon fruits. Their colour are yellowish brown or greenish and shape is oblong, length varies from 8 to 10 mm and width 3-4 mm. The mericarp often remains united, glabrous, bear 5 pelar; very prominent and primary ridges, and attached to a pedicel. In transverse section, on the dorsal surface of each mericarp four large vittae are present that can be easily seen by naked eye and two vittae on commissural surface. Moreover, the endosperm is not deeply grooved and its colour is dark and is oily. The odour of *F. vulgare* fruit is aromatic and recalling anise and taste is sweet, camphoraceous (Greenish, 1999).

Adulteration

It was said that fennel specially exposed to admixture with exhausted fruits. It includes the partly exhausted fruits which were distillated with the current of alcohol vapour for liqueur making or with steam or water. When steam or water exhausted the fruits they get darker in colour, contain less amount of oil and in water sink immediately, but when alcohol vapour exhausted the fruits, they maintain oil of about 1.0 to 2.0% and their appearance is little changed. However, they obtained the odour of special fusel oil. Moreover, the recoloured fennel is detected when the fruit is rubbed between the hands (Greenish, 1999).

Ethnobotany

The *F. vulgare* belongs to family Apiaceae and its Urdu name is sonf. Plant part used is leaves and seeds as antidiabetics (Shah and Khan, 2006).

Uses

The *F. vulgare* dried fruits are extensively used in medicines, cosmetics, French type alcoholic liqueurs, candies and in food preparations such as used in pastry and bread as a flavour (Farrel, 1985 and Hansel *et al*., 1993). *F. vulgare* and its herbal drugs are used for upper respiratory tract catarrh and dyspeptic problems i.e. flatulence, mild, bloating and spasmodic gastric intestinal problems (Maduas, 1976; Merkes, 1980; Forster *et al*., 1980; Czygane *et al*., 1989; Forster, 1983; Weib, 1991).

It has been reported that plant seeds are used to increase libido, reduced the female climacteric signs and also used as a menstruation promoter (Albert-Puleo, 1980). Moreover, the essential oil of fennel is used in some respiratory complaints and in pediatric colic because of its antispasmodic properties (Reynolds, 1994).

Constituents

Avicularin, Aspartic acid, Ascorbic acid, Arginine, Arachidates, Apiole, Anisic-ketone, Anisic acid, Anisaldehyde, Aluminium, α-phellandrene, α-thujene, α-terpineol, α-terpinene, α-pinene, Alanine, 8-methoxpsoralen, 5-methoxpsoralen, 3-carene, 1,8-cineole, cis-anethole, Camphor, 3-carene, α-phellandrene, β-myrcene, β-pinene, Camphene, Limonene, fenchone, *trans*-Anethole, Boron, β-pinene, β-sitosterol, β-carotene, β-phellandrene, Bergapten, Benzoic acid, Cystine, Cynarin, Copper, Columbiantin, Cobalt, Citric acid, cis-ocimene, cis-anethole, Cinnamic acid, Chromium, Choline, Ceryl-alcohol, Camphor, Camphene, Calcium, Caffic acid, Dipentine, Dillapiol, Dianethole, D-limonene, Estragole, Fumaric acid, Ferulic acid, Fenchyl alcohol, Fenchone, Golycolic acid, Glycine, Glutamic acid, Gentisic acid, Gamma-tocotrienol, Gamma-terpinene, Histidine, Isoquercitrin, Isopimpinelin, Isoleucine, Iodine, Imperatorin, Kaempferol-3-glucuronide, Kaempferol-3-arabinoside, Kaempferol, Linoleic acid, Limonene, L-limonene, Myristicin, Methionine, Malic acid, Marmesin, Magnesium, Maganese, Methyl-chavicol, Nickel, Osthenol, Oleic acid, O-coumaric-acid, Psoralen, Proline, Photoantheole, Phenylalanine, Pectin, P-hydroxycinnamic acid, P-hydroxybenzoic acid, P-cymene, P-coumaric acid, Palmitic acid, Petroselinic acid, Phosphorus, Potassium, Protocatechuic-acid, Quercetin-3-glucuronide, Quercetin-3-1-arabinoside, Quinic acid, Quercetin, Quercetin-3-arabinoside, Rutin, Riboflavin, Syringic acid, Sinapic acid, Stigmasterol palmitate, Silicon, Shikimic acid, Stigmasterol, Scoparone, Seselin, Serine, Selenium, Scopoletin, Tyrosine, Trans-anethole, Tryptophan, Trigonelline, Transocimene, Thiamin, Tocopherol, Tin, Threonine, Terpinolene, Terpinen-4-ol, Tartaric acid, Trans-1,8-terpin, Urease, Umbelliferone, Vanillin, Vanillic acid, Valine, Zinc, Xanthotoxin (Lawrence, 1994; Bernath *et al*., 1996; Simandi *et al*., 1999; Ozcan *et al*., 2001).

Pharmacology

The ethanol extract of *F. vulgare* dried ripe fruit (500 g/kg) was tested in order to know about its antimicrobial, antipyretic, analgesic, cytotoxic and diuretic effects and its influence on bile flow in rats. The extract revealed the antipyretic, analgesic and diuretic activities and improved bile flow. It also prevented the growth of *Bacillus subtilis* and *Staphylococcus aureus* as an antimicrobial agent and also indicated a marked mitodeppressive effect. Further, the acute toxicity was investigated after three doses i.e. 0.5, 1 and 3 g/kg. When administrated acutely to mice at a 3g/kg dose, it did not cause death but reduced the locomotor activity and caused piloerection (Tanira *et al.*, 1998). Celik and Isik (2008) studied the protective effect of water infusion of *F. vulgare* against tetrachloroacetic acid (TCA) exposure in rats which is a carcinogen chemical. To know about the chemopreventive potential of plant infusion, different serum marker enzymes were measured in various tissues of rats. Different effects were shown by TCA and plant infusion on antioxidant defense systems of tissues, lipid peroxidation and on serum marker enzymes when 50 days test ends against TCA exposed rat as compared the TCA exposed rats and control. The result showed that serum marker enzyme activity was significantly increased by both TCA and TCA + plant. And the non-enzymatic antioxidant GSH level in brain of TCA+FV was significantly increased but in erythrocytes and kidney it decreased than that of TCA group and control. After TCA treatment, it was found that different enzymes of antioxidant were triggered at different degrees and significant declined occur in tissues Malondialdehyde concentration that reduced the plant infusions enzymes activation. It might be suggested from the observation of 50 days protective exposure that the FV may possess antioxidant properties. Fennel seeds are used in traditional medicine and its methanol extract revealed the mechanism based inactivation characteristic mediated through CYP3A4 (microsomal cytochrome P450 3A4) of human liver on erythromycin N-demethylation (Subehan *et al*., 2007). Subehan *et al*. (2007) isolated 13 compounds from fennel methanol extract and their inhibitory activity were tested on CYP3A4. In these compounds, strongest inhibition was shown by 5-MOP with 18.3 μM IC 50 value and also showed an inhibition of mixed type. Moreover, only 5-MOP revealed the dependence on pre-incubation time, as its 18.3 μM IC50 value at 0 min pre-incubation was decreased to 4.6 μM at twenty min pre-incubation. Further analysis on 5-MOP indicated the time dependent prevention characteristic, NADPH necessity, lack of nucleophiles protective effect and through the competitive inhibitor the recovery of CYP3A4 activity. This showed that 5-MOP induced inhibitory activity on CYP3A4 was a mechanism based inactivation. The *F. vulgare* essential oil was tested in order to know about its antifungal activity. Essential oil was tested against *Sclerotinia sclerotiorum*. Than with the help of light and scanning electron microscope, the essential oil effect on morphological structure of sclerotia and hyphae of *S. sclerotiorum* were examined. The result showed that oil possessed a marked antifungal activity against *S. sclerotrum* (Soylu *et al*., 2007). Turkish plants 9 different species, including *F. vulgare* were examined to know about the antioxidant effects and chemical composition of essential oils of these plants. The antimycobacterial effect of 9 plants was examined because these plants were used in traditional medicine of Mexican to cure respiratory diseases such as tuberculosis. The best activity was shown by *Nasturtium officinale* against the sensitive *Mycobacterium tuberculosis*. And it was also found that the *F. vulgare* is also active Mycobactrium as compared to other plants (Camacho-Corona *et al*., 2007). *F. vulgare* also showed the immunomodulatory NFkappaB effects (Kaileh *et al*., 2007). Volatile oil of *F. vulgare* emerged from twenty-four essential oils pool because of its antiplatelet activities and also its coagulum destabilizing effect. In vivo, anethole and volatile oil of *F. vulgare* were orally given to mice for five days in a subacute treatment (30 mg kg^{-1} day^{-1}) revealed important antithrombotic action by inhibiting the paralysis (70% and 83% protective respectively) and paralysis is caused by injection of collagen epinephrine intravenous. The antithrombotic dosage at variance did not caused prohemorrhgic side effect with reference drug i.e. acetylsalicylic acid. Moreover, the oral administration of both anethole and volatile oil of *F. vulgare* at 100 kg^{-1} day^{-1} to rats, gave important protection from ethanol as ethanol induced gastric lesion. So, it was concluded that antheole and volatile oil was safe antithrombotic because of their blood spectrum antiplatelet property, coagulum destailizing effect and vasorelaxant activity (Tognolini *et al*., 2007). Material derived from *F. vulgare* fruit and its insecticidal activities were tested by direct contact appliance and by fumigation methods against adults of *Lasioderma serricorne, Callosobruchus chinensis* and *Sitophilus oryzae*. The active biologically components were monoterpene (+)-fenchone, phenylpropanes (E)-estragole and phenylpropanes (E)-anethole, characterized by spectroscopic analysis (Kim and Ahn, 2001).

The effect of different hydrodistillation methods was examined to know about its effect on antifungal activity, chemical composition and yield of essential oil from *F. vulgare* seeds. 3 hydrodistillation methods were studied. The essential oil main components were methyl chavicol (3.78% to 5.29%), fenchone (11.32% to 16.35%) and (E)-anethole (72.27% to 74.18%). The result showed that the essential oil quantitative composition and yield were significantly affected by distillation method but its antifungal activity was slightly changed against some fungi (Mimica-Dukid *et al*., 2003). Essential oil was extracted from *F. vulgare* fruit and its antibacterial activity was examined in vitro to *Bacillus megalerium* and *Eschericha coli*, these bacteria commonly used in antimicrobial examination for comparison, and 27 phytopthogenic and 2 mycopathogenic bacterial species as these bacteria caused diseases in cultivated mushrooms. The observation showed that the *F. vulgare* oil reduced the bacteria effect and the oil may be used for the management of plants bacterial pathogens and in organic agriculture for seed treatment as natural bactericides because the essential oil antibacterial activities was significant against the mushrooms bacterial diseases (Lo Cantore *et al*., 2004). Kwon *et al*. (2002) isolated six compounds from the stem of *F. vulgare*. Among these, dillapional, derivative of phenyl propanid, was found to be notable antimicrobial agent against *Cladosporium cladosporioides*, *Aspergillus niger, Bacillus subtilis* with MIC value125, 250 and 125 respectively. A coumarin derivative, scopoletin showed slight antimicrobial activity and other compounds, bergapten, imperatorin and psolaren were found inactive. But the isolated all six compounds were not active against *E. coli.* By GS-MS and GS, the *F. vulgare* essential oil were analyzed. It indicated that 35 constituents were present and it's the total amount 96.4%. and *trans*-anethole (70.1%) was the main constituent. Further, when its acetone extract was analyzed, it revealed that 9 constituents were present and it's the total amount 68.9% and the major constituents were palmitic acid (5.4%), Oleic acid (5.4%) and linoleic acid (54.9%). The antioxidative and antifungal properties were also studied through different methods. The essential oil revealed complete zone prevention at 6 µL dose in inverted petriplate technique against *Fusarium moniliforme, F. graminearum, Aspergillus flavus and A. niger* while it was found that at 4 µL dose it was also effective against *A. niger*. Moreover, good to moderate zone of prevention was shown by both essential oil and extract using food poison method. The antioxidant value was estimated at fixed time intervals for linseed oil by measuring the values of peroxide and thiobarbituric acid. The essential oil and extract both produced strong antioxidant action as compared the reference antioxidant i.e. BHT (butylated hydroxytoluene) and BHA (butylated hydroxyanisole). In addition, through ferric thiocyanate method, studied the inhibitory activity in linoleic acid of micellar system during incubation by monitoring the accumulation of peroxide in emulsion. The result was same to above result i.e. it also showed a strong antioxidant action (Singh *et al*., 2006). By GC-MS and GS, the *F. vulgare* essential oil was analyzed and also examined its antibacterial and antioxidant actions. Using 2 lipid model systems from linoleic acid in a micellar system to studied the antioxidative effect of oil. One is spectrophotometric hydroeroxydienes detection and other is modified TBARS (thiobarbituric acid reactive species). The oil showed antioxidant activities, comparable in some cases to reference antioxidants i.e. α-tocopherol and BHT. Further, the essential oil antimicrobial activity was studied against 25 bacteria genera, these include the spoilage bacteria, food poisoning bacteria and plant and animal pathogens. The greater and vast degree of prevention was shown by both oil samples of *F. vulgare* as compared the *C. maritimum* (Roberto *et al*., 2000). The GC-MS and GS analysis of chemical composition of ripe and unripe fruits and flower of *F. vulgare* showed that the oils of ripe and unripe fruits and flower main identified constituents were α-phellandrene (0.72%, 3.30% and 5.77%), fenchone (23.46%, 19.18% and 13.53%) and estragole (61.08%, 56.11% and 53.08%), respectively. The less qualitative and greater quantitative variations in some compounds were shown by essential oils obtained from different parts of *F. vulgare*. In addition, the antifungal activities of oils were variable on mycelial growth of *Rhizoctonia solani, Fusarium oxysporum* and *Alternaria alternate*. The study showed that the antifungal activities of *F. vulgare* oils were dose dependent because inhibitory effect was shown by fennel oils at 40 ppm concentrations against *A. alternata* mycelial growth while 10 ppm concentrations were ineffective (Ozcan *et al*., 2006).

Tissue culture

Many populations of fennel were determined for callus formation and morphogenic response by genotype and hormonal treatment and it was observed that plant regeneration was occur especially in population of Francia Pernod. Moreover, under the activity of kinetin and α-naphthaleneacetic or 2, 4-dichlorophenoxyacetic acid only the Ferncia Pernod population showed 100% callus formation. In addition, only kinetin hormonal treatment induced shoot regeneration. Furthermore, in the presences of these hormones treatment, the calluses growth revealed great differences at morphological and cytological level, these were associated to their different morphogenic ability (Anzidei *et al*., 1996). Fennel embryogenic suspension cell line was developed which is directly induced from hypocotyl. At 4 °C, cold preserved the suspension cells for twelve weeks. After that at 25 °C it was cultured for two weeks. The volume of packed culture cells was declined because the cold preservation duration became longer and as the cold preservation time passing the cells (cells possess the embryogenic potential and size is about size 32-82 μm) declined suddenly. However, the cells become able to formed normal somatic embryos when it was cold preserved for about two to six weeks and these somatic embryos were similar to those acquired from the cells not treated with cold preservation. Than on hormone free MS medium, cultured the somatic embryo under light, these were regenerated to grow into normal plantlets. Furthermore, when esterase isoenzyme of embryo, treated with cold preservation of about four to six weeks, were analysed on gel electrophoresis, it showed the appearance of different bands as compared to the embryo from the cells not treated with the cold preservation. And detected the anethole at the same level in methanolic extracts of cold preserved cells embryos and untreated cells embryos (Umetsu *et al*., 1995).

Mycorrhiza

F. vulgare growth and essential oil concentration was significantly enhanced by 2 arbuscular mycorrhizal fungi i.e. *Glomus fasciculatum and G. macrocarpum* However, the AM inoculation with phosphorus fertilizer significantly improved plant essential oil contents, P uptake and growth than that of either of the fungi used alone. Moreover, among these, the highest growth was shown by *G. fasciculatum* at both levels of phosphorus used and essential oil contents of fennel seeds increase up to 78% as compared to control (non- mycorrhizal). Furthermore, the gas liquid chromatography characterized the essential oil and they showed that myccorhization significantly improved anethole level (Kapoor *et al*., 2004).

Genetics

Fennel donor plant belongs to population of Francia Pernod, was studied to know about the cytological use and particularly the chloroplast DNA and nuclear DNA molecular analysis to determine the organogenesis and somatic embryogenic derived plants genetic stability or instability. Normal diploid chromosome number was shown by different types of morphogenic callus, organogenic and embryogenic plants. The study of RAPD analysis showed that there was no nuclear DNA polymorphism in all regenerated plants. It was noticed that these plants have no variation independently in two cpDNA regions of morphogenic origin that have same restriction profiles, base sequence and length. Furthermore, the cpDNA microsatellite region study including a single Art repeat in these regenerated plants showed no variation of repeat numbers. The results showed that the regenerated fennel plants were genetically stable and uniform and these results were also supported by a comparison of natural fennel plants and regenerated fennel plants cpDNA microsatellite region which showed that except Francia Pernod, they found variations in some of them (Bennici *et al*., 2004). The composition of ripe mericarp hexane extract of 11 native populations were selected where annual rainfall in the northern region from ca. 1000 mm and in the Negev Region down to 125 mm. 18 components were separated by GC-MS and the major components were ɑ-pinene, limonene, fenchone, TRANS anethole and estragole. Further, four different groups were characterized on the basis of TRANS-anethole and estragole levels i.e. (i) low TRANS-anethole (3%) and high estragole (63%) were characteristic of Mount Meron population, (ii) TRANS-anethole (17% to 29%) and estragole (39% to 47%) characterized the 3 mountainous populations, (iii) TRANS-anethole (38% to 49%) and estragole (21% to 29%) characterized the lowland and coastal populations, (iv) high TRANS-anethole (55% and 74%) and low estragole (ca. 8%) were characteristic of two exceptional populations. It was suggested that a reversed association was occurred between the TRANS-anethole and estragole contents induced by rainfall because lowest TRANS-anethole and highest estragole were found in high rainfall habitat and under limited precipitation its vice versa. In addition, it is suggested that environmental condition governed the oleoresins composition of *F. vulgare*. However, the recorded differences of genetic variation are not out of ruled.

Pathology

First time, the phytoplasma disease was reported on *F. vulgare* in experiment and also in Lucknow, CIMAP commercial field and its adjacent areas. Phytoplasma caused the little leaf disease and their typical symptoms are growth retardation with axillary shoots excessive proliferation and development of narrow and small leaves which formed appearance like witch’s broom. Moreover, the plants were changed to completely yellow colour and fail to developed inflorescences when it was severely infected. It has been found that the disease effect in commercial fields in the range of 5 to 12%. Further, the study of transmission electron microscope showed that only in sieve tube elements the pleomorphic bodies were present in infected plants and not found in uninfected plants. Their size was variable ranging from 110 to 970 mm and their ultrastructural details were closely resembled to other identified plant pathogenic phytoplasmas. The single membrane surrounded the phytoplasma bodies and these were spherical to oval and some with budding. When infected plants were treated with tetracycline hydrochloride the disease symptoms were temporarily suppressed. It has been the phytoplasma disease first report from India on *F. vulgare* (Samad *et al*., 2002).

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