



Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (9):200-205
(<http://scholarsresearchlibrary.com/archive.html>)



Study of antibacterial, antimycobacterial, antifungal, and antioxidant activities of *Foeniculum vulgare*: A review

Sepideh Miraj¹ and Sadegh Kiani^{2*}

¹Assistant Professor, Fellowship of Infertility, Cellular and Molecular Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran

²Student of Nursing, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran

ABSTRACT

Foeniculum vulgare (fennel) belong to Apiaceae family has been used in traditional medicine for many therapeutic purposes. The aim of this study was to overview its therapeutic effects than its nutritive and industrial effects. This review article was carried out by searching studies in PubMed, Medline, Web of Science, and IranMedex databases. The initial search strategy identified about 114 references. In this study, 39 studies were accepted for further screening and met all our inclusion criteria [in English, full text, therapeutic effects of *Foeniculum vulgare* L and dated mainly from the year 1985 to 2016. The search terms were “*Foeniculum vulgare* L.”, “therapeutic properties”, “pharmacological effects”. It is commonly used for its anti-bacterial, anti-fungal, antimicrobial and antimycobacterial, antioxidant effects. It was said to be good for insomnia and dyssomina. *Foeniculum vulgare* l is a rich plant .although in many studies, its anti-microbial activities were confirmed, more studies are still needed to verify its potent effect.

Keywords: *Foeniculum vulgare* L., Phytochemicals, Therapeutic effects, Pharmacognosy, Alternative and complementary medicine.

INTRODUCTION

Foeniculum vulgare (fennel) belong to Apiaceae family has been used in traditional medicine for a wide range of ailments related to digestive, endocrine, reproductive, and respiratory systems. Additionally, it is also used as a galactagogue agent for lactating mothers. Findings based on their traditional uses and scientific evaluation indicates that *Foeniculum vulgare* remains to be the most widely used herbal plant [1]. It has been used for more than forty types of disorders. Compiled data indicate their efficacy in several in vitro and in vivo pharmacological properties such as antimicrobial, antiviral, anti-infantile colic [2], anti-inflammatory [3], antimutagenic, antihirsutim [4, 5], antinociceptive, antipyretic, antispasmodic, antithrombotic, apoptotic, cardiovascular, chemomodulatory, antitumor [6], hepatoprotective, hypoglycemic, hypolipidemic, and memory enhancing ,osteogenic property [7]. It has antibacterial, antifungal, antiviral, antimycobacterial, and antiprotozoal activities [7-11]. It has antioxidant, antitumor, chemopreventive, cytoprotective, hepatoprotective, hypoglycemic, and oestrogenic activities antioxidant, cytotoxic, anti-inflammatory, antimicrobial, bronchodilatory, estrogenic, diuretic, lithontripic, galactogogue, emmenagogue, antithrombotic, hypotensive, gastroprotective, hepatoprotective, memory enhancing activities [12-17].

Foeniculum vulgare has emerged as a good source of traditional medicine and it provides a noteworthy basis in pharmaceutical biology for the development/formulation of new drugs and future clinical uses. Phytochemical studies have shown the presence of numerous valuable compounds, such as volatile compounds [18, 19], flavonoids, phenolic compounds, fatty acids, and amino acids [20, 21].

Antibacterial activities

The antibacterial effect of the aqueous extract of 12 medicinal plants of Apiaceae family including *F. vulgare* was investigated. An aqueous extract of the aerial part of *F. vulgare* inhibited the growth of *Agrobacterium radiobacter* pv. *tumefaciens*, *Erwinia carotovora*, *Pseudomonas fluorescens*, and *Pseudomonas glycinea*. An aqueous extract of seed sample inhibited the growth of *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella flexneri*, and *Bacillus cereus* with 13–22, 22–24, 14–24, 20–21, 21–24, 11–12, 14–18, 17–18, and 24–26 mm zone of inhibition, respectively [3, 4]. Gulfranz *et al.* [92] investigated the antibacterial effect of the essential oil as well as ethanolic and methanolic fruit extracts of *F. vulgare* against *Bacillus cereus*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Pseudomonas putida*, *Pseudomonas syringae*, and *Candida albicans*.

Shrivastava and Bhargava investigated the antibacterial effect of the crude, chloroform, and methanol extract of leaves and flowers of *F. vulgare* along with *Raphanus sativus* and *Brassica nigra* against *Escherichia coli* and *Staphylococcus aureus*. Methanol extract of flower of *F. vulgare* showed significant activity against *Escherichia coli*, whereas crude and chloroform extracts failed to exhibit antimicrobial activity against *Staphylococcus aureus* (Table 9). Among different tested bacterial strains, the methanolic fruit extract of *F. vulgare* inhibited the growth of *Staphylococcus aureus* and *Bacillus pumilus* with 11.27 and 12.67 mm zone of inhibition, respectively.

The characterization of seven different types of oxygenated monoterpenes, from methylene chloride crude extract of *F. vulgare* suggested that the crude extract containing monoterpenes could be a new medicinal resource for antibacterial agents.

A total of 78 compounds were identified from the active antimycobacterial fraction of *F. vulgare* with the help of gas chromatography-mass spectra (GC-MS). Out of these, twenty compounds were tested against one sensitive and three MDR strains of *Mycobacterium tuberculosis* using the Alamar Blue microassay. Compounds that showed some degree of antimycobacterial activity against all strains tested were the following: linoleic acid (MIC 100 µg/mL), oleic acid (MIC 100 µg/mL), 1, 3-benzenediol (MIC 100–200 µg/mL), undecanal (MIC 50–200 µg/mL), and 2, 4-undecadienal (MIC 25–50 µg/mL). 2, 4-Undecadienal was the most active compound against multidrug resistant *M. tuberculosis* species. Thus, the dietary intake of *F. vulgare* may lower the risk of *M. tuberculosis* infection.

Anti-viral activity

The antiviral activity of the essential oil of fruit sample of *F. vulgare* against the DNA virus Herpes simplex type-1 was studied. Most of the oils and compounds displayed strong antiviral effects against HSV-1, ranging between 0.8 and 0.025 µg/mL. However, the samples tested were less effective against PI-3, with results ranging between 1.6 and 0.2 µg/mL. Only the essential oils of *Anethum graveolens*, *Foeniculum vulgare* (fully mature), *Mentha piperita*, *Mentha spicata*, *Ocimum minutiflorum*, *Ocimum vulgare*, and *Satureja jacuneifolia* inhibited this virus significantly.

The scolocidal effects of amphotericin B, Silver nano particles, *Foeniculum vulgare* Mill, essential oil and hypertonic saline against protoscoleces of hydatid cyst on an *in vitro* model was examined. The results indicated weak scolocidal activity of AmB and Ag-NPs; whereas *F. vulgare* essential oil had potent scolocidal activity against protoscoleces of hydatid cyst that revealed the potential of *F. vulgare* as a natural source for the production of new scolocidal agent for use in hydatid cyst surgery. However, further studies will be needed to confirm these results by checking the essential oil and its active component in the *in vivo* model [22].

Anti-fungal activity

The antifungal effects of FSEO were investigated from varied aspects, such as MIC and minimum fungicidal concentration, mycelia growth, spore germination and biomass. The results suggested that the antifungal mechanism

of FSEO was to damage the plasma membrane and intracellular organelles. Further study revealed that it could also inhibit the mitochondrial enzyme activities, such as succinate dehydrogenase, malate dehydrogenase and ATPase. With better antifungal activity than the commonly used antifungal agents and less possibility of inducing drug resistance, FSEO could be used as a potential antidermatophytic agent [23].

In an in vitro study, fungal and aflatoxin contamination in stored tobacco leaves and the potential of *Foeniculum vulgare* (fennel) seed essential oil (EO) as a plant-based preservative in protection of tobacco during storage was examined and it showed that the fennel EO can thus be formulated as a plant-based preservative for food items [24].

the antibacterial and antifungal effects of *Foeniculum vulgare*, *Mentha spicata*, and *Rosmarinus officinalis* was investigated against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Candida albicans*, and phytopathogenic molds, *Aspergillus niger* and *Fusarium oxysporum*. Essential oil of *F. vulgare* showed significant antifungal activity against the food spoilage fungi *Aspergillus niger* and *Fusarium oxysporum* and may have important applications as food additives. The MIC values of *F. vulgare* essential oil were 250 µg/mL for *Fusarium oxysporum* and 750 µg/mL for *Aspergillus niger*.

In an in vitro study, aqueous and alcoholic seed extracts of *F. vulgare* exhibited inhibitory effect against *Alternaria alternata*, *Mucor rouxii*, and *Aspergillus flavus*. Interestingly, aqueous seed extract of *F. vulgare* showed strongest antifungal activity as compared to reference fungicidal agent, that is, griseofulvin.

Anthelmintic activity

The in vitro antischistosomal activity and cytotoxic effects against V79 cells of the essential oil of *F. vulgare* was investigated. This plant displayed moderate in vitro schistosomicidal activity against adult *S. mansoni* worms, exerted remarkable inhibitory effects on the egg development, and was of low toxicity [25].

Antimicrobial activity

The chemical composition and antimicrobial activity of essential oils obtained by hydrodistillation from fruits of six fennel accessions collected from wild populations occurring in the centre and south of Portugal was examined. *Escherichia coli* ATCC 25922; *Morganellamorganii* LFG 08; *Proteus mirabilis* LFG 04; *Salmonella enteritidis* LFG 05; *S. enteritidis* serovar typhimurium LFG 06 and *Pseudomonas aeruginosa* ATCC 27853 by the disc diffusion agar method; the minimal inhibitory concentration (MIC) was determined [14].

The composition and antifungal effect of volatile extracts obtained from the aerial parts of Sardinian wild fennel was investigated. The essential oil was more active against *Candida albicans*, whereas the supercritical fluid extract possesses higher activity against *Candida guilliermondii* and *Cryptococcus neoformans*, with MIC values of 0.32 µL/mL [26].

The anticoccidial effect of *Artemisia annua* and *Foeniculum vulgare* on *Eimeria tenella* infection was investigated. The effects of *A. annua* and oil extract of *A. annua* + *F. vulgare* on *E. tenella* infection were assessed by clinical signs, mortality, fecal oocyst output, faeces, lesion score, weight gain, and feed conversion results suggest that *A. annua* leaf powder (Aa-p), at 1.5% of the daily diet post-infection, can be a valuable alternative for synthetic coccidiostats, such as lasalocid [27].

In an animal study, a fatal nitrate toxicities in cattle associated with the consumption of fennels (*Foeniculum vulgare*) was reported. The fennels were grown in a polluted area of the Campania region in southern Italy and distributed in a public market for human consumption. The waste from the sale of the fennels was fed to the cows. The accumulation of nitrates in some vegetables poses a risk not only for animal health but also for human and environmental safety [28].

In vitro antifungal and anti-aflatoxigenic activities of *Cymbopogon martinii*, *Foeniculum vulgare*, and *Trachyspermum ammi* essential oils were examined and it showed that *C. martinii*, *F. vulgare*, and *T. ammi* oils as antifungals were found superior over synthetic preservative. Moreover, a concentration of 5336.297 µL/kg body weight was recorded for LC50 on mice indicating the low mammalian toxicity. In conclusion, the essential oils from *T. ammi* can be a potential source of safe natural food preservative for food commodities contamination by *Aspergillus* species [29].

The effect of planting date and plant spacing and their interactive effects on yield, yield components and growth of Fennel under irrigation was examined. Harvest index and thousand fruit weight was not significantly affected by planting date. Increase plant spacing to 30 cm led to more than 15% increase in fruit and biological yield. The early planting date with 30 cm plant spacing resulted in higher fruit and biological yield [30].

The chemical composition and biological activity of the essential oils obtained from the leaves of two different cultivars of Florence fennel cropped under three different fertilization treatments was examined. The antimicrobial activity expressed by assays on the examined oils indicates an appreciable effect, generally higher on Gram-positive bacteria. The various samples of Florence fennel analyzed did not show any results with FRAP test. The DPPH test showed a weak capacity of the samples to catch the free radicals from the solution, attributable to their content in Anatole.[16]

Concentrations of nanosized TiO₂ at 0, 5, 20, 40, 60 and 80 mg L⁻¹ with bulk TiO₂ for phytotoxic and stimulatory effects on fennel seed germination and early growth stage was determined. It was found that there was a considerable response by fennel seed to nanosized TiO₂ presenting the possibility of a new approach to overcome problems with seed germination in some plant species, particularly medicinal plants [31].

pharmacological properties, toxicity and adverse events, and drug interactions of vulgare was reviewed and brings conclusive results about the use of this plant in men, women and during pregnancy .the result showed that It is better not to use F. vulgare during pregnancy due to its estrogenic activity. Because of inhibition of cytochrome, the pharmacokinetic parameters of drugs mainly metabolized by this isozyme may be affected by F. vulgare. In addition, a significant interaction between ciprofloxacin and F. vulgare was demonstrated. The aim of current paper is to review [1].

Antimycobacterial effect of *Foeniculum vulgare* (Apiaceae) were tested against one sensitive and three MDR strains of *Mycobacterium tuberculosis* using the Alamar Blue microassay. Compounds that showed some degree of antimycobacterial activity against all strains tested were the following: linoleic acid, oleic acid, 1, 3-benzenediol, undecanal, and 2,4-undecadienal, the last being the most active compound[32].

In an animal study, the efficacy of fennel seed methanolic extract (FSME) for its antioxidant, cytotoxic, and antitumor activities was evaluated. It was found that FSME may reduce oxidative stress and protect mouse cells from damage caused by reactive oxygen species. In addition, it could be used as a safe, effective, and easily accessible source of natural antioxidants to improve the oxidative stability of fatty foods during storage. FSME also exhibited an antitumor effect by modulating lipid peroxidation and augmenting the antioxidant defense system in EAC-bearing mice with or without exposure to radiation [33].

Essential oil of F. vulgare showed significant antimicrobial activities against some of microorganisms as compared to the methanolic and ethanolic extracts. The diameters of growth inhibition zone ranged from 14 to 31 mm (including the diameter of the disc 6 mm) with the highest inhibition zone values observed against *Bacillus megaterium* (31 mm) and *Bacillus subtilis* (29 mm).

antimicrobial effect of the methanol, ethanol, diethyl ether, and hexane extracts of seed of F. vulgare was investigated against two species of Gram negative bacteria (*Escherichia coli* and *Salmonella typhi*), two species of Gram positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*), one species of yeast (*Candida albicans*), and one species of mold (*Aspergillus flavus*). The results from the disc diffusion method, followed by measurement of minimum inhibitory concentration (MIC), indicated that *Bacillus cereus* and *Aspergillus flavus* were the most sensitive microorganisms tested, showing the largest inhibition zones and the lowest MIC values. Least activity was exhibited against *Escherichia coli*, with the smallest inhibition zones and the highest MIC value.

Antioxidant activity

The effect of fennel and sage extracts and the influence of the egg yolk source (fresh or pasteurized) on the success of freezing boar epididymal spermatozoa was evaluated. Results showed that the interaction between fennel and sage antioxidants with fresh egg yolk significantly improved post thaw sperm quality and protected boar epididymal spermatozoa from cryopreservation damage as a result of oxidative stress [34].

the anti-inflammatory and antioxidant effects of coumarins isolated from *F. vulgare* in lipopolysaccharide (LPS)-stimulated macrophages and 12-O-tetradecanoylphorbol-13-acetate (TPA)-stimulated mice was assessed. It was demonstrated that although four coumarins isolated from the fruits of *F. vulgare* provide effective anti-inflammatory and antioxidant activities, imperatorin is most potent [34].

The effect of hydro-alcoholic extract of fennel on some hematological indices in male rats was evaluated and it showed that fennel increased red and white blood cells probably due to the presence of polyphenols and antioxidant activity of fennel and reduced negative effects of free radicals on blood cells [35].

In an animal study, the anti-inflammatory effects of fennel in model of lipopolysaccharide (LPS)-induced acute lung injury was investigated. Fennel significantly and dose-dependently reduced LDH activity and immune cell numbers in LPS treated mice. Fennel effectively blocked the inflammatory processes induced by LPS, by regulating pro-inflammatory cytokine production, transcription factors, and NO [36].

The antioxidant and hepatoprotective activities of the methanolic extract as well as the ethyl acetate fractions of the wild fennel was investigated. In addition, quantification of the total phenolic content in the 80% methanol extract of fennel wild and cultivated herbs is measured. The results obtained in this study indicated that the fennel (*F. vulgare*) herb is a potential source of natural antioxidant. Two phenolic compounds, i.e. 3,4-dihydroxy-phenethylalcohol-6-O-caffeoyl- β -D-glucopyranoside (A) and 3',8'-binaringenin (B) were isolated from the fennel wild herb for the first time [37].

In an animal study, therapeutic and antioxidant effects of Uygur Herb *Foeniculum Vulgare* Mill (FVM) in hepatic fibrosis rats was evaluated and it was found that *Foeniculum Vulgare* Mill declines liver inflammation response, and prevent the hepatic fibrosis progression, this may be due to its effects of antioxidative results [38].

The effect of different concentrations of fennel added to the freezing extender on boar semen quality and lipid peroxidation after thawing were examined. It was indicated that higher concentrations of fennel produced significant improvement in total motility. Thus, it works in a dose-dependent manner to increase sperm viability. In contrast, the addition of fennel had no effect on acrosome integrity or hypoosmotic swelling test (HOST) compared with the control [39].

CONCLUSION

In this study, firstly traditional usages of fennel and secondly its antioxidant, antifungal, antimicrobial properties were reviewed in detail. *Foeniculum vulgare* has been used as an ethnic remedy for the cure of numerous infectious disorders of bacterial, fungal, viral, and mycobacterial origin. Several studies have been carried out in the past validating its antimicrobial, antimycobacterial, and antiviral potential.

REFERENCES

- [1] Rahimi R, Ardekani MR. *Chin J Integr Med.* **2013**;19(1):73-9.
- [2] Alexandrovich I, Rakovitskaya O, Kolmo E, Sidorova T, Shushunov S. *Altern Ther Health Med.* **2003**;9(4):58-61.
- [3] Yaralizadeh M, Abedi P, Najar S, Namjoyan F, Saki A. *Maturitas.* **2016**;84:75-80.
- [4] Akha O, Rabiei K, Kashi Z, Bahar A, Zaeif-Khorasani E, Kosaryan M, et al. *Caspian J Intern Med.* **2014**;5(1):26-9.
- [5] Javidnia K, Dastgheib L, Mohammadi Samani S, Nasiri. *Phytomedicine.* **2003**;10(6-7):455-8.
- [6] Badgajar SB, Patel VV, Bandivdekar AH. *Biomed Res Int.* **2014**;2014:842674.
- [7] Malini T, Vanithakumari G, Megala N, Anusya S, Devi K, Elango V. *Indian J Physiol Pharmacol.* **1985**;29(1):21-6.
- [8] Zhu M, Wong PY, Li RC. *J Pharm Pharmacol.* **1999**;51(12):1391-6.
- [9] Cruz GS, Wanderley-Teixeira V, Oliveira JV, Lopes FS, Barbosa DR, Breda MO, et al. *J Econ Entomol.* **2016**.
- [10] Chatterjee S, Zahid MS, Awasthi SP, Chowdhury N, Asakura M, Hinenoya A, et al. *Jpn J Infect Dis.* **2016**.
- [11] Stefanello ME, Pascoal AC, Salvador MJ. *Chem Biodivers.* **2011**;8(1):73-94.
- [12] Rahali FZ, Lamine M, Gargouri M, Rebey IB, Hammami M, Sellami IH. *Phytochemistry.* **2016**;124:58-67.
- [13] Najdoska-Bogdanov M, Bogdanov JB, Stefova M. *Nat Prod Commun.* **2015**;10(9):1619-26.

- [14] Mota AS, Martins MR, Arantes S, Lopes VR, Bettencourt E, Pombal S, et al. *Nat Prod Commun.* **2015**;10(4):673-6.
- [15] Nguyen S, Huang H, Foster BC, Tam TW, Xing T, Smith ML, et al. *J Pharm Pharm Sci.* **2014**;17(2):254-65.
- [16] Senatore F, Oliviero F, Scandolera E, Tagliatalata-Scafati O, Roscigno G, Zaccardelli M, et al. *Fitoterapia.* **2013**;90:214-9.
- [17] Miguel MG, Cruz C, Faleiro L, Simoes MT, Figueiredo AC, Barroso JG, et al. *Nat Prod Commun.* **2010**;5(2):319-28.
- [18] Qiu J, Li H, Su H, Dong J, Luo M, Wang J, et al. *World J Microbiol Biotechnol.* **2012**;28(4):1399-405.
- [19] Stojanovic-Radic Z, Comic L, Radulovic N, Blagojevic P, Mihajilov-Krstev T, Rajkovic J. *Pharmaceutical biol.* **2012**;50(8):933-40.
- [20] Lixandru BE, Dracea NO, Dragomirescu CC, Dragulescu EC, Coldea IL, Anton L, et al. *Roum Arch Microbiol Immunol.* **2010**;69(4):224-30.
- [21] Gutierrez J, Rodriguez G, Barry-Ryan C, Bourke P. *J Food Prot.* **2008**;71(9):1846-54.
- [22] Lashkarizadeh MR, Asgaripour K, Saedi Dezaki E, Fasihi Harandi M. *Iran J Parasitol.* **2015**;10(2):206-12.
- [23] Zeng H, Chen X, Liang J. *J Med Microbiol.* **2015**;64(Pt 1):93-103.
- [24] Kedia A, Dwivedy AK, Pandey AK, Kumar RR, Regmi P, Dubey NK. *J Appl Microbiol.* **2015**;119(4):991-8.
- [25] Wakabayashi KA, de Melo NI, Aguiar DP, de Oliveira PF, Groppo M, da Silva Filho AA, et al. *Chem Biodivers.* **2015**;12(7):1105-14.
- [26] Piras A, Falconieri D, Porcedda S, Marongiu B, Goncalves MJ, Cavaleiro C, et al. *Nat Prod Res.* **2014**;28(21):1819-25.
- [27] Dragan L, Gyorke A, Ferreira JF, Pop IA, Dunca I, Dragan M, et al. *Acta Vet Scand.* **2014**;56:22.
- [28] Costagliola A, Roperto F, Benedetto D, Anastasio A, Marrone R, Perillo A, et al. *Environ Sci Pollut Res Int.* **2014**;21(9):6252-7.
- [29] Gameda N, Woldeamanuel Y, Asrat D, Debella A. *Int J Food Sci.* **2014**;2014:874135.
- [30] Al-Dalain SA, Abdel-Ghani AH, Al-Dala'een JA, Thalaen HA. *Pak J Biol Sci.* **2012**;15(23):1126-32.
- [31] Feizi H, Kamali M, Jafari L, Rezvani Moghaddam P. *Chemosphere.* **2013**;91(4):506-11.
- [32] Esquivel-Ferrino PC, Favela-Hernandez JM, Garza-Gonzalez E, Waksman N, Rios MY, del Rayo Camacho-Corona M. *Molecules.* **2012**;17(7):8471-82.
- [33] Mohamad RH, El-Bastawesy AM, Abdel-Monem MG, Noor AM, Al-Mehdar HA, Sharawy SM, et al. *J Med Food.* **2011**;14(9):986-1001.
- [34] Monton A, Gil L, Malo C, Olaciregui M, Gonzalez N, de Blas I. *Cryo letters.* **2015**;36(2):83-90.
- [35] Mansouri E, Kooti W, Bazvand M, Ghasemi Boroon M, Amirzargar A, Afrisham R, et al. *Jundishapur J Nat Pharm Prod.* **2015**;10(1):e18396.
- [36] Lee HS, Kang P, Kim KY, Seol GH. *Foeniculum vulgare* Mill. *Korean J Physiol Pharmacol.* **2015**;19(2):183-9.
- [37] Ghanem MT, Radwan HM, Mahdy el SM, Elkholy YM, Hassanein HD, Shahat AA. *Pharmacognosy res.* **2012**;4(2):104-8.
- [38] Zhang ZG, Lu XB, Xiao L, Tang L, Zhang LJ, Zhang T, et al. *Zhonghua Gan Zang Bing Za Zhi.* **2012**;20(3):221-6.
- [39] Malo C, Gil L, Cano R, Gonzalez N, Luno V. *Andrologia.* **2012**;44 Suppl 1:710-5.