FORMULATION OF HERBAL GEL FROM *Pimpenella anisum* PLANT SEEDS EXTRACT AND EVALUATE THE WOUND HEALING ACTIVITY

A THESISSUBMITTED TO

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Inthe partial fulfillment of the requirement for the award of the degree of Master of Pharmacy (M.Pharm) in Pharmaceutics

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I wish him all success in life.

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DECLARATION

I hereby declare that the thesis entitled **"FORMULATION OF HERBAL GEL FROM *Pimpenella anisum* PLANT SEEDS EXTRACT AND EVALUATE THE WOUND HEALING ACTIVITY"**is a bonafide and genuine research work carried out by me under the supervision of **Dr. Tapash Chakraborty, Assistant professor, Department of Pharmaceutics, Girijananda Chowdhury Institute of Pharmaceutical Science,** Azara, Guwahati-17.The work embodied in this thesis is original and has not been submitted in part or full for the award of degree, diploma, associateship or fellowship of any other university or institution.

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To whom it may concern

This is to certify that Naimul Hasan bearing Roll no.: **190520011010**, Regd. No.:- **387905219** is a 4th semester bonafide student of M.Pharm course in this Institute for the academic session 2019-2021.

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Affectionately

Dedicated

To

My BelovedParents

ABSTRACT

Pimpinellaanisum (anise), belonging to Umbelliferae family, is an aromatic plant which has been used in Iranian traditional medicine (especially its fruits) as carminative, aromatic, disinfectant, and galactagogue. So far, different studies were performed on aniseeds and various properties such as antimicrobial, antifungal, antiviral, antioxidant, muscle relaxant, analgesic and anticonvulsant activity as well as different effects on gastrointestinal system have been reported of aniseeds. It can also reduce morphine dependence and has beneficial effects on dysmenorrhea and menopausal hot flashes in women. In diabetic patients, aniseeds showed hypoglycemic and hypolipidemic effect and reduce lipid peroxidation. The most important compounds of aniseeds essential oil were trans-anetole, estragole, γ-hymachalen, para anis aldehyde and methyl cavicol. Anise oil showed excellent antioxidant activity, in comparison with the reference compounds. Anise oil has the potential to be used as a therapeutic, antimicrobial, and antioxidant agent. Increasing microbial resistance to chemical antibiotics and their probabilistic side effects cause popularity of medicinal plants, so there is an instantaneous and steady need for novel antimicrobial compounds from plants.

This experiment was conducted in order to compare the effects of *Pimpinella anisum*Extracts on healing of wounds in rats. Eighteen adult, male Wistar-albino rats were divided into three groups of six rats each. Group A received *Pimpinella anisum*extract (prepared in the lab) and Group B received a *Pimpinella anisum* oil (bought from market) and Group C, as the control group, didn't receive any treatment. The results show that *Pimpinella anisum*extract (prepared in lab) served to accelerate the wound healingprocess and specifically increased epithelialization in treatment groups compared to the other groups. Thus, thisstudy demonstrates that*Pimpinella anisum* may be effective in stimulating the enclosure of wounds. Results fromour investigation suggest a positive effect of *Pimpinella anisum*on aseptic surgical skin wound healing.

KEYWORDS:*Pimpinella anisum*, chemical composition, pharmacological properties, antimicrobial effect, anise, essential oil, wound healing

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Thanks to one an all . . . . .

Naimul Hasan

CHAPTER 1
INTRODUCTION

1.INTRODUCTION:

1.1 INTRODUCTION:

Anise, also called aniseed or rarely anix, is a flowering plant in the family Apiaceae native to the Eastern Mediterranean region and South-west Asia. The flavor and aroma of its seedshave similarities with some other species, such as star anise, fennel and liquorice.

Scientific name: *Pimpinella anisum.*

Higher classification: Pimpinella

Family: Apiaceae

Order: Apiales

Kingdom: Plantae

Many studies have been described that aniseeds extracts demonstrated some pharmacological effects such as fungicidal, antibacterial and antioxidant, anti -inflammatory antispasmodic and relaxant effects on rat anococcygeus smooth muscle, anesthetic and hypothermic activities.

Moreover, recent studies reported that P. anisum extract has great potential as a skin whitening agent. [1]

Fig 1: *Pimpinella anisum* plant Fig 2: *Pimpinella anisum*seed

Pimpinella anisum L., is a plant belonging to the Umbelliferae family, hails from the same family as carrots, celery and parsley; which is one of the oldest medicinal plants. It is an annual grassy herb with 30–50cm high, white flowers, small white fruits and small green to yellow seeds, which grows in the Eastern Mediterranean Region, West Asia, the Middle East, Mexico, Egypt, and Spain.

Pimpinella anisum is primarily grown for its fruits (aniseeds) that harvested in August and September. Aniseedscontain1.5–5%essentialoilandusedasfavoring, digestive, carminative, and relief of gastrointestinal spasms.Consumption of aniseed in lactating women increases milk and also reliefs their infants from gastrointestinal problems. Anise has a distinct, licorice-like taste. Hence,in the food industry, anise is used as flavoring and aromaticagentforfishproducts,ice-cream,sweets,andgums. So, the objective of this study was collecting all published articles aboutthe chemicalconstituents,antimicrobial properties and wound healing process of aniseeds with literature search of Googlescholar, PubMed, Sciencedirect, Scopus, and SID database from 1970 up to 2011.Anise seeds are used as analgesic in migraine and also as carminative, aromatic, disinfectant, and diuretic in traditional medicine. Aniseed has warm and dry nature and can increase milk production, menstruation, urine, and sweat secretion and also making good complexion. It is also eff ective in polishing of teeth. In some traditional texts, anise is mentioned for melancholy, nightmare, and also in treatment of epilepsy and seizure. It is also known for its powerful health-promoting properties and acts as a natural remedy for a wide variety of ailments. Anise seed is low in calories but contains a good amount of several important minerals, including iron, manganese and calcium.

Umbelliferae (Apiaceae) is the most temperate herbal plants, with umbellate inflorescences, consisting of more than 3,000 species and over 300 genera. There are pairs of fruits or seeds. Plants, as members of this family, are commonly utilized in the food industry and medicine. Many plants in this family, such as, parsley, celery, and carrots, are popular vegetable crops. In contrast, others, such as cumin, anise, and fennel, are known for their therapeutic and fragrant characteristics. Anise [Pimpinella anisum L. (P. anisum L.)], is an annual grassy plant with a white flower, which is grown in warm regions around the globe.

About 1.5 to 3.5% of the mass of anise seed are essential oil (volatile oil), consisting of mainly cis- and trans-anethole. The key ingredient of anise (trans-anethole) is used, in the

synthesis of different pharmaceutical products, as a substrate. Fatty oils (non-volatile) are derived from the Umbelliferae family. The potency of the seed oils obtained from Apiaceae fatty oils depends on the chemical structure of the fatty acid and the composition of minor constituents in the oil.The main fatty acid constituent in oils is petroselinic acid, which is common in the Apiaceae seeds, compared to oleic acid. Aside from oleic acid, a small residue of one moreisomer, cis-vaccenic acid is also noticed.

The volatile oil is applied as a carminative, in cough medicine, particularly for pediatric applications. The significant phenyl-propane, such as, estragole and trans-anethole, has a stabilization effect on the autonomic nervous system. In traditional medicine, anise was applied as a tranquilizer, a diuretic, and appetizer. It was documented that it has various healing properties in various cases, such as, gynecology, respiratory, neurology, and digestive illnesses. Several recent studies showed that oils with plant origin exhibit good antioxidant activity against several microorganisms. Nowadays, plant oils could be used as an alternative to synthetic packing materials that are harmful to human health. Moreover, spices are also considered as a source of antioxidants due to the large contents of bioactive compounds, such as, phenolic compounds, flavonoids, tannins, phenolic diterpenes, sulfur-containing compounds, alkaloids, and vitamins. Also, polyunsaturated fatty acid (PUFA) compounds can be obtained from some spices, such as the seeds of Apiaceae. Thus, this study aimed to examine the antimicrobial and antioxidant properties and chemical composition of essential and fixed oils in the anise seeds. [2]

1.2*Pimpinellaanisum* in Traditional Medicine of Iran:

Anise seeds are used as analgesic in migraine and alsoas carminative, aromatic, disinfectant, and diuretic in traditionalmedicine. Aniseed has warm and dry natureand can increase milk production, menstruation, urine, andsweat secretion and also making good complexion. It is alsoeffective in polishing of teeth. In some traditional texts,anise is mentioned for melancholy, nightmare, and also intreatment of epilepsy and seizure. [4]

1.3Uses of Aniseed:

Anise is used for upset stomach, intestinal gas, “runny nose,” and as an expectorant to

increase productive cough, as a diuretic to increase urine flow, and as an appetite stimulant.

Women use anise to increase milk flow when nursing, start menstruation, treat menstrual discomfort or pain, ease childbirth, and increase sex drive.

Men use anise to treat symptoms of “male menopause.” Other uses include treatment of seizures, nicotine dependence, trouble sleeping (insomnia), asthma, and constipation.

Anethole: Anethole also known as anise camphor in an organic compound that is widely used as a flavoring substance.It is derivative of phenylpropene.Anethole is only slightly soluble in water but exhibits high solubility in ethanol. [2]

1.4 PHARMACOLOGICAL EFFECTS OF ANISE SEED:

1.4.1 Antibacterial and Antifungal Effects:

The antibacterialactivities of the aqueous, 50% (v/v) methanol, acetone andpetroleum ether extracts of Pimpinella anisum L. fruitswere tested against 4 pathogenic bacteria (Staphylococcusaureus, Streptococcus pyogenes, Escherchia coli, and Klebsiellapneumoniae) by disc diffusion method. The results showedthat only aqueous and methanol extracts exhibited fairantibacterial activity against all of the test bacteria andthe aqueous extract was found to be more effective thanmethanolic extract, whereas acetone and petroleum etherextracts cannot inhibit the growth of the pathogenic testbacteria.

Antimicrobial effects of water and ethanolic extracts ofaniseed were studied by Gulcin et al. against 10 bacterialspecies and also Candida albicans with disc diffusion method.In this study, ethanolic extract showed significant inhibitoryactivity against all tested bacteria but not effective onCandida albicans. However, the antimicrobial effect of waterextract was not detected against Gram-negative bacteria,Pseudomonas aeruginosa, and Escherichia coli, but it waseffective against Candida albicans. The alcoholic extractsof Pimpinella anisum seeds also showed antibacterial activityagainst Micrococcus luteus and Mycobacterium smegmatis.In another study, synergic antibacterial activity betweenThymus vulgaris and Pimpinella anisum essential oil andmethanol extract was evaluated against 9 pathogenic bacteria.Essential oil and methanol extract of these plants exhibitedantibacterial activity against most tested

pathogens,and the maximum effect was observed against Staphylococcusaureus, Bacillus cereus, and Proteus vulgaris. However,combination of essential oil and methanol extracts of theseplants showed an additive effect against most tested bacteriaespecially Pseudomonas aeruginosa. The antibacterialpotential of aqueous decoctions of black pepper, bay leaf,aniseed, and coriander against 176 bacterial isolates belongingto 12 different genera were detected by the mean of discdiffusion technique. The findings showed that the aqueousdecoction of black pepper was the most bacterial-toxicexhibited 75% antibacterial activity and decoction of aniseedexhibited 18.1% antibacterial activity (maximum antibacterialactivities exhibited against Micrococcus roseus). Inaddition to antibacterial activity, the essential oil of aniseedshowed significant inhibitory activity against fungi, and themost active component of it was anethol. In a studyby Kosalec et al., antifungal activities of fluid extract andessential oil of anise fruits studied on seven species of yeastsand four species ofdermatophytes by the means of diffusionmethod with cylinders and the broth dilution method. Thefindings revealed that the fluid extract of aniseed showedantimycotic activity against Candida albicans, C. parapsilosis,C. tropicalis, C. pseudotropicalis, and C. krusei, and the largestinhibition zone was observed in C. albicans. It is also showedinhibitory effect against dermatophyte species (Trichophytonrubrum, T. mentagrophytes, Microsporum canis, and M.gypseum), and the largest inhibitory zone was detected forT. mentagrophytes. Theessential oil of anise exhibited strongantifungal activity against yeasts and dermatophytes, and thelargest inhibition zone was found in C. parapsilosis (30 mm),followed by C. albicans, C. glabrata, and Geotrichum spp. Inthis study, anise essential oil exhibited stronger antifungalactivities rather than its extract against yeasts and dermatophytes. Antifungal activity of anise essential oil alsowas reported against Aternaria alternata, Aspergillus niger,and Aspergillus parasiticus, and the most effected funguswas A. parasiticus. Investigation of antifungal activity ofmethanolic extracts of aniseed against four dermatophytespecies, and one saprophyte fungus showed that the extractinhibited only dermatophyte species included Candida albicans,Trichophyton mentagrophyte, and Microsporum canisbut demonstrated no inhibitory effect on saprophyte A. niger. [7]

1.4.2 Muscle Relaxant Effect:

The relaxant effect of *Pimpinellaanisum* on isolated guinea pig tracheal chains and its possiblemechanism was studied by Boskabady and Ramazani-Assari.In this research, the

bronchodilatory effects of aqueous andethanol extracts and essential oil of anise were examined onprecontracted isolated tracheal chains of the guinea pig by10mM methacholine in two different conditions includingnonincubated tissues (group 1) and incubated tissues with1mM propranolol and 1mM chlorpheniramine (group 2).

The results showed that aqueous and ethanol extracts,essential oil, and theophylline (1mM) showed significantrelaxant effects compared to those of controls. The relaxanteffects of aqueous and ethanol extracts were not significantlydifferent from that of theophylline, but the effect of essentialoil was significantly lower than theophylline. There wasalso no significant difference between the relaxant effectsobtained in group 1 and 2 experiments.The results alsoshowed that the relaxant effect of this plant is due toinhibitory effects on muscarinic receptors.

In another study, antispasmodic and relaxant effects ofthree hydroalcoholic extracts of the aerial parts of *Pimpinellaanisum* (ethanol: water; 40 : 60, 60 : 40, and 80 : 20) wereinvestigated on rat anococcygeus smooth muscle. Theentire three hydroalcoholic extracts attenuated acetylcholineinducedcontraction. The finding of this study described thatonly the extract contains 60% ethanol (HA60) showed concentrationdependently relaxedacetylcholine-precontractedtissues, but two other hydro alcoholic extracts cannot producerelaxation. Studying the possible mechanisms underlyingthe relaxant effect showed that this effect is mainlydependent on the activation of the NO-cGMP pathway. [3]

1.4.3 Anticonvulsant Effect:

Anticonvulsant effects of an essentialoil of the fruits of *Pimpinella anisum* were studied againstseizures induced by pentylenetetrazole (PTZ) or maximalelectroshock (MES) in male mice. This study revealed that *P.anisum* increases the threshold of clonic seizures induced byi.v. infusion of PTZ, and it can also block tonic convulsionsinduced by i.p. injection of PTZ.Moreover, *P. anisum* possessesanticonvulsant activity against tonic seizures inducedbyMES [34]. In another study byHeidari and Ayeli, the effectof methyl alcoholic extract of anise on picrotoxin-inducedseizure in mice was studied. The results showed that aniseextract caused an increased delay in the onset of seizure inthe mice which had been pretreated with different doses ofthe extract, and the most effective dose was 200 mg/kg (P <0.05). In addition, this dose delayed the time of death in mice(P <0.01) more satisfactory

than phenobarbital (40mg/kg)on delaying death time [35]. Results of the other study forinvestigating the effect of the aqueous extracts of leaves andstems of some Arab medicinal plants including *Pimpinellaanisum* on the picrotoxin-induced seizures in mice revealedthat the extracts of *Rosmarinus officinalis, Pimpinella anisum,Matricaria chamomilla,* and *Artemisia vulgaris* were foundto delay the onset of picrotoxin-induced seizures and todecrease the mortality rate.

In year 2008, Janahmadi et al. studied the cellularmechanisms of antiseizure effect of anise fruits. In this study,they determined whether the fruit essential oil of anise affectthe bioelectrical activity of snail neurons in control conditionor after pentylenetetrazol-(PTZ-) induced epileptic activity.The results indicate that the candidate cellular mechanismsprobably underlying the hyperexcitability produced by aniseoil include enhancement of Ca2+ channels activity or inhibitionof voltage and/or Ca2+ dependent K+ channels activityunderlying post-hyperpolarization potential. [3]

1.4.4 Anti-depressant effect:

Depression is a common yet debilitating condition that affects up to 25% of women and 12% of men around the world. Interestingly, some researches found that anise seed may help treat depression. Human and animal studies showed that anise seed extract exhibited powerful antidepressant properties in mice and was effective as a common prescription medication used to treat depression. Anise seed may help reduce symptoms of depression and may be as effective as some types of anti-depressants. [3]

1.4.5 Effect on gastric ulcer:

Stomach ulcers, also called gastric ulcers, are a painful sore that forms in the lining of your stomach, causing symptoms like indigestion, nausea and a burning sensation in your chest. Though traditional treatment typically involves the use of medications to decrease the production of stomach acid, preliminary research suggests that anise seed could help prevent stomach ulcers and reduce symptoms. For instance, one animal study noted that anise reduced stomach acid secretion, helping prevent the formation of stomach ulcers and protecting cells against damage. However, research on anise seed's effects on stomach ulcers

is still very limited. Though research is extremely limited, anise seed reduced stomach acid secretion and protected against stomach ulcer formation in one animal study.

For studying the effect ofaqueous suspension of anise against gastric ulcers in rat,acute gastric ulceration was produced by various noxiouschemicals and indomethacin. The results showed that anisesignificantly inhibited gastric mucosal damage induced bynecrotizing agents and indomethacin. The antiulcer effectwas further confirmed histologically. [3]

1.4.6 Palliation of Nausea:

In a case study, an aromatherapytreatment containing *Pimpinella anisum*, *Foeniculum vulgare,*var. *dulce*, *Anthemis nobilis,* and *Mentha piperita* was examinedin twenty-five patients suffering from the symptoms ofnausea in a hospice and palliative care program. A majorityof patients who used the aromatherapy treatments reportedrelief. However, all patients in this study were also using avariety of other treatments for their symptoms. [3]

1.4.7 Effect on Constipation:

The laxative efficacy of aphytotherapic compound containing *Pimpinella anisum* L.,*Foeniculum vulgare* Miller, *Sambucus nigra* L., and *Cassiaaugustifolia* was studied in a randomized clinical trialincluded 20 patients presenting with chronic constipationaccording to the criteria of the American Association ofGastroenterology. The primary endpoint was colonic transittime (CTT), measured radiologically. Secondary endpointsincluded number of evacuations per day, perception of bowelfunction, adverse effects, and quality of life. The results ofthe study revealed significant laxative effects of phytotherapiccompound when compared with placebo. This effect wasdemonstrated by a decrease in colonic transit time as wellas an increase in the number of daily evacuations. Althoughquality of life did not show significant differences among thestudy periods and no significant differences were observed interms of adverse effects throughout the study period, so thiscompound can be a safe alternative option for the treatmentof constipation. [3]

1.4.8 Analgesic and Anti-Inflammatory Effect:

The effects of essentialoil of *Pimpinella anisum* on the expression and acquisition ofconditioned place preference (CPP) induced by morphine inmice were studied. The findings showed that subcutaneousinjections of morphine (2–5 mg/kg) produced place preferencein a dose-dependent manner and injection of essentialoil of *P. anisum* may induce conditioned place aversion inmice, that is, the essential oil has some aversive effects asinvestigated by place conditioning paradigm. In addition,this oil has also a GABA ergic effect.

In a study by Tas, essential oil of Pimpinella anisum showed significant analgesic effect similar to morphine and aspirin. Also, fixed oil of anise was investigated for anti-inflammatory and analgesic activity in mice. The finding showed that the fixed oil of anise has anti-inflammatory effect as strong as indomethacin and it showed analgesic effect comparable to that of 100mg/kg aspirin and 10mg/kg morphine at 30th min. In many cases, inflammation is considered a normal response by your immune system to protect against injuries and infection. However, high levels of long-term inflammation are linked to chronic conditions, such as heart disease, cancer and diabetes. Animal and test-tube studies suggest that anise seed may reduce inflammation to promote better health and prevent disease. For example, one study in mice showed that anise seed oil reduced swelling and pain. Other research indicates that anise seed is high in antioxidants, which can reduce inflammation and prevent disease-causing oxidative damage. Animal and test-tube studies have found that anise seed is high in antioxidants and can reduce inflammation to help prevent chronic disease. [8]

1.4.9 Effect on Morphine Dependence:

The effects of essential oil of Pimpinella anisum on the expression and acquisition of conditioned place preference (CPP) induced by morphine in mice were studied. The findings showed that subcutaneous injections of morphine (2–5mg/kg) produced place preference in a dose-dependent manner and injection of essential oil of P. anisum may induce conditioned place aversion in mice, that is, the essential oil has some aversive effects as investigated by place conditioning paradigm. In addition, this oil has also a GABA ergic effect.

Opioid dependence is a major health problem worldwide that often requires long-term treatment and care. Morphine is one of the major opioid analgesics mainly used for alleviating pain and chronic diarrhea. But, its repeated use leads to physical dependence and

tolerance. Furthermore, after abrupt cessation of morphine or an administration of opioid antagonist (e.g. naloxone), withdrawal signs will appear. Today, pharmacotherapies for opioid addiction include administration of opioid agonists (e.g. methadone), partial agonists (e.g. buprenorphine), opioid antagonists (e.g. naltrexone), and alpha-2-adrenergic agonists (e.g. clonidine), which are targeted toward either detoxification or long-term agonistmaintenance. Several medicinal herbs including Cymbopogon citratus, Avena sativa, Carthamus tinctorius, Rosa damascena, Otostegia percica, Carum copticum, and Rodmarinus officinalis have been investigated for the treatment of some aspects of morphine dependence in animal models of drug dependence.

Pimpinella anisum L. form Apiaceae (Umbelliferae) family grows in different parts of Mediterranean countries and Iran. In Iranian traditional medicine, P. anisum is used for treating gastrointestinal and nervous system disorders. Previous phytochemical studies revealed that the prominent component of P. anisum is 1.5%–6.0% essential oil consisting primarily of trans-anethole. Other studies demonstrated the presence of eugenol,trans-anethole, methyl chavicol, anis aldehyde, estragole,coumarins, scopoletin, umbelliferone, estrols, terpenehydrocarbons, polyenes, and polyacetylenes as the othermajor components of the essential oil. P. anisumL. extract and essential oil showed different biologicaland pharmacological activities including antispasmodic,analgesic, anticonvulsant, antibacterial and laxativeeffects, as well as alleviating morphine dependenceproperty. Furthermore, our recent study revealedthe antidepressant - like effects of P. anisum in animalmodels of depression. Hence, the aim of the presentstudy was to investigate the effect of P. anisum ethanolicextract on morphine physical dependence in mice. [11]

1.4.10 Effect on Menopausal Hot Flashes:

In a double blind clinical trial, the effect of anise extract on menopausal hot flashes in 72 postmenopausal women was examined. In this study, consumption of 3 capsules of anise extract (each capsule contains 100mg of extract) for 4 weeks leads to significant reduction in hotflash frequency and intensity and in postmenopausal women. [3]

1.4.11 Effect on Dysmenorrhea:

In a study by Khoda Karami et al., the effectiveness of a herbal capsule containing dried extracts of celery, saffron, and anise was compared with mefenamic acid capsule in 180 female students (with age 17–28) with primary dysmenorrhea. The results showed significant reduction in pain intensity in both herbal and mephnamic acid group compare to placebo group. Also, the results revealed that the effectiveness of herbal capsule was better than mephnamic acid in pain relief and can be a suitable alternative in primary dysmenorrhea. [3]

1.4.12 Antioxidant Activity:

In a study by Gulcin et al., theantioxidant properties of water and ethanolic extracts ofaniseeds were evaluated using different antioxidant tests, andantioxidant activities were compared with synthetic antioxidantssuch as butylated hydroxyanisole (BHA), butylatedhydroxytoluene (BHT), and α-tocopherol. Both extracts ofaniseeds showed strong antioxidant activity, reducing power,DPPH radical and superoxide anion scavenging, hydrogenperoxide scavenging, and metal chelating activities comparedto BHA, BHT, and α-tocopherol, and water extract exhibitedgreater antioxidant capacity than ethanolic extract.Also, investigation of in vitro and in vivo antioxidantpotential of aniseeds showed that ethanolic extract of aniseeddisplayed scavenging activity against nitric oxide, superoxideand 1,1-diphenyl, 2-picryl hydrazyl (DPPH) radicals andreducing power in a concentration-dependent manner.The antioxidant potential of essential oil and oleoresinsfrom anise seeds was studied. The antioxidant activitieswere assessed by inhibition of linoleic acid peroxidation,1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging,Fe3+ reducing power, and various lipid peroxidation assays.The findings showed that the anise oil and its methanololeoresin showed highest antioxidant activity, even higherthan BHA and BHT. However, the antioxidant activities ofother oleoresins were somewhat lower.Screening of antioxidant properties of some Umbelliferaefruits from Iran (including *Pimpinella anisum*) by theDPPH (2,2_-diphenyl-1-picrylhydrazyl) radical scavengingtest showed that all the extracts exhibited antioxidantcapability, and *P. anisum* extract (IC50 = 109.80) exhibitedthe strongest activity and the ethyl acetate fraction of theextract exhibited the highest activity and flavonoid content.A positive correlation was found between the antioxidantpotency and flavonoid content of the fractions. Inanother study the antioxidant activity of water and alcoholextracts of chamomile flowers, anise seeds, and dill seedswas investigated. The extracts showed marked antioxidantactivity in both linoleic acid and liposome model

systems,although the water extracts showed higher antioxidant activitythan the corresponding alcohol extracts. Also, antioxidantactivity of aniseeds was lower than chamomile flowers anddill seeds.The antioxidant properties of thirteen herbal teas includinganise tea was assessed in vitro. The finding indicatethat anise tea showed weak antioxidant activity in Troloxequivalentantioxidant capacity (TEAC assay) and moderateeffect in hypochlorite quenching assay and peroxynitritequenching assay rather than other herbal teas. [7]

1.4.13 Insecticidal Effects:

Plant essential oils from 40 plantspecies including *Pimpinella anisum* were tested for theirinsecticidal activities against larvae of *Lycoriella ingenua*using a fumigation bioassay. Some of the essential oil includinganise and garlic essential oil showed good insecticidalactivity against the larvae. Among the identified compoundin effective essential oils, allyl isothiocyanate was the mosttoxic against larvae of *L. ingenua* followed by *trans*-anethole,diallyl disulfide, and *p*-anisaldehyde.Prajapati et al. showed that the essential oils of *Juniperusmacropoda* and *Pimpinella anisum* were highly effectiveas larvicidal and ovicidal against three mosquito species. Also, the anise essential oil showed repellency againstmosquito *Culex pipiens*. In another study, the exposureto vapours of essential oils from anise and cumin resultedin 100% mortality of the eggs of two stored-product insects(the confused flour beetle, *Tribolium confusum*, and theMediterranean flour moth, *Ephestia kuehniella*).The acaricidal activity of *p*-anisaldehyde derived fromanise seed oil and commercially available componentsof anise seed oil were studied against the house dustmites, *Dermatophagoides farina*, and *D. pteronyssinus*. Theresults showed that the compound most toxic to thesedermatophagoides were *p*-anisaldehyde followed by benzylbenzoate and, therefore, *p*-anisaldehyde may be useful as alead compound for the selective control of house dust mites. [3]

1.4.14 Antiviral Effects:

The effects of the essential oil of*Foeniculum vulgar* and *Pimpinella anisum* were examinedagainst PVX (potato virus), TMV (tobacco mosaic virus) andTRSV (tobacco ring spot virus), on the hypersensitive host*Chenopodium amaranticolor*. The essential oil is at

3000 ppmcompletely inhibited PVX, TMV, and TRSV.Three lignin-carbohydrate-protein complexes (LC1, LC2,and LC3) with antiviral and immunostimulating activitywere isolated from a hot water extract of seeds of *Pimpinellaanisum* by combination of anion exchange, gel filtration, andhydrophobic interaction column chromatographies. Thesecomplexes showed antiviral activities against herpes simplexvirus types 1 and 2, human cytomegalo virus, and measlesvirus. Also, the effects of these complexes in activation ofmacrophage were investigated. After RAW 264.7, murinemacrophage cells had been incubated with these compoundsfor 20 h, and the nitric oxide (NO) production was enhancedin dose-dependent manner. The effects of these compoundson mRNA and protein expression of inducible nitric oxidesynthase (iNOS) in RAW 264.7 cells showed that theyinduced mRNA iNOSexpression in a time-dependentmanner. Furthermore, they induced expression of both IL-1β and IL-10 mRNAs. These results suggest that the lignin carbohydrate-protein complexes from *P. anisum* possessedpotency as functional food ingredients against infectiousdiseases. [7]

1.4.15 Effects on Diabetic Patients:

The antidiabetic, hypolipidemic, and antioxidant activities of aniseeds and coriander seeds were compared in type 2 diabetic patients. The seed powders (5g/day) were administered to two groups of type 2 diabetes patients for 60 days. The results indicated 11% rise of fasting blood glucose in control and 36% decrease in aniseed-treated, and 13% decrease in coriander-treated type 2 diabetics. Also, significant decrease in serum cholesterol and triglycerides in aniseed treated and coriander seed-treated patient was observed. Protein oxidation in serum and lipid peroxidation in erythrocytes and plasma was decreased in both treated groups as compared with the initial values. Both the groups showed rise in serum β-carotene and vitamin A levels which could have resulted in a significant decrease in lipid peroxidation in RBC and plasma, and also rise in vitamin C was detected in both anise and coriander group. So, both the seeds have antidiabetic, hypolipidemic and antioxidant effects in diabetic patient. [3]

1.4.16 Effect on Glucose Absorption:

The effect of aniseed oilon the absorption of glucose from the jejunum and waterfrom the

colon and kidney tubules and also its mechanism ofaction was studied by Kreydiyyeh et al. The findings showedthat aniseed oil increased significantly glucose absorptionin the rat jejunum, because the oil enhanced the activityof the Na+-K+ ATPase which increases the sodium gradientthat gears the mucosal glucose transport. However, addingof anise oil at a 0.05% concentration did not have anysignificant effect on colonic water absorption, and it seemsthus that the ATPase in the colon is resistant to the oil effect.Furthermore, adding aniseed oil to drinking water, reducedthe volume of urine produced in the rat and increased theactivity of the renal Na+-K+ ATPase even at extremely lowconcentrations. [3]

1.4.17 Effect on Broiler Performance:

In the study by Ciftci etal., adding 400 mg/kg of anise oil to the diet of day-old broilerswas improved feed conversion ratio by approximately 6%compared to antibiotic group. So aniseoil can be consideredas a natural growth promoter for poultry. [3]

1.4.18 Effect on Milk Production:

The effects of diet supplementation with aniseed and fenugreek seeds on the performance does and kits were studied. Finding revealed that the daily milk intake of kits in anisum-fenugreek group was equivalent to that of control rabbits. Also, the 17 days body weight did not differ significantly between two groups. At 35 days of lactation, the differences between anisum fenugreek group and control groups were not significant in litter size, litter weight, kit weight and 1–35day weight, gain. In conclusion, further studies are needed to investigate the palatability and optimal level of these spices in the feed of lactating rabbits. [3]

1.4.19 Antimicrobial effect:

Test-tube studies show that anise seed and its compounds possess potent antimicrobial properties that prevent infections and block the growth of fungi and bacteria. One test-tube study demonstrated that anise seed and anise essential oil were especially effective against certain strains of fungi, including yeasts and dermatophytes, a type of fungus that can cause

skin disease. Anethole, the active ingredient in anise seed, inhibits bacterial growth as well. In one test-tube study, anethole blocked the growth of a specific strain of bacteria that causes cholera, an infection characterized by severe diarrhea and dehydration. However, further research is needed to examine how anise seed may affect the growth of fungi and bacteria in humans. Test-tube studies show that anise seed and its components may decrease the growth of certain strains of fungi and bacteria. Antimicrobial effects of water and ethanolic extracts of aniseed were studied by Gulcin et al. against 10 bacterial species and also Candida albicans with disc diffusion method. In this study, ethanolic extract showed significant inhibitory activity against all tested bacteria but not effective on Candida albicans. However, the antimicrobial effect of water extract was not detected against Gram-negative bacteria, Pseudomonas aeruginosa, and Escherichia coli, but it was effective against Candida albicans. The alcoholic extracts of Pimpinella anisum seeds also showed antibacterial activity against Micrococcus luteus and Mycobacterium smegmatis. [8]

TABLE NO. 1:PHARMACOLOGICAL EFFECTS OF ANISE SEED

System	Effect	Preparation
Organism	Anti-bacterial	Aqueous and 50% (v/v) methanol extract
		Ethanol extract
		Essential oil and methanol extract (in combination with Thymus vulgaris)
		Aqueous decoction
	Antifungal	Essential oil
		Fluid extract
		Methanol extract
	Insecticidal	Essential oil
		p-Anis aldehyde from aniseed oil
	Antiviral	Essential oil
		Lignin-carbohydrate-protein complexes from hot water extract

Muscle	Muscle relaxant of tracheal chain	Aqueous extract
		Ethanolic extract
	Antispasmodic and relaxant of anococcygeus smooth muscle	Essential oil
		Hydro alcoholic extract (60% ethanol)
Nervous system	Anticonvulsant	Essential oil
		Methanol extract of seeds
		Aqueous extract of leaves and stem extract
	Analgesic	Essential oil
		Fixed oil
	Conditioned place aversion in morphine dependence	Essential oil
Gastrointestinal	Antiulcer	Aqueous suspension
	Palliation of nausea	Essential oils of aniseeds, foeniculum vulgar, Anthemis nobilis, and Mentha piperita
	Laxative	Phytotherapic compound of anise and foeniculum vulgar, Sambucus nigra, Cassia angustifolia
	Increase glucose absorption from the jejunum	Essential oil
Renal	reduce volume of urine by increased activity of the renal Na+-K+-ATPase	Essential oil
Endocrine	Anti-diabetic	Seed powder
	Hypolipidemic	Seed powder
Immune system	Antioxidant	Ethanol extract
		Water extract
		Essential oil
		Oleoresin
		Ethyl acetate fraction of ethanol extract
		Anise tea

	Increase of β-carotene, vitamins A, C	Seed powder
Other	Reduction of menopausal hot flashes	Capsules of anise extract
	Growth promoter of day-old broilers	Essential oil
	Reduction of pain in dysmenorrhea	Herbal capsule (extracts of anise, celery, saff ron)

1.5 WOUND HEALING:

Wound healing is a normal biological process of repair, following injury to the skin and other soft tissues, which is achieved through overlapping phases such as haemostasis, inflammation, proliferation and maturation. A fibrous scar containing large amounts of collagen is an end product of this process. Well-vascularised granulation tissue that is made within the wound area is responsible for synthesis of collagen and provides strength and integrity to the dermis. Many important factors such as malnutrition, ageing, medication, radiation, and some diseases including diabetes, hypertension and obesity are associated with delayed wound healing.

Wound care can be traced back to early civilisations, when use of herbal remedies were the basis of many treatments. Some plants have a high content of tannins, flavonoids, saponins, naphthaquinone, alkaloids, and triterpenes, which can increase the quality and the rate of wound healing.

Iran has a rich flora widely distributed throughout the country, particularly in the west. In Iranian traditional medicine, herbal medicines have been the basis of treatments and cures many physiological conditions and diseases. *Pimpinella anisum* (anise), also known as ‘Badian Roomi’, from the umbelliferae family is one of the most important herbal medicines and is widely consumed in the west of Iran.

Concurrent with these findings, it seems that the *Pimpinella anisum* genus can cope with the oxidative and inflammatory changes involved in diabetic wounds, stimulate fibroblast

deposition and enhance wound closure by its compounds.

The present study was conducted to assess the dermal wound healing potential of *Pimpinella anisum* after topical application of its methanolic extract on experimentally-induced cutaneous wounds in Wistar-albino rat model. [9]

CHAPTER 2
LITERATURE REVIEW

2. LITERATURE REVIEW:

- **Gilligan NP. stated that:** The treatment contains P. anisum and oils from Foeniculum vulgare var. dulce, Anthemis nobilis, and Mentha piperita, which was examined in twenty-five patients who were suffering from the symptoms of nausea in a hospice and palliative care program. The symptoms of majority of patients who used this treatment were significantly relieved. However, all patients in this study were also using a variety of other treatments for their symptoms. [8]
- **Nahidi F, Taherpoor M, Mojab F, Alavi Majd H. stated that:** The effect of anise oil on menopause was investigated in 72 postmenopausal women. In this clinical study, consumption of 3 capsules of anise oil (each capsule contains 100 mg of P. anisum) for 4 weeks leads to significant reduction in a period, frequency and intensity of hot flush in menopausal and postmenopausal women. [10]
- **Khaled Mohamed Mohamed Koriem stated that:** P. anisum increases glucose absorption and reduces urine output in the rat. The plant oil has pharmacological (antimicrobial, hepatoprotective, anticonvulsant, anti-inflammatory, antispasmodic, bronchodilator, estrogenic, expectorant and insecticidal) effects and clinical effects on nausea, constipation, menopausal period, virus, diabetes, obesity and sedative action. It is recommended for more clinical trails to discover a new medication from the active constituents of the plant oil in the future to treat human diseases especially chronic ones. [11]
- **Chaudhry and Tariq stated that:** The aqueous decoction of P. anisum seed showed no activity against both gram positive and gram-negative bacteria. This result is different from that reported by Chaudhry and Tariq, (2006) who found that the aqueous extract possesses broad antibacterial spectrum against gram positive and gram-negative bacteria. [12]
- **Asie Shojaii and Mehri Abdollahi Fard stated that**: Pimpinella anisum (anise), belonging to Umbelliferae family, is an aromatic plant which has been used In Iranian traditionalmedicine (especially its fruits) as carminative, aromatic, disinfectant, and galactagogue. Because the wide traditional usage of*Pimpinella anisum* for treatment of

diseases, in this review published scientific reports about the composition and pharmacologicalproperties of this plant were collected with electronic literature search of Google Scholar, PubMed, ScienceDirect, Scopus, and SIDfrom 1970 to 2011. So far, different studies were performed on aniseeds and various properties such as antimicrobial, antifungal,antiviral, antioxidant, muscle relaxant, analgesic and anticonvulsant activity as well as different effects on gastrointestinal systemhave been reported of aniseeds. It can also reduce morphine dependence and has beneficial effects on dysmenorrhea andmenopausal hot flashes in women. In diabetic patients, aniseeds showed hypoglycemic and hypolipidemic effect and reduce lipid peroxidation. The most important compounds of aniseeds essential oil were trans-anetole, estragole, γ-hymachalen, paraanisaldehyde and methyl cavicol. Due to broad spectrum of pharmacological effects, and very few clinical studies of Pimpinella anisum, more clinical trials are recommended to evaluate the beneficial effects of this plant in human models and synthesis of new drugs from the active ingredients of this plant in future. [13]

- **Maryam Rezaei, Mohamad Dalvand, Forozan Hadipor, Mahbobe Pirhemat, Mohamad Alipor, Omolbanin stated that:** This experiment was conducted in order to compare the effects of Achillea millefolium and Pimpinella anisum Extracts on healing of wounds in rats. Twenty-four adults, male Wistar-albino rats were divided into four groups of six rats each. The results show that P. anisum extract served to accelerate the wound healing process and specifically increased epithelialization in treatment groups compared to the other groups. Thus, this study demonstrates that it may be effective in stimulating the enclosure of wounds. Results from our investigation suggest a positive effect of P. anisum on aseptic surgical skin wound healing. [14]

- **Muthanna J. Mohammed1, Hiyam A. Ebraheem stated that:**Essential and fixed oils of anise plant *Pimpinella anisum* growing in Iraq have been investigated regarding their chemical components, antioxidant, and antimicrobial properties. Essential oils were extracted using the Clevenger-apparatus, while fixed oil was extracted using a Soxhlet apparatus. Gas chromatography-mass spectrometry (GC-MS) was used for the analysis of the oil components. Six strains of bacteria, namely *S. epidermidis, S. aureus, E. coli, B. cereus, P. vulgaris,* and *S. typhimurium* were tested against the antimicrobial activity of each oil. Anise oil demonstrated a broad antibacterial property range, against gram-positive and gram-negative bacteria, through the inhibition zone. The antibiotic sensitivity

test was performed by disk diffusion process against the test organisms. The agar dilution method was used at five different concentrations (12.5, 25, 50, 100, and 200 mg/mL) throughout the test. The minimum inhibitory concentration (MIC) was determined for each volatile and fixed oil. The DPPH radical scavenging assay was used to test the antioxidant activities of essential and fixed oils. Anise oil showed excellent antioxidant activity, in comparison with the reference compounds. Anise oil has the potential to be used as a therapeutic, antimicrobial, and antioxidant agent.[15]

- **Azadeh Foroughi, Pouya Pournaghi, Reza Tahvilian, Mohammad Mahdi Zangeneh, Akram Zangeneh, Rohallah Moradi stated that:** Increasing microbial resistance to chemical antibiotics and their probabilistic side effects cause popularity of medicinal plants, so there is an instantaneous and steady need for novel antimicrobial compounds from plants. As we know, there is no documented proof on antibacterial effects of *Pimpinella anisum* (PA) hydroalcoholic extract in west of Iran. Gas chromatography mass spectrometry was done to determine chemical composion. As a screen test to discover antibacterial properties of the extract, agar disk and agar well diffusion methods were employed. Macrobroth tube test was performed to specify MIC. The findings show that the most substance found in PA was anethole. The results indicated the MIC and MBC values was 0.031 g/ml for PA except in case of *B. subtilis* which was 0.062 g/ml. Thus, the present research demonstrates the antibacterial effects of the medical plant on *E. coli*, *S. aureus* and *B. subtilis*, suggesting to use as antibacterial supplement in the developing countries towards the development of new therapeutic agent.[16]
- **Mehmet Musa Özcan, Jean Claude Chalchat said that:**The composition of the essential oil of *Pimpinella anisum* L fruit is determined by GC and GC-MS. The volatile oil contentobtained by hydro distillation was 1.91%. Ten compounds representing 98.3% of the oil was identified. The main constituents of the oilobtained from dried fruits were trans-anethole (93.9%) and estragole (2.4%). The olfactorially valuable constituents that were foundwith concentration higher than 0.06% were (E)-meth eugenol, α-cuparene, α-himachalene, β-bisabolene, p-anis aldehyde and cis-anethole.Also, the different concentrations of anise oil exerted varying levels of inhibitory effects on the mycelial growth of *Alternaria alternata,Aspergillus niger* and *Aspergillus parasiticus* used in experimental. The results showed that the most effected fungus from aniseoil was *A.*

parasiticus, which is followed by *A. niger* and *A. alternata*. Individual of this plant oil may provide a useful to achieve adequateshelf-life of foods.[17]

- **Zohra Ghlissi, Rim Kallel, Fatma Krichen, Ahmed Hakim, Khaled Zeghal, Tahiya Boudawara, Ali Bougatef, Zouheir Sahnoun said that:**The polysaccharide preparation from *Pimpinella anisum* seeds (PAP) was isolated andcharacterized to evaluate its laser burn wound-healing and anti-inflammatory activities inmice. The structure characterization of PAP by Infra-red spectrometry (IR), Nuclear magneticresonance (NMR), Gas chromatogram-Mass spectrometer (GC-MS) and colorimetric methodsrevealed an optimum yield of 8.84 %, a high quantity of carbohydrate (64.75 %) and lowlevels of lipids, protein and sulfate. Galactose (33.47 %), β-D-Glucose (26.71 %) and α-DMannose(18.21 %) were the major monosaccharides components presenting in PAP, and asmaller amount of β-D-Galactose, D-Fructose, α-D-Glucose, α-L-Galactose and arabinosewere detected. PAP showed noticeable antioxidant and antibacterial properties. The anti-inflammatoryactivity of PAP in the carrageenan-induced paw edema model in mice,demonstrated by reduced edema and cellular infiltration, and oxidative stress markers inmuscle tissue. A beneficial wound healing effect was also revealed. The topical application ofPAP based gel on laser burn lesions accelerates wound contraction, the re-epithelization andremodeling phases after seven days of treatment. The results demonstrated that PAP is a novelpromising source of natural wound healing and anti-inflammatory drugs. The high contentand varied PAP monosaccharides seem to be responsible for the observed biologicalactivities.[18]

- **Shojaii, Asie; Abdollahi Fard, Mehri said that:**Pimpinella anisum (anise), belonging to Umbelliferae family, is an aromatic plant which has been used In Iranian traditionalmedicine (especially its fruits) as carminative, aromatic, disinfectant, and galactagogue. Because the wide traditional usage ofPimpinella anisum for treatment of diseases, in this review published scientific reports about the composition andpharmacological properties of this plant were collected with electronic literature search of GoogleScholar, PubMed, Sciencedirect,Scopus, and SID from 1970 to 2011. So far, different studies were performed on aniseeds and various properties such asantimicrobial, antifungal, antiviral, antioxidant, muscle relaxant, analgesic and anticonvulsant activity as well as different effectson gastrointestinal system have been

reported of aniseeds. It can also reduce morphine dependence and has beneficial effects ondysmenorrhea and menopausal hot flashes in women. In diabetic patients, aniseeds showed hypoglycemic and hypolipidemiceffect and reduce lipid peroxidation. The most important compounds of aniseeds essential oil were trans-anetole, estragole, Î³-hymachalen, para-anisaldehyde and methyl cavicol. Due to broad spectrum of pharmacological effects, and very few clinicalstudies of Pimpinella anisum, more clinical trials are recommended to evaluate the beneficial effects of this plant in humanmodels and synthesis of new drugs from the active ingredients of this plant in future. [19]

- **Park, Ii-Kwon; Choi, Kwang-Sik; Kim, Do-Hyung; Choi, In-Ho; Kim, Lee-Sun; Bak, Won-Chull; Choi, Joon-Weon; Shin, Sang-Chul said that:** Plant essential oils from 40 plant species were tested for their insecticidal activities against larvae of Lycoriella ingÃ©nue(Dufour) using a fumigation bioassay. Good insecticidal activity against larvae of L. ingenua was achieved with essential oils ofChenopodium ambrosioides L., Eucalyptus globulus Labill, Eucalyptus smithii RT Baker, horseradish,aniseand garlic at 10 and 5microL L(-1) air. Horseradish, aniseand garlic oils showed the most potent insecticidal activities among the plant essential oils.At 1.25 microL L(-1), horseradish,aniseand garlic oils caused 100, 93.3 and 13.3% mortality, but at 0.625 microL L(-1) air thisdecreased to 3.3, 0 and 0% respectively. Analysis by gas chromatography-mass spectrometry led to the identification of one majorcompound from horseradish, and three each fromaniseand garlic oils. These seven compounds and m-anisaldehyde and o-anisaldehyde, two positional isomers of p-anisaldehyde, were tested individually for their insecticidal activities against larvae ofL. ingenua. Allyl isothiocyanate was the most toxic, followed by trans-anethole, diallyl disulfide and p-anisaldehyde with LC(50)values of 0.15, 0.20, 0.87 and 1.47 microL L(-1) respectively.[20]

- **Tavallali, Vahid; Rahmati, Sadegh; Bahmanzadegan, Atefeh said that:**The antioxidant activity and essential oil content of plants may vary considerably with respect to environmental conditions,especially nutrient availability. Among micronutrients, zinc (Zn) is needed by plants in only small amounts but is crucial to plantdevelopment. This study aimed to evaluate the effects of Zn fertilization on the antioxidant activity,

polyphenolic contents andessential oil composition ofPimpinellaanisumfruit. Foliar application of Zn fertilizer considerably increased the number ofdetected essential oil components from 27 to 45. Zinc application at a rate of 0.2% (w/v) significantly enhanced the levels of Î²-bisabolene, germacrene D, n-decane and Î±-zingiberene, whereas the opposite trend was observed for (E)-anethole and geijerene.Application of 0.2% Zn considerably increased the levels of phenolic compounds, with chlorogenic acid showing the highestcontent among eight phenolic compounds detected in treated plants. The maximum antioxidant activity was achieved throughapplication of 0.2% Zn fertilizer. These findings indicated that the quality and quantity ofanisefruit essential oil componentswere significantly altered by application of low levels of Zn. After foliar application of Zn, polyphenolic contents as well asantioxidant activity ofanisefruit increased. Using Zn fertilizer is an efficient method to improve the pharmaceutical and foodproperties ofanisefruit. Â© 2017 Society of Chemical Industry. Â© 2017 Society of Chemical Industry.[21]

- **Soto-Argel, Camilo; Hidalgo, Diego; Palazon, Javier; Corchete, PurificaciÃnsaid that:**To explore the potentiality of undifferentiated Pimpinella anisum L. cell cultures for the production of secondary metabolites bymeans of elicitation. Two chromone compounds were secreted to the medium of undifferentiated cultures of P. anisum: 4-methoxyfuro[3,2-g] chromen-7-one, known as bergapten, which is constitutive to anise, and 5-hydroxy-7-methoxy-2-methylchromen-4-one, the rare chromone eugenin, not yet described in P.Â anisum. Caffeoyl quinic acid species were also identified in the biomass. Elicitation with methyl jasmonate enhanced chromone accumulation in the medium and stimulated phenolic acid metabolism in the biomass (11Â mg caffeoyl quinic acids g -1 DW cells). The application of 2,6-dimethyl-Î²-cyclodextrins to cultures led to an intense accumulation of chromones, with nearly 10Â mgÂ l -1 bergapten and 150Â mgÂ l -1eugenin being accumulated extracellularly after optimal elicitation conditions. The significant amounts of eugenin obtained in the anise cultures and the stability of production over long periods of time can be of interest for its biotechnological production andfor future studies on biosynthesis regulation.[22]

- **Fitsiou, Eleni; Mitropoulou, Gregoria; Spyridopoulou, Katerina; Tiptiri-Kourpeti,**

Angeliki; Vamvakias, Manolis; Bardouki,Haido; Panayiotidis, Mihalis Î™; Galanis, Alex; Kourkoutas, Yiannis; Chlichlia, Katerina; Pappa, Aglaia said that: Natural products, known for their medicinal properties since antiquity, are continuously being studied for their biologicalproperties. In the present study, we analyzed the composition of the volatile preparations of essential oils of the Greek plantsOcimum basilicum (sweet basil), Mentha spicata (spearmint), Pimpinella anisum (anise) and Fortunella margarita (kumquat).GC/MS analyses revealed that the major components in the essential oil fractions, were carvone (85.4%) in spearmint, methylchavicol (74.9%) in sweet basil, trans-anethole (88.1%) in anise, and limonene (93.8%) in kumquat. We further explored theirbiological potential by studying their antimicrobial, antioxidant and antiproliferative activities. Only the essential oils fromspearmint and sweet basil demonstrated cytotoxicity against common foodborne bacteria, while all preparations were activeagainst the fungi Saccharomyces cerevisiae and Aspergillus niger. Antioxidant evaluation by DPPH and ABTS radical scavengingactivity assays revealed a variable degree of antioxidant potency. Finally, their antiproliferative potential was tested against apanel of human cancer cell lines and evaluated by using the sulforhodamine B (SRB) assay. All essential oil preparationsexhibited a variable degree of antiproliferative activity, depending on the cancer model used, with the most potent one being sweetbasil against an in vitro model of human colon carcinoma.[23]

- **Abdul-Hamid, Manal; Gallaly, Sanaa Rida said that:** The study aims to investigate the protective effect of Pimpinella anisum oil on aspartame (ASP) which resulted in cerebellarchanges. The rats were divided into four equal groups: Group 1: (control group): served as control animals. Group 2: control P.anisum oil received .5â€‰mL/kg/d/b wt. once daily. Group 3 (ASP group): received daily 250â€‰mg/kg/b wt. of ASP dissolvedin distilled water and given orally to the animals by intra-gastric tube for 2 months. Group 4: received .5â€‰mL/kg/b wt. ofprophylactic P. anisum oil once daily, followed by ASP after 2â€‰h for 2 months. The histopathological approach revealedmarked changes in the Purkinje cells, myleinated nerve fibers and granular cells of ASP-treated animals. Some of these cellsappeared with deeply stained cytoplasm. Ultrastructural examination showed Purkinje cells with dilated rough endoplasmicreticulum and condensed mitochondria. Granular cells appeared with less c

nuclei and surrounded by dissolution of most Mossyrosettes structures. Most myelinated nerve fibers showed thickening of myelinated sheath and others showed splitting of theirmyelin sheath. The histopathological, immunohistochemical and ultrastructural alterations were much less observed inconcomitant use of P. anisum oil with ASP. Cerebellar cortex is considered target areas of ASP neurotoxicity, while P. anisum oil,when used in combination with ASP displays a protective action against neurotoxicity.[24]

- **PubMed Central said that:** Background Essential oil of Pimpinella anisum L. Apiaceae (anise oil) has been widely used in traditional Persian medicine totreat a variety of diseases, including some neurological disorders. This study was aimed to test the possible anti-seizure and anti-hypoxia effects of anise oil. Methods The effects of different concentrations of anise oil were tested on seizure attacks induced bypentylenetetrazol (PTZ) injection and neuronal hypoxia induced by oxygen withdrawal as well as on production of dark neuronsand induction of long-term potentiation (LTP) in in vivo and in vitro experimental models of rat brain. Results Anise oilsignificantly prolonged the latency of seizure attacks and reduced the amplitude and duration of epileptiform burst dischargesinduced by injection of intraperitoneal PTZ. In addition, anise oil significantly inhibited production of dark neurons in differentregions of the brain in epileptic rats. Anise oil also significantly enhanced the duration of the appearance of anoxic terminalnegativity induced by oxygen withdrawal and inhibited induction of LTP in hippocampal slices. Conclusions Our data indicate theanticonvulsant and neuroprotective effects of anise oil, likely via inhibition of synaptic plasticity. Further evaluation of anise oil touse in the treatment of neurological disordersis suggested. [25]

- **PubMed Central said that:** Background A phytotherapic compound containing Pimpinella anisum L., Foeniculum vulgare Miller, Sambucus nigra L., andCassia augustifolia is largely used in Brazil for the treatment of constipation. However, the laxative efficacy of the compound hasnever been tested in a randomized clinical trial. The aim of this study was to evaluate the efficacy and safety of the product.Methods This randomized, crossover, placebo-controlled, single-blinded trial included 20 patients presenting with chronicconstipation according to the criteria of the American Association

of Gastroenterology. The order of treatments wascounterbalanced across subjects: half of the subjects received the phytotherapic compound for a 5-day period, whereas the otherhalf received placebo for the same period. Both treatment periods were separated by a 9-day washout period followed by thereverse treatment for another 5-day period. The primary endpoint was colonic transit time (CTT), measured radiologically.Secondary endpoints included number of evacuations per day, perception of bowel function, adverse effects, and quality of life.Results Mean CTT assessed by X ray was 15.7 hours (95%CI 11.1-20.2) in the active treatment period and 42.3 hours (95%CI33.5-51.1) during the placebo treatment ($p < 0.001$). Number of evacuations per day increased during the use of active tea;significant differences were observed as of the second day of treatment ($p < 0.001$). Patient perception of bowel function wasimproved ($p < 0.01$), but quality of life did not show significant differences among the study periods. Except for a small reductionin serum potassium levels during the active treatment, no significant differences were observed in terms of adverse effectsthroughout the study period. Conclusions The findings of this randomized controlled trial allow to conclude that the phytotherapiccompound assessed has laxative efficacy and is a safe alternative option for the treatment of constipation. [26]

- **Gamberini, Maria Thereza; Rodrigues, Domingos SÃ¡vio; Rodrigues, Daniela; Pontes, Victoria Bottino said that:** Pimpinella anisum L. is considered one of the first plants used for medicinal purposes. Pharmacological actions of the plant on thecentral nervous system have been proven but previous analyses have focused on anticonvulsant and neuroprotective actions. Intraditional medicine worldwide, the use of Pimpinella is commonly recommended as a tranquilizer, although no scientificinformation supporting this use is available. Therefore, it was decided to investigate the central actions of the plant to observebehavioral responses, with an emphasis on the emotional component. To investigate the effects of the aqueous extract ofPimpinella seeds on exploratory activity and emotional behavior in rats using the open field and elevated plus maze tests. Seeds ofPimpinella were extracted with distilled water, concentrated and freeze-dried yielding the aqueous extract(AE). Rats were dividedinto four groups: control(water 5 mL/kg, p.o.) and AE 0.5, 1.0 and 2.0 g/kg, p.o. Individual observations were performed in anopen field and the parameters locomotor activity, rearing, grooming and defecation were recorded.

In elevated plus maze test, ratswere divided into four groups: control(water 5 mL/kg, p.o.) and AE 0.5, 1.0 and 2.0 g/kg, p.o. The parameters arm entries, totaltime spent in open and closed arms; and total number of arrivals at the end of an open or closed arm were recorded for each rat.Among the parameters assessed with the open field test, only rearing was reduced in the AE 0.5 g/kg group. When AE 1.0 g/kgwas administered, only the initiation of exploratory activity was delayed, without impairing the animals' general activity. Thehighest dose of AE (2.0 g/kg) induced a reduction in the animals' habituation during the open field test within the same session, asevidenced by the maintenance of high levels of peripheral locomotion and rearing throughout the test. [27]

- **Obando Pacheco, Pablo; MartÃ nez-MartÃ nez, Patricia Luisa; PÃ©rez de Eulate BazÃ¡n, Yolanda; de la Mota Ybancos, JosÃ©Luis; Milano Manso, Guillermo; Sierra Salinas, Carlos said that:** Intoxications in pediatric age represent a frequent cause of visit to the hospital emergency unit. Herb-made products can be toxicfor the infant. The neurotoxic properties of the star anise (Illicium verum) have been widely described, although it is a classicproduct used to treat the infantile colic. Hepatic failure due to the consumption of anise herb elaborated infusions is presented asan exceptional finding in our environment. A case of a 4-month-old infant with hypertransaminasemia, severe coagulopathy, nonketotic hypoglycemia, moderated metabolic acidosis and neurologic symptoms such as seizures and nistagmus is described. Afterdiscarding infectious, metabolic and autoimmune etiology and through a meticulous anamnesis, the family referred havingadministered in the last two months a daily star anise and green anise (Pimpinella anisum) infusion to the patient. It is important toemphasize the serious risk of administering homemade herb infusions to infants.[28]

- **Koeduka, Takao; Baiga, Thomas J.; Noel, Joseph P.; Pichersky, Eran said that:** The phenylpropene t-anethole imparts the characteristic sweet aroma of anise (Pimpinella anisum, family Apiaceae) seeds andleaves. Here we report that the aerial parts of the anise plant accumulate t-anethole as the plant matures, with the highest levels oft-anethole found in fruits. Although the anise plant is covered with trichomes, t-anethole accumulates inside the leaves and not inthe trichomes or the epidermal cell layer. We have obtained anise cDNA encoding t-anol/isoeugenol synthase 1 (AIS1), anNADPH-

dependent enzyme that can biosynthesize t-anol and isoeugenol (the latter not found in anise) from coumaryl acetate andconiferyl acetate, respectively. In addition, we have obtained a cDNA encoding S-[methyl-14C]adenosyl-l-methionine:t-anol/isoeugenol O-methyltransferase 1 (AIMT1), an enzyme that can convert t-anol or isoeugenol to t-anethole ormethylisoeugenol, respectively, via methylation of the para-OH group. The genes encoding AIS1 and AIMT1 were expressedthroughout the plant and their transcript levels were highest in developing fruits. The AIS1 protein is 59% identical to petunia(Petunia hybrida) isoeugenol synthase 1 and displays apparent Km values of 145 Î¼m for coumaryl acetate and 230 Î¼m forconiferyl acetate. AIMT1 prefers isoeugenol to t-anol by a factor of 2, with Km values of 19.3 Î¼m for isoeugenol and 54.5 Î¼mbasilicum) and Clarkia breweri phenylpropene O-methyltransferases, but unlike these enzymes, which do not show largediscrimination between substrates with isomeric propenyl side chains, AIMT1 shows a 10-fold preference for t-anol over chavicoland for isoeugenol over eugenol. [29]

- **Koeduka, Takao; Baiga, Thomas J; Noel, Joseph P; Pichersky, Eran said that:** The phenylpropene t-anethole imparts the characteristic sweet aroma of anise (Pimpinella anisum, family Apiaceae) seeds andleaves. Here we report that the aerial parts of the anise plant accumulate t-anethole as the plant matures, with the highest levels oft-anethole found in fruits. Although the anise plant is covered with trichomes, t-anethole accumulates inside the leaves and not inthe trichomes or the epidermal cell layer. We have obtained anise cDNA encoding t-anol/isoeugenol synthase 1 (AIS1), anNADPH-dependent enzyme that can biosynthesize t-anol and isoeugenol (the latter not found in anise) from coumaryl acetate andconiferyl acetate, respectively. In addition, we have obtained a cDNA encoding S-[methyl-14C]adenosyl-l-methionine:t-anol/isoeugenol O-methyltransferase 1 (AIMT1), an enzyme that can convert t-anol or isoeugenol to t-anethole ormethylisoeugenol, respectively, via methylation of the para-OH group. The genes encoding AIS1 and AIMT1 were expressedthroughout the plant and their transcript levels were highest in developing fruits. The AIS1 protein is 59% identical to petunia(Petunia hybrida) isoeugenol synthase 1 and displays apparent Km values of 145 microm for coumaryl acetate and 230 micromfor coniferyl acetate. AIMT1 prefers isoeugenol to t-anol by a factor of 2, with Km values of 19.3 microm for isoeugenol and 54.5microm for S-[methyl-14C]adenosyl-l-methionine. The AIMT1 protein sequence is

approximately 40% identical to basil(Ocimum basilicum) and Clarkia breweri phenylpropene O-methyltransferases, but unlike these enzymes, which do not showlarge discrimination between substrates with isomeric propenyl side chains, AIMT1 shows a 10-fold preference for t-anol overchavicol and for isoeugenol over eugenol.[30]

- **Erler, F; Ulug, I; Yalcinkaya, B said that:** Essential oils extracted from the seeds of anise (Pimpinella anisum), dried fruits of eucalyptus (Eucalyptus camaldulensis), driedfoliage of mint (Mentha piperita) and basil (Ocimum basilicum) and fresh foliage of laurel (Laurus nobilis) were tested for theirrepellency against the adult females of Culex pipiens. All essential oils showed repellency in varying degrees, eucalyptus, basiland anise being the most active.[31]

- **De, Minakshi; De, Amit Krishna; Sen, Parimal; Banerjee, Arun Baran said that:** Star anise (Illicium verum Hook f) has been shown to possess potent antimicrobial properties. Chemical studies indicate that amajor portion of this antimicrobial property is due to anethole present in the dried fruit. Studies with isolated anethole (comparedwith standard anethole) indicated that it is effective against bacteria, yeast and fungal strains. Copyright 2002 John Wiley & Sons,Ltd.[32]

- **Howes, Melanie-Jayne R; Kite, Geoffrey C; Simmonds, Monique S J said that:** The volatile compounds from the pericarps of Illicium anisatum L., Illicium brevistylum A.C.Sm., Illicium griffithii Hook.f. &Thomson, Illicium henryi Diels, Illicium lanceolatum A.C.Sm., Illicium majus Hook.f. & Thomson, Illicium micranthum Dunn,and Illicium verum Hook.f. were examined by thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS). The volatiles desorbed from the pericarps of I. verum (Chinese star anise), the species traded for culinary purposes, were generallycharacterized by a high proportion of (E)-anethole (57.6-77.1%) and the presence of foeniculin; the latter was otherwise onlydetected in the pericarps of I. lanceolatum. In the pericarps of all other species analyzed, the percentage composition of (E)-anethole was comparatively lower (anise) were characterized by the presence of asaricin, methoxyeugenol, and two other eugenolderivatives, none of which were detected in any of the other species examined. TD-GC-MS enables the direct analysis of

thevolatile components from the pericarps of Illicium and can assist with differentiating the fruits of I. verum from other species ofIllicium, particularly the more toxic I. anisatum.[33]

- **Topuz, Osman Kadir; Ã–zvural, Emin BurÃ§in; Zhao, Qin; Huang, Qingrong; Chikindas, Michael; Muharrem said that:** The purpose of this research was to investigate antimicrobial effects of nano emulsions of anise oil (AO) on the survival ofcommon food borne pathogens, Listeria monocytogenes and Escherichia coli O157:H7. Series of emulsions containing differentlevel of anise oil as potential antimicrobial delivery systems were prepared. Antimicrobial activities of bulk anise oil and itsemulsions (coarse and nano) was tested by the minimum inhibitory concentration and time kill assay. Our results showed that bulkanise oil reduced the population of E. coli O157:H7 and L. monocytogenes by 1.48 and 0.47 log cfu/ml respectively after 6 h ofcontact time. However, under the same condition anise oil nanoemulsion (AO75) reduced E. coli O157:H7 and L. monocytogenescount by 2.51 and 1.64 log cfu/ml, respectively. Physicochemical and microbial analyses indicated that both nano and coarseemulsions of anise oil showed better and long-term physicochemical stability and antimicrobial activity compared to bulk aniseoil. Copyright Â© 2016 Elsevier Ltd. All rights reserved.[34]

- **Gradinaru, Adina Catinca; Miron, Anca; Trifan, Adriana; Spac, A; Brebu, M; Aprotosoaie, Ana Clara said that:** This study aimed to investigate the effects of anise essential oil alone and in combination with conventional antibiotics(amoxicillin, ciprofloxacin) against Streptococcus pneumoniae clinical isolates. MATERIAL AND METHODs: Anise essentialoil (AEO) was isolated by hydrodistillation from dried powdered fruits. Its chemical composition was investigated by GC-MS andGC-FID. Broth dilution assay was used to evaluate the antibacterial effects of anise essential oil. The interactions with antibioticswere studied by the checkerboard assay. Trans-anethole (90.18%) was identified as major constituent in anise essential oil. Almostall combinations AEO-amoxicillin and AEO-ciprofloxacin showed indifferent interactions (1 < FIC index < or = 2). Positive interactions (addition and weak synergism) were found only for four combinations AEO-amoxicillin (FIC index = 1,0.62, 0.75and 0.5) and one combination AEO-ciprofloxacin (FIC index = 0.62). Herbal products containing anise

essential oil may be usedas expectorants in combination with amoxicillin or ciprofloxacin in Streptococcus pneumoniae infections without diminishingantibiotic efficacy.[35]

- **Bluma, R; Amaiden, M R; Daghero, J; Etcheverry, M said that:** The antifungal effect of Pimpinella anisum (anise), PÃ«umus boldus (boldus), Mentha piperita (peppermint), Origanum vulgare(oregano) and Minthosthachys verticillata (peperina) essential oils against Aspergillus section Flavi (two isolates of Aspergillusparasiticus and two isolates of Aspergillus flavus) was evaluated in maize meal extract agar at 0.982 and 0.955 water activities, at25 degrees C. The percentage of germination, germ-tube elongation rate, growth rate and aflatoxin B(1) (AFB(1)) accumulation atdifferent essential oils concentrations were evaluated. Anise and boldus essential oils were the most inhibitory at 500 mg kg(-1) toall growth parameters of the fungus. These essential oils inhibited the percentage of germination, germ-tube elongation rate andfungal growth. AFB(1) accumulation was completely inhibited by anise, boldus and oregano essential oils. Peperina andpeppermint essential oils inhibited AFB(1) production by 85-90% in all concentrations assayed. Anise and boldus essential oilscould be considered as effective fungitoxicans for Aspergillus section flavi. Our results suggest that these phytochemicalcompounds could be used alone or in conjunction with other substances to control the presence of aflatoxigenic fungi in storedmaize. [36]

- **Bristol, D W said that:** Estragole is a natural organic compound that is used as an additive, flavoring agent, or fragrance in a variety of food, cleaning,and cosmetic products; as an herbal medicine; as an antimicrobial agent against acid-tolerant food microflora; and to producesynthetic anise oil. Estragole was nominated for toxicity testing by the National Institute of Environmental Health Sciences tocharacterize its toxicity when administered by gavage to F344/N rats and B6C3F1 mice and to determine how similar its effectsmight be to those of the structurally related compound, methyleugenol. Male and female F344/N rats and B6C3F1 mice weregiven estragole (greater than 99% pure) in corn oil by gavage for 3 months. Genetic toxicology studies were conducted inSalmonella typhimurium and mouse peripheral blood erythrocytes. Core and special study (rats only) groups of 10 male and 10female rats and mice were administered 37.5,

75, 150, 300, or 600 mg estragole/kg body weight in corn oil by gavage, 5 days perweek. The core study groups were given estragole for 3 months and the special study groups for 30 days. All core study ratssurvived the 3-month exposure period. Mean body weights of the 300 and 600 mg/kg groups were 73% to 92%, respectively, ofthose of the vehicle control groups. A staining pattern on the ventral surface anterior to the genitalia beginning at week 9 in the300 and 600 mg/kg groups was attributed to residue of estragole or metabolites in the urine. Alterations in the erythron related toestragole administration occurred in male and female rats; male rats demonstrated a stronger response. The changes in theerythron were characterized as a microcytic, normochromic, nonresponsive anemia. There were decreases in serum ironconcentration in the 300 mg/kg females and 600 mg/kg males and females. The average percent saturation of total iron bindingcapacity was decreased in the 600 mg/kg males and females. [37]

- **Calsamiglia, S; Busquet, M; Cardozo, P W; Castillejos, L; Ferret, A said that:** trans-Anethole (anethole), a major component of anise oil, has a broad antimicrobial spectrum and a weaker antimicrobial potencythan other available antibiotics. When combined with polygodial, nagilactone E, and n-dodecanol, anethole has been shown toexhibit synergistic antifungal activity against a budding yeast, Saccharomyces cerevisiae, and a human opportunistic pathogenicyeast, Candida albicans. However, the mechanism underlying this synergistic effect of anethole has not been characterized. Westudied this mechanism using dodecanol-treated S. cerevisiae cells and focusing on genes related to multidrug efflux. Althoughdodecanol transiently reduced the number of colony forming units, this recovered to levels similar to those of untreated cells withcontinued incubation beyond 24h. Reverse transcription polymerase chain reaction analysis revealed overexpression of an ATP-binding cassette (ABC) transporter gene, PDR5, in addition to a slight increase in PDR11, PDR12, and PDR15 transcriptions indodecanol-treated cells. In the presence of anethole, these effects were attenuated and the fungicidal activity of dodecanol was extended. Dodecanol showed longer lasting fungicidal activity against a Î”pdr5. In addition, Î”pdr3 and Î”lge1, lack transcriptionfactors of PDR5 and PDR3, were partly and completely susceptible to dodecanol, respectively. Furthermore, combination ofanethole with fluconazole was also found to exhibit synergy on C. albicans. These results indicated that although anethole reducedthe transcription of several transporters, PDR5 expression was particularly relevant to dodecanol efflux. Anethole is expected tobe

a promising candidate drug for the inhibition of efflux by reducing the transcription of several ABC transporters. CopyrightÂ© 2016 Elsevier B.V. All rights reserved.[38]

- **Chang, Kyu-Sik; Ahn, Young-Joon said that:** The insecticidal activities of materials derived from the fruit of star anise, Illicium verum, against adults of Blattella germanicawere examined by direct contact application and fumigation methods, and compared with those of DDVP, deltamethrin andhydramethylnon. The biologically active constituent of the Illicium fruit was characterized as the phenylpropene, (E)-anethole, byspectroscopic analysis. In a filter paper diffusion test with females, (E)-anethole caused 80.3% mortality at 0.159 mg cm-2 at 1and 3 days after treatment (DAT), whereas 16.7% mortality at 3 DAT was achieved at 0.079 mg cm-2. DDVP and deltamethringave > 90% mortality at 0.019 mg cm-2 at 1 DAT. At 0.009 mg cm-2, DDVP and deltamethrin showed 73.3 and 60% mortality at1 DAT, respectively, but 93.3 and 76.7% mortality at 3 DAT. Hydramethylnon exhibited 0 and 93.3% mortality at 0.159 mg cm-2at 1 and 3 DAT, respectively, whereas 6.7% mortality at 3 DAT was observed at 0.079 mg cm-2. In a fumigation test with females,(E)-anethole was much more effective in closed cups than in open ones, indicating that the insecticidal activity of the compoundwas largely attributable to fumigant action. (E)-Anethole and DDVP caused 100% mortality at 0.398 and 0.051 mg cm-2 4 and 1h after treatment, respectively. (E)-Anethole showed 46.7% mortality at 0.199 mg cm-2 at 3 DAT, whereas deltamethrin andhydramethylnon at 0.796 mg cm-2 was ineffective for 3-day period. As naturally occurring insect-control agents, the I verumfruit-derived materials described could be useful for managing populations of B germanica.[39]

- **GaztaÃ±aga, Mirari; Aranda-FernÃ¡ndez, P Ezequiel; Chotro, M Gabriela said that:** Rat fetuses can perceive chemosensory stimuli derived from their mother's diet, and they may learn about those stimuli. Inprevious studies we have observed that prenatal exposure to alcohol during the last days of gestation increases the acceptance andliking of an alcohol flavor in infant and adolescent rats. While these results were not found after prenatal exposure to vanilla,cineole or anise, suggesting that the pharmacological properties of alcohol, mediated by the opioid system, underlie the effectsobserved with this drug. Considering that other studies report enhanced acceptance of non-alcohol flavors experienced prenatallywhen subjects were tested before infancy, we explore the

possibility of observing similar results if testing 1-day old rats exposedprenatally to vanilla. Using an "odor-induced crawling" testing procedure, it was observed that neonates exposed prenatally tovanilla or alcohol crawl for a longer distance towards the experienced odor than to other odors or than control pups. Blocking mu,but not kappa opioid receptors, reduced the attraction of vanilla odor to neonates exposed to vanilla in utero, while the response toalcohol in pups exposed prenatally to this drug was affected by both antagonists. Results confirm that exposure to a non-alcoholodor enhances postnatal responses to it, observable soon after birth, while also suggesting that the mu opioid receptor systemplays an important role in generating this effect. The results also imply that with alcohol exposure, the prenatal opioid system iswholly involved, which could explain the longer retention of the enhanced attraction to alcohol following prenatal experiencewith the drug. Copyright Â© 2014 Elsevier Inc. All rights reserved.[40]

CHAPTER 3
AIM & OBJECTIVE

3.1 AIM:

This study aims to formulate the herbal gel from *Pimpenella anisum* plant seed extract and to evaluate the wound healing activity.

3.2 OBJECTIVE:

- Literature survey in the context of national/international scientific journals, books, e-sources etc.
- Extraction of oil from *Pimpinellaanisum* seeds using ethanol as solvent.
- Identification test
- Phytochemical Screening
- Formulation of gel
- Evaluation of gel
- In-vitro antimicrobial study
- In-vivo wound healing activity

3.3PLAN OF WORK:

Collection of plant

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Preparation of plant extract

↓

Determination of the antibacterial activity of extract

↓

Standardization of plant Extract

↓

Phytochemical screening

↓

Formulation of gel

↓

Evaluation of Gel Formulation

↓

In vitro antimicrobial study

↓

In-vivo Animal Study for Wound healing

CHAPTER 4

MATERIALS & METHODS

4. MATERIALS AND METHODS:

4.1 CHEMICALS USED:

All the chemicals are used of analytical grade and the manufacturers for respective chemicals are listed below:

TABLE NO.2 : LIST OF CHEMICALS USED:

Sl no.	Chemicals	Suppliers
1.	Ethanol	Infinity solution
2.	Methanol	Infinity solution
3.	Liquid CO_2	Rankem, Mumbai.
4.	Hydro Chloric acid	Rankem, Mumbai.
5.	KOH	Infinity solution
6.	Anise tincture	B S Trading, Kolkata
7.	Acetone	Infinity solution
8.	Estragole	B S Trading, Kolkata
9.	Trans-anethole	B S Trading, Kolkata
10.	4-methoxy benzoic acid	Rankem, Mumbai.
11.	Sodium hydroxide	Infinity solution
12.	Chloroform	Infinity solution

4.2 INSTRUMENTS USED:

All the instruments are used of analytical grade and the manufacturers for respective chemicals are listed below:

TABLE NO.3 : LIST OF INSTRUMENTS USED:

Sl. No.	Instruments
1.	Clevenger apparatus
2.	Separating funnel
3.	Digital weighing balance
4.	Mechanical grinder
5.	Magnetic stirrer with a hot plate
6.	Hot air oven
7.	Refrigerator

4.3 CHEMICAL COMPOSITION OF ANISE SEED:

TABLE NO. 4: CHEMICAL COMPOSITION OF ANISE SEED:

CHEMICALS	AMOUNT PRESENT IN ANISE OIL
Trans-anethole	87- 94.0%
Estragole	0.5-5.0%
Anis aldehyde	0.1- 1.4%
Linalol	< 1.5%
Alpha-terpineol	< 1.2%
Cis-anethole	0.1-0.4%
Pseudoisoeugeny l, 2-methylbutirate	0.3-2.0%
Fenchone max	0.01%

Aniseed contains 1.5–6.0 mass % of a volatile oil consisting primarily of trans-anethole and also as much as 8–11 mass % of lipids rich in fatty acids, such as palmitic and oleic acids, as well as approximately 4 mass % of carbohydrates, and 18 mass % of protein. Other studies have demonstrated the presence of eugenol trans-anethole, methyl chavicol, anis aldehyde, estragole, coumarins, scopoletin, umbelliferone, estrols, terpene hydrocarbons, polyenes, and polyacetylenes as the major compounds of the essential

oil of anise seed. Study of the essential oil of *Pimpinellaanisum* L. fruits by GC and GC-MS showed the presence of trans-anethole (93.9%) and estragole (2.4%).

Other compounds that were found with concentration higher than 0.06% were (E)-methyl eugenol, α-cuparene, α-himachalene, β-bisabolene, p-anis aldehyde, and cis-anethole. In another study for determination of the composition of essential oil of Pimpinella anisum L. fruits obtained from different geographical areas of Europe, in addition to the major components (trans-anethole (76.9–93.7%) and γ-himachalene (0.4–8.2%), some other compounds such as trans-pseudoisoeugenyl 2-methylbutyrate, p-anis aldehyde, and methyl chavicol were also identified in essential oil. Study of components of the whole plants and the seeds of Pimpinella anisum from Alberta showed that the major oil constituent (trans-anethole) was 57.4% of whole plant and 75.2% of seed oil. The other constituents of plant oil, present in amounts of 1–5% were cis-anethole, carvone, β-caryophyllene, dihydrocarvyl acetate, estragole and limonene. The chemical constituents of aniseed extract obtained by Supercritical extraction using CO2 were determined by GCMS.

The major compounds were anethole (∼90%), γ-himachalene (2−4%), p-anis aldehyde (<1%), methyl chavicol (0.9−1.5%), cis-pseudoisoeugenyl 2-methylbutyrate (∼3%), and trans-pseudoisoeugenyl 2-methylbutyrate (∼1.3%). A new terpene hydrocarbon called neophytadiene was isolated from aniseed in 1978 [12]. 4-(β-d-glucopyranosyloxy) benzoic acid which is one of the phenolic glycosides of the Umbelliferae family was also isolated from aniseed. In a study by Fujimato et al., four aromatic compound glucosides, an alkyl glucoside, and a glucide were isolated as new compounds from the polar portion of methanolic extract of anise fruits. The structures of the new compounds were clarified as (E)-3-hydroxy-anethole β-d-glucopyranoside, (E)-10-(2-hydroxy-5-methoxyphenyl) propane β-d-glucopyranoside, 3-hydroxyestragole β-d-glucopyranoside, methyl syringate 4-O-β-d-glucopyranoside, hexane-1,5-diol 1-O-β-d-glucopyranoside, and 1-deoxy-l-erythritol 3-O-β-dglucopyranoside. Isolation and structure elucidation of flavonoid constituents from anise, caraway, coriander, and fennel by means of chromatography on cellulose columns lead to isolation of quercetin 3-glucuronide, rutin, luteolin 7-glucoside, isoorientin, and isovitexin as crystalline compounds and apigenin 7-glucoside and a luteolin glycoside as noncrystalline compounds from anise. In another study, a silver ion HPLC procedure was used to determine the fatty acids composition of aniseed oil.

The results showed the positionally isomeric 18:1 fatty acids oleic acid (cis 9–18:1),

petroselinic acid (cis 6–18:1), and cisvaccenic acid (cis 11–18:1), in aniseed oil by a single gradient run on a single cation exchange column laboratory converted to the silver ion form. Also, three lignin-carbohydrate protein complexes were isolated from a hot water extract of the seeds of Pimpinella anisum by combination of anion exchange, gel filtration, and hydrophobic interaction column chromatographies. [3]

4.4 PREPARATION OF PLANT EXTRACT:

Several techniques had been implemented for the extraction of oils like turbo distillation, super critical fluid extraction, Solvent extraction, hydro distillation, use of superheated and supercritical water and other such as microwave assisted processes, Steam distillation etc.

Out of these techniques the Steam distillation is one of the promising techniques for extraction of oil.

Aniseed, on steam distillation, yields an essential oil, known as `Oil of Aniseed`, which has now replaced the fruits for medicinal and flavoring purposes. Aniseed oil is a colorless or pale-yellow liquid having the characteristic odor and taste of the fruit. The yield of oil generally varies from 1.9 to 3.1 percent. Higher values up to 6 per cent have been reported from Syrian aniseed. Crushing of fruits prior to distillation gives better yields of oil. The material should be distilled soon after the crushing to prevent any loss of oil due to evaporation. Aniseed oil is a highly refractive liquid, which solidifies on cooling. The congealing point depends much on the anethole content and is a valuable criterion for evaluating the oil. Exposure of the oil to air causes polymerization, and some oxidation also takes place with the formation of anisaldehyde and anisic acid. The chief constituent of aniseed oil is anethole, which is present to the extent of 80 to 90 per cent and is mainly responsible for the characteristic flavor of the oil. The oil also contains methyl chavicol, p-methoxyphenyl acetone, and small amount of terpenes and sulfur containing compounds of disagreeable odor.

Fig 3: Clevanger apparatus

Clevanger apparatus consists of a steam generator connected to the round bottom flask through a glass inlet tube. The flask is connected to a water condenser through a glass outlet tube. Condenser is further attached to a receiver through an adaptor. [45]

4.4.1 PROCEDURE OF STEAM DISTILLATION:

i. Take about 750 ml of water in the steam generator and start heating to produce steam.
ii. In the round bottom flask take crushed anise seed.
iii. A vigorous current of steam from steam generator is passed through the round bottom flask.
iv. A part of the steam condenses in the round bottom flask. As more and more steam is passed, the steam volatile components of anise seed pass through the condenser along with steam. These contents on condensation are collected in the receiver.
v. The contents in the round bottom flask may be heated by a heating mental to prevent excessive condensation of steam.
vi. The process of steam distillation is continued for about half an hour.
vii. Transfer the distillate to a separating funnel.
viii. Wait for one day allow settling down to found two layer of phases of different density, raffinate and extract phases.
ix. Separate the two layers.
x. Find the weight of the extracted essential oil. Note the color, odor and weight of the essential oil.

4.4.2 RESULTS AND DISCUSSION:

Batches:

I) first batch of distillation and results:

Quantity of aniseed = 150 gm

Water quantity = 500 ml

Temperature = 70^{o}C

Oil recovery = 2 ml

2) Second batch of distillation

Quantity of aniseed = 150 gm

Water quantity = 400 ml

Oil recovery = 3 ml

3) Third batch of distillation

Quantity of aniseed = 250 g

Water quantity = 300 ml

Oil recovery = 2 ml

4) Fourth batch of distillation

Quantity of aniseed = 350 gm

Water quantity= 350 ml

Oil recovery= 4 ml

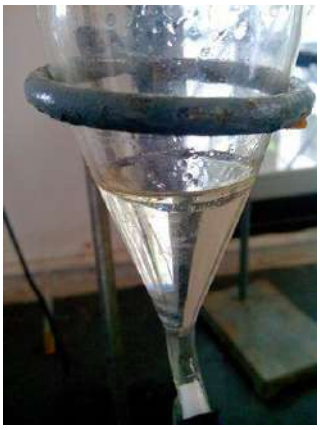

Fig 4: Collected Oil & water

The color of Anise oil was measured using spectrophotometer. The Saponification

value of oil is determined by laboratory method using HCl, KOH chemical. Acid value determines free fatty acid content in oil by laboratory method. Refractive Index was measured by using Refractometer at 25^{o}C. [46]

TABLE NO.5 : PROPERTIES OF ANISE SEED OIL

Sl. no.	Parameter	Results
i.	Color	Pale yellow
ii.	Specific gravity	0.987
iii.	Saponification value	168.3
iv.	Acid value	2.55
v.	Iodine value	99
vi.	Refractive index	1.55
vii.	Odor	Sweet like Anethole

4.4.3 OBSERVATION:

It was observed that very less work was done in obtaining Oil from Aniseed by Steam distillation method. Volume of Essential Oil increases with increases in time of heating keeping the temperature constant. On decreasing the pressure, we can extract Essential Oils at relatively less temperature and within less time of heating.

4.5 ANIMALS REQUIRED:

i. Species and Strain: Wistar albino rats
ii. Age and Weight: Adult (150-180g) rats
iii. Gender: Male

iv. Number to be used (Year-wise breakups and total figures needed to be given in tabular form):

TABLE NO.6 : NUMBER OF RATS TO BE USED

ANIMAL	NUMBER OF ANIMALS
Control	6
Standard	6
Test	6

Total number of animals: 18

v. Number of days each animal will be housed: 30 days

vi. Duration of the animal experiment:

Date of initiation (Proposed): - 01/04/2021

Date of completion (Proposed): - 30/4/2021

4.6 NECESSITY OF ANIMAL USAGE IN SUCH STUDIES:

- Animal models are the next step when assessing product efficacy.
- Animal models are beneficial to wound research because of their compliance towards wound healing studies [50]

4.7 REASON FOR SELECTING PARTICULAR ANIMAL SPECIES:

- Albino rats are used for this project because they will eat any food typically eaten by humans
- They have a digestive system similar to humans; they are small, clean, gentle and easily caged animals
- They can safely and easily be handled; they do no transmit disease to humans; and

they grow quickly.

- The estimated number of animals is required to study the wound healing activity and statistical analysis of data. [50]

4.8 PROCEDURE OF WOUND CREATION:

- The rats were weighed prior to the surgical procedure.
- The animals were anaesthetized prior to the infliction of the experimental wound.
- The backs of the animals were shaved and then prepared for aseptic surgery.
- Under sterile conditions, a square shape, full thickness incision (2×2cm) was made in the skin with sharp scalpel and the incised piece was removed.
- The wound was left undressed and no local or systemic antimicrobial drugs were administrated.
- Then, the 3 groups were applied with equal aliquots of formulation, standard solution and water for injection respectively.
- Each group of rats received a particular treatment regimen house separately in a ventilated cage with appropriate bedding, food, and water.
- Rats were checked twice daily during wounds and treatment to ensure no adverse reactions took place and closely observed the wound activity of the gel formulation against the experimental wounds on the experimental rats. [51]

4.9 STUDY DESIGN:

The rats were randomly allocated into three main groups (n=6) as follows:

- **control**
- **Standard,***Pimpinella anisum* oil (bought from market)
- **Treatment,***Pimpinella anisum* 10% (prepared in lab)

Once in there main groups, the rats were representing days seven, 14 and 21 post-injury (n=6).

The groups were defined wound dressings were not used in the wound area of rats in the control group.

In the standard groups, the injured area was covered with 1ml*Pimpinella anisum* daily, for 14 days post-injury.

In the treatment group, the wound area was covered with 1ml *Pimpinella anisum* 10% (*Pimpinella anisum* powder, 10g was suspended in 90g Eucerin) for 14 days post-injury.

From each group, three animals were euthanised at days seven, 14 and 21 post-injury by intravenous injection of pentobarbital (50mg/kg). [51]

4.10 RATE OF WOUND CLOSURE:

Wounds were serially photographed using a digital camera on days seven, 14, and 21 post-injury. The wound area was measured using Image J software (Java 1.6.0.20; National Institute of Health, US). The following formula was used to calculate the rate of wound closure:

$$\% \text{ wound closure} = \frac{\text{wound size day 0} - \text{wound size day (n)}}{\text{wound size day 0}} \text{ x100}$$

n=numbers of days (seven, 14 and 21).

Statistical analysis:

Descriptive statistics including the median, mean, standard error (SE), minimum and maximum were calculated for all variables [51]

CHAPTER 5

RESULTS & DISCUSSIONS

5.1RESULTS:

From the oil extraction process, the results are as follows:

Batches:

I) first batch of distillation and results:

Quantity of aniseed = 150 gm

Water quantity = 500 ml

Temperature = 70^{o}C

Oil recovery = 2 ml

2) Second batch of distillation

Quantity of aniseed = 150 gm

Water quantity = 400 ml

Oil recovery = 3 ml

3) Third batch of distillation

Quantity of aniseed = 250 g

Water quantity = 300 ml

Oil recovery = 2 ml

4) Fourth batch of distillation

Quantity of aniseed = 350 gm

Water quantity= 350 ml

Oil recovery= 4 ml

The color of Anise oil was measured using spectrophotometer. The Saponification value of oil is determined by laboratory method using HCl, KOH chemical. Acid value determines free fatty acid content in oil by laboratory method. Refractive Index was measured by using Refractometer at 25^{o}C.

Since the experiment of wound healing was not performed, a literature review on "Wound healing activity of *Pimpinella anisum* methanolic extract in streptozotocin-inducedWistar Albino rats"by**Mohammad Hashemnia, Zahra Nikousefat, Adel Mohammadalipour, Mohammad-Mahdi Zangeneh,Akram Zangeneh**, from JOURNAL OF WOUND CARESILK ROAD SUPPLEMENT VOL 28, NO 10, OCTOBER 2019, was referred for the observation and discussion part.

5.2GENERAL OBSERVATION:

A total of 18 rats were used in the study, with eachgroup containing 6 rats. At day seven post-injury, thewounds exhibited the formation of a scar coveringthick granulation tissue in all rats. However, treatmentwith *Pimpinella anisum*(which was prepared in lab) produced more scar tissue thanother groups when examined macroscopically.

Nore-epithelialisation was evident in the control, *Pimpinella anisum*standardgroups, while rats in the*Pimpinella anisum*10% (prepared in lab) treatment group indicated minimalre-epithelialisation. At this stage, less cellularity,perivascular oedema and fibrin deposition and morecollagen fibres were observed in Pimpinella anisumtreatedwounds compared with the other groups.

At day 14 post-injury, a more organised pattern inthe collagen fibres and better tissue alignment wereseen in the *Pimpinella anisum*treated group comparedwith the other groups. In all groups, although theepidermis was disorganised and thick, particularlywhen compared with the adjacent normal skin, but itssize was decreased and its alignment was better in the*Pimpinella anisum*treated lesions in comparison withthe *Pimpinella anisum* standard groups and control lesions. At this stage,evidence of pus accumulation, fibrin deposition,polymorphonuclear cells infiltration or oedema werenot observed in the lesions of animals in all groups.

At day 21 post-injury, the wounds in groups weretotally closed in the *Pimpinella anisum*treated lesions.The size of scar tissue was also smaller and the collagenfibre alignment was better than in those of the *Pimpinella anisum* standard groups and control group lesions. Additionally, thenumber of lymphocytes and macrophages wasdecreased in the Pimpinella anisum and tetracyclinetreated groups, and considerably greater tissuematuration and large capillary-sized blood vessels wereobserved compared with the other groups. [52]

5.3 QUANTITATIVE ANALYSIS:

At days seven and 14 post-injury, the diabetic rats inthe control group displayed a significant delay inwound healing compared with those treated with*Pimpinella anisum* ($p<0.05$).

At day 21 post-injury, theclosure of the wounds treated with *Pimpinella anisum* (94.07±1.21%) was significantly faster thanthose of the control (77.19±2.97%)and *Pimpinella anisum* standard group (83.24±2.53%) groups($p<0.05$).

The wounds were almost closed or greatlyimproved (between 98–100%) in two rats in the*Pimpinella anisum* group, while the wounds were notclosed in other groups. [52]

5.4 DISCUSSION:

Cutaneous wounds are diverse, multifactorial, complex and persistent in patients. Several mechanisms can cause cutaneous wounds, such as poor blood flow and oxygen release due to increased blood sugar. This condition is believed to be caused byimpaired blood flow and oxygen release from increased blood sugar, decreased collagen and fibronectin synthesis from protein malnutrition, impaired local immune and cell defences, and decreased anabolic activity with decreased insulin and growth hormone.

The study of wound healing medicines for patients and the quest for better, more effective remedies is possibly one of the main challenges for investigators. The enormous costs of modern medicines lead the researchers to seek alternative strategies for the better management of wounds in their patients with diabetes. Medicinal plants are common remedies used by people in many countries. [53]

Due to its reported hypoglycaemic, anti-inflammatory and antioxidant effects of *Pimpinella*

anisum, and also its anti-ulcer activity and healing properties, we hypothesised that this plant could be used as a compound to accelerate wound healing process.

The results of this study indicate that topical application of *Pimpinella anisum* improved the quality of wound contraction, re-epithelialisation and scar formation in Wistar albino rats in the short-term.

Over a longer time-period, topical application of this compound appeared to decrease total cellularity, improve maturation of fibroblast, increase fibroblast differentiation rate in lesion and ultimately reduce scar tissue size. [54]

A main result of this experiment was the clear difference in wound contraction rate and re-epithelialisation rate between the *Pimpinella anisum* group and the other groups.

Wound contraction is the procedure of mobilising normal skin around the wound to cover the denuded area and contains complex and coordinated interactions of cells, extracellular matrix (ECM) and cytokines.

The higher rate of wound contraction and decrease in healing time in the wounds treated with *Pimpinella anisum* may be due to the plant's anti-inflammatory effects and its effect on the maturation and organisation of the granulation tissue. In this regard, recent studies have established that phytochemical constituents such as flavonoids can stimulate wound contraction and increase the rate of re-epithelialisation. Furthermore, the high level of trans-anethole composition with proven wound healing activity may represent the restorative effect of this compound. [56]

In these wounds, high intracellular reactive oxygen species (ROS) generation comes from hyperglycaemia via activation of protein kinase C (PKC) signalling which suppresses the antioxidant system, similar to glutathione peroxidase. In this current study, imbalance of redox potential was observed by a significantly lower total antioxidant capacity level and glutathione peroxidase activity, and a higher MDA concentration in wound area.

Interestingly, oxidative stress appeared to be ameliorated by the *Pimpinella anisum* throughsignificant reversing of the previously mentioned oxidant/antioxidant parameters, particularly in the later phases of the study. It has been reported that ROS intervenes in proliferation of fibroblasts and keratinocytes in the later stages of wound healing. Therefore, the findings of our study confirm the restorative effect of *Pimpinella anisum* with an

antioxidant mechanism. Similarly, its antioxidant properties against CCl_4 and gentamycin-induced oxidative stress have been previously reported. [55]

Based on histopathological findings, granulation tissue formation appeared to be accelerated by applying *Pimpinella anisum*. The ability to accelerate wound healing was very prominent, especially when these observations were compared with other groups. Moreover, the increase in weight of dry granulation tissue in the *Pimpinella anisum*-treated rats suggested a higher content of protein at the wound site and ability to remodel the wound.

Generally, fibroblasts, collagen and new small blood vessels are the most important components of granulation tissue formed in the final part of the proliferative phase. Our results indicate that there is a positive correlation between the fibroblast count, number of blood vessels and collagen content.

In granulation tissue formation, the adherent cells, defined as fibroblasts, have a capacity to synthesise a new, impermanent ECM by excreting collagen and fibronectin. In the granulation tissue, collagen is the principal extracellular protein that is produced immediately after injury in the wound area. Furthermore, collagen plays an important role in providing strength and integrity to a tissue matrix, as well as in haemostasis. Better collagen maturity and alignment in this present study in wounds treated with *Pimpinella anisum* showed its novel healing effect.

Proliferation, maturation and migration of the epithelial cells and fibroblasts are dependent on an adequate oxygen supply. Therefore, vasculature in the wound area could supply nutrients and oxygen to the cells, enhance fibroblast maturation and support the production of considerably higher amounts of collagen. In addition, better healing in the wound area may also result from a better blood flow due to enhanced smooth muscle relaxation, the muscle relaxant properties of *Pimpinella anisum* have been previously reported. [58]

The presence of a large number of blood vessels in the *Pimpinella anisum*-treated wounds at the earlier stages of wound healing, indicate that the *Pimpinella anisum* is an angiogenic agent that enabled proper circulation of red blood cells for wound healing. In addition, a higher content of hydroxyproline, a specific marker of collagen, in the *Pimpinella anisum*-treated wounds indicate that *Pimpinella anisum* can accelerate production of collagen by fibroblasts and also their release into the ECM.

The wounds treated with *Pimpinella anisum* also showed a decreased total cellularity together

with smaller macrophage and lymphocyte counts in days seven, 14 and 21 post-injury, while the presence of higher levels of macrophages and lymphocytes in the control group, even at the end of experiment, indicate the presence of chronic inflammation in these lesions.

The inflammatory phase is characterised by inflammation and haemostasis, followed by angiogenesis, collagen deposition and re-epithelialisation in the proliferative phase.8,9 Inflammatory cells, particularly macrophages, play an important role in facilitating fibroplasia during the inflammation phase. Several cytokines, pro-inflammatory mediators and growth factors are released by the macrophages that accelerate mesenchymal cell migration, proliferation, maturation and matrix production. [59]

The lower levels of inflammation in the *Pimpinella anisum*-treated group can be due to the presence of some phytoconstituents, including antioxidants and flavonoids such as isovitexin and epigenin, in this plant species which inhibited the activities of macrophages and production of chemical mediators by inhibiting NF-kappaB as mentioned before.Accordingly, in our study, *Pimpinella anisum* appeared to decrease inflammation and subsequently promote tissue organisation.

Limitations:

The higher rate of the wound healing in rats, small population size, and the anatomical and physiological variations between rats and humans should be considered as limiting factors.

Plants have been the origins of many drugs from the ancienttimes. Pharmacological evaluations of plants may be due tonew natural agents for treatment of disease. Furthermore,identification of the active principles of these medicinalplants has an important role in introducing new drugs.*Pimpinella anisum* is one of the medicinal plants whichhave been used for different purposes in traditionalmedicineof Iran. So far, different studies were performed on theextracts and essential oil of *Pimpinella anisum* to identifythe chemical compounds and pharmacological propertiesof this plant, and various properties such as antimicrobial,antifungal, antiviral, antioxidant, and insecticidal effectshave been reported of aniseeds.

The findings also revealedthat aniseeds can cause gastric protection, muscle relaxant,and affect digestive system. In diabetic patients, it hashypoglycemic and hypolipidemic effects and reduces lipidperoxidation. Furthermore, aniseeds showed anticonvulsanteffect, reduced morphine dependence, and induced conditionedplace aversion in mice. Aniseed also has

beneficialeffects on dysmenorrhea and menopausal hot flashesin women. The most important compounds of aniseedsessential oil were *trans*-anethole, estragole, *γ*-hymachalen, *p*anisaldehyde,and methyl chavicol. Due to broad spectrumof pharmacological effects of this plant, and very few clinicalstudies performed on this plant, more clinical trials arerecommended to evaluate the beneficial effects of *Pimpinellaanisum* in human models and identification of active compoundsof this plant which can lead to synthesis of new drugsfrom the active ingredients in future. [60]

CHAPTER 6
CONCLUSION

6. CONCLUSION:

This present study established topical application of *Pimpinella anisum* can stimulate wound healing activity in Wistar albino rats. It was also observed that in the group treated with *Pimpinella anisum*, wound size, inflammation and cellularity were significantly reduced, and the re-epithelialisation rate, collagen content, fibroblastic response were enhanced.

More scar tissue in the early stages of wound healing was also produced.

In the later stages, *Pimpinella anisum* significantly decreased oxidative stress, scar tissue formation, and improved tissue maturity and remodelling. The wound-healing activity of *Pimpinella anisum* may be due to the individual or the additive effects of phytoconstituents present in the plant.

In this study, the effect of the phytoconstituents of *Pimpinella anisum* were not investigated. More phytochemical studies are needed to characterise and identify the specific active compounds of this plant that are responsible for wound healing activity.

Even the exact mechanisms and modes of action of thePAP extract described in this work have not yet been fully determined; its wound healingeffects could presumably be attributed to their bioactive molecules and their associated antiinflammatoryand antioxidant activities. Further investigations are warranted to investigatetheir structure and thus to elucidate a clear structure–effect relationship.

CHAPTER 7
REFERENCES

7. REFERENCES:

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