Introduction

INTRODUCTION

Plants have been an integral part of traditional medicine all over the world since time immemorial. Although synthetic drugs are essential for current medical practice, plants provide major contribution to pharmaceutical industry [1]. However, due to nonregulated collections, many species have become threatened or endangered [2]. So, the current study will focus on production of good quality beneficial secondary metabolites and to determine some of their medicinal effects.

The current work includes two important members of medicinal aromatic plants, *Ocimum basilicum* L. (sweet basil) of family Lamiaceae (Labiatae) and *Pimpinella anisum* L. (anise) of family Umbelliferae (Apiaceae).

Family Lamiaceae (Labiateae)

Lamiaceae, also known as the mint family, consisting of 180 genera and 3500 species. It is widely distributed in the world, but the chief centre is the Mediterranean region.The family is characterized by terrestrial herbs or under shrubs. Leaves are decussate, simple, exstipulate, often hairy and with epidermal glands secreting volatile oil. Inflorescence is cymose or racemose. Flowers are bisexual, irregular. Calyx is formed of 5 sepals, united into a campanulate or a funnel-shaped tube, sometimes 2-lipped persistent in fruit. Corolla constitutes 5 united petals, usually 2-lipped. Stamens usually four, didynamous and epipetalous, with introrse anthers. Ovary superior, of two united carpels, on a nectar-secreting disc [3] .

Ocimum basilicum **L. (*O. basilicum* L.)**

The genus *Ocimum* contains between 50 to150 species of herbs and shrubs found in the tropical regions of Asia, Africa, Central and South America [4,5]. Basil is an annual herb with erect branched quadrangular stem arising to the height of 30 to 60 cm. Bearing opposite, ovate entire to toothed leaves which are smooth above and hairy on the veins beneath. Flowers are small, white sometimes tinged purple with an unequally toothed calyx and two-lipped corolla. Fruit is a sub-globose nutlet [6].

Family Umbellifereae (Apiaceae)

Apiaceae, also called parsely or carrot family, consisting of 400 genera and 3500 species worldwide [7]. It is widely distributed in the world, but the chief centre is temperate region.The family is characterized by herbs with stout stems, hollow internodes, and alternate, exstipulate, sheathing leaves with their blades much divided pinnately. Only few members have entire leaves. Inflorescence usually a compound umbel (often subtended by an involucre of bracts) formed from partial umbels (each one often subtended by an involucre of bracteoles termed an involucel) sometimes simple umbel, or cymose head. Flowers usually bisexual, regular and epigenous. Calyx is formed of 5 free or united usually very small sepals. Corolla constitutes 5 free petals, usually white or yellow, rarely absent. Stamens are five, with introrse anthers. Ovary inferior, composed of two united carpels, bilocular, with one pendulous, anatropous ovule in each loculus; epigenous disc present prolonged into two short styles [3].

Pimpinella anisum L. (*P. anisum* L.)

Anise originated in the eastern Mediterranean region and is native to Asia Minor, Greece and Egypt. Principal anise-growing regions are Spain, the Soviet Union, France and North Africa as well as some parts of Germany [8]. It is an annual herb, with erect, cylindrical, striated, smooth stem, arising to the height of 50 cm. The leaves are alternate below, opposite above, the lower being long petioled, ovate, upper with short petioles, pinnatifid or ternately pinnate, with long entire or cut segments. The inflorescence is a long stalked 8 to 14 rayed, compound umbel, with small flowers, white and each with a long hairy pedicel, the fruit is a pubescent cremocarp [9].

Owing to the new attraction for natural products like essential oils, despite their wide use and being familiar to us as fragrances, it is important to develop a better understanding of their biological action for new applications in human health, agriculture and the environment. Some of them constitute effective alternatives or complements to synthetic compounds of the chemical industry, without showing the same secondary effects [10].

Since the current study includes two plants producing essential oils of high medicinal and biological value, follows will be a list of some information about these essential oils.

Essential oil of *O. basilicum* L.

O. basilicum is an aromatic herb that has been used traditionally as medicinal herb in the treatment of headaches, coughs, diarrhea, constipation, warts, worms and kidney malfunctions [11]. It is also a source of aroma compounds and essential oil containing biologically active constituents that possess insecticidal [12], nematicidal[13], fungistatic [14] and antimicrobial properties[15].

Intensive investigations around the world have revealed that the concentrations of the main active substances of *O. basilicum* oil which are estragole, linalool, 1, 8-cineol, camphor, eugenol, and geraniol vary among basil chemotypes [16-18].

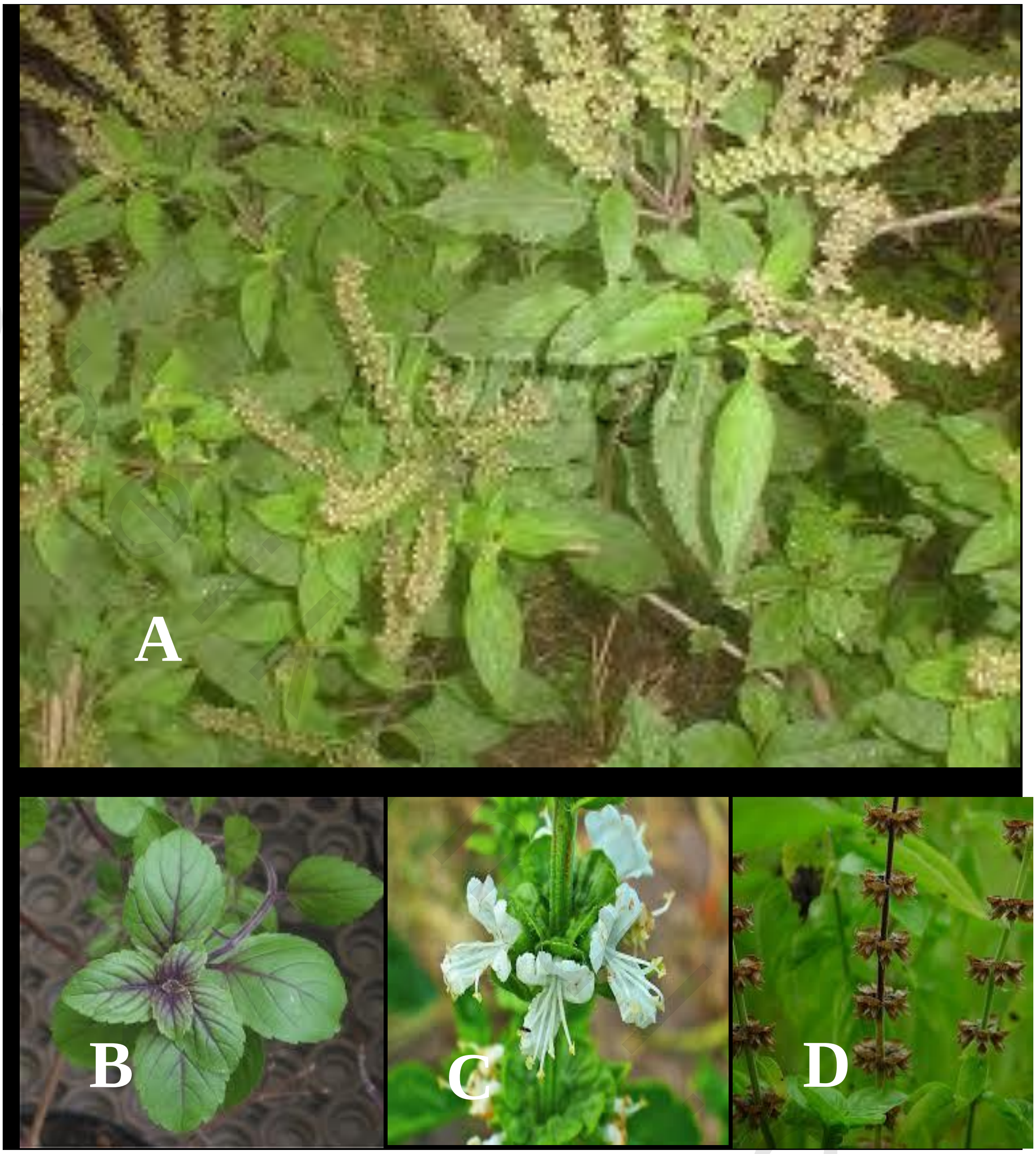


Figure 1. ***Ocimum basilicum*** **L.**

A. Whole herb.
B. Leaves.
C. Flowers.
D. Fruits.

Figure 2. ***Pimpinella anisum*** **L.**

A. Whole herb.
B. Leaves.
C. Flowers.
D. Fruits.

Table I . Chemical composition of *O. basilicum* L. essential oil in Egypt using GC/MS [19].

Retention Time (t_R) (min)	Components	Relative Percentage
5.22	α-Pinene	0.69
5.59	Camphene	0.88
6.31	β-Pinene	1.11
6.68	β-myrcene	0.82
7.89	1,8-Cineol	13.65
8.29	trans- β Ocimene	1.61
8.59	γ-Terpin	1.81
11.38	Linalool	44.18
12.55	Camphor	0.67
12.71	Myrtenol	1.33
16.08	α-Cubebene	4.97
19.23	Eugenol	8.59
19.57	Methyl cinnamate	4.26
19.99	Iso-caryophyllene	3.10
20.9	α-caryophyllene	1.75
21.8	Azulene	1.31
22.9	α-Farnesene	1.70
23.5	Germacrene B	1.62
23.9	GermacreneD	0.82
24.6	Naphthalene	2.01
	Compounds not identified	3.12

Table II. Chemical structure of compounds identified in *O. basilicum* L. essential oil .

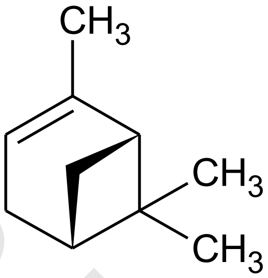
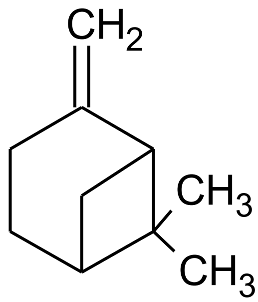
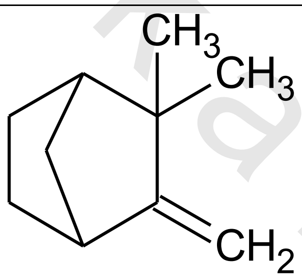
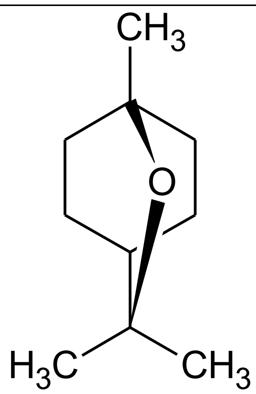
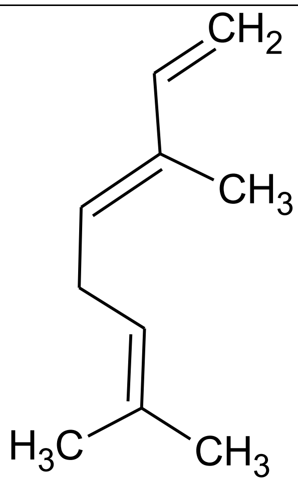
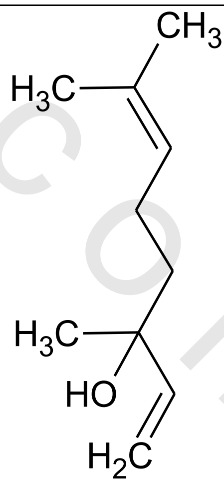
Compound name	Chemical structure	Compound name	Chemical structure
α-Pinene		**β-Pinene**	
Camphene		**1, 8-Cineol**	
***Trans* β-ocimene**		**Linalool**	

Table II. Continued

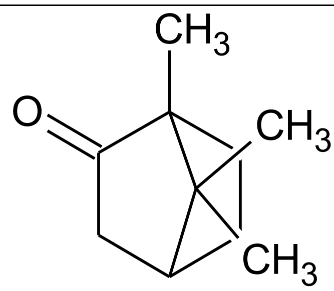
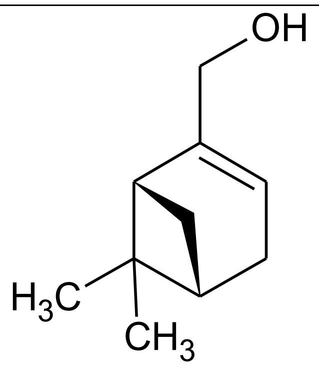
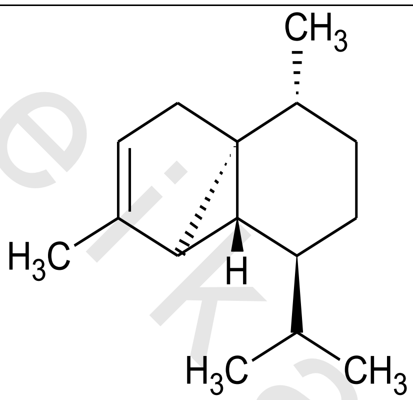
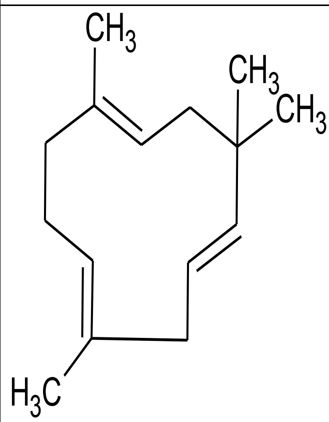
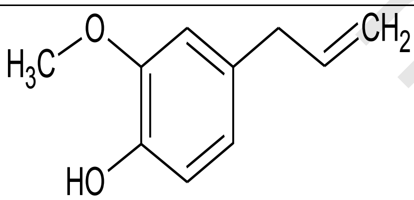
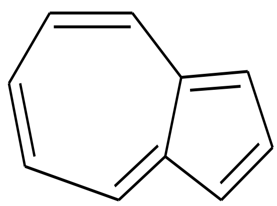
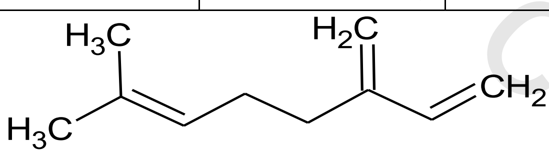
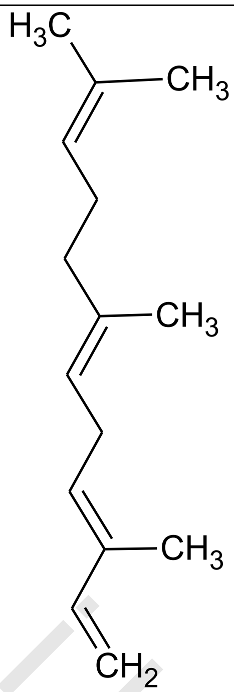
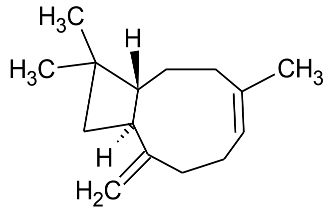
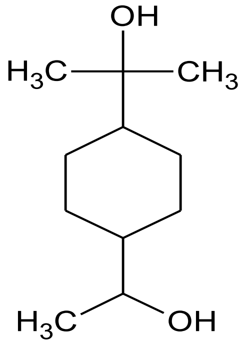
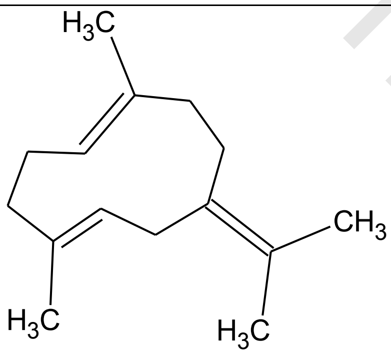
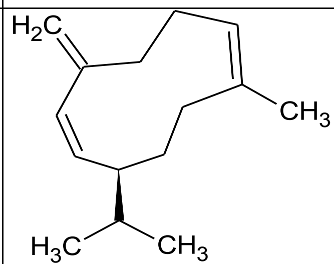
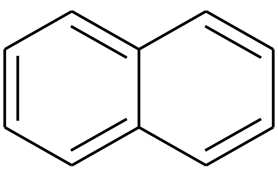
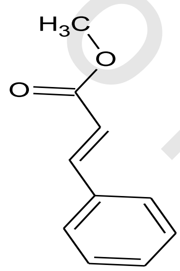
Camphor		**Myrtenol**	
α-Cubebene		**α-Caryophyllene**	
Eugenol		**Azulene**	
β-Myrecene			

Table II. Continued

α-Farnesene		**Iso-caryophyllene**	
		γ -Terpin	
Germacene B		**Germacene D**	
Naphthalene		**Methyl cinnamate**	

Biological and pharmacological effects of *O. basilicum* L. and its essential oil

Central nervous system effects

The essential oil of *O. basilicum* has inhibited convulsions induced by strychnine at higher doses, close to those associated with motor impairment. Thus, the anticonvulsant activity of *O. basilicum* essential oil is close to that of members of barbiturates or benzodiazepines, which possess multiple mechanisms of action and display broad anticonvulsant activity [20, 21].

GC/MS analysis showed that most of the essential oil was composed of three main terpenes including linalool, 1, 8-cineol, and eugenol. It has been reported that terpenes have a protective effect against pentylenetetrazole and picrotoxin-induced convulsions [22]. Modulation of glutamergic and GABAergic transmission are mechanisms indicated for anticonvulsant action of monoterpenes [23]. Linalool (44.18%) as the most abundant constituent of *O. basilicum* essential oil was found to have anticonvulsant activity against pentylenetetrazole-induced convulsion [24]. Anticonvulsant activity of linalool is through inhibition of glutamergic transmission [25] and suppression of voltage-gated currents [26]. Additionally, it has been reported that linalool has a locomotor inhibitory activity as well as hypnotic action [27]. Cineole constitutes (13.65%) the second major terpene present in the essential oil of *O. basilicum*. It has been reported that 1,8-cineole exerts anticonvulsant activity, potentiates phenobarbitone sleeping time, and has an inhibitory effect on loco-motor activity[28]. Eugenol comprised 8.59% of *O. basilicum* essential oil. Eugenol is reported to exert an anticonvulsant effect in mice [29]. Eugenol exerts anticonvulsant activity through potentiating the binding of GABA to its receptor by increasing the affinity of these receptors to bind GABA [30]. Additionally, it has been reported that eugenol has anesthetic, sedative, and muscle relaxant effects [31, 32].

Antioxidant activity

There have been great efforts to find safe and potent natural antioxidants from various plant sources. As harmless sources of antioxidants, wild herbs, spices, fruits, nuts, and leafy vegetables have been investigated, for their antioxidant properties [33]. *O. basilicum* contains high levels of phenolic acids that contribute to its strong antioxidant capacity and the substantial concentrations of rosmarinic acid, in particular, have been associated with the herb's medicinal qualities [34]. Rosmarinic acid is noted in the literature to be the most prevalent basil phenol component [35]. However, other caffeic acid derivatives, such as chicoric acid, have been also found in high concentrations [36]. The antioxidant activity of ethanol extract of *O. basilicum* was investigated by electrochemical measurements [37].

Anti microbial, antifungal and insect repelling activities

Production of essential oils by plants is believed to be predominantly a defense mechanism against pathogens and pests [38], and indeed, essential oils have been shown to possess antimicrobial and fungicidal properties.

Ethanol, methanol, and hexane extracts from *O. basilicum* were investigated for their *in vitro* antimicrobial properties. A total of 146 microbial organisms belonging to 55 bacteria, and four fungi, and a yeast species were studied using a disk-diffusion and minimal inhibition concentration (MIC) method. The result showed that none of the three extracts tested have antifungal activities, but anticandidal and antibacterial effects. Both the hexane and methanol extracts, but not the ethanol extracts, inhibited three isolates out of 23 strains of *Candida albicans* studied. All three extract of *O. basilicum* were different in terms of their antibacterial activities [39].

The activity of *O. basilicum* against multidrug resistant clinical isolates from the genera *Staphylococcus*, *Enterococcus* and *Pseudomonas* has been reported [40]. The essential oil of *O. basilicum* 22 ml/l completely inhibited the mycelial growth of species of fungi, including the mycotoxin-producing strains of at a dose of 1.5 *Aspergillus flavus* and *Aspergillus parasiticus*. Additionally it has been proved to repel the insect *Allacophora foveicollis*. The toxic dose of the oil against the test fungi was much lower than that of some commercial fungicides and fumigants, and it remains unaffected by temperature treatment, storage, and increased inoculums [41].

Nematicidal activity

Bioassay tests proved that essential oil of *O. basilicum* has a nematicidal activity against *Meloidogyne incognita* [42] .

Antiinflammatory effect

Investigation of antiinflammatory activity of crude extracts of *O. basilicum* using peripheral blood mononuclear cells (PBMC) of healthy individuals showed a good inhibitory effect on the proliferative response of PBMC in mitogenic lymphocyte proliferation assays. Furthermore, gene expression studies on lipopolysaccharide (LPS) induced production of proinflammatory cytokines like Tumor necrosis factor (TNF-42), Interleukin-1B (IL-I/3) and IL-2 showed down regulation of the markers. Furthermore, it suppressed the induction of inducible nitric oxide synthase (iNOS) and the subsequent production of nitric oxide (NO) in LPS-stimulated RAW 264.7 macrophages in a time-dependent manner. Results showed that *O. basilicum* crude methanolic extract inhibits the key proinflammatory cytokines and mediators, which accounts for its antiinflammatory effect [43].

Anticancer activity

The use of dietary antimutagens and anticarcinogens has been seen as a promising approach to the protection of human health. *O. basilicum* as a well-known medicinal and aromatic plant, with a range of newly discovered biological activities possibly important for chemoprevention. In the preliminary experiments, toxic and mutagenic potential of essential oil (EO) from *O. basilicum* and pure substances: linalool, ß-myrcene and 1,8-cineole were tested using *Salmonella typhimurium* TA98, TA100 and TA102, with and without S9 mix (microsomal fraction of rat liver). No mutagenic effect of *O. basilicum* derivatives was detected in any tested strain. Antimutagenic effects of essential oil from *O .basilicum* and its pure constituents were further evaluated in the Ames test using *S. typhimurium* TA100. UVC irradiation and three chemical mutagens, 4-nitroquinoline-N-oxide (4NQO), 2-nitropropane (2-NP) and benzo(a)pyrene were used to induce mutagenesis. All tested *O. basilicum* derivatives significantly reduced UV-induced mutations. The maximum inhibition was in the range of 64–77 %. Inhibitory potential against direct acting model mutagen/carcinogen 4NQO was similar to UV (52–67 %). In the presence of S9, EO and 1,8-cineole showed moderate inhibition of 2-NP induced mutagenesis, while the remaining two substances had no effect. Linalool exhibited high co-mutagenic effect with benzo(a)pyrene, 1,8-cineole showed moderate inhibitory effect against benzo(a)pyrene -induced mutations, while EO and ß-myrcene were ineffective [44].

The anticancer activity of *O. basilicum* extract and its fractions was evaluated using human cancer cell lines; active compound(s) residing in it were identified and mechanism of their anti-proliferative action was explored. Methanolic extract was fractionated into petroleum ether soluble (PE-S) and insoluble (PE-I) fractions. These were evaluated on HT-144, MCF-7, NCI-H460 and SF-268 cell lines using Sulforhodamine B assay. Immunofluorescence microscopy was employed to study their effects on the cytoskeleton and nuclei of MCF-7 cells. Fractionation of PE-I (GI50: 5 μg/ml; LC50: 71μg/ml against MCF-7) led to the isolation of four compounds, mainly ursolic acid (LC50: 18.6 μg/ml). Ursolic acid (100 μM) induced a significant decrease in the percentage of cells in anaphase/ telophase stages along with F-actin aggregation and mitotic spindle distortion. These results support anti-proliferative activity of *O. basilicum* extract against MCF-7 cells which may partly be due to effects of ursolic acid on F-actin and microtubules [45].

Essential oil of *P. anisum* L.

In the middle Ages, it was taken as a medicine for cough and cancer, as well as for cases of snake and scorpion bites, mental diseases and epilepsy; it was even used as a diuretic. The first legal certification of anise oil dates back to the beginning of the 16^{th} century [46].

P. anisum fruit and its essential oil are used as a spicy seasoning, as a flavor additive in the field of oral hygiene (in toothpastes and gargles), in the confectionery industry and for the production of alcoholic beverages, such as herb liqueur or anise brandy [47,48]. In medicine, the carminative, spasmolytic and expectorant effects of the drug and oil are of interest [49]. Moreover, the plant's importance is shown nowadays in its adaptation into essential European pharmacopeias: DAB 8 (FRG), DAB 7 (GDR), ÖAB 9 (Austria), Helv VI (Switzerland) and Ph. Eur. III (Europe).

The minimum content of essential oil according to the pharmacopeias is 2% (Ph. Eur. III), and the maximal yields are gained from the ripe central umbels [50].

Study of the essential oil of *P. anisum* 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)-methyleugenol, α-cuparene, α-himachalene, *β*-bisabolene, *p*-anisaldehyde, and *cis*-anethole [51].

Table III. Chemical composition of *P. anisum* L. essential oil using GC/MS [52] .

Retention time (t_R) (min)	Component	Relative percentage (%)
4.78	α-pinene	0.12
5.91	α-Phellandrene	0.19
6.5	Limonene	0.28
8.03	Linalool	2.26
8.25	Fenchone	2.44
8.74	Camphor	0.86
10.54	Carvone	0.24
10.75	Estragole	1.67
10.82	Cis- Anethole	0.15
11.11	Trans –Anethole	82.74
11.38	Anisyl acetone	5.2
11.40	Dihydrocarvyl-acetate	0.34
11.8 5	p-Anisic acid	1.8
11.91	Eugenol	0.5
12.24	β-Caryophyllene	0.27

Table IV. Chemical structure of compounds identified in *P. anisum* L. essential oil.

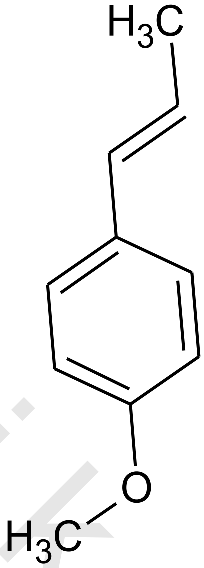
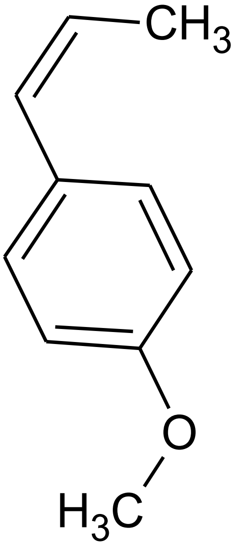
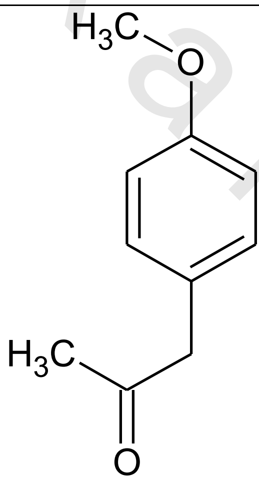
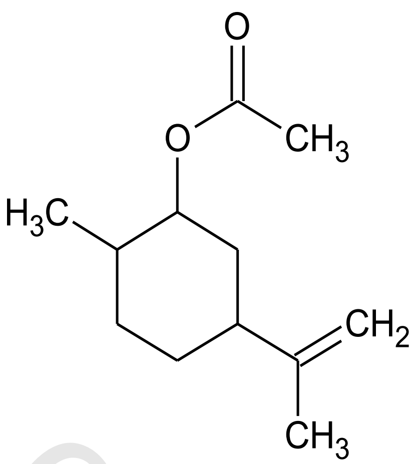
Compound name	Chemical structure	Compound name	Chemical structure
Trans-anethole		**Cis-anethole**	
Anisyl acetone		**Dihydrocarvyl-acetate**	

Table IV. Continued

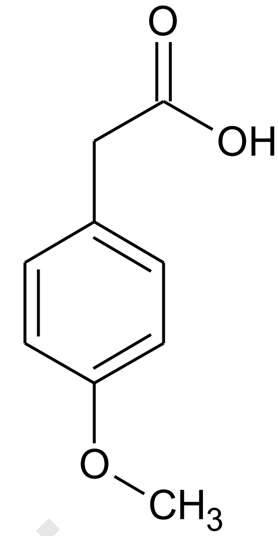
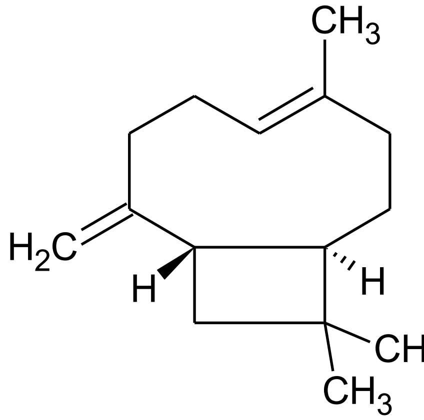
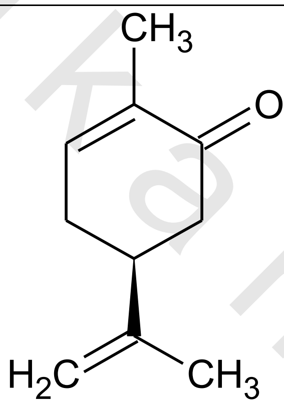
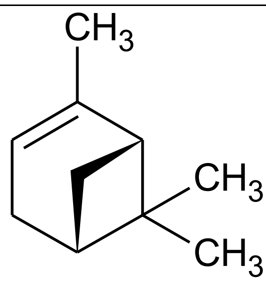
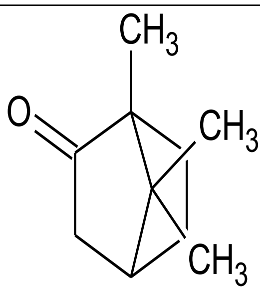
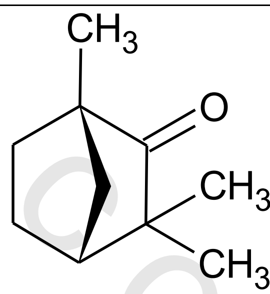
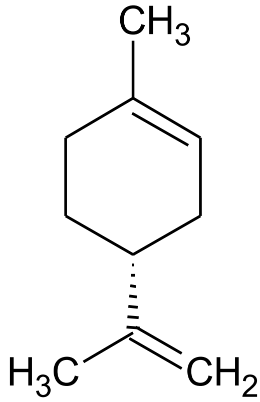
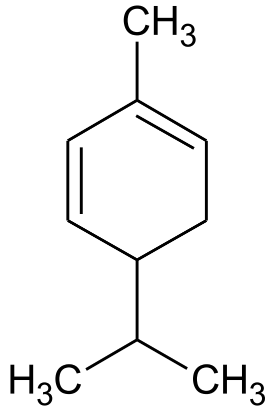
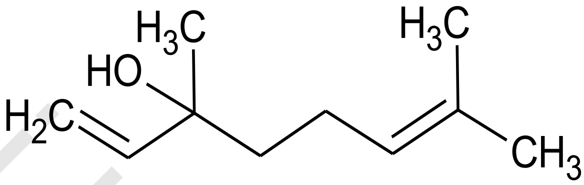
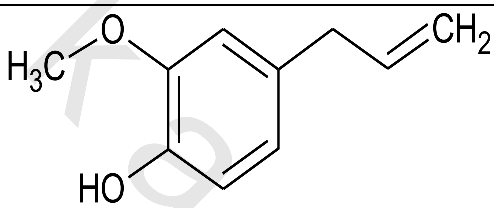
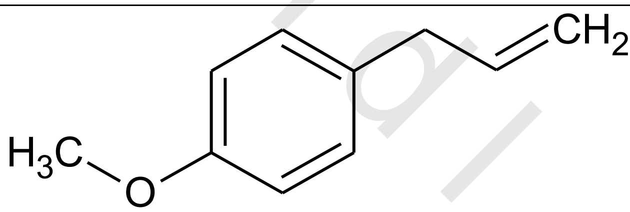
p-Anisic acid		**β-Caryophyllene**	
Carvon		**α-Pinene**	
Camphor		**Fenchone**	

Table IV. Continued

Limonene		**α-Phellandrene**	
Linalool			
Eugenol			
Estragole			

Biological and pharmacological effects of *P. anisum* L. and its essential oil

Antibacterial effect

The antibacterial activities of the aqueous, 50% (v/v) methanol, acetone and petroleum ether extracts of *P. anisum* fruits were tested against 4 pathogenic bacteria (*Staphylococcus aureus, S. pyogenes, Escherchia coli, and Klebsiella pneumoniae*) by disc diffusion method. The results showed that only aqueous and methanol extracts exhibited fair antibacterial activity against all of the test bacteria and the aqueous extract was found to be more effective than methanolic extract, whereas acetone and petroleum ether extracts cannot inhibit the growth of the pathogenic test bacteria [52]. Furthermore, alcoholic extracts of *P. anisum* seeds also showed antibacterial activity against *Micrococcus luteus* and *Mycobacterium smegmatis* [53].

Antifungal effect

Essential oil of *P. anisum* showed significant inhibitory activity against fungi, and anethol was its most active component , antifungal activities of fluid extract and essential oil of *P. anisum* fruits studied on seven species of yeasts and four species of dermatophytes by the means of diffusion method with cylinders and the broth dilution method. The findings revealed that the fluid extract of *P. anisum* showed antimycotic activity against *Candida albicans*, *C. parapsilosis*, *C. tropicalis*, *C. pseudotropicalis*, and *C. krusei*. Additionally, it showed inhibitory effect against dermatophyte species (*Trichophyton rubrum, T.mentagrophytes*, *Microsporum canis*, and *M. gypseum*). The essential oil of *P. anisum* exhibited strong antifungal activity against yeasts and dermatophytes. In that study, *P. anisum* essential oil exhibited stronger antifungal activities rather than its extract against yeasts and dermatophytes [55]. Antifungal activity of *P.anisum* essential oil also was reported against *Aternaria alternata*, *Aspergillus niger*, and *Aspergillus parasiticus*, and the most effected fungus was *A. parasiticus* [56].

Muscle relaxant effect

Antispasmodic and relaxant effects of three hydroalcoholic extracts of the aerial parts of *P. anisum* (ethanol : water; 40 : 60, 60 : 40, and 80 : 20) were investigated, the entire three hydroalcoholic extracts attenuated acetylcholine-induced contraction. Studying the possible mechanisms underlying the relaxant effect showed that this effect is mainly dependent on the activation of the NO-cGMP pathway [57].

Anticonvulsant effect

Anticonvulsant effects of an essential oil of the fruits of *P. anisum* were studied against seizures induced by pentylenetetrazole (PTZ) or maximal electroshock (MES) in male mice. This study revealed that *P. anisum* increases the threshold of clonic seizures induced by I.V. infusion of PTZ, and it can also block tonic convulsions induced by I.P. injection of PTZ. Moreover, *P. anisum* possesses anticonvulsant activity against tonic seizures induced by MES [58] .

Effect on gastrointestinal system

Effect on gastric ulcer

In studying the effect of aqueous suspension of *P. anisum* against gastric ulcers, results showed that *P. anisum* significantly inhibited gastric mucosal damage induced by necrotizing agents and indomethacin. The antiulcer effect was further confirmed histologically [59].

Effect on constipation

The laxative efficacy of a phytotherapic compound containing *P. anisum* L., *Foeniculum vulgare* Miller, *Sambucus nigra* L., and *Cassia augustifolia* was studied in a randomized clinical trial; results revealed that this compound can be a safe alternative option for the treatment of constipation [60].

Effect on morphine dependence

Studies revealed that 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 [61] .

Analgesic and antiinflammatory effect

Essential oil of *P. anisum* showed significant analgesic effect similar to morphine and aspirin [62]. Additionally fixed oil of *P. anisum* was investigated for anti-inflammatory and analgesic activity in mice. The finding showed that the fixed oil of *P. anisum* has anti-inflammatory effect as strong as indomethacin and it showed analgesic effect comparable to that of 100 mg/kg aspirin and 10 mg/kg morphine at the 30^{th} min [63].

Effect on menopausal hot flashes

In a study, consumption of 3 capsules of *P. anisum* extract (each capsule contains 100 mg of extract) for 4 weeks leads to significant reduction in hot flash frequency and intensity in postmenopausal women [64].

Effect on dysmenorrheal

The effectiveness of a herbal capsule containing dried extracts of celery, saffron, and *P. anisum* was compared with mefenamic acid capsule. The results showed significant reduction in pain intensity in both herbal and mefenamic acid group comparing to placebo group. Furthermore, the results revealed that the effectiveness of herbal capsule was better than mefenamic acid in pain relief and can be a suitable alternative in primary dysmenorrhea [65].

Antioxidant activity

Investigation of *in vitro* and *in vivo* antioxidant potential of aniseeds showed that ethanolic extract of *P. anisum* displayed scavenging activity against nitric oxide, superoxide and 1,1-diphenyl, 2-picryl hydrazyl radicals and reducing power in a concentration-dependent manner [66].

The antioxidant potential of essential oil and oleoresins from *P. anisum* seeds was studied. The antioxidant activities were assessed by inhibition of linoleic acid peroxidation, 1, 1-diphenyl-2-picrylhydrazyl radical scavenging, Fe^{3+} reducing power, and various lipid peroxidation assays. The findings showed that *P. anisum* oil and its methanol oleoresin posses the highest antioxidant activity, even higher than butylated hydroxyanisole and butylated hydroxytoluene. However, the antioxidant activities of other oleoresins were somewhat lower [67].

Insecticidal effects

P. anisum essential oil showed repellency against mosquito *Culex pipiens* [68]. However, the exposure to vapours of essential oils from *P. anisum* and cumin resulted in 100% mortality of the eggs of two stored-product insects (the confused flour beetle, *Tribolium confusum*, and the Mediterranean flour moth, *Ephestia kuehniella*) [69] .

The acaricidal activity of *p*-anisaldehyde derived from *P. anisum* seed oil and commercially available components of *P. anisum* seed oil were studied against the house dust mites, *Dermatophagoides farina,* and *D. pteronyssinus*. The results showed that, the most toxic compound of these compounds was *p*-anisaldehyde followed by benzyl benzoate and, therefore, *p*-anisaldehyde may be useful as a lead compound for the selective control of house dust mites [70].

Antiviral effects

The effects of the essential oil of *P. anisum* was examined against PVX (potato virus X), TMV (tobacco mosaic virus) and TRSV (tobacco ring spot virus), on the hypersensitive host *Chenopodium amaranticolor*. 3000 ppm of the essential oil is completely inhibited PVX, TMV, and TRSV [71].

Three lignin-carbohydrate-protein complexes (LC1, LC2, and LC3) with antiviral and immunostimulating activity were isolated from a hot water extract of seeds of *P. anisum* by combination of anion exchange, gel filtration, and hydrophobic interaction column chromatographies. These complexes showed antiviral activities against herpes simplex virus types 1 and 2, human cytomegalo virus, and measles virus [72].