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Farukh Sharopov

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Phytochemistry and bioactivities of selected plant species with volatile secondary metabolites

Referees: Prof. Dr. Michael Wink

Prof. Dr. Stefan Wölfl

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Zusammenfassung

Arzneipflanzen stellen noch immer ein gleichermaßen interessantes wie auch herausforderndes Gebiet der Wissenschaft dar. Sie enthalten eine komplexe Mixtur biologisch aktiver sekundärer Pflanzenstoffe mit diversen Wirkungen und oftmals geringer Toxizität.

In dieser Studie wurde unter Verwendung konventioneller analytischer, chemischer und biologischer Methoden die Phytochemie und Bioaktivität sekundärer Pflanzenstoffe von 28 Arzneipflanzen der Flora Tadschikistans und Deutschlands untersucht. Viele dieser Pflanzen sind in Tadschikistan und Zentralasien endemisch und kommen innerhalb der traditionellen Medizin häufig zum Einsatz.

Erstmals wurde die chemische Zusammensetzung und die Bioaktivität der ätherischen Öle von *Ferula clematidifolia, Galagania fragrantissima, Philadelphus x purpureomaculatus, Polychrysum tadshikorum* und *Salvia discolor* untersucht. Der Hauptbestandteil der Öle von *Ferula clematidifolia, Galagania fragrantissima, Philadelphus x purpureomaculatus, Polychrysum tadshikorum* und *Salvia discolor* wurden mittels GLC-MS analysiert und als Pinene, 2E-Dodecenal, Viridiflorol, Terpinen-4-ol trans-Caryophyllen identifiziert.

Die antioxidative Aktivität der ätherischen Öle ist stark vom Vorhandensein phenolischer Metaboliten abhängig. Das Öl von *Origanum tyttanthum*, welches Phenole enthält (Carvacrol und Thymol) zeigte mit einer IC_{50} von 0,1-0,3 mg/ml hohes antioxidatives Potential. Ebenso sind die antioxidativen Effekte der Methanolextrakte direkt proportional zum Phenolgehalt.

Das ätherische Öl von *Galagania fragrantissima* (GFEO) zeigte starke Aktivität gegenüber Gram-positiven Bakterien (methicillin-resistenter *Staphylococcus aureus*). Die MHK betrug 0,04 und die MBK 0,08 mg/ml. GFEO verfügt über hohe antientzündliche Aktivität; es inhibierte 5-LOX mit einer IC_{50} von 7,3 g/ml.

Die ätherische Öle aus *Origanum tyttanthum*, *Galagania fragrantissima* und *Mentha longifolia* verfügten über starke zytotoxische Aktivität gegen fünf humane Krebszelllinien (HeLa, CaCo-2, MCF-7, CCRF-CEM und CEM/ADR 5000). Ihre IC_{50}-Werte lagen im Bereich von 7,5 und 78 µg/ml. Die Kombinationen aus Doxorubicin mit ätherischen Ölen aus *Mentha longifolia, Anethum graveolens, Origanum tyttanthum, Galagania fragrantissima* und *Artemisia absinthium* zeigten synergistische Wirkung. Das Verhältnis der IC_{50}-Werte von Doxorubicin konnte durch gleichzeitige Behandlung mit zwei ätherischen Ölen um den Faktor 3 bis 15 verbessert werden. Synergistische Effekte von ätherischen Ölen scheinen ein vielversprechender Bereich für künftige Studien zu sein.

Methanolextrakte aus *Polychrysum tadshikorum* und *Tanacetum parthenium* zeigten die stärksten zytotoxischen Effekte gegen CCRF-CEM- und CEM/ADR 500-Zellen. Ihre IC_{50}-Werte lagen zwischen 7,3 und 32,5 µg/ml. Die Ergebnisse des Gesamtextrakts zeigten, dass CCRF-CEM-Zellen, welche keine ABC-Transporter exprimieren, sensitiver waren, als die abgeleiteten CCRF/ADR 5000-Zellen (multiresistent). Dies lässt vermuten, dass einige Bestandteile der Extrakte als Substrate von ABC-Transportern fungieren.

Schlussendlich sind ätherische Öle aus *Galagania fragrantissima, Origanum tyttanthum* und Methanolextrakte von *Polychrysum tadshikorum* und *Tanacetum parthenium* interessante Kandidaten hinsichtlich der Anwendung in der Phytotherapie.

Summary

Medicinal plants still represent an interesting and a challenging field of science. Plants generally contain complex mixtures of biologically active secondary metabolites with multiple target effects and often low toxicity.

In this study, well-known analytical, chemical and biological methods were used in order to investigate the phytochemistry and bioactivities of secondary metabolites of 28 medicinal plant from the flora of Tajikistan and Germany. Many of these plants are endemic plants of Tajikistan and Central Asia, which are widely used in traditional medicine.

For the first time, the chemical composition and bioactivity of the essential oils of *Ferula clematidifolia, Galagania fragrantissima, Philadelphus x purpureomaculatus, Polychrysum tadshikorum* and *Salvia discolor* were investigated. The main component of the essential oils of *Ferula clematidifolia, Galagania fragrantissima, Philadelphus x purpureomaculatus, Polychrysum tadshikorum* and *Salvia dsicolor* were analysed by GLC-MS and identified as pinene, 2E-dodecenal, viridiflorol, terpinen-4-ol and trans-caryophyllene, respectively.

The antioxidant activity of essential oils are strongly dependent to the presence of phenolic metabolites. Essential oil of *Origanum tyttanthum* which contain phenols (carvacrol and thymol) exhibited a high antioxidant activity with an IC_{50} value of 0.1-0.3 mg/ml. Also the antioxidant activity of methanol extracts is directly proportional to their phenol contents.

Galagania fragrantissima essential oil (GFEO) was very active against gram-positive bacteria (methicillin resistant *Staphylococcus aureus*). MIC and MBC values were 0.04 and 0.08 mg/ml respectively. GFEO shows high anti-inflammatory activity, it inhibited 5-LOX enzyme with an IC_{50} value of 7.3 µg/ml.

Essential oils from *Origanum tyttanthum, Galagania fragrantissima* and *Mentha longifolia* exhibited a high cytotoxic effect against five human tumour cell lines (HeLa, CaCo-2, MCF-7, CCRF-CEM and CEM/ADR 5000). Their IC_{50} values ranged between 7.5-78 µg/ml. The combinations of doxorubicin with essential oils of *Mentha longifolia, Anethum graveolens, Origanum tyttanthum, Galagania fragrantissima* and *Artemisia absinthium* exhibit synergistic activity. The ratio of IC_{50} values of doxorubicin could be enhanced in dual combinations with essential oils 3-15 fold. Synergistic effects of essential oils seem promising area for future research.

Methanol extracts from *Polychrysum tadshikorum* and *Tanacetum parthenium* were most cytotoxic against CCRF-CEM and CEM/ADR 5000 cell lines. Their IC_{50} ranged between 7.3-32.5 µg/ml. Results of cytotoxicity for the total extracts show that CCRF-CEM cells, which do not express ABC transporters were more sensitive than the derived CEM/ADR5000 (multidrug resistant) cells. This indicate that some components of the extracts are substrates of ABC transporters.

In general, essential oils from *Galagania fragrantissima, Origanum tyttanthum* and methanol extracts from *Polychrysum tadshikorum* and *Tanacetum parthenium* are interesting candidates for a use in phytotherapy.

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2. **Sharopov F.S.**, Wink M., Khalifaev D.R., Zhang H., Dosoky N.S., Setzer W.N., 2013. Chemical composition and antiproliferative activity of the essential oil of *Galagania fragrantissima* Lipsky (Apiaceae). *American Journal of Essential Oils and Natural Products* 1, 11-13.

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11. **Sharopov F.S.,** Satyal P., Wink M. Chemical composition of the essential oil of *Salvia discolor, Salvia officinalis* and *Salvia sclarea*. *Natural Product Communication* (submitted)

12. **Sharopov F.S.,** Braun M.S., Isupov S., Wink M. Antimicrobial, antioxidant and anti-inflammatory activities of essential oils of the selected aromatic plants from Tajikistan. (in preparation)

13. **Sharopov F.S.,** Sobeh M., Satyal P., Wink M. Alkaloid contents, antioxidant and cytotoxic activities of the different parts of *Papaver somniferum.* (in preparation)

14. **Sharopov F.S.,** Sobeh M., Richter E., Wink M. Antioxidant and cytotoxic activities, total phenolic and flavonoid contents of some *Salvia* species (in preparation)

15. **Sharopov F.,** Satyal P., Wink M. Composition of the essential oil of *Philadelphus purpureomaculatus* (in preparation)

16. **Sharopov F.,** Wink M., Kukaniev M.A., Setzer W.N., 2015, The essential oil composition of *Ocimum basilicum* from three different regions: Nepal, Tajikistan, and Yemen (in preparation)

Posters

1. **Sharopov F.S.**, Wink M. Cytotoxicity of the essential oils from Tajikistan plants against HeLa cells. 61st International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research, September 1-5, 2013, Münster, Germany in *Planta Medica, Journal of Medicinal Plant and Natural Product Research* 79, 1138-1139.

2. Setzer W.N., **Sharopov F.S.**, Wink M. Antioxidant activities of essential oil components – An experimental and computational investigation. 45th International Symposium on Essential Oils, September 7-10, 2014, Istanbul, Turkey.

3. **Sharopov F.S.,** Wink M. Antioxidant activity, total phenol and flavonoid contents of selected medicinal plants from Tajikistan**.** International Conference "Natural Products and Drug Discovery – Future Perspectives", November 13–14, 2014, Vienna, Austria.

Chapter 1. General Introduction

1.1. Medicinal Plants

Plants have been and continue to be valuable natural treasures, providing an important source of nutrients and therapeutic agents. Plants must defend themselves against herbivory and microbial infections, and over the last 400 million years they have evolved a high diversity of secondary metabolites that are toxic to animals and microorganisms. Because of this evolutionary background, most secondary metabolites are biologically active. This did not escape our ancestors who started to use plants as a means to treat infections and health problems (van Wyk and Wink, 2004; Wink and Van Wyk, 2008). In this context, traditional medicine and our understanding of the pharmacological properties of plants were developed.

Plants have the ability to produce a wide variety of chemical substances. These chemicals are divided into primary and secondary metabolites. Primary metabolites such as sugar and fats are found in all plants but secondary metabolites are produced in small range of plants which have specific functions. Each plant species on Earth produces a number of specific secondary metabolites. In addition, there are a number factors such as climate, soil, season, environment, pesticides, fertilizers, plant disease, age and etc. whole affect to the quality and quantity of secondary metabolites.

Plants have been used since ancient times in traditional medicine. Avicenna described more than 750 pharmaceutical substances of vegetative, animal, and mineral origin, several of them from Central Asia, in his book Al-Qanoon fi al-Tibb (The Canon of Medicine). Many medicines (drugs) described by Avicenna have entered the pharmacopeia and are still in use (Eisenman et al., 2013). His experience was influenced from his years in Central Asia, but he was also aware of the important *Materia Medica* of Dioscorides (who had lived 900 years earlier) (van Wyk and Wink, 2004; Wink and Van Wyk, 2008). The *Materia Medica* of Avicenna comprised more than 50 cardiac, 70 antiasthmatic, and 75 antidiabetic plants; 110 plants were described as useful for the treatment of kidney and gallstones, more than 40 plants for the treatment of vitiligo, dozens for wound healing, and others with antitoxic, antitumor, hemostatic and several other activities (Nuraliev, 2008).

Aromatic plants have attracted human attention for a long time because of their mostly pleasant fragrances. As a consequence, many of them are used as raw materials for the production of perfumes and cosmetics; others have found application in aromatherapy and phytotherapy. Many aromatic plants serve as spices because they can reduce the load of

microbial pathogens in food, improve the taste, and support digestion (e.g., as carminatives and choleretics).

1.2. Plant secondary metabolites

Secondary metabolites are recognized for their medicinal value and they are very interesting and powerful natural products. Today, they are widely used as antibacterial, anticancer, antidiabetic, anti-inflammatory, antimicrobial, antioxidative, blood pressure regulator, sedative drugs and etc.

The defense chemistry of plants includes a surprisingly wide diversity of biologically active compounds, such as alkaloids, glucosinolates, cyanogenic glucosides, flavonoids, tannins, coumarins, lignans, terpenoids, saponins, organic acids, and many others. A number of secondary metabolites, especially mono- and sesquiterpenes but also phenylpropanoids, are volatile. These compounds serve not only as deterrents against herbivory and often against microbial infection, but also as signal compounds to attract pollinators or predators.

Essential oils have gained special attention among secondary metabolites because many of them are used as raw materials for the production of perfumes, cosmetics, pharmaceuticals, pesticides. In addition they are exploited in aromatherapy and in phytotherapy, furthermore, as spices and for nutrition (Baser and Buchbauer, 2010; van Wyk and Wink, 2004).

Essential oils are complex systems of volatile components. They represent great interest for their multitarget properties, including cancer prevention. For example, authors (Warnke et al., 2009) reported that application of antibacterial essential oils reduced tumor smell and inflammation in cancer patients with necrotic ulcers.

1.3. Biological activity and mode of action of secondary metabolite with special reference to essential oil

Medicinal plants generally contain complex mixtures of biologically active compounds. They affect multiple targets. In general, plants used in phytotherapy show low toxicity. Some of the active secondary metabolites can have advantages in treating chronic diseases.

According to the Wink (2012b) phytochemicals have three major cellular targets: 1. the biomembrane; 2. proteins (including receptors, ion channels, enzymes, transporters, regulatory proteins, structure proteins, cytoskeleton, mitotic spindle (microtubules), transcription factors, hormones); 3. nucleic acids (DNA, RNA) (Wink and Schimmer, 2010). (Table 1.1).

Table 1.1. Interaction of representative secondary metabolites with molecular targets (Wink, 2012b)

Target	Activity	Secondary metabolite (SM) (Examples)
Biomembrane	Membrane disruption	Saponins
	Disturbance of membrane fluidity	Small lipophilic SM
	Inhibition of membrane proteins (→ change of protein conformation)	Small lipophilic SM
Proteins Specific interaction	Inhibition of enzymes	HCN from cyanogens, many substrate mimics
	Modulation of regulatory proteins	Phorbol esters, caffeine
	Inhibition of ion pumps	Cardiac glycosides
	Inhibition of microtubule formation	Vinblastine, colchicine, podophyllotoxin, paclitaxel (taxol)
	Inhibition of protein biosynthesis	Emetine, lycorine, lectins
	Inhibition of transporters	Nonprotein amino acids
	Modulation of hormone receptors	Genistein, many other isoflavonoids
	Modulation of neuroreceptors	Nicotine, many alkaloids, NPAA
	Modulation of ion channels	Aconitine, many other alkaloids
	Modulation of transcription factors	Cyclopamine, hormone mimics
Nonselective interactions	Noncovalent bonding (→ change of protein conformation)	Polyphenols such as phenylpropanoids, flavonoids, catechins, tannins, lignans, quinones, anthraquinones, some isoquinoline alkaloids
	Covalent bonding (→ change of protein conformation)	Isothiocyanates, sesquiterpene lactones, allicin, protoanemonine, furanocoumarins, iridoids (aldehydes), SM with aldehyde groups, SM with exocyclic CH_2 groups, SM with epoxide groups, SM with cyclopropane groups
DNA/RNA	Covalent modifications (alkylation) (→ point mutations)	Pyrrolizidine alkaloids, aristolochic acids, furanocoumarins, SM with epoxide groups
	Intercalation (→ frame shift mutations)	Planar, aromatic, and lipophilic SM, sanguinarine, berberine, emetine, quinine, furanocoumarins, anthraquinones
	Inhibition of DNA topoisomerase I	Camptothecin, berberine
	Inhibition of transcription	Amanitine

In aromatic plants, part of their therapeutic effects comes from their essential oils. Most secondary metabolites in essential oils are small lipophilic natural products, which allow them to readily enter body tissues by free diffusion.

Terpenoids, as part of essential oils, can disturb cells (modulate membrane fluidity, increase membrane permeability or solubilise biomembranes). In an aequous environment, small lipophilic secondary metabolites will be trapped by biomembranes. The lipophilic secondary metabolites will dive into the membrane and form hydrophobic interactions with the lipophilic side chains of phospholipids or cholesterol. Higher concentrations can influence membrane fluidity. In addition, these molecules can disturb the interaction of membrane proteins with membrane lipids, which are important for their correct three dimensional conformation. A change in protein confirmation will most likely modulate protein activity (Wink, 2008; 2012a; Wink and Van Wyk, 2008).

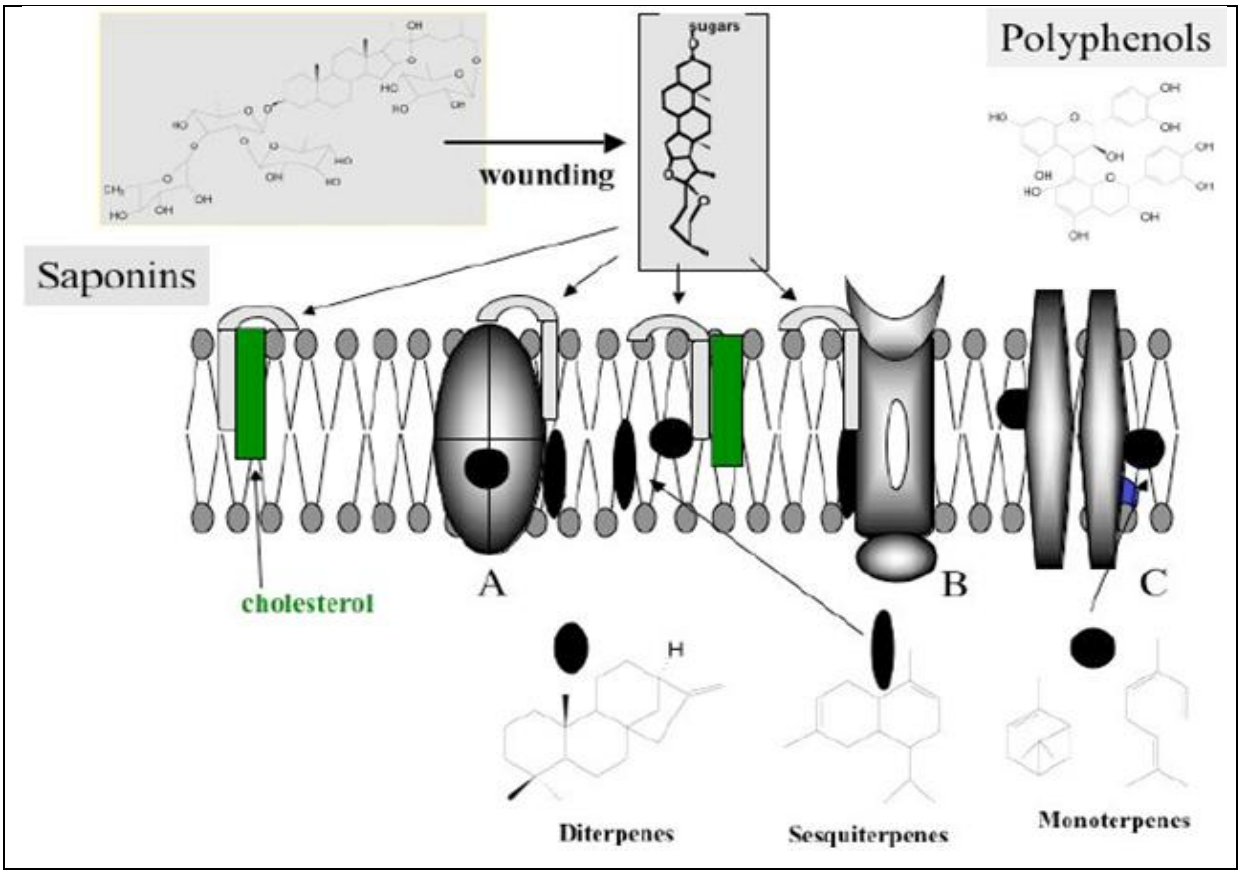


Figure 1.1. Interaction of terpenoids with biomembranes and membrane proteins. *Lipophilic terpenoids make hydrophobic interactions with lipids and membrane proteins. Saponins will complex cholesterol (in green) and can permeabilise cells. Membrane proteins (transporters, A; receptors, B; ion channels, C) (Wink, 2008).*

The lipophilic components can interact with biomembranes and membrane proteins. They thus influence membrane fluidity and permeability. This explains why many components of essential oils exhibit antibacterial, antifungal, antiviral, and cytotoxic activities.

A majority of secondary metabolites including essential oils, can interfere with several targets (multitarget) in a pleiotropic fashion (Wink, 2008). The cytotoxic activity of essential oils is based on their individual components (Reichling et al., 2009). In addition, as result of disturbed cellular membranes will be reduce ATP production, alteration of pH gradient, and loss of mitochondrial potential (Russo et al., 2015). Essential oils and their constituents can effect on tumour suppressor proteins (p53 and Akt), transcription factors (NF-κB and AP-1), MAPK-pathway, and detoxification enzymes like SOD, catalase, glutathione peroxidase, and glutathione reductase (Gautam et al., 2014).

Many essential oil components have reactive functional groups, such as epoxide and aldehyde groups which can covalently bind to functional groups of the proteins and nucleic acids. In results of interaction, DNA can be modified by those compounds (Wink and Schimmer, 2010). Covalent modifications can lead to point mutations and deletion of single bases or several bases if the modified bases are not exchanged by repair enzymes (Wink and Schimmer, 2010). Compounds with aromatic rings and lipophilic properties intercalate DNA, which can lead to frameshift mutations. They fit between the planar stacks of adenine–thymine (AT) and guanine–cytosine (GC) base pairs (Wink and Schimmer, 2010).

Different mechanisms are responsible for chemopreventive properties of essential oil. These include antioxidant, antimutagenic and antiproliferative, enhancement of immune function and surveillance, enzyme induction and enhancing detoxification, modulation of multidrug resistance and synergistic mechanism of volatile constituents (Bhalla et al., 2013). Essential oils have preventive properties to oxidative damage of lipids, low density of lipoproteins, proteins and DNA. Essential oils as natural antioxidant can provide electrons or hydrogens and neutralize free radicals and reactive oxygen species. In eukaryotic cells, some essential oils can act as prooxidants affecting inner cell membranes and organelles such as mitochondria (Bakkali et al., 2008). Depending on type and concentration, they exhibit cytotoxic effects on living cells but are usually non-genotoxic (Bakkali et al., 2008). In some cases, changes in intracellular redox potential and mitochondrial dysfunction induced by essential oils can be associated with their capacity to exert antigenotoxic effects (Bakkali et al., 2008).

1.4. Objective of the present study

There are many different factors influence on the biosynthesis and accumulation of plant secondary metabolites. In this work, we collected plants material from two different geographical locations: Tajikistan and Germany (Heidelberg).

Tajikistan is diverse in terms of environmental conditions including climate, high altitudes, mountainous soil and minerals, relatively large number of sunny days per year, which can all affect plant growth, biosynthesis, and accumulation of biological active secondary metabolites. The high-mountain ecosystems of Tajikistan have been regarded as biodiversity hotspots with around 4550 species of higher plants recorded in Tajikistan and about 30% endemism (Nowak et al., 2014; Rahmonov et al., 2013). The high degree of biodiversity and endemism in Tajikistan is due to the presence of high mountain ranges that serve as barriers to migration of plants.

Tajikistan has a rich flora including large numbers of herbs and aromatic plants. According to preliminary estimates, about 1500 species of Tajik plants are used in folk medicine, but only a small number of them are important in modern medicine.

Besides an investigation of Tajikistan plants, we investigated a number of Heidelberg plants, some of them very popular, like *Papaver somniferum, Salvia officinalis, Salvia sclarea.* However we investigate phytochemistry and bioactivity of *Salva discolor* and *Philadelphus x purpureomaculatus*. Very limited information about these species exist in the literature.

The aim of the present study was searching medicinal plants and plant derivatives with favourable bioactivities. While pursuing this goal, we investigated phytochemistry, antioxidant, antibacterial, anti-inflammatory and cytotoxic activities of the number medicinal plants.

The following were the main objectives of the present study:

- isolation of secondary metabolites from the selected plant species from flora of Tajikistan and Germany
- chemical characterization of complex SM mixtures by using different chromatographic techniques, such as GLC, GLC-MS, and HPLC
- evaluate antioxidant activity of SM by using DPPH, ABTS, FTC, FRAP and Folin-Ciocalteu methods
- evaluate cytotoxicity of extracts with MCF-7 (human breast adenocarcinoma), HeLa (human cervical cancer), Caco-2 (human colorectaladenocarcinoma), CCRF-CEM (human T lymphoblast leukaemia) and CEM/ADR5000 (adriamycin resistant leukaemia) cell lines using the MTT assay. Evaluate the effect of SM in multidrug resistance cancer cells (Caco-2 and CEM/ADR5000) with a high P-gp expression.
- screen antimicrobial activity against two gram positive and gram negative bacteria by using broth microdilution method

- evaluate anti-inflammatory activity by inhibition of 5-lipoxygenase enzyme
- finally, investigate mode of action SM by using microscopy and hemolysis in red blood cells.

Chapter 2. Literature review of the investigated plants

2.1. *Anethum graveolens* L. - Apiaceae

Anethum graveolens L. (dill), an important member of the Apiaceae, is widely used for flavoring foods and beverages due to its pleasant spicy aroma (Mabberley, 2008). It has been extensively used as a traditional herbal medicine throughout Europe, Asia and America (Babri et al., 2012; Huopalahti et al., 1988; van Wyk and Wink, 2004; Vera and Chane-Ming, 1998). *A. graveolens* oils exhibit important biological activities including antibacterial, antimicrobial and antifungal activities (Abed, 2007; Badar et al., 2008; Tian et al., 2011).

In Tajik folk medicine, water extracts (tea and infusion) of *A. graveolens* are widely applied for improving appetite, treating flatulence, stomach problems, digestive disorders, insomnia, cramps, inflammations of the respiratory tract, and for stimulating the release of milk in nursing mothers (Nuraliev, 1989; Williams, 2010).

The biosynthesis of secondary metabolites in medicinal and aromatic plants is strongly influenced by environmental factors. Chemical composition of the essential oil of *Anethum graveolens* growing in different geographical location has been extensively studied. According to these studies, the major components of the *Anethum graveolens* oil are phellandrene, dill ether, carvone, limonene, apiol, dihydrocarvone, and myristicin (Babri et al., 2012; Huopalahti et al., 1988; Sefidkon, 2001; Vokk et al., 2011).

The major componenets of the essential oil of Tajik *A. graveolens* are carvone (52%), *trans*-dihydro carvone (15%), dill ether (13%), α-phellandrene (8%) and limonene (7%). These results are qualitatively consistent with those previously reported by Jirovetz et al. (2003), Babri et al. (2012), and, probably Sefidkon (2001).

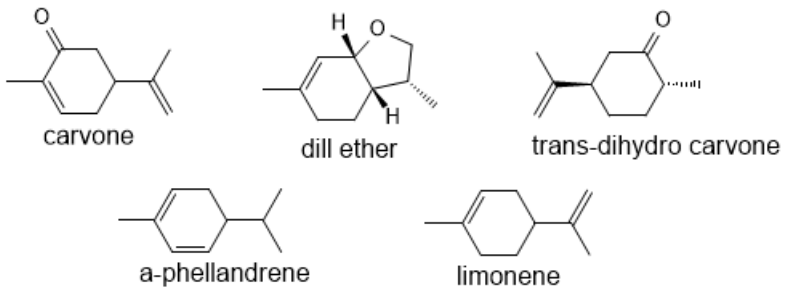


Fig. 2.1. Chemical structures of the main components of the essential oil of *A. graveolens*

2.2. *Ferula clematidifolia* Koso-Pol. - Apiaceae

F. clematidifolia is a herbaceous perennial plant growing to 1.5 m tall with yellow flowers. It is distributed in Tajikistan and Afghanistan. Plant is used for the treatment of flu, fever and colds in children. The plant produces an essential oil (Sokolov, 1988). Three crystalline compounds (giferolide (diversolide-C), malaphyll and malaphyllin (diversolide-D)) have been isolated from an ethanolic extract of the roots (Sagitdinova et al., 1990).

No previous data are reported about the composition and bioactivity of the essential oil of *F. clematidifolia*. Results of our work on essential oil composition of the *F. clematidifolia* are represented on chapter 4. Antioxidant activity, cytotoxicity of the plant extracts are documented on chapters 5, 6 and 7.

2.3. *Foeniculum vulgare* Millar - Apiaceae

Foeniculum vulgare (fennel**)** is a large perennial herb up to 2.5 m high with dark green to bronze-colored leaves and yellow flowers (umbels). It is native in the Mediterranean region. Fennel, is widely used for flavouring foods and beverages due to its pleasant spicy aroma (Mimica-Dukic et al., 2003; Rather et al., 2012). In traditional medicine, the plant and its essential oil have been extensively used as carminative, digestive, lactogogue and diuretic and to treat respiratory and gastrointestinal disorders (van Wyk and Wink, 2004). Plant extracts commonly used for gurgling from indigestion and kidney problem (Williams, 2010). Antimicrobial, antiviral, anti-inflammatory, antimutagenic, antinociceptive, antipyretic, antispasmodic, antithrombotic, apoptotic, cardiovascular, chemomodulatory, antitumor, hepatoprotective, hypoglycemic, hypolipidemic, and memory enhancing properties of *F. vulgare* have been demonstrated (Badgujar et al., 2014; Rather et al., 2012; van Wyk and Wink, 2004).

Volatile compounds, flavonoids, phenolic compounds of the plant have reported (Badgujar et al., 2014). Trans-anethole, estragole, limonene and fenchone were as principal compound in the essential oil of F. vulgare. Eriodictyol-7-rutinoside, quercetin-3-rutinoside, and rosmarinic acid have been isolated (Badgujar et al., 2014).

Results of our work on essential oil composition of the *F. vulgare* growing in Tajikistan are represented on chapter 4. Bioactivity of plant extracts are documented on chapters 5, 6 and 7.

2.4. Galagania fragrantissima Lypsky - Apiaceae

Galagania fragrantissima is a perennial herb with long branched steam up 1-2 m and with small yellow flowers. It is distributed in Afghanistan, Kyrgyzstan, Uzbekistan and Tajikistan.

The leaves and young shoots are used as a spice for soups and other dishes (Dudchenko et al., 1989). Plant is used against stomach problems.

The essential oil, which may be important for the perfumery industry, has a light yellow color and exhibits a spicy pleasant odor. The oil yield was 0.16-0.3% (Goryaev, 1952). Information about the chemical composition of *G. fragrantissima* was missing.

In chapter 4 are represented our results of essential oil composition of the *G. fragrantissima.* Antioxidant, cytotoxic, antimicrobial and anti-inflammatory activities of plant extracts are documented on chapters 5, 6, 7 and 8. Plant extracts show promising biological activity.

2.5. *Pastinaca sativa* L. - Apiaceae

Pastinaca sativa (parsnip) is a flowering plant (Mabberley, 2008). It is native to Eurasia between the western Mediterranean region and the Caucasus mountains (Cain et al., 2010). Plant is useful in arthritis treatment (Mabberley, 2008). *P. sativa* is a rich source of coumarins, especially furanocoumarins (Waksmundzka-Hajnos et al., 2004). Furanocoumarins are often the cause of inflammation (Wink and Van Wyk, 2008). Terpinolene (40–70%), octyl butyrate (79.5%) and myristicin (17–40%) have previously been reported as the main constituents in the root and seed essential oil of *P. sativa* (Kurkcuoglu et al., 2006).

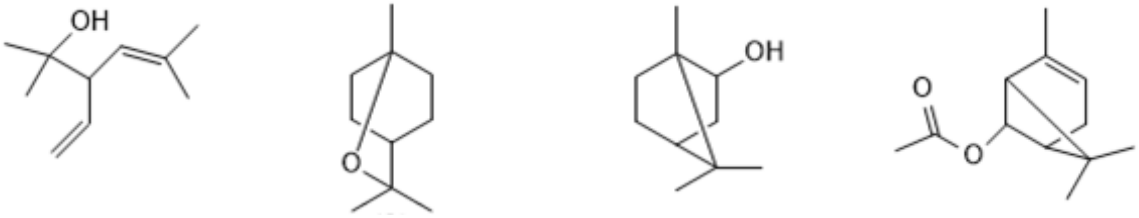
Results of our work on essential oil composition of the *P. sativa* are represented on chapter 4. Bioactivity of plant extracts is documented on chapters 5, 6 and 7.

2.6. *Achillea filipendulina* Lam. - Asteraceae

There are around 130 species in the genus *Achillea* distributed mainly in Eurasia (Mabberley, 2008), seven of have been recognized in Tajikistan. *Achillea filipendulina* Lam. is distributed in West and Central Asia (Mabberley, 2008), the Caucasus and Iran (Abdusalyamova et al., 1988). This plant grows 40-120 cm and flowers from June to September, and has been used since ancient times in traditional herbal medicines for a variety of ailments (Hojimatov, 1989; Kobilov, 1962). Decoctions of *A. filipendulina* have been used to treat sciatica, gout, arthritis, gastrointestinal disturbances, congestion, cardiovascular diseases, and malaria, as well as a diuretic, anthelmintic and purgative. Externally, the plant has been used to treat scabies and wounds. According to the "Canon of Medicine", decoctions of *A. filipendulina* have been used to treat for "breaking the muscles" and chronic inflammation of the sciatic nerve (sciatica). In Tajik folk medicine, a decoction from the dried flowers of *A. filipendulina* is used for gastric problems, a children digestive, to treat

stomachaches and cough (Kurbanov, 1992; Williams, 2010). This plant is also traditionally used as an emmenagogue, expectorant, and antitussive (Nuraliev, 1989). A high antibacterial activity of the essential oil of *A. filipendulina* against seven gram positive and gram negative bacteria was observed (Kiyanpour et al., 2011).

The plant contains 0.07–0.26% essential oil, alkaloid traces and nitrogen containing substances (Eisenman et al., 2013). The essential oil of Tajik *A. filipendulina* is rich in santolina alcohol (43-46%), 1,8-cineole (9-11%), borneol (5-6%), isoborneol (5%) and *cis*-chrysanthenyl acetate (7-9%).



santolina alcohol **1,8-cineole** **borneol** ***cis*-chrysanthenyl acetate**

Figure 2.2. Chemical structure of the main components of the essential oil of *A. filipendulina*

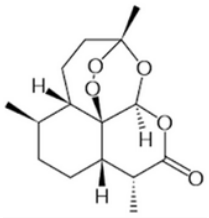
1,8-Cineole has a number of biological activities that makes it particularly useful in the treatment of respiratory tract infections (Harris, 2010). *A. filipendulina* leaf oil from Iran has shown antibacterial activity (Kiyanpour et al., 2011). The major flavonoids from the leaf exudates of *A. filipendulina* are quercetagetin and centaureidin (Valant-Vetschera and Wollenweber, 1996). Quercetagetin has shown anti-HIV activity (inhibitor of HIV reverse transcriptase and HIV integrase) (Farnet et al., 1996), while centaureidin has shown cytotoxic activity (tubulin polymerization inhibitor) (Beutler et al., 1993).

2.7. *Artemisia absinthium* L. - Asteraceae

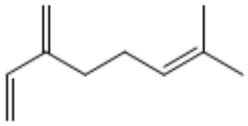
Artemisia absinthium L. (wormwood) is a herbaceous, perennial plant of up to 1.5 m high, with straight and branched stems. The leaves are pinnately compound and silvery-green in colour, with deeply dissected leaflets. Numerous small, pale yellow flower heads are borne along the branch ends (van Wyk and Wink, 2004). Flowers in June - September. *A. absinthium* is native to temperate regions of Europe, Asia and northern Africa (Mabberley, 2008; van Wyk and Wink, 2004). *Artemisia* species are most commonly used in the traditional folk medicine, notably in treatment of malaria (Nibret and Wink, 2010). *A. absinthium* is used as digestive, antihypertensive, and antipyretic, regardless of geographical origin or chemotype. *A. absinthium* is an herb used traditionally in Tajikistan. This plant is known to possess ethnomedical and biological properties related to anthelmintic activity,

digestive, antifungal, antimicrobial activity, choleretic, antiseptic, balsamic, depurative, diuretic, emmenagogue and in treating leukaemia and sclerosis. (Hojimatov, 1989; Kurbanov, 1992; Mabberley, 2008; Nazarov et al., 2002). *A. absinthium* has a vast range of biological activities including cytotoxic, antihepatotoxic, antibacterial, antifungal, antioxidant, antimalarial properties (Bora and Sharma, 2010). This plant is known for the production of various types of sesquiterpene lactones, including the antimalarial artemisinin (Nibret and Wink, 2010).

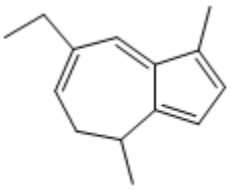
The major components of *A. absinthium* oil from Tajikistan are myrcene (9-23%), *cis*-chrysanthenyl acetate (8-18%), a dihydrochamazulene isomer (6-12%), germacrene D (2-8%), β-thujone (up to 7%), linalool acetate (up to 7%), α-phellandrene (1-5%), and linalool (5-7%).



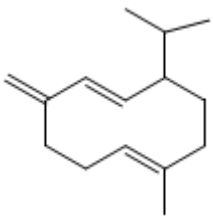
artemisinin



myrcene



dihydrochamazulene isomer



germacrene D

Figure 2.3. Chemical structure of the main components of *A. absinthium*

Antifungal, antimicrobial activity, choleretic, antiseptic and balsamic properties of plant can be explained by the composition of its essential oil. Myrcene-containing *A. absinthium* essential oil is used in the manufacture of alcoholic beverages and in pharmaceutical preparations as a mild sedative in the treatment of insomnia (Jäger, 2010).

2.8. *Artemisia rutifolia* Stephan ex Spreng. - Asteraceae

Artemisia rutifolia is plant with shrub15-80 cm tall, branched thick stems, strongly leafy branches. Flowers in August (Goryaev, 1952). It is distributed in Central Asia and Siberia. This plant is used as a tonic, febrifuge and anthelmintic in traditional medicine. A powder of the plant mixed with honey is useful against worms. A tea prepared from the dried and chopped herb is drunk to treat asthma, weakness of the heart, and also as anti-inflammatory, diuretic, and anthelmintic (Eisenman et al., 2013; Kurbanov, 1992; Nuraliev, 2008). Essential oil of *Artemisia rutifolia* has antibacterial, antihelminthical and fungicidal activity. It is recommended for cosmetic products (Azimova et al., 2011). Sesquiterpenes, sesquiterpene lactones, costic acid derivative, eudesmanolide and guaianolide were found in the aerial parts of *A. rutifolia* (Tan and Jia, 1992). *A. rutifolia* from Tajikistan belongs to the thujone-rich

chemotype, in contrast to the cineole/camphor chemotype found in Mongolia, and was dominated by α-thujone (21-37%), and β-thujone (36-47%), as well as 1,8-cineole (3-12%), and germacrene D (2-3%).

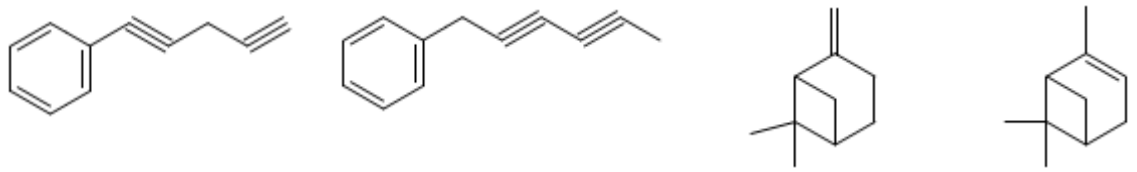
α-thujone **β-thujone**

Figure 2.4. Chemical structure of the main components of the essential oil of *A. rutifolia*

The bioactivity of *A. rutifolia* is most likely due to the thujones that are present in its essential oil. However, thujone has psychotropic effects, acting on the γ-aminobutyric acid-gated chloride channel, a member of the superfamily of ligand-gated ion channel receptors (Deiml et al., 2004). In addition to the essential oil, *A. rutifolia* is rich in guaianolide, germacranolide, and eudesmanolide sesquiterpenoids (Jakupovic et al., 1991; Tan and Jia, 1992; Tan et al., 1991). In general, α-methylene lactones have shown potent antitumor, antischistosomal, anthelmintic, and antimicrobial properties (Maries et al., 1995; Simonsen et al., 2013).

2.9. *Artemisia scoparia* Waldst & Kit. - Asteraceae

Artemisia scoparia (redstem wormwood) is a faintly scented annual or biennial herb with a vertical root. Stems single or few. 30-90 cm tall. Flowers in July, fruits in August - September (Eisenman et al., 2013). It is widespread and common throughout the word, particularly in southwest Asia and Central Europe (Rahman, 2012). In the folk medicine of Tajikistan, decoctions and infusions from the tops of the shoots of *A. scoparia* are used to treat kidney disorders, as well as a diaphoretic, diuretic, and anthelmintic. Decoctions of the plant are considered useful against epilepsy, rheumatism, fever, and inflammation of the respiratory tract (Hojimatov, 1989). According to some authors (Kurbanov, 1992; Nazarov et al., 2002) the aerial parts of *A. scoparia* are useful as an expectorant. It has antibacterial, anti-inflammatory, anticholesterolemic, antipyretic, anticrptic, cholagogue, diuretic, purgative and vasodilator activity (Rahman, 2012). *A. scoparia* essential oils from different geographical locations have shown great variation, but that from Tajikistan is dominated by the diacetylenes 1-phenyl-2,4-pentadiyne (34%) and capillene (5%), as well as β-pinene (21%), methyl eugenol (6%), α-pinene (5%), myrcene (5%), limonene (5%), and (*E*)-β-ocimene (4%).



1-phenyl-2,4-pentadiyne | **capillene** | **β-pinene** | **α-pinene**

Figure 2.5. Chemical structure of the main components of the essential oil of *A. scoparia*

Polyacetylenes from plants are known to be highly toxic against fungi, bacteria, and mammalian cells, and to display neurotoxic, anti-inflammatory and anti-platelet-aggregation effects and to be responsible for allergic skin reactions (Christensen and Brandt, 2006). Because of the reactive triple bonds they can alkylate a variety of proteins, thus changing their activity (Rivas da Silva et al., 2012; Wink, 2008). β-Pinene has also shown antimicrobial activity (Rivas da Silva et al., 2012).

2.10. *Polychrysum tadshikorum* (Kudr.) Kovalevsk. - Asteraceae

Polychrysum tadshikorum (Kudrj.) Kovalevsk. (family Asteraceae) has several synonyms in the literature including *Cancrinia tadshikorum* (Kudr.) Tzvelev, *Chrysanthemum myriocephalum* Rech., and *Tanacetum tadshikorum* Kudr. This herb is an endemic plant of Central Asia (Tajikistan and Afghanistan) (Schloeder and Jacob, 2010; Tzvelev, 1961). The herb is used traditionally against toothache and to reduce fever by Tajik people.

To our best knowledge, there no information on the chemical composition of this species has been published. Results of our work on essential oil composition of the *P. tadshikorum* are represented on chapter 4. Antioxidant activity, cytotoxicity of plant extracts are documented on chapters 5, 6 and 7.

2.11. *Tanacetum parthenium* L. Schultz-Bip. - Asteraceae

Tanacetum parthenium L. (feverfew) is a traditional medicinal plant in Southeast Europa, North Africa and Central Asia (Mabberley, 2008). Feverfew is a medicinal plant traditionally used for the treatment of fevers, migraine headaches, rheumatoid arthritis, stomach aches, toothaches, insect bites, infertility, and problems with menstruation and labor during childbirth (Pareek et al., 2011; van Wyk and Wink, 2004).

Parthenolide (Heptinstall et al., 1992) and tanetin (Williams et al., 1995) are the principal non-volatile active chemicals of *T. parthenium*. Parthenolide is one the principal sesquiterpene lactone with the most responsible for biologically active of feverfew. It is

found in the superficial leaf glands (0.2%–0.5%), but not in the stems, and comprises up to 85% of the total sesquiterpene content (Bohlmann and Zdero, 1982).

Our results about essential oil composition of the *T. parthenium* growing in Tajikistan are represented on chapter 4. Antioxidant activity, cytotoxicity of the extracts is documented on chapters 5, 6 and 7. Plant extracts show high bioactivity.

2.12. *Tanacetum vulgare* L.- Asteraceae

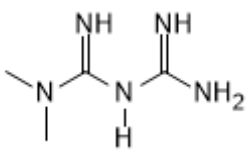
Tanacetum vulgare L. (tansy) is a perennial herb and growing widely in Europe and Asia (Mabberley, 2008). Tea from plant used as a tonic, abortifacient (Mabberley, 2008). It is aromatic plants has traditionally been used as a spicy additive for food, in cosmetics, and as a herbal remedy due to its biologically active compounds (van Wyk and Wink, 2004). Plant extracts and essential oils of tansy are known for their distinct medicinal, antimicrobial, antioxidant, insecticidal, and attractant properties (Rohloff et al., 2004). Thujone, camphor, chrysanthenyl acetate, chrysanthenol, chrysanthenone, artemisia ketone, artemisia alcohol, and 1,8-cineole were the main component in the essential oil of *T. vulgare* (Rohloff et al., 2004).

Results of our work on essential oil composition of the *T. vulgare* growing in Germany are represented on chapter 4. Antioxidant activity, cytotoxicity of the plant extracts are documented on chapters 5, 6 and 7.

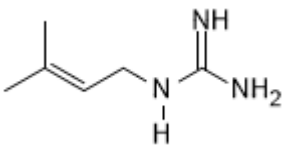
2.13. *Galega x hartlandii* - Fabaceae

Galega x hartlandii known by many other names including goat's rue, Spanish sanfoin, false indigo, Italian fitch, French lilac and professor-weed (Shenfield, 2013). It is a perennial up to 1 m in height, with pinnately compound leaves and attractive white or pink legume flowers arranged in dense, many-flowered clusters (van Wyk and Wink, 2004).

Plant is distributed in Europe and northern Arabia. The herb is cultivated to some extent, but mainly as a fodder plant (van Wyk and Wink, 2004). It used as diuretic, antidiabetic, also to treat skin ulcer. It has the diuretic and hypoglycaemic effects (van Wyk and Wink, 2004).



metformin



galegine

Figure 2.6. Chemical structure of isolated compounds from *Galega officinalis*

Metformin was developed from an herb, *Galega officinalis*, which was used for centuries to treat many ailments including polyuria. It is a rich source of the toxic substance guanidine. A less toxic alkaloid, galegine (Shenfield, 2013).

2.14. *Geranium macrorrhizum* L.- Geraniaceae

Geranium macrorrhizum (Bulgarian geranium) is native from the Balkans, Carpathian and Alps mountains (Ivancheva et al., 2006; Mabberley, 2008). It is a perennial with 20 - 60 cm stem, orbicular leaves. In autumn, the fragrant green leaves turn red. Inflorescence with 2 - 7 flowers in a corymb or umbel. Flowers and fruts in May and June (Öner et al., 2010). Shades of pink or purple flowers are borne in small clusters.

Plant was used to treat diarrhea, dysentery and hemorrhages. *G. macrorrhizum* oil is an excellent astringent and antiseptic. The herb can help treat bruises, cuts, eczema, scrapes, sunburns and varicose veins (Herbdatabase, 2014). The essential oil from *G. macrorrhizum* inhibits very high and selective activity against *Bacillus subtilis* (Radulovic et al., 2010).

Gallic acid, ellagic acid, 4-galloyl quinic acid, quercetin, quercetin-3-β-glucopyranoside, quercetin-3-β-galactopyranoside and quercetin-4'-β-glucopyranoside were isolated and identified in *G. macrorrhizum* (Giedrius, 2006). The major component of the essential oil of *G. macrorrhizum* was germacron sesquiterpene (Ivancheva et al., 2006; Radulovic et al., 2010).

Results of our work on the chemical composition of the essential oil of the *G. macrorrhizum* growing in Germany are represented on chapter 4. Antioxidant activity, cytotoxicity of the plant extracts are documented on chapters 5, 6 and 7. Plant extracts show high antioxidant activity.

2.15. *Philadelphus x purpureomaculatus* - Hydrangeaceae

A genus Philadelphus of about 80 species (Mabberley, 2008), mainly from East Asia and the Himalaya, North America, South Europe and the Caucasus (Cullen et al., 2011). Common names of *P.* x *purpureomaculatus* are Sybille or Mock orange Sybille. *P.* x *purpureomaculatus* is a flowering plant in the family Hydrangeaceae. It is hybrid between *P.* x *lemoinei* and *P. mexicanus* (Rose Syringa) (Mabberley, 2008). Its flowers are strongly fragrant.

Literature data (Czigle et al., 2006) reported that *epi*-13-manool (48%), *iso*-longifolol (15%), (*E,E*)-farnesole (37%) were as major component in *P. coronarius* L. species. To our best knowledge, no previous work on chemical composition of the volatile oil of *P.*

x *purpureomaculatus* have been reported.

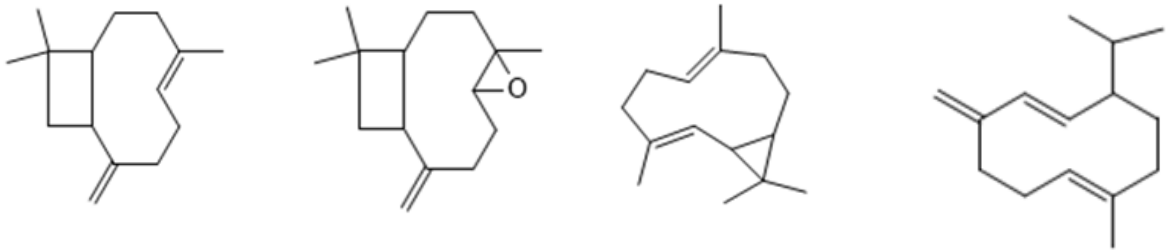
Results of our work on the chemical composition of the essential oil of the *P.* x *purpureomaculatus* growing in Germany are represented on chapter 4. Antioxidant activity, cytotoxicity of the plant extracts are documented on chapters 5, 6 and 7.

2.16. *Hypericum perforatum* L. - Hypericaceae

Hypericum perforatum (St. John's wort) is a perennial herb with stems 30–120 cm tall. Leaves are opposite, ovate to oblong or linear in shape. Flowers are bright yellow, 1–2 cm in diameter. Its roots can penetrate the soil to a depth of 1 m (Csurhes and Zhou, 2008). It is native to Europe and Mediterranean, until to south China (Mabberley, 2008).

Plant is poisonous to stock through photo-sensitization especially in sunny countries (Mabberley, 2008). *H. perforatum* has a long history of use as an anti-inflammatory, astringent and antiseptic. It has been used for centuries to treat mental disorders and nerve pain. In ancient times, it was used as a sedative and a treatment for malaria, as well as a balm for wounds, burns and insect bites (Csurhes and Zhou, 2008). The people in Russia called *H. perforatum* "means for the ninety-nine disease." According to Russian national doctors, "As without flour it is impossible to bake bread, so without *H. perforatum* it is impossible to treat many illnesses of people and animals." (Nuraliev, 1989).

H. perforatum contains many biologically active compounds including rutin, pectin, choline, sitosterol, hypericin and pseudohypericin, hyperforin (Csurhes and Zhou, 2008). Tajik *H. perforatum* oil are characterized with predominantly germacrene D (14%), α-pinene (5%), β-caryophyllene (5%), caryophyllene oxide (4%), bicyclogermacrene (4%), dodecanol (5%), and spathulenol (3%).



β-caryophyllene **caryophyllene oxide** **bicyclogermacrene** **germacrene D**

Figure 2.7. Structure of the main components of *H. perforatum* essential oil

The antidepressant activity of *H. perforatum* has been linked to the presence of hypericin and pseudohypericin (Butterweck et al., 1998), along with hyperforin (Müller et al., 2001). Germacrene D has shown antifungal activity against *Aspergillus niger* as well as cytotoxic

activity in PC-3 cells (Stranden et al., 2003). β-Caryophyllene exhibits several biological properties including anti-inflammatory, antibiotic, antioxidant, anticarcinogenic and local anaesthetic activities. It has a potentiating effect on the anticancer activity of several compounds (Legault and Pichette, 2007). Caryophyllene oxide shows *in vitro* cytotoxicity against MCF-7, PC-3, and Hep-G2 cells (Schmidt et al., 2006).

2.17. *Hypericum scabrum* L. - Hypericaceae

Hypericum scabrum L. is perennial herb with numerous stems, 20–70 cm tall, brown or reddish, covered with small, rigid papillae. Leaves opposite, sessile, oblong to lanceolate or elongate-linear, apex rounded or mucronate, covered with glands. In florescence a dense, corymbiform cyme. Sepals 5, partially connate. Petals 5, yellow with marginal black glands. Fruit a brown, ovoid to elongate-elliptical capsule. Seeds 1.5 mm long, brown (Eisenman et al., 2013). It distributed in Central Asia**.**

In traditional medicine *Hypericum scabrum* is used same as *H. perforatum* (Eisenman et al., 2013). *H. scabrum* is used in traditional medicine to treat a variety of disorders of liver, heart, stomach, intestines, bladder, cough, *etc.* A "marham" (poultice made from the herb and butter) is applied externally to treat sores, ulcers, abscesses, furuncles, and mastitis. An infusion of the flowers is recommended against jaundice (Hojimatov, 1989; Nuraliev, 2008).

The plant has antioxidant, antimicrobial, antidepressant activities (Abdollahi et al., 2012). A biflavonoid 5, 5″-dihydroxy-7, 4′, 7″, 4‴-tetramethoxybiflavone isolated from aerial parts of *Hypericum scabrum* (Abdollahi et al., 2012). It is essential oil dominated by α-pinene (71.6%) (Cakir et al., 1997). The essential oil of *H. scabrum* from Tajikistan is dominated by α-pinene (45%), with lesser amounts of spathulenol (7%), verbenone (6%), *trans*-verbenol (4%), and γ-muurolene (4%). Both *H. scabrum* essential oil (Kızıl et al., 2004) and methanol extracts (Erdoğrul et al., 2004) have shown antimicrobial activity. Interestingly, the anti-inflammatory and chondroprotective activity of (+)-α-pinene is believed to be greater than that of (–)-α-pinene or β-pinene (Rufino et al., 2014).

2.18. *Hyssopus seravschanicus* Pazij - Lamiaceae

Hyssopus seravschanicus (hyssop) is a perennial, branched, semi-shrub. Stems 20–50 cm tall. Leaves opposite, linear, 1–3.5 cm long, 1–3 mm wide. Flowers in July-August, fruits in September. (Eisenman et al., 2013). Plant distributed in Kyrgyzstan, Tajikistan, Uzbekistan.

Hyssop has been used in Tajik folk medicine for a long time ago. Avicenna prescribed that hyssop has antiseptic, anti-inflammatory, wound healing, analgesic, antitussive and

exciting action (Sino, 1982). A decoction of hyssop was used to treat bronchial asthma, chronic bronchitis, flu and diseases of the respiratory tract, it helps to relieve inflammation of the urinary tract. The ointment containing essential oil of *H. seravschanicus* shows antimicrobial activity against gram positive and gram negative bacteria (Gulmurodov et al., 2013). The activity of *H. seravschanicus* essential oil likely due to the high concentration of *cis*-pinocamphone (Cvijovic et al., 2010; Kizil et al., 2008).

Hyssop plant contains flavonoids apigenin, quercetin, diosmin, luteolin and other phenolic compounds chlorogenic, protocatechuic, ferulic, syringic, p-hydroxybenzoic and caffeic acids (Fathiazad and Hamedeyazdan, 2011). Pinocamphone, β -pinene, 1,8-cineol, camphene are the major components in the Tajik *H. seravschanicus* essential oil.

2.19. *Melissa officinalis* L. - Lamiaceae

Melissa officinalis (lemon balm) is a perennial bushy plant and is upright, reaching a height of about 1 m. The soft, hairy leaves are 2 to 8 cm long and either heart-shaped. The leaves have a gentle lemon scent, related to mint. During summer, small white flowers full of nectar appear. Flowers in June - July, fruits August - September (Eisenman et al., 2013; Moradkhani et al., 2010). *Melissa officinalis* is native to the southern Europe (Mabberley, 2008), eastern Mediterranean Region and western Asia (Moradkhani et al., 2010).

This herb has been traditionally used for different medical purposes as tonic, antispasmodic, carminative, diaphoretic, surgical dressing for wounds, sedative-hypnotic strengthening the memory, and relief of stress induced headache (Mabberley, 2008; van Wyk and Wink, 2004). In modern pharmacology, herb is used as a cardiac remedy and acts by slowing down the rate of breaths and heartbeats and by reducing tachycardia, palpitations, shortness of breath, and chest pain (Eisenman et al., 2013). Beside this it has moderate activity against Alzheimer, migraine and rheumatism (Moradkhani et al., 2010).

The plant contains tannins, rosmarinic, caffeic, oleanolic, ursolic acids, and essential oil (Eisenman et al., 2013; van Wyk and Wink, 2004). The literature data indicated that the major components of the essential oil of *M. officinalis* were geranial, neral, citronellal, (*E*)-caryophyllene, caryophyllene oxide, linalool, geraniol, thymol, α-pinene, β-pinene, carvacrol, *iso*-menthone, decadienal and *trans*-carveol.

The main constituents of the essential oils of *M. officinalis* from Tajikistan are geranial (43%), neral (32%), *trans*-anethole (12%), (E)-caryophyllene (4%) and citronellal (3%).

geranial **neral**

citronellal ***trans*-anethole**

Fig. 2.8. Chemical structures of the main components of the essential oil *M. officinalis*

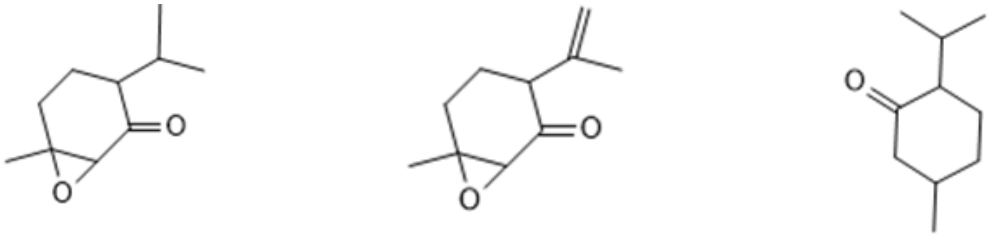
The chemical composition of *M. officinalis* oil from Tajikistan is qualitatively similar to several earlier reports, indicating the main components to be the citral isomers geranial and neral (Sari and Ceylan, 2002; Taherpour et al., 2012).

2.20. *Mentha longifolia* (L.) Huds. - Lamiaceae

Mentha longifolia (mint, silver mint or horse mint) is a herbaceous perennial plant with a peppermint-scented aroma. It has an erect stems 40–120 cm tall. The leaves are oblong-elliptical to lanceolate, 5–10 cm long and 1.5–3 cm broad. The flowers are 3–5 mm long with lilac, purplish, or white colors (Eisenman et al., 2013). It is native to Europe and Mediterranean, until to Himalayan and to South Africa (Mabberley, 2008).

Mentha species have been use by humans since ancient times in many parts of the world (van Wyk and Wink, 2004). The aerial parts of *Mentha* species are commonly used in many processed foods as well as in herbal teas. In folk medicine, mint is widely utilized as a tea or as a gargle against both acute and chronic conditions of the upper respiratory tract. It is prescribed against liver disorders (jaundice, chronic hepatitis), intestinal spasms, biliary tract infections (acute and chronic cholecystitis), and cholangitis (Hojimatov, 1989; Nuraliev, 1989). Mint juice from freshly picked leaves as well as dried and crushed mint leaves are widely used to improve appetite and digestion, as anti-inflammatory, diaphoretic, carminative, antiemetic, antitussive, and analgesic agents. Infusions of mint are employed to prepare washes and lotions for treating spasms, rheumatic pains, arthritis, itching and inflammation of the skin (Makhlayuk, 1967). Mint is also used to repel insects, snakes, and worms (Hojimatov, 1989). Mountain mint has been used externally to treat cracks in the skin caused by dry skin as well as bone fractures and internally to relieve muscular aches and sciatica. A compress treated with a hydroalcoholic extract mountain mint is used to treat bruises and animal bites (Nuraliev, 1989). A chemical composition of *M. longifolia* essential

oils from different geographical locations varies considerably, even within Tajikistan. Wild populations of Tajik *M. longifolia* can be dominated by *cis*-piperitone epoxide (up to 78%), piperitenone oxide (up to 49%), carvone (up to 22%), menthone (up to 17%), as well as pulegone (1%–5%) and thymol (2%–4%).



***cis*-piperitone oxide** **piperitenone oxide** **carvone**

Figure 2.9. Structure of the main components of *M. longifolia* essential oil

The essential oil has shown moderate antimicrobial activity, supporting the traditional use of this plant to treat wounds and skin infections (Viljoen et al., 2006).

2.21. *Ocimum basilicum* L. - Lamiaceae

Ocimum basilicum L., also called sweet basil, is a half-hardy annual plant in the Lamiaceae and is native to the tropical and subtropical regions of Asia (Mabberley, 2008; van Wyk and Wink, 2004). Sweet basil can grow to a size of 0.5-0.9 m in height and prolifically produce large green leaves that are around 5 cm in length throughout the summer (Back and Boxer, 1980; van Wyk and Wink, 2004).

O. basilicum plays a major role in many Asian cuisines and is also used medicinally in treating ailments like headaches, coughs, diarrhea, worms, and kidney malfunctions. Sweet basil is rich in essential oils, which mainly contain cinnamate, citronellol, geraniol, linalool, estragole, myrcene, pinene, ocimene, and terpineol; the quantity of each component varies based on the part of the plant being examined. Sweet basil's essential oils have been widely used in flavoring confectionery products, baked goods, condiments, sausages and other meat, nonalcoholic beverages, and ice creams. This oil also has use in perfumery, dental, and oral products (Simon et al., 1990).

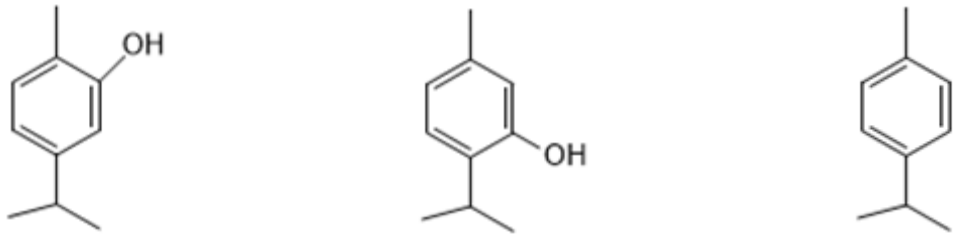
The leaves and flowering tops of basil are used at inflammatory diseases of upper respiratory ways infection (bronchitis, laryngitis, pharyngitis, etc.), chronic gastritis, enterocolitis, and food poisoning. Additionally, hot basil tea is taken for treating nausea, flatulence, and dysentery. The volatiles of basil have been found to repel flies and other insects (Hojimatov, 1989; Jurbi, 1988; Nuraliev, 1989; van Wyk and Wink, 2004).

The major components in basil oil, linalool and methylchavicol, have shown anti-inflammatory activities (Moretti et al., 1997), supporting the rationale for the basil traditional use in inflammatory diseases of the upper respiratory tract. Linalool has also shown antibacterial (Bassole et al., 2010) and antiviral (Chiang et al., 2005) activities.

2.22. *Origanum tyttanthum* Gontsch. - Lamiaceae

The species *Origanum tyttanthum* Gontsch. is synonymous with *Origanum vulgare* L. subsp. *gracile* (K. Koch) Ietsw. and is the most widely distributed medicinal plant in Central Asia (Borisova, 1954). In Tajikistan, where *O. tyttanthum* is a common species, this plant has commercial value. Cultivation of *O. tyttanthum* covers a total area of over 140,000 hectares, yielding annually a total of 6490 tons of air-dried raw materials (Denisenko et al., 2008). As a medicinal plant, *O. tyttanthum* has traditionally been used as an expectorant, carminative, diaphoretic, stimulant, stomachic, and tonic. In addition, it has been used as a folk remedy against colic, coughs, headaches, nervousness, toothaches, and irregular menstrual cycles. Teas prepared from the aerial parts of *O. tyttanthum* have been used to treat tuberculosis and against human intestinal parasites. They are also used for sedative purposes and widely used against flatulence and as a gargle against laryngitis, stomatitis, and angina (Hojimatov, 1989; Jurbi, 1988; Nuraliev, 1989; Sakhobiddinov, 1948).

The major components of *O. tyttanthum* essential oil from Tajikistan are carvacrol (34-59%), thymol (11-46%), and *p*-cymene (1-7%). The ointment "Subinak" was created on the base of essential oil of *O. tyttanthum* growing in Tajikistan (Kholnazarov, 2004).



carvacrol **thymol** ***p*-cymene**

Figure 2.10. Structure of the main components of *O. tyttanthum* essential oil

The presence of phenolic monoterpenes (carvacrol and thymol) with known antiseptic properties (Kholnazarov, 2004) as the major oil components is responsible for its potent antioxidant, antibacterial, fungicidal, insecticidal, herbicidal, and nematicidal activities (Yaniv and Dubai, 2014).

2.23. *Salvia discolor* Kunth- Lamiaceae

The genus *Salvia* is the largest genus in the family Lamiaceae with 800-900 species (Mabberley, 2008; Walker et al., 2004) and distributed throughout tropical and temperate regions of the world (Li et al., 2010). *Salvia discolor* is an herbaceous perennial growing in a much localized area in Peru. Plant is traditionally used for respiratory illness treatment, especially for cough (Bussmann and Glenn, 2010).

There are no previous work on chemical composition of essential oil of *S. discolor* have been reported. Results of our work on essential oil composition of the *S. discolor* are represented on chapter 4. Antioxidant activity, cytotoxicity of the plant extracts are documented on chapters 5, 6 and 7.

2.24. *Salvia officinalis* L. - Lamiaceae

Salvia officinalis or common sage, is an aromatic herb that has been used in medicine and cooking since ancient times (van Wyk and Wink, 2004). This plant is native to Europe and Mediterranean region (Mabberley, 2008). Common sage is used worldwide as flavoring spices as well as traditional herbal medicine. It has been traditionally used for the relief of pain, protecting the body against oxidative stress (Mabberley, 2008), free radical damages, angiogenesis, inflammation, bacterial and virus infection, etc. Several studies suggest that sage might potentially provide novel natural treatments for the relief or cure of many serious and life threatening diseases in addition to treating minor common illnesses such as depression, dementia, obesity, diabetes, lupus, heart disease and cancer (Hamidpour et al., 2013; van Wyk and Wink, 2004). It is a source of essential oils and its constituents include cinerol, camphor, borneol, thujone, rosmarinic acid, flavonoids, tannins, saponins and estrogenic substances. The diterpenes carnosol and carnosic acid which are found in *S. officinalis,* potently suppressed microsomal PGE_2 synthase-1 (Bauer et al., 2012). Pre-clinical studies have suggested that carnosol selectively targets tumorigenic cell as opposed to non-tumorigenic cells and is safe and tolerable in animals (Johnson, 2011).

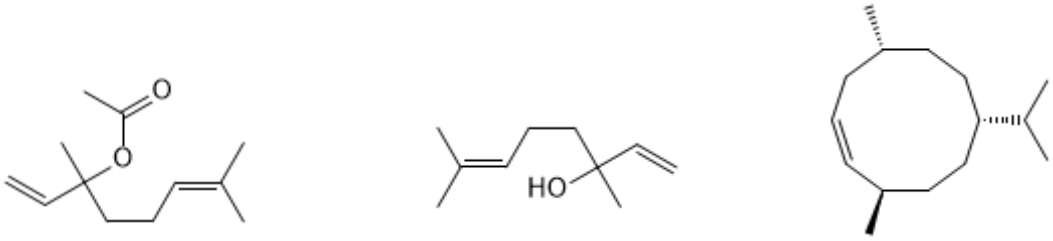
2.25. *Salvia sclarea* L. - Lamiaceae

Salvia sclarea (clary sage) is an important aromatic plant that is mainly cultivated in Europe and North America (Clebsch, 2003). The major components of traded clary sage oil are linalool and linalyl acetate. This plant has been used as a folk remedy for the treatment at palpitation, for improvement of digestion, against colds and throat disturbances, and also as a tonic against fatigue. An infusion from the aerial parts is employed to treat conditions of the kidney and to reduce fever. A tea prepared from the aerial parts of *S. sclarea* is taken to

improve digestion and appetite, and also as a diuretic. *S. sclarea* fruits are utilized to treat dysentery and bloody diarrhea. *S. sclarea* is applied externally to soften the skin (Hojimatov, 1989; Jurbi, 1988; Nuraliev, 1989).

Plants from the genus *Salvia* are a rich source of polyphenols (more than 160 polyphenols have been identified in *Salvia*) which are believed to be responsible for the many biological activities of sage and their use in traditional medicine (Lu and Foo, 2002). Sclareol, the major component of the clary sage concrete (Lawrence, 1986), is used as starting material for Ambrox synthesis, an important synthetic ambergris-like product used in perfumery (Moulines et al., 2004).

Clary sage is a traditional medicinal plant in Europe and Asia (Eisenman et al., 2013). Commercial grade clary sage oil is rich in linalyl acetate and linalool, and Tajik oil is comparable with 39% linalyl acetate and 13% linalool, as well as germacrene D (11%), α-terpineol (6%), geranyl acetate (4%), and β-caryophyllene (2%).

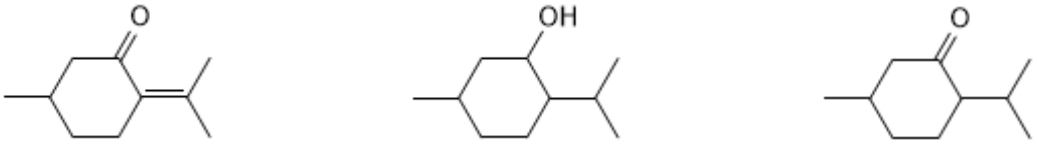


linalyl acetate **linalool** **germacrene D**

Figure 2.11. Structure of the main components of *Salvia sclarea* essential oil

2.26. *Ziziphora clinopodioides* Lam. - Lamiaceae

The genus *Ziziphora* belongs to the Lamiaceae and consists of about 30 species that are distributed in Southern Siberia, Central Asia and the Mediterranean (Mabberley, 2008; Yuzepchuk, 1954). *Z. clinopodioides* (blue mint) is an edible medicinal plant that is widely distributed in Tajikistan. The leaves, flowers and stem of the plant are frequently used as a wild vegetable or as an additive to foods. The plant has been used since ancient times in traditional herbal medicines for the treatment of colds and cough, stomach ache, nausea, poor appetite, sexually transmitted diseases, and as an antiseptic and to promote wound healing (Hojimatov, 1989; Kurbanov, 1992; Nuraliev, 1989). Tajik *Z. clinopodioides* oil is a pulegone-rich oil, composed mainly of pulegone (73%–35%), neomenthol (7%–23%), and menthone (6%–13%).



pulegone **neomenthol** **menthone**

Figure 2.12. Structure of the main components of *Z. clinopodioides* essential oil

Three chemotypes of *Z. clinopodioides* have been recognized according to the composition of the essential oil, namely pulegone-rich, thymol-rich, and cineole-rich chemotypes (Setzer, 2011). Pulegone-rich essential oils have shown antiviral activity (Buchbauer, 2010). Pulegone can irritate mucosal tissues of the GI-tract and externally the skin. It can cause spasms and cramps (Wink and Van Wyk, 2008). Like most essential oils, *Z. clinopodioides* oil has lipophilic properties, targeting biomembranes, which would explain its antiseptic properties (Wink, 2008).

2.27. *Ficus carica* *L.*- Moraceae

Ficus carica (common fig) is shrub or small tree 5-10 m high with several spreading branches. Leaves are alternate and borne on 5-12 cm long. It has male and female flowers. Fruits are pyriform-obovoid to subglose, 2-5 cm in diameter (Lim, 2012). Plant are distributed in south-western Asia to northwest India, Mediterranean area (Mabberley, 2008). Fig are used as a mild laxative, expectorant, diuretic and diabetic (Joseph and Raj, 2011). Its roots are used in treatment of leucoderma and ringworms. Its fruits have antipyretic, purgative, aphrodisiac properties (Kalaskar et al., 2010). *F. carica* has been traditionally used for its medicinal benefits as metabolic, cardiovascular, respiratory, antispasmodic, and anti-inflammatory remedy (Mawa et al., 2013). Anticancer, antiviral, hepatoprotective, hypoglycaemic, antihyperlidemic/hypocholesterolemic, immunomodulatory, antipyretic, antimicrobial, blood coagulation, antiplatelet, antiinflamatory, antiwarts, anthelmintic, larvicidal activities of *F. carica* were documented (Lim, 2012).

The plant has numerous bioactive compounds such as mucilages, flavonoid, vitamins, essential oil (Joseph and Raj, 2011; Mawa et al., 2013). Psoralen, bergapten, umbelliferone, campesterol, stigmasterol, fucosterol, 6-(2-methoxy-Z-vinyl)-7-methyl-pyranocoumarin, 9,19-cycloarlane triterpenoid, 6-O-acyl-*β*-D-glucosyl-*β*-sitosterol, calotropenyl acetate and lupeol acetate were found in this plant (Kalaskar et al., 2010; Khodarahmi et al., 2011).

2.28. *Papaver somniferum* L. - Papaveraceae

The Papaveraceae is a family comprising of 43 genera and about 820 species (Kapoor, 1995; Walsh and Kellermann, 2011). One well-known and medicinally important member of this family is Opium poppy *Papaver somniferum*. It is an annual herb, which originated in southeast Europe and western Asia. Poppy has been used by humankind for thousands of years and is still used around the world today. The isoquinoline alkaloids isolated from this plant have played a special role in history (Roberts and Wink, 1998). The worldwide demand for opiates is nearly 200 tons of alkaloids per year (van Wyk and Wink, 2004). Opium, the dried latex from *P. somniferum* contains more than 30 individual alkaloids (Baros et al., 2012) and the main alkaloid constituents are morphine, thebaine, codeine, papaverine, noscapine (Baros et al., 2012; Dittbrenner et al., 2009).

morphine **codeine**

Figure 2.13. Structure of the main alkaloids of *P. somniferum*

Opium poppy and opium have antioxidant properties; furthermore extracts and isolated compounds have been used as analgesic, narcotic, CNS stimulant, antitussive, anti-diarrheic, and sedative (Alok et al., 2014; Roberts and Wink, 1998). In addition, *P. somniferum* is the valuable source for several medicinally used alkaloids including the narcotic and analgesic morphine, the antitussive codeine, the spasmolytic papaverine and several derived semi-synthetic drugs such as oxycodone, buprenorphine and naltrexone (Wijekoon and Facchini, 2012). Morphine is considered the "gold standard" for relieving pain and is currently one of the most effective drugs available clinically to relieve severe pain associated with cancer (Gach et al., 2011). Additionally, morphine has been discussed as a regulator of tumour growth (Bimonte et al., 2013).

Conclusions

This chapter reviews the phytochemical and bioactivity of 28 medicinal plant from Tajikistan and Germany. Many of them are well-know species which are widely used in traditional and modern medicine. However, no data about some species such as *F. clematidifolia*, *G. fragrantissima*, *P. x purpureomaculatus*, and *P. tadshikorum.*

Chapter 3. Materials and Methods

3.1. Plant Material

The plants were collected mainly from two places: a) from different region of Tajikistan and b) from an ornamental garden in Heidelberg, Germany. The species name, family, the voucher specimen numbers, the collection time and location of plants are summarized in Table 3.1. The voucher specimens of the plant material were deposited at the Department of Biology, Institute of Pharmacy and Molecular Biotechnology, Heidelberg University. A total of 28 medicinal plant species were collected mainly from the central-southern part of Tajikistan (18 plant species) and Heidelberg, Germany (10 plant species). The Tajik plants have been compared with voucher specimens which have been deposited in the herbarium of the Institute of Botany, Plant Physiology and Genetics of the Tajikistan Academy of Sciences.

3.2. Reagents and chemicals

All chemicals and reagents were analytical grade or purest quality purchased from Aldrich, AppliChem, Fluka, Merck, Roth and Sigma.

Table 3.1. Origin of plant samples

Species	Family	IPMB accession number	Collection time	Location (Altitude m above sea level)
Achillea filipendulina Lam**.**	Asteraceae	P8582	9. 06.2013, 11.06.2014	Chormaghzak pass, Yovon region, Tajikistan (TJ), 1300 m
Anethum graveolens L.	Apiaceae	P8577	7.06.2013, 10.06.2014	Chormaghzak pass, Yovon region, TJ, 1300 m
Artemisia absinthium L.	Asteraceae	P8583	10. 06.2013, 11.06.2014	Chormaghzak pass, Yovon region, TJ, 1300 m
Artemisia rutifolia Stephan ex Spreng.	Asteraceae	P8584	9.06.2013, 12.06.2014	Chormaghzak pass, Yovon region, TJ, 1300 m
Artemisia scoparia Waldst. & Kit.	Asteraceae	P8585	27.06. 2013, 11.06.2014	Chormaghzak pass, Yovon region, TJ, 1300 m
Ferula clematidifolia Koso-Pol.	Apiaceae	P8580	9.06.2013, 12.06.2014	Chormaghzak pass, Yovon region, TJ, 1300 m
Ficus carica L.	Moraceae	P8559	30.03.2014	Heidelberg , Germany
		P8560		
Foeniculum vulgare Millar	Apiaceae	-	2012	Ziddeh pass, Varzob Region, TJ, 2000 m
Galagania fragrantissima Lipsky	Apiaceae	P8578	8.06.2013, 1.05.2014	Chormaghzak pass, Yovon region, TJ, 1300 m
Galega x hartlandii	Fabaceae	P8603	22.07.2013	Heidelberg , Germany
Geranium macrorrhizum L.	Geraniaceae	P8474	12.08.2013	Heidelberg , Germany
Hypericum perforatum L.	Clusiaceae	P8592	9.06.2013, 11.06.2014	Teppahoi sharqi, Dushanbe, TJ, 800 m; Chormaghzak pass, Yovon region, TJ, 1300-1500 m
Hypericum scabrum L.	Clusiaceae	P8593	10.06.2013, 11.06.2014	Chormaghzak pass, Yovon region, TJ, 1300-1500 m
Hyssopus seravschanicus Pazij	Lamiaceae	-	2012	Ziddeh pass, Varzob Region, TJ, 2000 m

Table 3.1. Continued.

Melissa officinalis L.	Lamiaceae	P8594	2012, 12.06.2014	Teppahoi sharqi, Dushanbe, TJ, 800 m; Chormaghzak pass, Yovon region, TJ, 1300 m
Mentha longifolia (L.) Huds.	Lamiaceae	P8595	8.06.2013, 12.06.2014	Chormaghzak pass, Yovon region, TJ, 1300 m
Ocimum basilicum L.	Lamiaceae	P8597	27.06.2013, 14.10.2013	Chormaghzak pass, Yovon region, TJ, 1300 m
Origanum tyttanthum Gontsch.	Lamiaceae	P8596	8.06.2013, 11.06.2014	Chormaghzak pass, Yovon region, TJ, 1300 m
Papaver somniferum L.	Papaveraceae	P8611	22.07.2013	Heidelberg , Germany
Pastinaca sativa L.	Apiaceae	P 8472	12.08.2013	Heidelberg , Germany
Philadelphus x purpureomaculatus Limoine	Hydrangeaceae	P8605	22.07.2013	Heidelberg , Germany
Polychrysum tadshikorum (S. Kudr) Kaval)	Asteraceae	P8588	8.06.2013, 12.06.2014	Chormaghzak pass, Yovon region, TJ, 1300 m
Salvia discolor Kunth	Lamiaceae	P8610	9.10.2013	Heidelberg , Germany
Salvia officinalis L.	Lamiaceae	P8609	22.07.2013	Heidelberg , Germany
Salvia sclarea L.	Lamiaceae	P8608	22.07.2013	Heidelberg , Germany
		P8598	9.06.2013, 11.06.2014	Chormaghzak pass, Yovon region, TJ, 1300 m
Tanacetum parthenium L. Schultz-Bip.	Asteraceae	P8587	8.06.2013, 12.06.2014	Chormaghzak pass, Yovon region, TJ, 1300 m
Tanacetum vulgare L.	Asteraceae	P8586	7.06.2013, 11.06.2014	Chormaghzak pass, Yovon region, TJ, 1300-1500 m
		P8471	12.08.2013	Heidelberg , Germany
Ziziphora clinopodioides Lam.	Lamiaceae	P8899	10.06.2013, 11.06.2014	Chormaghzak pass, Yovon region, TJ, 1300 m

3.3. Preparation of crude MeOH and water extracts

The dried or fresh plant material is ground or cut into small pieces and macerated (ratio 1 g/12.5 ml) in methanol and water. Figure 3.1 summarizes the process of extraction and a brief summary of the experimental conditions of extraction is shown in Table 3.2.

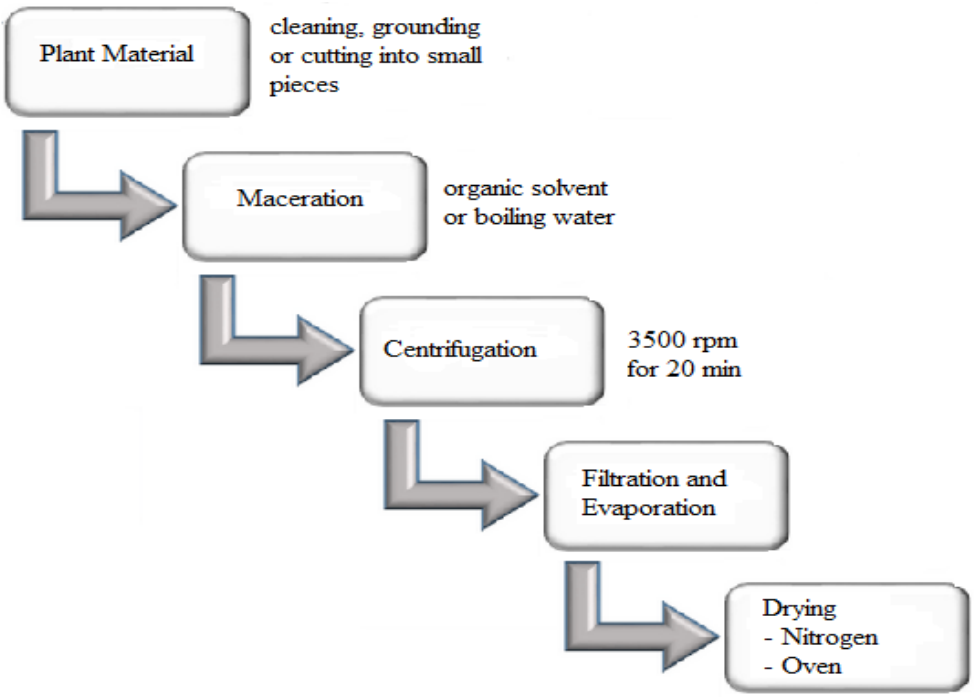


Figure 3.1. Schematic diagram of the extraction process

Table 3.2.

A brief summary of the experimental conditions of extraction by water and methanol

Solvent	Water	Methanol
Plant material	Dry	Fresh or dry
Ratio of PM/solvent	1 g/12.5 ml	1 g/12.5 ml
Time	12 h	24-48 h
Temperature	Added boiling water and kept at room temperature	Room temperature
Drying of extracts	60-70 °C	in N2

3.4. Isolation of essential oils

Several techniques can be used to extract essential oils from different parts of the aromatic plants, including water or steam distillation, solvent extraction, expression under pressure,

supercritical fluid and subcritical water extraction. Among these methods hydrodistilation have practical advantages that it is an inexpensive, easy to construct and suitable for field operation. Essential oils are obtained by hydrodistillation using three type apparatus: Ginsberg, Clevenger and Likens-Nickerson (Fig. 3.2). The essential oils are kept at 4 ^{o}C for further analysis.

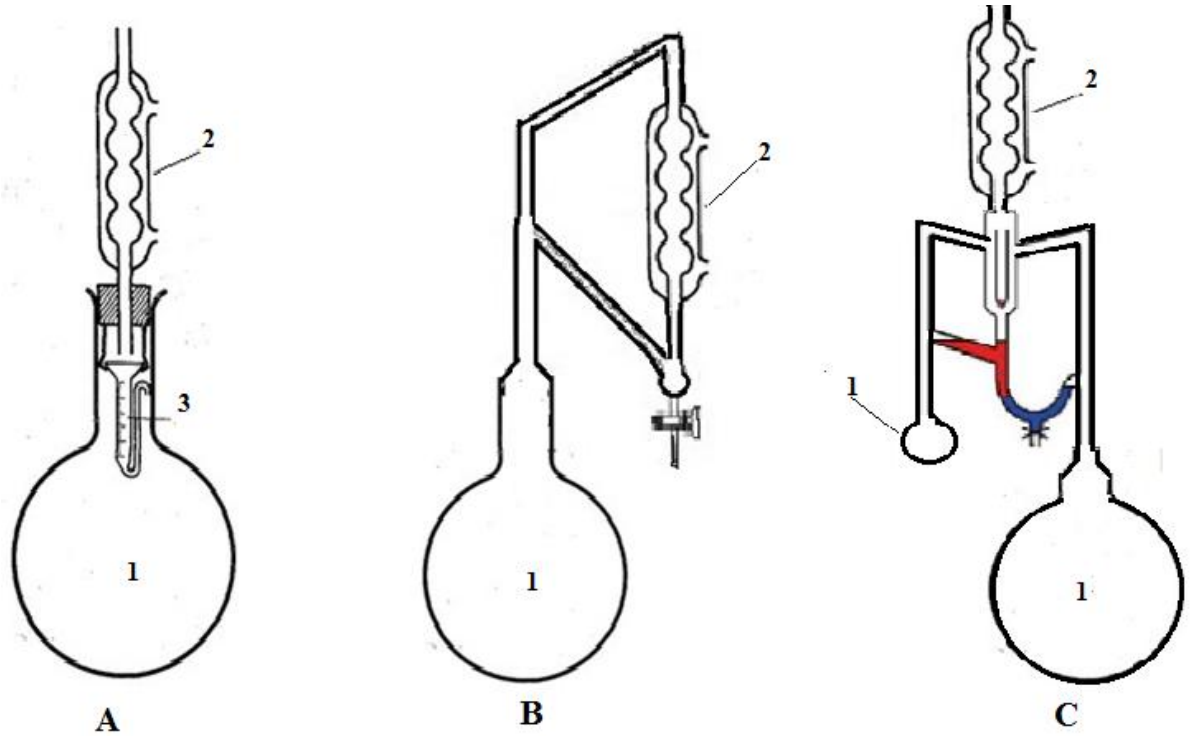


Fig. 3.2. Essential oil distiller A). Ginsberg type apparatus B). Clevenger type apparatus C). Likens-Nickerson type apparatus. *1 - round-bottom flask; 2 - backflow condenser; 3 - Ginsberg collector*

The following formula is used to determine the yield of plant extracts (essential oils, methanol and water extracts):

$$\textbf{Plant extract yield } (\%) = \frac{\mathbf{a}}{\mathbf{b}} \times \mathbf{100}$$

where, **a** and **b** are net weight of extracts (grams) and total weight of plant materials (grams), respectively.

3.5. Separation of alkaloids of the *Papaver somniferum*

The methanol extracts from different parts of *P. somniferum* were mixed with 40 ml 1 M HCl in ratio 1:20 and were placed on a shaker for 12 h. Then the mixture was filtered. The pH of the filtrates was changed to pH 10-12 by adding ammonium hydroxide. Then alkaloids were

obtained by solid phase extraction using Hydromatrix Bulk Material (Agilent) and 100 ml dichloromethane. After evaporation of the solvent a pure alkaloid fraction was obtained. This isolation protocol follows closely that used by other researchers (Knapp et al., 1967).

3.6. Gas liquid chromatography/flame ionization detector (GLC/FID)

The analysis of the essential oils and alkaloid fraction were performed using a GC-2010 plus gas chromatograph (Shimadzu), equipped with a non-polar ZB-5 fused bonded column (Phenomenex) and FID detector. The column parameters were 30 m length, 0.25 mm inner diameter and 0.25 µm film thickness. The carrier gas was helium (purity 99.99%) with a flow rate of 1.5 ml / min with split mode. The operating temperature conditions were initial temperature 120°C for 2 min isothermal followed by linear temperature increase till 320°C at a rate of 8°C / min, and then for 10 min at isothermal mode at 320°C. Detector and injector temperatures were 320°C and 310°C, respectively. GLC solution by Shimadzu was used for recording and integration. Samples were injected with the auto sampler AOC-20i, Auto Injector, Shimadzu.

3.7. Gas liquid chromatography/mass spectrometry (GLC/MS) analysis

GLC/MS analysis was carried out on:

1. a Shimadzu GCMS-QP2010 Ultra operated in the EI mode [(electron energy = 70eV), scan range = 3.0 scans/sec], and GLCMS solution software. The GLC column was ZB-5 fused silica capillary column with a (5% phenyl)-polymethyl siloxane stationary phase a film thikness of 0.25 mm. The career gas was helium with a column head pressure 80 psi and flow rate of 1.37 ml/min. Injector temperature was 250 ^{o}C and the ion source temperature was 200 ^{o}C, increase in temperature rate 2 ^{o}C/min to 260 ^{o}C. The GLC oven temperature program was programmed for 50 ^{o}C initial temperature, increase in rate 2 ^{o}C/min to 260 ^{o}C. A 5% w/v solution of the sample in CH_2Cl_2 was prepared and 0.1 µL was injected in splitting mode (30:1). GLG-FID experiments are performed by help of Mr. Mansour Sobeh.

2. an Agilent 6890 GLC with Agilent 5973 mass selective detector [MSD, operated in the EI mode (electron energy = 70 eV), scan range = 40-400 amu, and scan rate = 3.99 scans/sec], and an Agilent ChemStation data system. The GLC column was an HP-5ms fused silica capillary with a (5% phenyl)-polymethylsiloxane stationary phase, film thickness of 0.25 µm, a length of 30 m, and an internal diameter of 0.25 mm. The carrier gas was helium with a column head pressure of 48.7 kPa and a flow rate of 1.0 mL/min. Inlet temperature was 200°C and interface temperature was 280°C. The GLC oven temperature program was used as follows: 40°C initial temperature, hold for 10 min; increased at 3°C/min to 200°C;

increased 2°/min to 220°C. A 1 % w/v solution of the sample in CH_2Cl_2 was prepared and 1 µL was injected using a splitless injection technique.

Identification of the oil components was based on their retention indices determined by reference to a homologous series of *n*-alkanes (Kovats RI), and by comparison of their mass spectral fragmentation patterns with those reported in the literature (Adams, 2007) and stored on the MS library (NIST 11, WILEY 10, FFNSC version 1.2). The percentages of each component are reported as raw percentages based on total ion current without standardization (set 100%). GLG-MS experiments are performed by help of Prof. William Setzer, Dr. Prabodh Satyal and Mr. Frank Sporer.

3.8. High performance liquid chromatography analysis

For a phytochemical analysis of non volatile compounds a high performance liquid chromatography (HPLC) was employed using an YL9100 HPLC system (Younglin company, Korea). Samples were injected automatically by using a Midas autosampler with an injection loop of 100 µl from which only 20 µl was injected via partial loop fill. Standard and sample solutions were filtered through a Sartorius syringe filter before injection. The separation was achieved by using a C18 reversed-phase column (Merck Lichrocart 250-4, Lichrospher 100 RP-18e, 5 µm, Sorbent lot number L010077333). The mobile phase consisted of acetonitrile and water/formic acid 0.1%; gradient elution from 10 to 100% in 45 min, and then 100% acetonitrile for 5 min and back again to initial condition for 10 min to recondition of the column. The flow rate was kept at 1.0 ml/min. All analyses were performed at 30 $^{\circ}$C and detection was carried out at several wavelengths (220 nm, 254 nm, 280 nm, 320 nm, 350 nm) using a UV-DAD detector. HPLC experiments are performed by help of Mr. Mansour Sobeh and Dr. Bernhard Wetterauer.

3.9. Determination of total phenolic contents

The total phenolic content was determined according to the Folin-Ciocalteu method as described before (Folin and Ciocalteu, 1927; Vermerris and Nicholson, 2007). Extracts were dissolved in methanol in the concentration of 10 mg/ml. 20 µl of serial dilutions with a concentration range from 0 to 200 µg/ml were pipetted into wells of a 96 well microtiter plate to which 100 µl Folin-Ciocalteu reagent were then added. After 5 min 80 µl of 7.5% sodium carbonate solution was added and the plate was mixed well. The plate was kept in the dark at room temperature for 2 h (Fujita et al., 2012; Zhang et al., 2006). The absorbance was measured at 750 nm with a Biochrom Asys UVM 340 Microplate Reader (Biochrom

company). Based on absorbance measurements of a standard solution (caffeic acid) a calibration curve was constructed. Results were expressed as mg caffeic acid equivalents (CAE)/100 g of fresh or dry weight.

3.10. Determination of total flavonoid contents

Total flavonoid contents were determined by the calorimetric aluminum method (Harborne, 1973) using quercetin as a reference standard. Briefly, the extract (150 µl, 0.4 mg/ml) was mixed with 2% (w/w) $AlCl_3$ (100 µl) in 96-well plates. Absorbance was measured at 430 nm with a Biochrom Asys UVM 340 Microplate Reader after 30 min of incubation at room temperature. All determinations were performed in triplicates with reference to a sample blank value (without aluminum chloride). The calibration curve was plotted versus concentrations of quercetin (4 to 80 µg/ml). Results were expressed as mg quercetin equivalents (QE)/100 g of fresh or dry weight.

3.11. DPPH radical scavenging assay

In the DPPH radical scavenging assay (Blois, 1958), 100 µl of extracts (diluted in methanol in a concentration range 7.8 to 1000 µg/ml) was mixed with 100 µl of 0.2 mM DPPH in methanol in wells of a 96-well plate. The plate was kept in the dark for 15 min, after which the absorbance of the solution was measured at 515 nm in a Biochrom Asys UVM 340 Microplate Reader. Appropriate blanks (methanol) and standards (ascorbic acid in methanol) were run simultaneously. This method follows closely that used by previous workers (Brand-Williams et al., 1995; Clarke et al., 2013). The inhibitory activity (in %) was calculated by using the following expression:

$$\textbf{DPPH scavenging}\ (\%) = \frac{\textbf{100} \times\ [(\textbf{Abs sample} + \textbf{DPPH}) - (\textbf{Abs sample blanke})]}{[(\textbf{Abs DPPH}) - (\textbf{Abs methanol})]}$$

The IC_{50} value is defined as the amount of extract needed to scavenge 50% of DPPH radicals.

3.12. Free radical scavenging capacity using the ABTS assay

The $ABTS^{\bullet+}$ free radical was prepared by dissolving 38 mg ABTS reagent in 10 ml deionised purified water (final concentration was 7.0 mM). Then 6.5 mg potassium persulphate was added to the $ABTS^{\bullet+}$ solution and allowed to react for 16 h to form the stable $ABTS^{\bullet+}$ radical cation. The $ABTS^{\bullet+}$ solution was further diluted with water to obtain a final absorbance value between 2.0 and 2.4 at 645 nm. A 20 mM Trolox solution was prepared in absolute ethanol as a positive control. A 96-well microplate was loaded with 100 µl of extract (in a concentration range 7.8-1000 µg/ml) or Trolox or water blank to which 100 µl of $ABTS^{\bullet+}$ solution were

added. The absorbance was measured at 645 nm, using a Biochrom Asys UVM 340 Microplate Reader (Biochrom company). This method follows closely that used by previous workers (Brangoulo and Molan, 2010). The inhibitory activity was calculated as for the DPPH assay.

3.13. Antioxidant activity using the linoleic acid peroxidation method

The ferric thiocyanate method (FTC) method is based on the determination of peroxide in the first stage of linoleic acid peroxidation (Noorhajati et al., 2012). The peroxide reacts with ferrous chloride to form a reddish ferric chloride pigment. The linoleic acid emulsion was prepared by homogenizing 0.28 g linoleic acid, 0.28 g Tween-20 as emulsifier and 50 ml phosphate buffer (0.2 M, pH 7.0). 100 µl of each sample (10 mg/ml in methanol) was mixed with 2.0 ml of the linoleic acid emulsion, 2.0 ml of phosphate buffer (0.2 M, pH 7.0) and incubated at 37° C for 24 h. The mixture, prepared as above without test sample, served as control. The incubation solution (100 µl) was mixed with 2.0 ml 75% ethanol, 50 µl 30% ammonium thiocyanate, and 50 µl 20 mM ferrous chloride in 3.5% hydrochloric acid and allowed to stand at room temperature for 5 min. A 96-well microplate was loaded with 150 µl of the mixed solution and the absorbance was measured at 500 nm using a Biochrom Asys UVM 340 Microplate Reader. The degree of linoleic acid peroxidation was calculated using the following formula:

$$\textbf{Antioxidant activity} = \left[1 - \frac{\mathbf{A_1}}{\mathbf{A_0}}\right] \times \mathbf{100}$$

where $\mathbf{A_0}$ is the increase in absorbance of control and $\mathbf{A_1}$ is the increase in absorbance of the sample. Trolox and caffeic acid were used as positive controls for comparison. All tests and analyses were carried out in triplicate and averaged.

3.14. Ferric reducing antioxidant power

For determination of FRAP response, 20 µL of oil sample, diluted appropriately in DMSO, was mixed with 180 µL FRAP reagent in wells of a 96-well plate, left for 6 minutes, and the absorbance measured at 595 nm in a microplate reader. FRAP reagent was prepared freshly by mixing 300 mM acetate buffer pH 3.6, 10 mM tripyridyltriazine in 40 mM HCl, and 20 mM $FeCl_3 \bullet 6H_2O$ in the volume ratio 10:1:1. The FRAP working solution was warmed at 37 ^oC for 30 min prior to the assy. $FeSO_4$ (1200 µM - 6.25 µM) was used to generate the standard curve. FRAP values were expressed as µM Fe (II)/mg of samples.

3.15. Inhibition of 5-lipoxygenase (anti-inflammatory activity)

Inhibition of 5-lipoxygenase (5-LOX), a key enzyme in the inflammation pathway, was determined spectrophotometrically (Baylac and Racine, 2003). Briefly, 0.1 M phosphate buffer (1 ml) pH 9.0, containing 6.25 µl of 5-LOX from soybean (7.9 U/ml) and 20 µl of 10 different concentrations (0.04-10 mg/ml extracts in ethanol) were incubated at room temperature for 10 min. The reaction was initiated by adding 20 µl of 62.5 µM sodium linoleate and the reaction kinetics were monitored at 234 nm at 10-second intervals using a Biowave II UV/VIS spectrophotometer. 5-LOX experiments are performed by Mrs. Sonja Kristin help.

3.16. Cytotoxicity assay

Cytotoxicity of extracts was determined with MCF-7 (human breast adenocarcinoma), HeLa (human cervical cancer), Caco-2 (human colorectaladenocarcinoma), CCRF-CEM (human T lymphoblast leukaemia) and CEM/ADR5000 (adriamycin resistant leukaemia) cell lines using the MTT assay. The cells were seeded at a density of 2×10^4 cells/well (MCF-7, HeLa and Caco-2) and 3×10^4 cells/well (CCRF-CEM and CEM/ADR5000). The methanol extracts were serially diluted from 5 mg/ml to 0.004 mg/ml, and 100 µl liquid of each concentration was applied to the wells of a 96-well plate. Cells were incubated with the samples for 24 h (MCF-7 and HeLa) and 48 h (CCRF-CEM and CEM/ADR5000) before the medium was removed and replaces with fresh medium containing 0.5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The formazan crystals were dissolved in DMSO 2 - 4 h later; the absorbance was measured at 570 nm with a Biochrom Asys UVM 340 Microplate Reader.

3.16.1. Cytotoxicity of combination of doxorubicin with essential oils

Non-toxic concentration of essential oils (2-3 µg/ml) were combined with doxorubicin in order to determine whether a dual combination of doxorubicin with an essential oil. The CCRF-CEM cells were seeded at a density of 3×10^4 cells/well. Cells were incubated with serial dilutions of doxorubicin in addition to a non-toxic concentration of each essential oil for 48 h. MTT assays were carried out as mentioned above.

3.17. TLC bioautography assay

TLC bioautography method was used to separate the chemical constituents and to screen the antioxidant capacity of the extracts (Dehshahri et al., 2012; Jin Wang et al., 2012). The method employed silica gel 60 F254 plates (Merck, Darmstadt, Germany) as stationary phase

and mixture of ethyl acetate-formic acid-water (84:1:5, v/v/v) as mobile phase. After developing, the plates were sprayed with DPPH reagent (0.2% in MeOH).

3.18. Antibacterial activity

The essential oils were stabilized in Tween-80 and screened against two gram positive and gram negative bacteria at concentrations between 9.8 µg/ml to 20 mg/ml. The final Tween concentration did not exceed 0.5%. The tested bacteria were MRSA NTCT 10442 and *Escherichia coli* ATCC 25922 and provided in courtesy of the Department of Infectious Diseases, Medical Microbiology and Hygiene, Heidelberg University, Heidelberg, Germany. The organisms were cultured on Columbia Agar supplemented with 5% sheep blood and in Müller-Hinton broth. The minimum inhibitory concentration (MIC) was obtained by means of broth microdilution following the method of CLSI (2012) with incubation at 35°C for 18 h. For the determination of the minimum bactericidal concentration (MBC), 3 µl of each well with concentrations at and above the MIC was streaked on agar plate and incubated for 24 h. The least concentration of the oil killing at least 99.9% of the initial inoculum was considered the MBC. Tween-80 growth and sterility controls were included in the tests and Ampicillin served as positive control. The tests were conducted in duplicate per plate and performed three times. The following bacterial species were used: Methicillin - resistant *Staphylococcus aureus* (MRSA 10442) and *Escherichia coli* (ATCC 25922). Antibacterial activity experiments are performed by help of Mr. Markus Braun.

3.19. The hemolytic activity of essential oils

The hemolytic activity was investigated by incubating 20 μL of serially diluted essential oil in phosphate-buffered saline (PBS; 400, 200, 100, 50, 25, 12.5, 6.25, and 3.12 $\mu g \cdot mL^{-1}$) with 80 μL of a suspension of 5% red blood cells (human O^{+}) for 1 h at $37^{o}C$ in assay tubes. The reaction was slowed by adding 200 μL of PBS, and then the suspension was centrifuged at 1000 g for 10 min. Cell lysis was then measured spectrophotometrically at 540 nm. The absence of hemolysis (blank control) or total hemolysis (positive control) was determined by replacing the essential oil solution with an equal volume of PBS or distillate water, respectively. The results were determined by the percentage of hemolysis compared to the positive control (100% hemolysis), and the experiments were performed in triplicate.

3.20. Microscopy

The images of the treated or untreated CCRF cells were obtained and photographed using a by fluorescence microscopy BZ-9000 microscope (Keyence) in order to investigate morphological changes.

3.21. Statistics

All experiments were carried out three times. The IC_{50} was determined as the drug concentration which resulted in a 50% reduction in viability or inhibition of the biological activity. IC_{50} values were calculated using a four parameter logistic curve (Sigma Plot 11.0).

Chapter 4. Phytochemistry part

4.1. Chemical composition of the essential oil of *Ferula clematidifolia*

To our best knowledge, no previous studies have been reported on the chemical composition of the essential oil of *F. clematidifolia*.

Essential oils of the Tajik *F. clematidifolia* were obtained from the aerial parts (leaves) and roots by hydrodistillation. Their compositions were analyzed by gas liquid chromatography – mass spectrometry (GLC-MS). The chemical composition of the *F. clematidifolia* oils is summarized in Table 4.1.

A total of 42 compounds were identified representing 99.8% of total oil composition. The major components of *F. clematidifolia* oil were β-pinene (2-37%), myrcene (4-34%), limonene (1-31%), α-pinene (2.5-29%), sabinene (8-17%), and β-phellandrene (up to 7%). The chemical structures of the main components of the *F. clematidifolia* oil are presented in Fig. 4.1A. GLC profile of the essential oil of *F. clematidifolia is presented in Fig. 4.1B.*

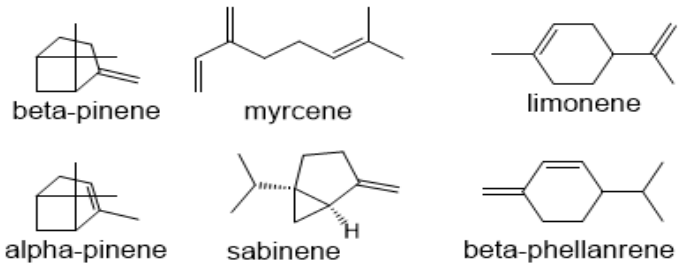


Fig. 4.1A. Chemical structures of the main components of the essential oil of *F. clematidifolia*

Essential oil from leaves of *F. clematidifolia* is dominated with sabinene and myrcene. That is differs from its root essential oil which is dominated with α- and β-pinenes.

Fig. 4.1B. **GLC-MS profile of the essential oil of *F. clematidifolia* (leaves).** 1. α-pinene 2. sabinene 3. myrcene 4. limonene 5. β-phellandrene

Table 4.1. Chemical composition of the essential oil of *F. clematidifolia*

RI*	Compound	Leaves (in %)	Roots (in %)	RI*	Compound	Leaves (in %)	Roots (in %)
860	2-Methyloctane	0.4	0.1	1186	Cryptone	0.2	-
925	α-Thujene	0.1	0.1	1242	Cuminal	0.1	-
933	α-Pinene	2.5	29.3	1331	δ-Elemene	-	0.2
949	Camphene	0.2	0.2	1357	Methylundecanal	-	0.2
973	Sabinene	16.5	8.1	1375	α-Copaene	0.1	1.0
978	β-Pinene	1.6	36.9	1387	β-Cubebene	0.2	1.0
991	Myrcene	34.3	3.9	1389	β-Elemene	0.1	0.3
1007	α-Phellandrene	0.3	0.1	1417	β-Funberene	-	0.2
1009	3-Carene	0.2	-	1419	*trans*-Caryophyllene	0.4	0.5
1017	α-Terpinene	0.4	-	1449	β-Barbatene	-	0.3
1025	*p*-Cymene	0.9	-	1452	*(E)*-β-Farnesene	-	0.1
1031	Limonene	30.1	1.0	1455	α-Humulene	-	0.3
1032	β-Phellandrene	7.0	0.3	1480	Germacrene D	0.7	3.2
1035	*Z*-β-Ocimene	0.2	1.5	1483	β-Selinene	-	0.1
1046	*E*-β-Ocimene	0.2	0.9	1495	Bicyclogermacrene	0.1	5.5
1058	γ-Terpinene	1.0	0.1	1507	β-Bisabolene	-	0.3
1085	Terpinolene	0.9	-	1517	δ-Cadinene	-	0.3
1098	Perillene	0.5	-	1576	Spathulenol	-	0.2
1159	*2E* - Nonenal	0.2	-	1597	Unidentified	-	0.1
1162	Lavandulol	0.3	-	1951	Grilactone	-	0.7
1175	(*3E,5Z*) -1,3,5 - Undecatriene	-	2.0	1964	Ferula lactone I	-	0.4
1181	Terpinen-4-ol	0.8	-				

*Kovats retention index in ZB-5 column.

4.2. Chemical composition of the essential oil of *Foeniculum vulgare*

The chemical composition of the essential oil of *F. vulgare* growing in different geographical locations has been extensively studied (Chowdhury et al., 2009; Dadaliogylu and Evrendilek, 2004; Ouariachi et al., 2014; Zellagui et al., 2011). According to these studies, the major

components of fennel oil are *trans*-anethole, estragole, fenchone, and limonene depending on the chemotype (Aprotosoaie et al., 2010; Radulovic and Blagojevic, 2010; Stefanini et al., 2006).

The main components of the composition of Tajik *F. vulgare* oil are *trans*-anethole (37%), *para*-anisaldehyde (8%), α-ethyl-p-methoxy-benzyl alcohol (9%), carvone (5%), 1-phenyl-penta-2,4-diyne (5%) and fenchyl butanoate (4%). The chemical structures of the main components of the *F. vulgare* oil are presented in Fig. 4.2A. GLC profile of the essential oil of *F. vulgare is presented in Fig. 4.2B.* Three major components (*trans*-anethole, *para*-anisaldehyde, α-ethyl-p-methoxy-benzyl alcohol) of the essential oil belong to aromatic ethers.

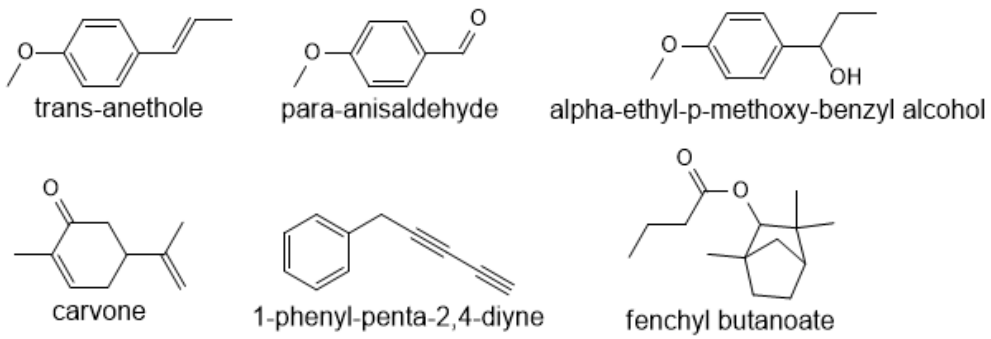


Fig. 4.2A. Chemical structures of the main components of the essential oil of *F. vulgare*

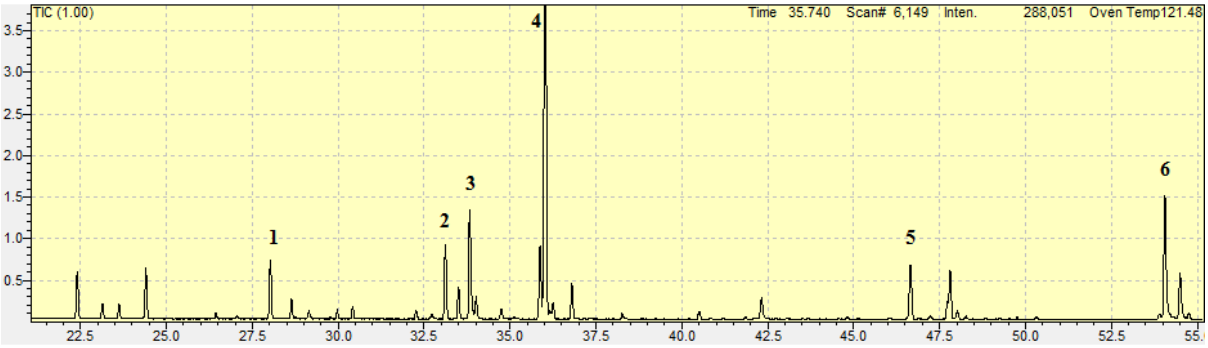


Figure 4.2B. GLC-MS profile of the essential oil from *Foeniculum vulgare.* 1. neomenthol 2. carvone 3. *para*-anisaldehyde 4. *trans*-anethole 5. fenchyl butanoate 6. α-ethyl-p-methoxy-benzyl alcohol

In accordance with previously published date, the main component of the composition of *F. vulgare* oil was *trans*-anethole (Mimica-Dukic et al., 2003; Singh et al., 2006). Its content varied from 5 to 86%. However, also estragole (Barazani et al., 2002; Gross et al., 2002), fenchyl acetate (Shahat et al., 2011) and limonene (Ouariachi et al., 2014) have been reported

as main component of the fennel oil from other origins. Fennel essential oil is known as sources for anethole (Franz and Novak, 2010).

Table 4.2. Chemical composition of the essential oil of *Foeniculum vulgare* according to a GLC-MS analysis

RI	Compounds	%	RI	Compounds	%
1286	*trans* - Anethole	36.8	1179	E- β-Terpineol	1
1569	α-Ethyl-p-methoxy-benzyl alcohol	9.1	1100	Linalool	0.75
1254	*para*-Anisaldehyde	7.73	1107	*cis* - Thujone	0.74
1243	Carvone	4.87	1204	*E*-Dihydrocarvone	0.64
1283	1-Phenyl-penta-2,4-diyne	4.75	1470	Unidentified	0.64
1448	Fenchyl butanoate	4.23	1267	Geranial	0.56
1170	Neomenthol	3.62	1288	Myrtenyl acetate	0.54
1467	2*E* - Dodecenal	3.44	1231	*exo*-Fenchyl acetate	0.48
1577	β-Ethyl-*p*-methoxy-benzyl alcohol	3.27	1353	Penta-1,3-diynylbenzene	0.46
1118	*trans* - Thujone	2.95	1186	Dill ether	0.44
1089	Fenchone	2.75	1198	Chavicol methyl	0.42
1297	Carvacrol	2.15	1567	Unidentified	0.3
1249	Linalyl acetate	1.88	1581	Caryophyllene oxide	0.25
1380	Unidentified	1.39	1147	Camphor	0.23
1256	*E* -Chrysanthenyl acetate	1.38	1156	*iso* - Menthone	0.1
1289	Thymol	1.03	1474	1-Hexadecene	0.08
1465	Fenchyl isobutanoate	1.03			

F. vulgare is subdivided it into three main chemotypes according to their relative compositions: 1: estragole chemotype, 2: estragole/anethole chemotype and 3: anethole chemotype (Muckensturm et al., 1997). The essential oil of *F. vulgare* from Tajikistan belongs to the anethole chemotype, which is widely distributed (Barazani et al., 2002).

4.3. **Chemical composition of the essential oil of *Galagania fragrantissima***

Because information about the chemical composition of *Galagania fragrantissima* was missing, we have analysed plant essential oil using high-resolution capillary GLC and GLC-MS which are the methods of choice for such analyses.

The chemical composition of the *G. fragrantissima* oil is summarized in Table 4.3. Ten components were identified representing 99.1% of total oil composition.

The main constituents of the essential oil *of the G. fragrantissima* were a series of aliphatic aldehydes and alcohols such as (2*E*)-dodecenal (84%), (2*E*)-dodecenol (8%), (2*E*)-tetradecenal (3%) and dodecanal (2%). The chemical structures of the main components of the *G. fragrantissima* oil are presented in Fig. 4.3A. GLC profile of the essential oil of *G. fragrantissima* is presented in Fig. 4.3B. Authors (Kim et al., 2011) had described the microbial metabolism of (2*E)*-dodecenal which resulted in two microbial metabolites: (2*E)*-dodecenol and (3*E*)-dodecenoic acid.

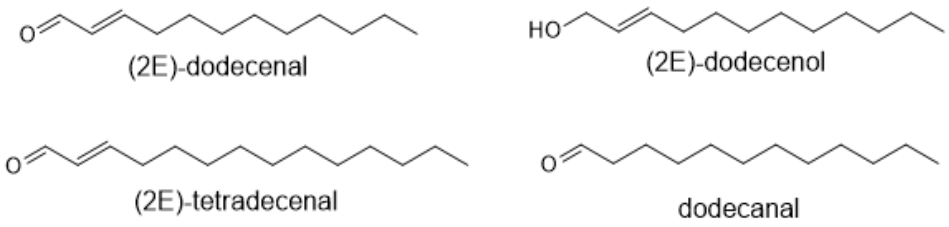


Fig. 4.3A. Chemical structures of the main components of the essential oil of *G. fragrantissima*

Table 4.3. Composition of the essential oil of *Galagania fragrantissima* determined by GLC-MS

RI	Compound	%
1206	Decanal	0.5
1261	(2*E*)-Decenal	Trace
1400	(4*E*)-Dodecenal	1.0
1411	Dodecanal	2.3
1453	Unidentified	0.9
1473	(2*E*)-Dodecenal	83.6
1480	(2*E*)-Dodecenol	7.8
1589	1-Hexadecene	0.1
1613	Tetradecanal	0.1
1673	(2*E*)-Tetradecenal	3.4
	Total identified	99.1

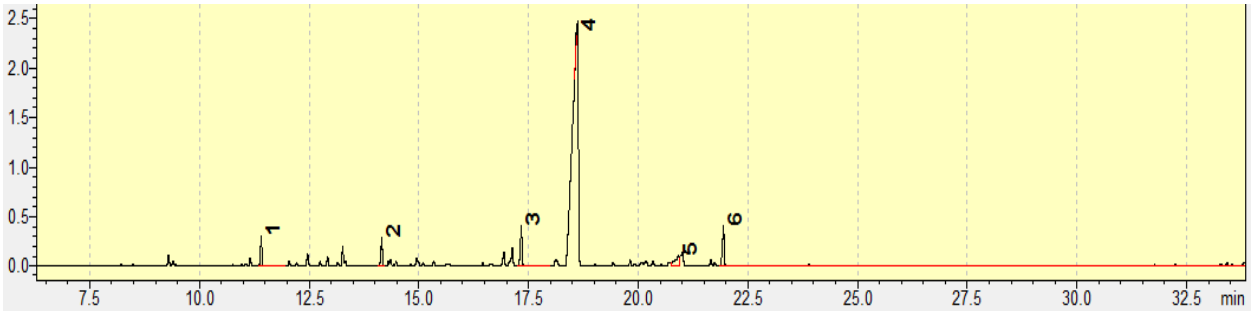


Fig. 4.3B. GLC profile of the essential oil of *Galagania fragrantissima.* 1. decanal 2. (4*E*)-dodecenal 3. dodecanal 4. (2*E*)-dodecenal 5. (2*E*)-dodecenol 6. (2*E*)-tetradecenal.

4.4. Chemical composition of the essential oil of *Geranium macrorrhizum*

Chemical composition of the essential oil German *G. macrorrhizum* is mainly dominated by germacrone (60%), following by *trans*-β-elemenone (5%), α-eudesmol (4%), germacrene B (4%), and 10-epi-β-acoradiene (4%). A result of analysis of *G. macrorrhizum* oil is presented in Table 4.4. The chemical structures of the main components of the *G. macrorrhizum* oil are presented in Fig. 4.4A. GLC profile of the essential oil of *G. macrorrhizum* is presented in Fig. 4.4B.

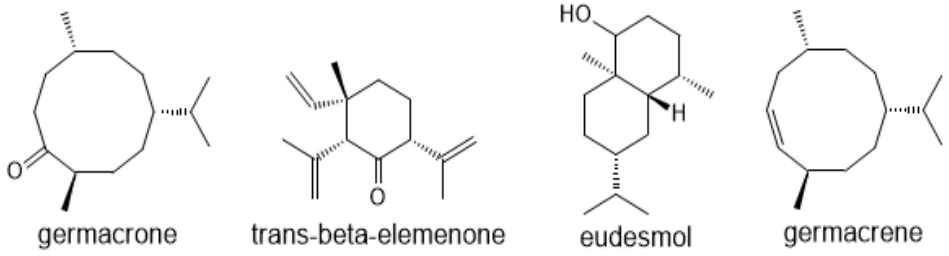


Fig. 4.4A. Chemical structures of the main components of the essential oil of *Geranium macrorrhizum*

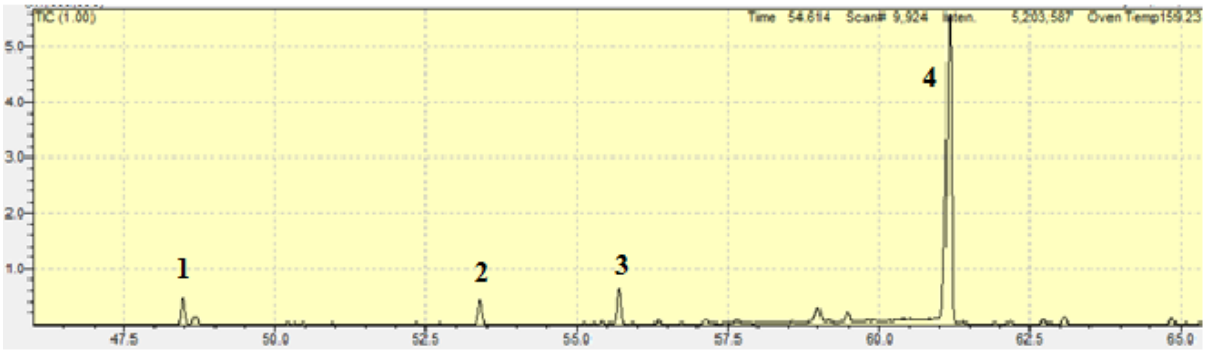


Figure 4.4B. GLC-MS profile of the essential oil from *Geranium macrorrhizum.* 1. γ-curcumene 2. germacrene B 3. *trans-β*-elemenone 4. germacrone.

Table 4.4. Chemical composition of the essential oil of *Geranium macrorrhizum*

RI	Compound	%	RI	Compound	%
978	β-Pinene	0.1	1658	Selin-11-en-4-alpha-ol	0.81
1024	p-Cymene	0.09	**1663**	**Germacrone isomer**	**2.53**
1058	γ-Terpinene	0.18	1669	Unidentified	1.25
1389	Elemene beta	0.34	1671	Unidentified	0.8
1405	Italicene	0.09	1675	Unidentified	0.84
1429	γ-Elemene	0.74	1676	Germacrone isomer	0.26
1477	γ-Curcumene	3.59	1680	Unidentified	1.03
1480	Ar-Curcumene	1.01	1681	Unidentified	1.21
1505	Z- α-Bisabolene	0.18	1685	Unidentified	0.93
1547	α -Elemol	0.11	1687	Eudesma-4(15),7-dien-1-ol	1.1
1558	**Germacrene B**	**3.62**	1689	Unidentified	0.83
1597	***trans-β*-Elemenone**	**5.34**	**1693**	**Germacrone**	**60.14**
1608	Unidentified	0.46	1697	Juniper camphor	0.34
1622	Unidentified	0.64	1721	Unidentified	0.55
1631	γ-Eudesmol	0.73	1728	Unidentified	1.09
1642	Muurolol	0.26	1761	Unidentified	0.75
1647	Unidentified	0.58	1765	Benzyl benzoate	0.11
1655	**α-Eudesmol**	**3.9**	1769	Hydroxy muurolene	0.11

283 constituents were identified in the essential oils of *G. macrorrhizum* (Radulovic et al., 2010). Germacrone (50% in the oil from aerial parts) and d-guaiene (49% in rhizome oil) were the main oils components (Radulovic et al., 2010). Composition of the essential oils from *G. macrorrhizum* is in agreement with those of previous studies (Ivancheva et al., 2006; Radulovic et al., 2010). Germacrone as the main prominent component of the oil, has been found in relatively high amounts in the essential oils of *Curcuma leucorhiza* (Devi et al., 2012), *Eugenia uniflora* (Bicas et al., 2011).

4.5. Chemical composition of the essential oil of *Pastinaca sativa*

Essential oil is obtained from the aerial parts of the German *Pastinaca sativa* by using Likens-Nickerson type apparatus. Results of GLC MS analysis of *P. sativa* essential oil is presented in Table 4.5. The major components of the composition of the essential oil *P. sativa* were octyl butyrate (40.9%), octyl acetate (32.4%), hexyl butanoate (4.6%), Z-β-

ocimene (4.3%), E-β-farnesene (3.4%) and γ-stearolactone (3.4%). The chemical structures of the main components of the *P. sativa* oil are presented in Fig. 4.5A. GLC MS profile of the essential oil of *P. sativa is presented in Fig. 4.5B.*

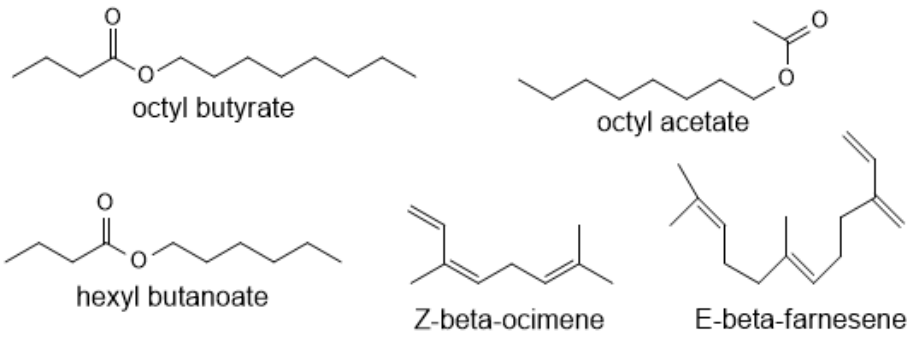


Fig. 4.5A. Chemical structures of the main components of the essential oil *P. sativa*

Terpinolene (40–70%), octyl butyrate (79%) and myristicin (17–40%) have previously been reported as the main constituents in the root and seed essential oil of *P. sativa* (Kurkcuoglu et al., 2006).

The essential oil from crushed seeds of *P. sativa* has previously been reported to contain octyl butyrate (80%) and octyl hexanoate (5%) as the major constituents.

According to the present results and previous reports the principal component is octyl butyrate. Octyl butyrate was also as the major constituents of the essential oils of *Malabaila aurea* (Vuckovic et al., 2014), *Heracleum sphondylium* (Maggi et al., 2014).

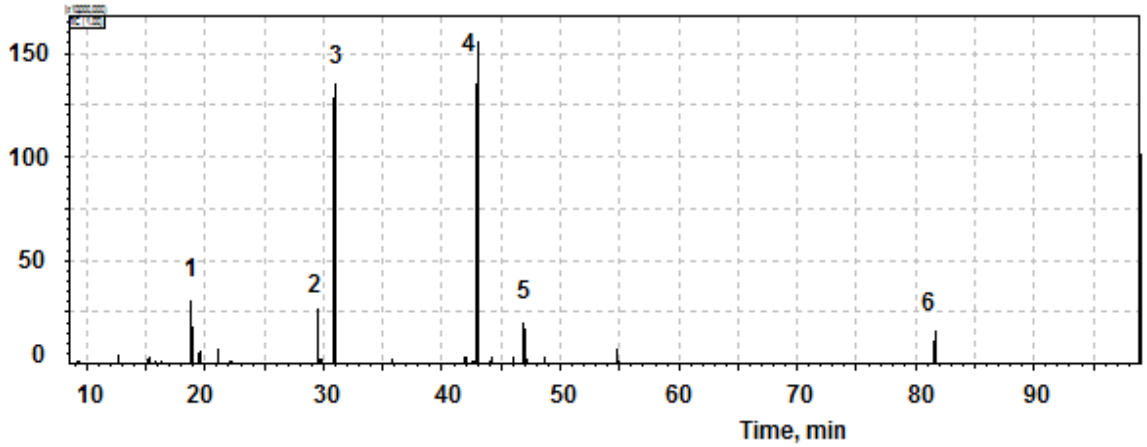


Figure 4.5B. GLC-MS profile of the essential oil from *Pastinaca sativa*. 1. Z-β-ocimene 2. hexyl butanoate 3. octyl acetate 4. octyl butyrate 5. *E-β*-farnesene 6. γ-stearolactone.

Table 4.5. Chemical composition of the essential oil of *Pastinaca sativa*

RI	Compounds	%	RI	Compound	%
860	2-Methyl octane	0.15	1283	Lavandulyl acetate	0.35
900	Nonane	0.02	1345	Benzyl butanoate	0.07
933	α-Pinene	0.47	1375	Pentadecenyl acetate	0.57
949	Camphene	0.03	1377	2Z-Octenol butanoate	0.09
972	Sabinene	0.07	1383	α-Bourbonene	0.07
978	β-Pinene	0.44	1386	Hexyl hexanoate	0.4
989	Myrcene	0.24	**1391**	**Octyl butyrate**	**40.95**
995	Butyl butanoate	0.22	1409	Decyl acetate	0.58
1003	Octanal	0.1	1439	Phenylethyl butanoate	0.66
1007	α-Phellandrene	0.03	**1452**	***E*-*β*-Farnesene**	**3.37**
1024	p-Cymene	0.04	1457	Neryl-propanoate	0.38
1029	Limonene	0.04	1480	Germacrene D	0.59
1035	**Z-β-Ocimene**	**4.28**	1490	Farnesene <isomer>	0.12
1045	E-β-Ocimene	0.89	1503	(E,E)-α-Farnesene	0.07
1057	γ-Terpinene	0.02	1519	Myristicin	0.04
1069	Octanol	1.18	1560	*trans*-Nerolidol	0.09
1085	Terpinolene	0.32	1570	Heptadecenyl acetate	0.03
1100	Linalool	0.03	1582	Octyl hexanoate	1.27
1103	Hotrienol	0.03	1587	Decyl isobutyrate	0.04
1128	*allo*-Ocimene	0.08	1615	Uuidentified	0.11
1185	3Z-Hexenyl butyrate	0.08	1640	Phenyl ethyl hexanoate	0.07
1187	p-Cymen-8-ol	0.03	1692	Germacrone	0.02
1192	**Hexyl butanoate**	**4.57**	2092	Methyl octadecanoate	0.03
1196	Nonenyl acetate	0.36	**2099**	**γ-Stearolactone**	**3.37**
1199	2Z-Octenol acetate	0.02	2106	Phytol	0.06
1206	Decanal	0.08	2121	Unidentified	0.13
1212	**Octyl acetate**	**32.44**	2500	Pentacosane	0.02
1278	Octen-3-ol butanoate	0.04			

4.6. Chemical composition of the essential oil of *Philadelphus x purpureomaculatus*

To our best knowledge, no previous work has been reported on the chemical composition of the volatile oil of *P. x purpureomaculatus*, and so this work is a first and original study of this deciduous shrub.

Table 4.6 presents the results of chemical composition of the essential oil of *P. x purpureomaculatus.* A total of 30 compounds were identified representing 99.9% of total oil compositions. The major components of *P. x purpureomaculatus* oil were viridiflorol (44%), manool (31%), pentadecanal (5%), and borneol (5%). The chemical structures of the main components of the *P. x purpureomaculatus* oil are presented in Fig. 4.6A. GLC profile of the essential oil of *P. x purpureomaculatus is presented in Fig. 4.6B. P. coronarius* L. (Czigle et al., 2005) contains a different set of volatile substances with *epi*-13-manool (48%), *iso*-longifolol (15%), and (*E*,*E*)-farnesole (37%) as major components.

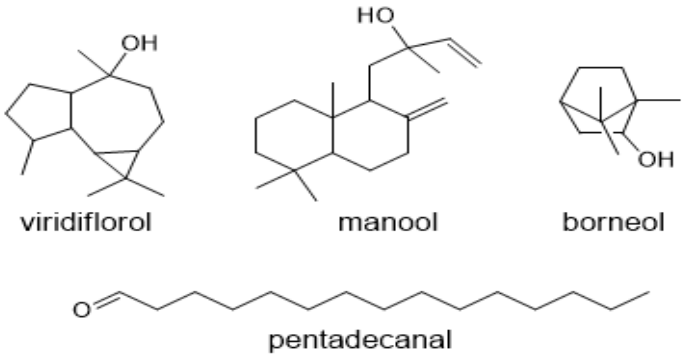


Fig. 4.6A. Chemical structures of the main components of the essential oil *P. x purpureomaculatus*

Figure 4.6B. GLC-MS profile of the essential oil from *P. x purpureomaculatus.* 1. borneol 2. viridiflorol 3. pentadecanal 4. manool.

Table 4.6. Chemical composition of the essential oil of *Philadelphus x purpureomaculatus*

RI	Compounds	%	RI	Compounds	%
933	α-Pinene	0.14	1513	δ-Amorphene	0.13
972	Sabinene	0.18	1515	δ-Cadinene	0.1
978	**β-Pinene**	**1.56**	1518	δ-Amorphene	0.72
989	Myrcene	0.12	1576	Spathulenol	0.97
1024	o-Cymene	0.42	1585	Gleenol	0.46
1029	Limonene	1.26	**1594**	**Viridiflorol**	**44.44**
1032	1.8-Cineole	1.16	1606	5-epi-7-epi-α-Eudesmol	0.18
1058	γ-Terpinene	0.25	1609	Humulene epoxide II	0.98
1100	Linalool	0.12	1613	Tetradecanal	0.05
1107	Z-Thujone	0.46	**1715**	**Pentadecanal**	**5.37**
1147	Camphor	1.39	1799	α-dimer- Phellandrene	0.27
1172	**Borneol**	**4.94**	1817	Hexadecanal	0.1
1284	Bornyl acetate	0.11	1886	Unidentified	0.13
1419	*E*-Caryophyllene	0.11	1919	3-Nonadecene	1.13
1455	α-Humulene	1.49	**2051**	**Manool**	**31.15**
1474	*Cis*- Cadina-1(6),4-diene	0.11			

4.7. Chemical composition of the essential oil of *Polychrysum tadshikorum*

To our best knowledge, there no information on the chemical composition of this species has been published.

The chemical composition of the essential oil of *P. tadshikorum* growing wild in Tajikistan is summarized in Table 4.7. A total of 72 compounds were identified representing 97.01% of total essential oil of *P. tadshikorum* composition. The major components of the oil were terpinen-4-ol (15%), sabinene (13%), *p*-cymene (7%), linalool (5%), and γ-terpinene (4%) which are typical secondary metabolites of Asteraceae. The chemical structures of the main components of the *P. tadshikorum* oil are presented in Fig. 4.7A. GLC profile of the essential oil of *P. tadshikorum* is presented in Fig. 4.7B.

Table 4.7. Chemical composition of the essential oil of *Polychrysum tadshikorum* as determined by GLC-MS

RI*	Compounds	%	RI*	Compounds	%
1180	Terpinen-4-ol	14.5	1504	Lavandulyl isovalerate	0.4
972	Sabinene	13.0	1320	Unidentified	0.4
1024	p-Cymene	7.4	1142	*E*-*p*-Menth-2-en-1-ol	0.4
1099	Linalool	5.4	1206	Decanal	0.3
1057	γ-Terpinene	4.4	1603	Geranyl isovalerate	0.3
1641	α-Muurolol	3.4	1623	Unidentified	0.3
1654	α-Eudesmol	3.3	1707	2*E*,6*Z*-Farnesal	0.3
1195	*α*-Terpineol	2.8	1282	Lavandulyl acetate	0.3
1466	*2E*-Dodecenal	2.8	1017	*α*-Terpinene	0.3
1375	α-Copaene	2.6	1514	γ-Cadinene	0.3
1480	Germacrene D	2.5	1147	Camphor	0.3
977	*β*-Pinene	2.4	1452	*E*-*β*-Farnesene	0.3
1032	1.8-Cineole	2.4	1631	γ-Eudesmol	0.3
1045	*E*-*β*-Ocimene	2.1	1186	*p*-Cymen-8-ol	0.3
1029	Limonene	1.6	1824	Unidentified	0.2
1581	Caryophyllene oxide	1.5	1208	*E*-Piperitol	0.2
1575	Spathulenol	1.5	1429	*β*-Copaene	0.2
1488	*β*-Selinene	1.4	1963	Pimaradiene	0.2
1372	Unidentified	1.2	1409	Dodecanal	0.2
1419	*trans*-Caryophyllene	1.2	1301	Unidentified	0.2
1273	*E*-Ascaridol glycol	1.1	1828	Unidentified	0.2
988	Myrcene	1.0	1284	Bornyl acetate	0.2
1030	*β*-Phellandrene	0.9	1840	Phytone	0.2
1512	γ-Cadinene	0.9	1224	Ascaridole	0.2
932	*α*-Pinene	0.9	1472	Unidentified	0.2
1101	*trans*-Sabinene hydrate	0.9	1085	Terpinolene	0.2
1124	*p*-Menth-2-en-1-ol	0.7	1107	*Z*-Thujone	0.2
1069	*trans*- 4-Thujanol	0.6	1297	3-Methoxy-acetophenone	0.2
1291	Ascaridol glycol	0.6	1474	Muurolene	0.2
1735	2*E*,6*Z*-Farnesal	0.6	1901	Unidentified	0.2
1105	Nonanal	0.6	2300	Tricosane	0.2
1860	Platambin	0.6	1497	α-Muurolene	0.1
1035	*Z*-*β*-Ocimene	0.6	1646	Unidentified	0.1
1494	*epi*-Cubebol	0.5	1455	*α*-Humulene	0.1
1517	δ-Amorphene	0.5	1459	*allo* –Aromadendrene	0.1
1486	*p*-Menthane-1,2,4-triol	0.5	1949	Geranyl α-terpinene	0.1
1172	Borneol	0.4	1140	*trans*-Sabinol	0.1
1470	*2E*-Dodecen-1-ol	0.4	1249	Linalylacetate	0.1
1163	Lavandulol	0.4	1608	Juneol	0.1

*Kovats retention index on ZB-5 column.

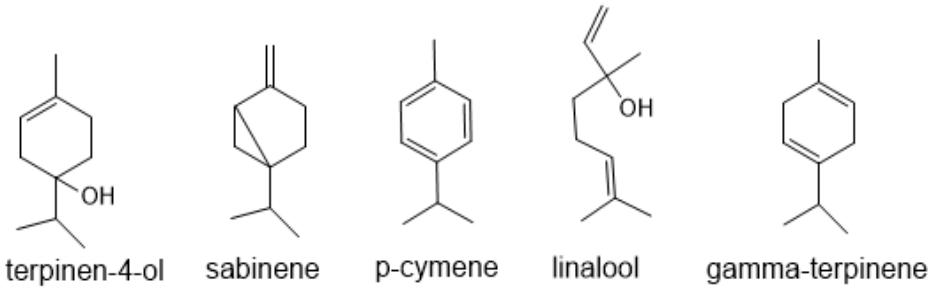


Fig. 4.7A. Chemical structures of the main components of the essential oil *P. tadshikorum*

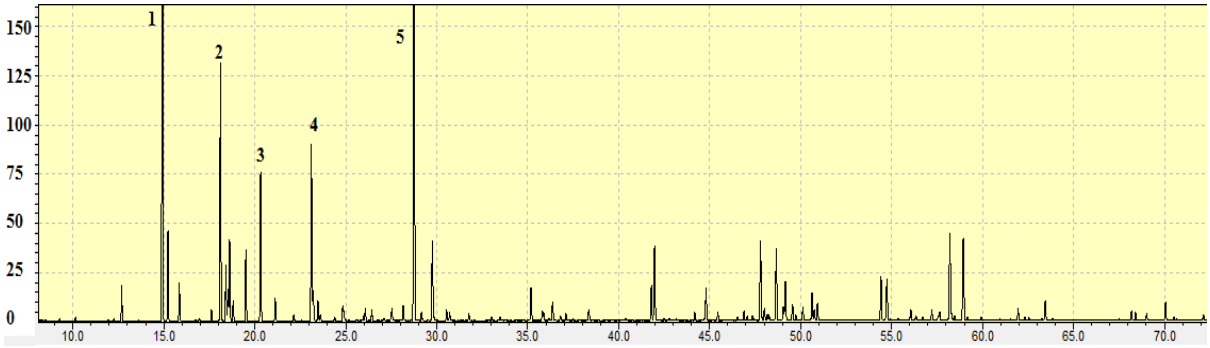


Figure 4.7B. GLC-MS profile of the essential oil from *P. tadshikorum.* 1. sabinene 2. p-cymene 3. γ-terpinene 4. linalool 5. terpinen-4-ol.

4.8. Chemical composition of the essential oil of *Salvia discolor*

Essential oil is obtained from the aerial parts of the *S. discolor* growing in Germany by using Likens-Nickerson type apparatus.

No previous work has been reported on chemical composition of essential oil of *S. discolor*.

Chemical composition of the *S.discolor* essential oil is presented in Table 4.8. The major components were *trans*-caryophyllene (18%), germacrene D (4%), α-humulene (3%) and linalool (3%). Chemical structures of the main components of the essential oil *S. discolor* are presented in Fig. 4.8A. GLC-MS profile of the essential oil *S.discolor* is presented in Fig. 4.8B. However, the most abundant component (57%) with retention index 1667 could not be identified (Figure 4.8C).

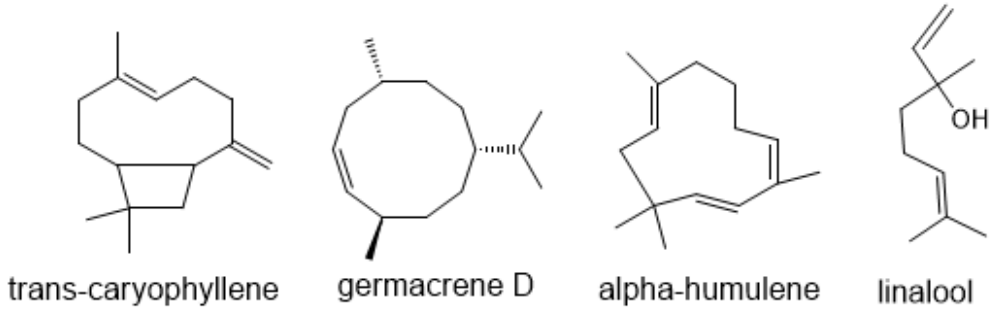


Fig. 4.8A. Chemical structures of the main components of the essential oil *Salvia discolor*

Table 4.8. Chemical composition of the essential oil of *Salvia discolor*

RI	Compounds	%
933	Cyclofenchene	0.34
972	Sabinene	0.24
978	β-Pinene	1.94
1024	p-Cymene	0.32
1029	Limonene	0.89
1032	1.8-Cineole	0.4
1058	γ-Terpinene	0.26
1099	**Linalool**	**3**
1107	Octen-3-yl acetate	0.54
1119	3-Octyl acetate	1.68
1389	β-Elemene	0.36
1419	***trans*-Caryophyllene**	**17.81**
1455	α-**Humulene**	**3.11**
1480	**Germacrene D**	**3.98**
1494	α-Zingiberene	1.21
1507	**β-Bisabolene**	**2.54**
1523	β-Sesquiphellandrene	0.55
1524	Unidentified	0.21
1547	α-**Elemol**	**2.07**
1581	Caryophyllene oxide	0.3
1633	Unidentified	0.51
1667	**Unidentified**	**57.37**
1782	Agarospirol acetate	0.37

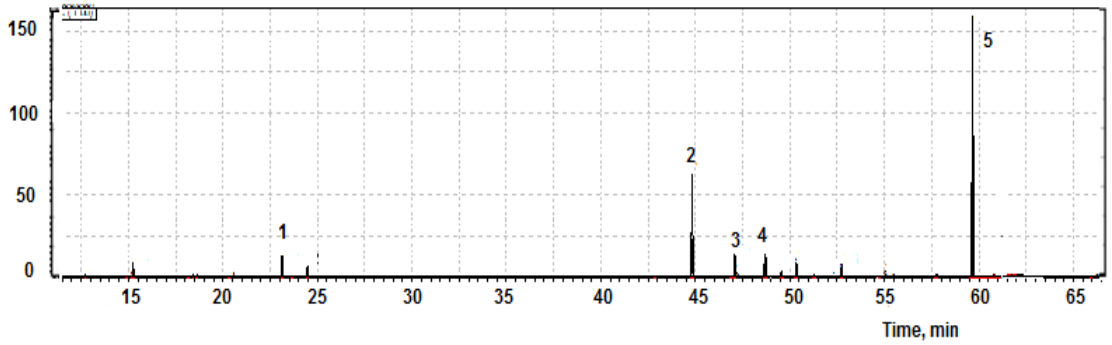


Figure 4.8B. GLC-MS profile of the essential oil from *Salvia discolor.* 1. linalool 2. *trans*-caryophyllene 3. α-humulene 4. germacrene D 5. unknown.

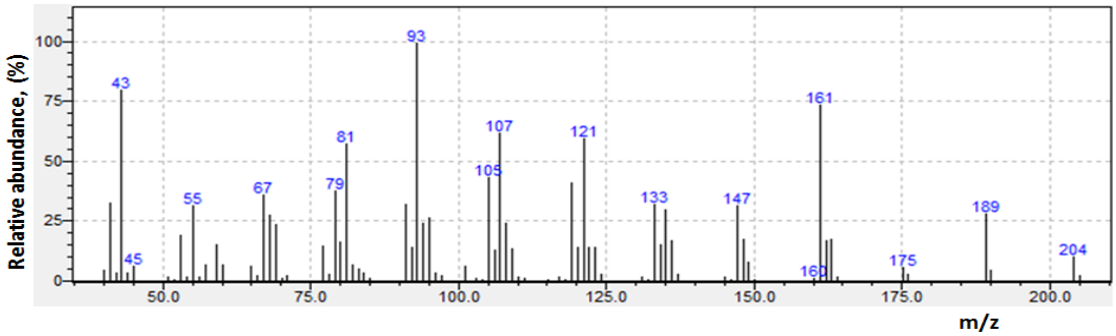


Figure 4.8C. Mass spectra of unknown compound (RT - 59.668; RI - 1667) from the essential oil of *Salvia discolor*

4.9. Chemical composition of the essential oil of *Salvia officinalis*

Chemical compositions of the essential oils of *S. officinalis* intensively are studied. Literature data indicate that α-thujone, β-thujone, 1,8-cineole, camphor, α-humulene, linalool, germacrene D, viridiflorol, α-pinene, limonene, and borneol are the major constituents of the essential oil of *S. officinalis* (Menghini et al., 2013).

Three chemotypes have been recognized in European *S. officinalis* (Franz and Novak, 2010): **1**. α-pinene, camphor, β-thujone; **2**. α-thujone, camphor, 1,8-cineole; **3**. β-thujone, camphor. Also, three other chemotypes: a thujone-rich chemotype; intermediate contents of thujones, α-pinene, camphene, camphor and borneol-rich chemotype and camphor, camphene, α-pinene rich chemotype have been discovered in the populations of Montenegro (Stesevic et al., 2014).

The essential oil of *S. officinalis* growing in Germany, is dominated by oxygen-containing monoterpenes. Results of GLC-MS analysis is documented in Table 4.9. 1,8-cineole (16%), camphor (13%), borneol (8%), α-humulene (8%), and Z-thujone (8%) were major components. Chemical structures of the main components of the essential oil *S. officinalis* are presented in Fig. 4.9A. GLC-MS profile of the essential oil *S. officinalis* is presented in Fig. 4.9B. The oil composition was in agreement with that of previous analysis from Iran (Badiee et al., 2012) and Lithuania (Mockute et al., 2003).

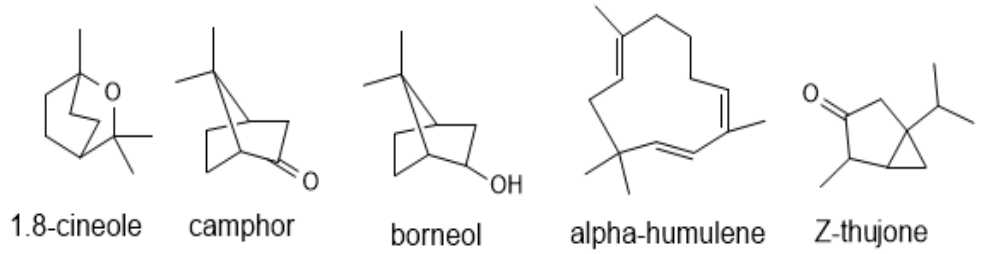


Fig. 4.9A. Chemical structures of the main components of the essential oil *S. officinalis*

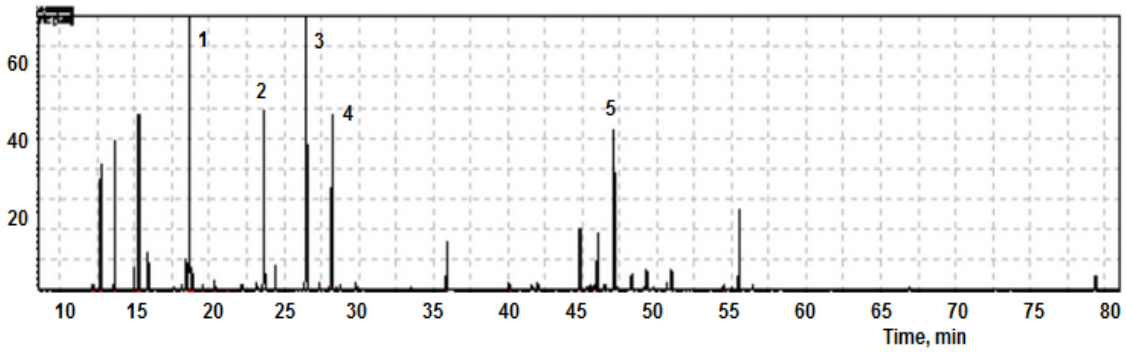


Figure 4.9B. GLC-MS profile of the essential oil from *Salvia officinalis.* 1. 1,8-cineol 2. Z-thujone 3. camphor 4. borneol 5. α-humulene.

Table 4.9. Chemical composition of the essential oil from *Salvia officinalis*

RI	Compounds	%	RI	Compounds	%
861	2-Methyl octane	0.07	**1284**	**Bornyl acetate**	**2.31**
923	Tricyclene	0.2	1346	α-Terpinyl acetate	0.48
925	α-Thujene	0.18	1369	α-Ylangene	0.24
933	α-**Pinene**	**4.27**	1370	iso-Ledene	0.12
949	**Camphene**	**5.26**	1375	α-Copaene	0.36
972	Sabinene	0.82	1407	α-Gurjunene	0.1
978	**β-Pinene**	**6.54**	1419	*trans*-Caryophyllene	3.4
989	Myrcene	1.38	1427	β-Copaene	0.21
1017	α-Terpienene	0.15	1430	β-Gurjunene	0.34
1024	p-Cymene	0.21	1434	Unidentified	0.29
1029	Limonene	1.32	1438	Aromadendrene	2.86
1032	**1.8-Cineole**	**15.52**	1446	Myltayl-4(12)-ene	0.27
1035	Z-β-Ocimene	0.66	**1456**	α-**Humulene**	**8.21**
1046	E-β-Ocimene	0.22	1460	(-)-Alloaromadendrene	0.16
1058	γ-Terpinene	0.41	1474	*trans*-Cadina-1(6),4-diene	0.81
1070	*cis*-Sabinene hydrate	0.12	1479	α-Amorphene	0.07
1085	α-Terpinolene	0.23	1488	β-Selinene	0.21
1100	Linalool	0.36	1490	Viridiflorene	1.23
1101	*trans*-Sabinene hydrate	0.11	1495	α-Selinene	0.13
1107	**Z-Thujone**	**8.05**	1498	α-Muurolene	0.16
1118	E-Thujone	1.08	1512	γ-Cadinene	0.31
1148	**Camphor**	**13.49**	1518	δ-Cadinene	1.05
1161	*trans*-Pinocamphone	0.37	1541	α-Calacorene	0.1
1169	3-Thujanol	0.14	1562	Sesquiterpeneol II	0.08
1171	δ-Terpineol	0.2	1576	Spathulenol	0.25
1173	**Borneol**	**8.47**	1585	Globulol	0.14
1176	Isopinocamphone	0.11	**1594**	**Viridiflorol**	**4.15**
1181	Terpinen-4-ol	0.23	1609	Humulene epoxide II	0.23
1195	α-Terpineol	0.47	1799	α-Humulene (dimer)	0.21
1249	Linalyl acetate	0.17	2051	Manool	0.71

4.10. Chemical composition of the essential oil of *Salvia sclarea*

A cluster analysis of the composition of essential oils of 39 *S. sclarea* studies have shown that most of the essential oils belong to the chemotype rich in linalyl acetate and linalool (Setzer, 2012). In addition, other chemotypes of *S. sclarea* such as geraniol/geranyl acetate-rich chemotype (Elnir et al., 1991), a methyl chavicol-rich chemotype (Moretti et al., 1997), a germacrene-D-rich chemotype (Carrubba et al., 2002), and α-thujone, thujene, and manool oxide/phytol chemotypes (Taarit et al., 2011) have been identified.

The composition of the essential oil of *S. sclarea* growing in Germany is shown in Table 4.10. Thirty six components were identified representing 98.25% of total components, which was dominated by linalyl acetate (36%), linalool (23%), α-terpineol (8%), and sclareol (15%). Chemical structures of the main components of the essential oil *S. sclarea* are presented in Fig. 4.10A. GLC-MS profile of the essential oil *S. sclarea* is presented in Fig. 4.10B. Apparently, the essential oil of this *S. sclarea* from Heidelberg belongs to chemotype rich in linalyl acetate and linalool (Setzer, 2012).

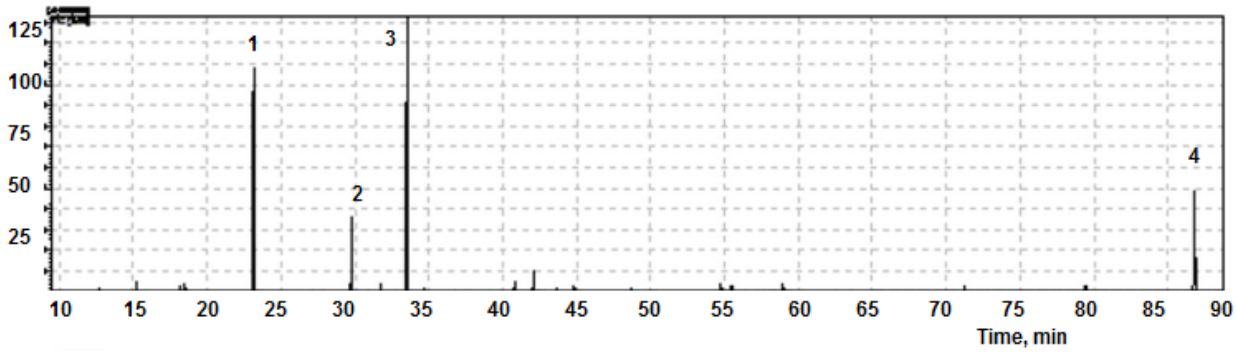


Figure 4.10B. GLC-MS profile of the essential oil from *Salvia sclarea.* 1. linalool 2. α-terpineol 3. linalyl acetate 4. sclareol.

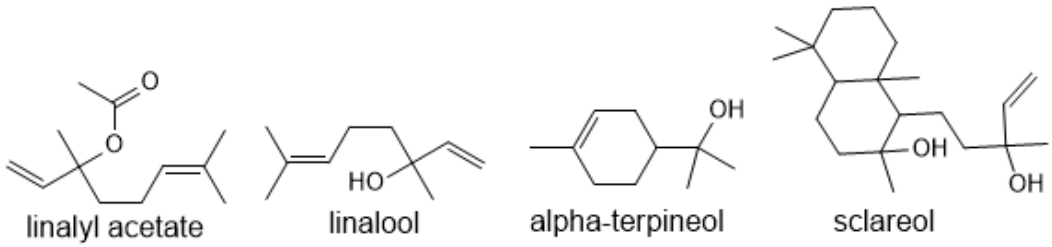


Fig. 4.10A. Chemical structures of the main components of the essential oil *Salvia sclarea*

Table 4.10. Chemical composition of the essential oil of *Salvia sclarea*

RI	Compounds	%	RI	Compounds	%
882	2-Butyl furan	0.03	1358	Neryl acetate	1.05
932	α-Pinene	0.21	1375	α-Copaene	0.28
972	Sabinene	0.17	1377	Geranyl acetate	2.3
977	β-Pinene	0.97	1379	*E-p*-Menth-6-en-2,8-diol	0.08
989	Myrcene	0.2	1400	γ-4-Dimethyl benzenebutanal	0.4
1024	o-Cymene	0.52	1419	*trans*-Caryophyllene	0.62
1029	Limonene	0.66	1426	Carvone hydrate	0.11
1032	1,8-cineole	0.36	1455	α-Humulene	0.09
1045	*trans*-β-Ocimene	0.05	1480	Germacrene D	0.52
1070	*cis*- Linalool oxide	0.21	1487	Linalool hydroxy	0.17
1086	*trans*- Linalool oxide	0.17	1576	Spathulenol	0.18
1100	**Linalool**	**23.47**	1581	Caryophyllene oxide	1.11
1103	Unidentified	0.06	1593	Viridiflorol	0.78
1107	*Z*-Thujone	0.09	1609	Humulene epoxide II	0.09
1118	*E*-Thujone	0.04	1638	(2S,5E)-caryophyll-5-en-12-al	0.03
1147	Camphor	0.19	1646	Unidentified	0.04
1169	*Z*-Linalool oxide	0.07	1654	α-Eudesmol	1.22
1174	*E*-Linalool oxide	0.08	1678	Unidentified	0.08
1187	3*Z*-Hexenyl butyrate	0.19	1702	δ-Dodecalactone	0.16
1195	α-**Terpineol**	**8.12**	1789	Unidentified	0.06
1211	Linalyl formate	0.04	1828	Unidentified	0.18
1223	Nerol	0.76	1884	Sclareol oxide	0.51
1237	Neral	0.06	1901	Unidentified	0.09
1249	**Linalyl acetate**	**36.33**	1927	Unidentified	0.1
1267	Geranial	0.31	1949	Geranyl α-terpinene	0.17
1307	Unidentified	0.04	1991	Manoyl oxide	0.12
1331	Terpendiol	0.11	2051	Manool	1.01
1343	Verbenyl acetate	0.04	2057	epi-7-Manool	0.13
1346	Unidentified	0.04	2082	Unidentified	0.03
1354	Terpen- diol II	0.08	**2214**	**Sclareol**	**14.62**

4.11. Chemical composition of the essential oil of *Tanacetum parthenium*

Chemical composition of the essential oil of Tajik *T. parthenium* is presented in Table 4.11. Eight components were identified representing 99.8% of total oil composition. The major components were the monoterpenes camphor (70-94%), camphene (2-12%), and bornyl

acetate (4-9%). Chemical structures of the main components of the essential oil *T. parthenium* are presented in Fig. 4.11A. GLC profile of the essential oil *T. parthenium* is presented in Fig. 4.11B.

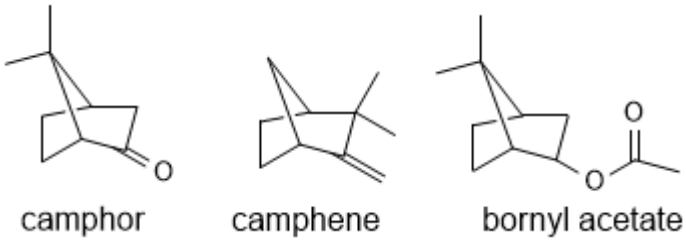


Fig. 4.11A. Chemical structures of the main components of the essential oil *Tanacetum parthenium*

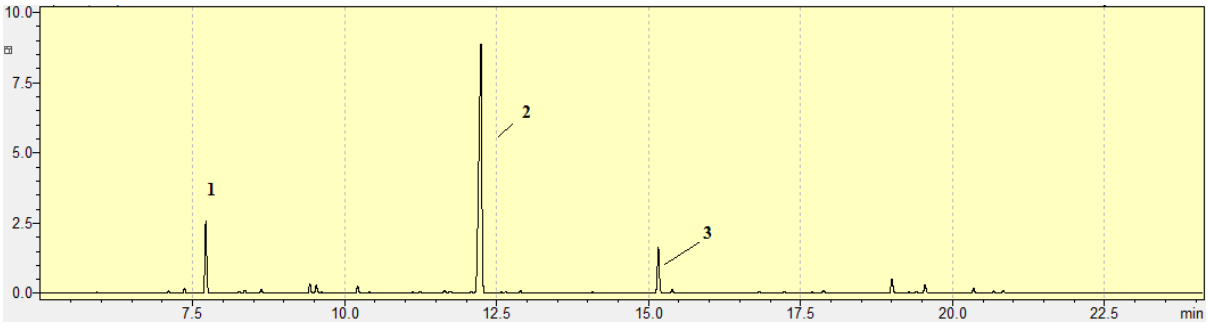


Figure 4.11B. GLC profile of the essential oil from *Tanacetum parthenium*. 1. camphene 2. camphor 3. bornyl acetate.

Table 4.11. Chemical composition the essential oil from of *Tanacetum parthenium* collected in Tajikistan in 2012 and 2013

RI	Compound	% of total peak area	
		2012	2013
954	Camphene	1.7	12.2
1021	*p*-Cymene	-	1.7
1029	Limolene	-	1.6
1059	γ-Terpinene	-	1.3
1144	Camphor	94.0	69.7
1285	Bornyl acetate	4.2	8.7
1443	β-Farnesene	-	2.9
1484	Germacrene D	-	1.9

4.12. Chemical composition of the essential oil of *Tanacetum vulgare*

T. vulgare is a potential source of schistosomicidal compounds (Godinho et al., 2014). The chemical composition of essential oils of *T. vulgare* from different geographical origins has been intensively investigated. More than *30* chemotypes of *T. vulgare* have been reported in literature (Schearer, 1984). α-Pinene, β-pinene, 1,8-cineole, γ-terpinene, artemisia ketone, thujone, camphor, borneol were reported as major components of the essential oil of *T. vulgare* (Keskitalo et al., 2001).

Results of analysis of the essential oil of German *T. vulgare* are presented in Table 4.12. The major components of the composition of the essential oil *T. vulgare* were camphor (52%), artemisia ketone (9%), camphene (6%), sabinene (4%), acetoxy-exo-camphene (4%), and germacrene D (3%). GLC-MS profile of the essential oil *T. vulgare* is presented in Fig. 4.12A. Chemical structures of the main components of the essential oil *T. vulgare* are presented in Fig. 4.12B. The most chemotypes of *T. vulgare* are containing camphor, which is in agreement with present work (Keskitalo et al., 2001; Mockutea and Judzentienea, 2004; Schearer, 1984).

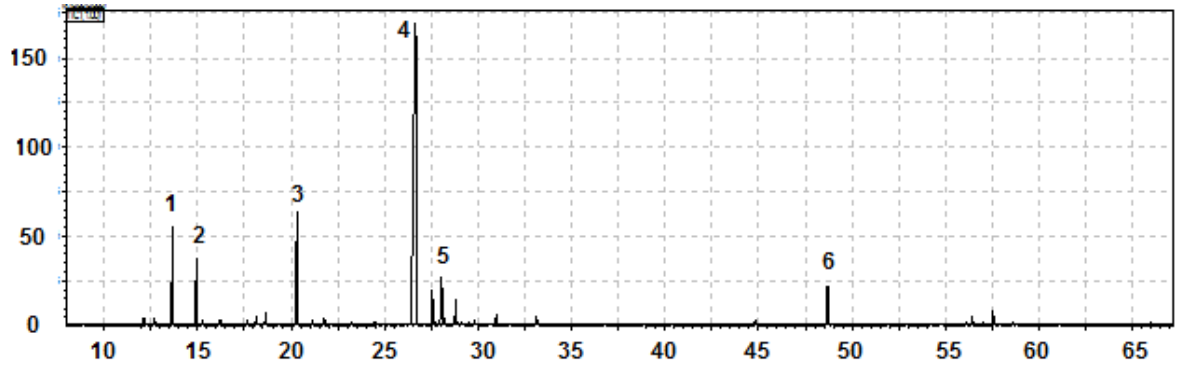


Figure 4.12A. GLC-MS profile of the essential oil from *Tanacetum vulgare.* 1. camphene 2. sabinene 3. artemisia ketone 4 . camphor 5. acetoxy-exo-camphene 6. germacrene D.

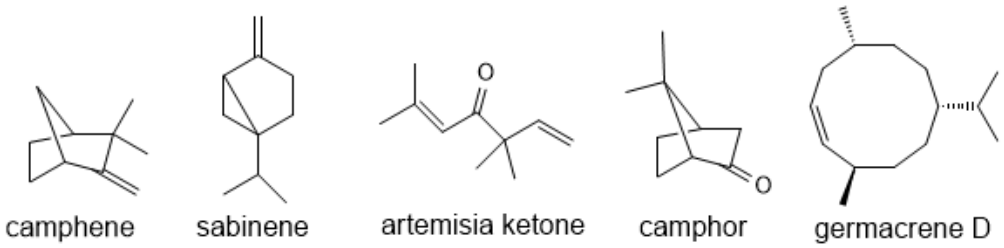


Fig. 4.12B. Chemical structures of the main components of the essential oil

Table 4.12. Chemical composition of the essential oil of *Tanacetum vulgare*

RI	Compounds	%	RI	Compounds	%
850	3Z-Hexenol	0.06	1173	Borneol	0.57
915	Ethylidenenorbornene	0.06	1181	Terpinen-4-ol	1.86
922	Tricyclene	0.48	1183	Thuj-3-en-10-al	0.26
925	α-Thujene	0.13	1185	2-Methyl-2-butene angelate	0.27
933	α-Pinene	0.43	1191	Myrtenol	0.22
949	**Camphene**	**6.03**	1195	Myrtenal	0.46
972	**Sabinene**	**4.26**	1209	Unidentified	0.11
978	β-Pinene	0.34	1212	Unidentified	0.85
990	Dehydro-1,8-cineole	0.06	1242	Cuminal	0.14
995	Yomogi alcohol	0.41	1244	Carvone	0.76
1017	α-Terpinene	0.39	1297	Carvacrol	0.05
1024	p-Cymene	0.66	1328	Mentha-1,4-dien-7-ol	0.06
1032	1,8-Cineole	0.84	1350	Eugenol	0.13
1043	Benzeneacetaldehyde	0.11	1419	trans-Caryophyllene	0.39
1057	**Artemisia ketone**	**9.08**	**1481**	**Germacrene D**	**3.44**
1070	*cis*-Sabinene hydrate	0.35	1495	Bicyclogermacrene	0.23
1079	Artemisia alcohol	0.59	1534	Unidentified	0.08
1085	Terpinolene	0.13	1572	Unidentified	0.09
1101	*trans*-Sabinene hydrate	0.34	1581	Caryophyllene oxide	0.18
1119	Dehydro- sabina ketone	0.37	1604	Ledol	0.23
1121	Chrysanthenone	0.07	1609	Unidentified	0.83
1125	*cis*-p-Menth-2-en-1-ol	0.11	1620	Muurola-4,10(14)-dien-1-alpha-ol	0.33
1127	α-Campholenal	0.12	1629	Unidentified	1.31
1143	*trans*-(-)-Pinocarveol	0.18	1636	Caryophylla-4(12),8(13)-dien-5-b-ol	0.16
1150	**Camphor**	**52.38**	1648	Butadecenol	0.26
1157	Sabina ketone	0.12	1654	α-Cadinol	0.09
1163	*cis*-Chrysanthenol	3.16	1664	Selin-11-en-4-alpha-ol	0.22
1164	Unidentified	0.33	1673	Jatamansone	0.09
1168	Acetoxy-exo camphene	0.17	1692	Germacrone	0.12
1170	**Acetoxy-exo camphene**	**4.2**	1782	Unidentified	0.25

4.13. Alkaloid contents and composition of *Papaver somniferum*

Total alkaloid contents of different organs of *P. somniferum* are documented in Table 4.13A, which ranged between 0.2 - 6 mg /g fresh weight (FW) plant material. The highest amount of alkaloids was found in the capsules, a common source of morphinane alkaloids.

Composition of the main poppy alkaloids has been analysed by different techniques such as gas liquid chromatography - mass spectrometry (GLC/MS) (Cole, 2003), liquid chromatography - mass spectrometry (LC/MS) (Sproll et al., 2006), high performance liquid chromatography (HPLC) (Dittbrenner et al., 2009), and capillary electrophoresis (Baros et al., 2012). GLC-MS is a simple, sensitive and specific technique with high resolution for the detection of opioid alkaloids. It is commonly used for drug analysis in blood, urine and hair of opioid abusers (Abdi et al., 2004).

Table 4.13. Total alkaloid contents in 100 g plant material (fresh weight) of *Papaver somniferum**

Plant parts	**Total alkaloid contents, mg /100 g**
All parts	135.0 ± 5.3
Roots	16.8 ± 0.2
Capsules (without seeds)	646.8 ± 15.1
Seeds	97.2 ± 2.4
Flowers	44.3 ± 0.8
Stems	267.5 ± 4.9
Leaves	110.9 ± 5.0

*Results are represented as means ± standard deviation (n=3)

Alkaloid contents of different parts (leaves, stems, roots, capsules, flowers, seeds, and whole plants) were analysed by GLC-FID and GLC-MS (Table 4.13B). The major components of the alkaloid fraction were papaverine (37.7-2062.9 µg/g), codeine (7.4-1280.5 µg/g) and morphine (3.25-929.3 µg/g). Meconine (0.05-52.4 µg/g) and β-hydroxy damascone (1.85-49.15 µg/g) also were identified in alkaloid fractions. A typical GLC profile of the alkaloid extract is illustrated in Figure 4.13. High contents of morphine, papaverine and codeine were found (as expected) in capsules. These results are consistent with most of previously published results (Baros et al., 2012; Dittbrenner et al., 2009; Sproll et al., 2006). The

maximum morphine and codeine concentrations were determined in the seeds with 33.2 and 13.7 mg/g seeds respectively (Meadway et al., 1998).

Table 4.13B. Chemical composition of the alkaloid extract of *Papaver somniferum*

Compounds	RI*	Composition, µg/g						
		all part	root	capsule	seed	flower	stem	leave
β-Hydroxy damascone	1607	7.9	4.3	49.1	32.1	1.8	7.7	4.8
Meconine	1732	15.5	0.1	2.6	52.4	6.6	34.5	0.6
Codeine	2415	260.9	7.4	1280.5	15.3	82.3	1248.8	327.8
Morphine	2466	107.1	3.2	929.3	20.7	6.2	280.8	96.4
Papaverine	2965	418.2	37.7	2062.9	129.7	150.9	875.2	368.5

*Retention Index

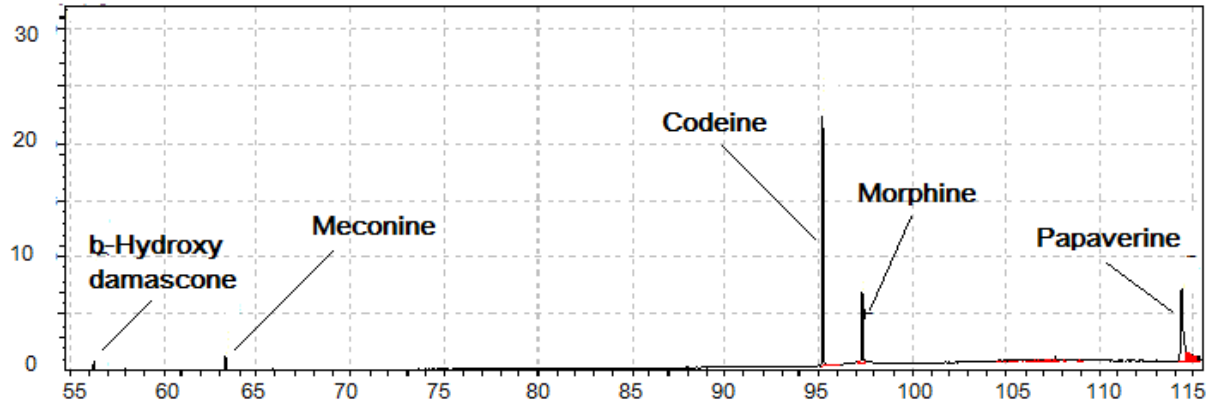


Figure 4.13. GLC-MS profile of an alkaloid extract of complete poppy plants

4.14. Content of polyphenols in methanol extracts of *Salvia sclarea, S. officinalis,* and *S. discolor*

Chemical and pharmacological properties of *Salvia* species have been extensively studied. The genus is a rich source of phenolic compounds with an excess of 160 polyphenols (Lu and Foo, 2002). Polyphenols act as multi-target drugs, they can inhibit the function of various enzymes, receptor proteins, transcription factors, transporters or cytoskeletal proteins (Wink, 2008). Because of their interaction with several proteins, drugs containing polyphenols have been used to treat a wide variety of illnesses and health disorders in which proteins play a role (van Wyk and Wink, 2004).

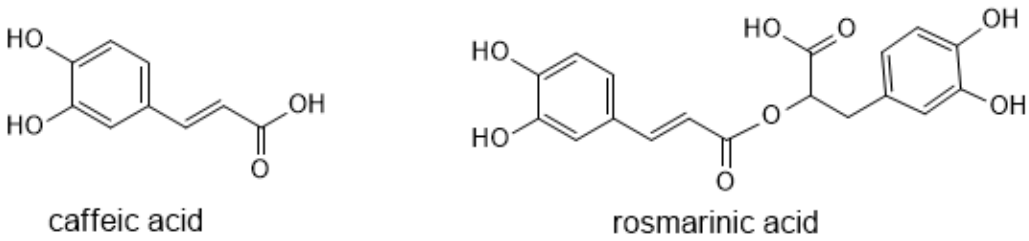


Figure 4.14A. Structures of caffeic and rosmarinic acid.

Caffeic acid and its abundant dimer - rosmarinic acid have been identified in most *Salvia* species. Caffeic acid is considered as the building block of a variety of other plant metabolites. Caffeic and rosmarinic acid contain a number of phenolic hydroxyl groups which can dissociate under physiological conditions. The resulting phenolate ions can form ionic and hydrogen bonds with positively charged amino groups of proteins and peptides and thus modulate their 3D-structures (Wink, 2008).

HPLC was used for the separation and identification of caffeic, p-coumaric and rosmarinic acid in the extracts, using standards (p-coumaric, caffeic and rosmarinic acid) for peak matching. HPLC profile of methanol extracts of *S. discolor*, *S. officinalis* and *S. sclarea* are illustrated in Figure 4.14B; the components and amounts of p-coumaric, caffeic and rosmarinic acid are represented in Table 4.14.

Table 4.14. Content of polyphenols and flavonoids in methanol extracts of *Salvia sclarea, S. officinalis,* and *S. discolor*

Plant name	Plant part	Total number of secon-dary meta-bolites	Identified polyphenols (%)		
			caffeic acid	p-cou-maric acid	rosma-rinic acid
Salvia sclarea	leaves, stems and blossoms	49	3.1	9.4	54.5
Salvia sclarea	Roots	43	8.8	0.5	1.2
Salvia officinalis	leaves, stems and blossoms	51	1.0	1.6	25.9
Salvia discolor	leaves, stems and blossoms	43	2.2	2.4	57.4

The major component of aerial parts is rosmarinic acid (1 – 57%) which is hardly present in root extracts of *S. sclarea*. These results are consistent with results previously reported by (Cosin et al., 2012).

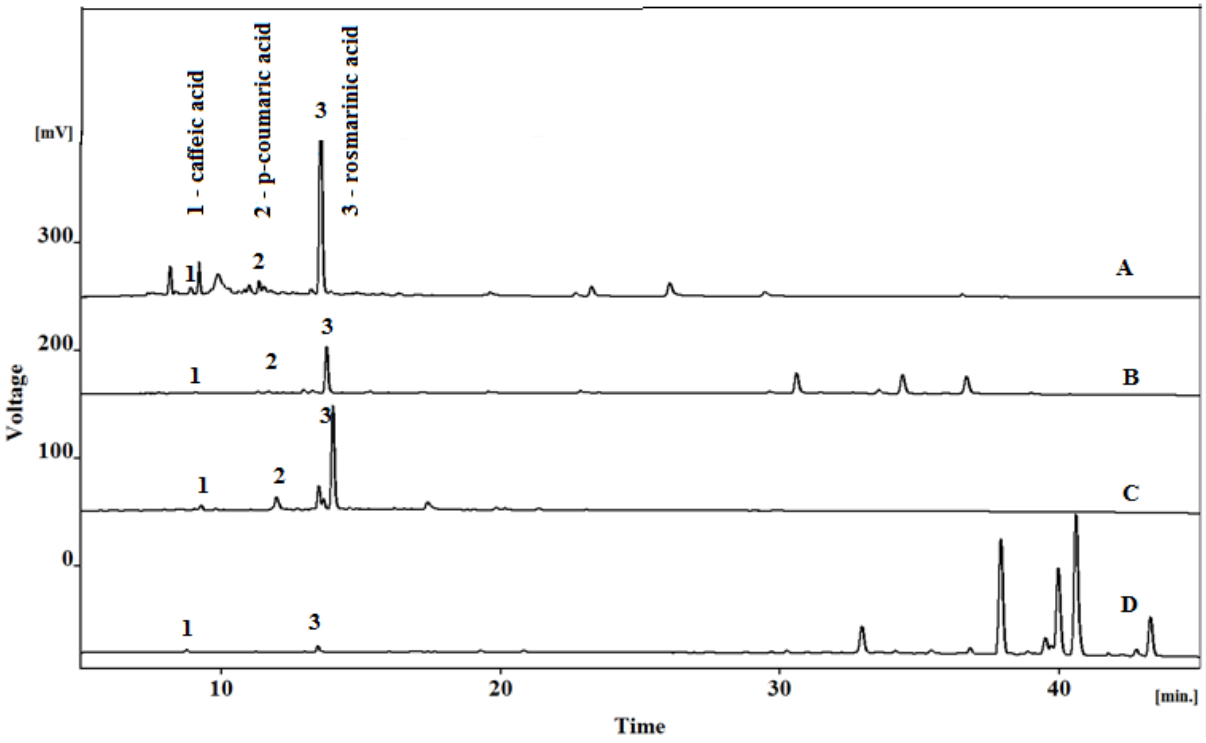


Figure 4.14B. HPLC profile of the methanol extracts: A - *S. discolor*; B – S. *officinalis*; C – *S. sclarea* aerial parts; D – *S. sclarea* (roots); 1. caffeic acid 2. p-coumaric acid 3. rosmarinic acid

Conclusions

In this chapter is illustrated data of the chemical composition of the essential oils of the 12 plant species collected from Tajikistan and Germany. Alkaloid contents of the *P. somniferum* and polyphenol composition of the three *Salvia* species collected from Germany are also represented. For the first time, chemical composition of *F. clematidifolia, G. fragrantissima, P. x purpureomaculatus, P. tadshikorum* and *S. discolor* were investigated.

Chapter 5. Antioxidant activity

5.1. Antioxidant activity of the essential oil

The investigation of antioxidant activity of natural compounds became an interesting aspect of food and pharmaceutical research (Amorati et al., 2013; Reichling, 2010; Schmidt, 2010). Synthetic food additives are increasingly replaced with plant-based natural ingredients, due to the safety, effectiveness and consumer acceptance (Amorati et al., 2013). This is particularly relevant because most common synthetic antioxidants are suspected to be potentially harmful to human health (Lanigan and Yamarik, 2002).

Production of reactive oxygen species (ROS) such as superoxide anion ($O2•^-$), hydroxyl (•OH), peroxyl (ROO•), and alkoxyl radicals (RO•), hydrogen peroxide (H_2O_2), singlet oxygen and etc. is one of the main reason for protein, lipid, and DNA damage, cell aging, oxidative stress-originated diseases (cardiovascular and neurodegenerative diseases), and cancer (Liu, 2010; Prior et al., 2005).

Antioxidants are molecules which small amount of them can prevent or reverse oxidation presses. They are scavenger of reactive oxygen species. In biological systems, oxidation processes occurs by a radical chain reaction mediated by peroxyl radicals (ROO•). The process, generalized in Figure 5.1, is initiated by some radical species (Amorati et al., 2013; Prior et al., 2005).

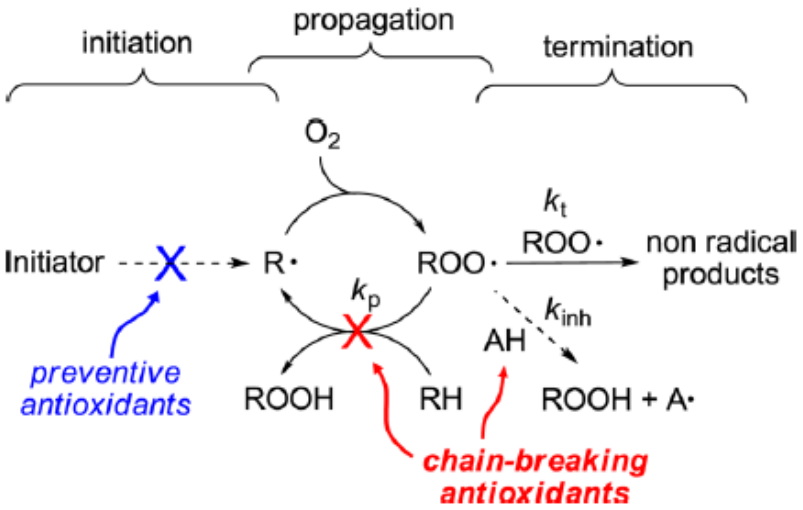


Figure 5.1. Simplified mechanism of hydrocarbon autoxidation and antioxidant protection (Amorati et al., 2013)

It is clear that preventive antioxidant can block oxidation initiator and chain breaking antioxidant can attack to the chain reaction cycles in termination and propagation steps.

Essential oils and their chemical, biological and pharmacological activities have been widely studied; an interesting aspect include their potential antioxidant activity (Amorati et al., 2013; Ashour et al., 2009). In addition, essential oils are represent interest to prevent autoxidation and prolong shelf life of edible products (Schmidt, 2010).

In this relation, the antioxidant activity of the essential oil from 22 plant species (30 samples) were evaluated by DPPH, ABTS, FTC, FRAP assays. The antioxidant activity values of the essential oils are represented in Table 5.1. The oils of *Origanum tyttanthum*, *Artemisia absinthium, Salvia officinalis* and *Tanacetum vulgare* are inhibited the higher antioxidant activity.

The antioxidant potential of an essential oil depends on its composition. Phenolics and secondary metabolites with conjugated double bonds usually show substantial antioxidative properties (Cabrera and Prieto, 2010). In chapter 2 and 4, we have already discussed about the chemical composition of the essential oils. Most of the essential oils are dominated by oxygenated monoterpenes: - alcohols (*Achillea filipendulina*), aldehydes (*Galagania fragrantissima*), ketones (*Anethum graveolens, Artemisia rutifolia, Hyssopus seravschanicus, Mentha longifolia, Ziziphora clinopodioides)* and esters *(Salvia sclarea). Artemisia absinthium* and *Artemisia scoparia* predominantly contain monoterpene hydrocarbons. Phenolic terpenoids, such as thymol or carvacrol, are present in *Origanum tyttanthum* and *Mentha longifolia* which would explain, that both plants exhibited the strongest antioxidant activity. These results are consistent with those previously reported by other authors (Baricevic and Bartol, 2002; Charles, 2013) that the phenolic monoterpenes thymol and carvacrol which are predominant in *Origanum tyttanthum* are also responsible for the antioxidant activity of several other essential oils (e.g. *Mentha longifolia, Thymus serpyllus*) which contain them. The biological activities of *Origanum tyttanthum* and *Mentha longifolia* essential oils may be attributed to the presence of phenols (carvacrol and thymol) as the major oil components.

Table 5.1. Antioxidant activity of the essential oils

Species	DPPH, IC_{50}, mg/ml	ABTS, IC_{50}, mg/ml	FRAP, µM Fe (II)/mg sample	Degree of linoleic acid peroxidation, %; Csam. = 0.9 mg/ml
Achillea filipendulina	4.83	2.01	214.2	0.5
Anethum graveolens	4.98	4.12	47.9	0.4
Artemisia absinthium	**1.35**	**0.87**	**338.9**	**33.3**
Artemisia rutifolia	7.91	0.25	74.2	17.6
Artemisia scoparia	2.55	0.28	43.1	18.5
Ferula clematidifolia[1]	15.7	1.55	124.5	n.d.
Ferula clematidifolia[2]	14.72	0.45	434.75	n.d.
Foeniculum vulgare	15.6	10.9	193.5	n.d.
Galagania fragrantissima[3]	8.34	11.68	208.33	n.d.
Galagania fragrantissima[1]	8.21	6.46	315.83	n.d.
Galagania fragrantissima[4]	8.13	4.74	67.2	n.d.
Hypericum perforatum	3.71	0.48	98.25	n.d.
Hypericum scabrum	6.69	5.67	22.5	n.d.
Hyssopus seravschanicus	4.9	1.39	53.8	54.2
Mentha longifolia	2.31	0.67	76.9	30.6
Ocimum basilicum	5.94	7.98	51.6	n.d.
Origanum tyttanthum	**0.28**	**0.12**	**699.2**	**79.7**
Pastinaca sativa	n.a.	n.a.	3.53	n.d.
Philadelphus purpureomaculatus	n.a.	n.a.	19.53	n.d.
Polychrysum tadshikorum	5.14	6.7	239.17	n.d.
Salvia officinalis	**1.12**	n.a.	**313.2**	n.d.
Salvia sclarea[5]	12.5	5.03	54	28.5
Salvia sclarea [3]	n.a.	0.3217	277.25	n.d.
Salvia sclarea[1]	14.5	7.9	121.42	n.d.
Salvia sclarea[6]	n.a.	0.45	113.8	n.d.
Tanacetum parthenium	4.82	0.96	n.d.	37.1
Tanacetum vulgare	7.69	2.56	70.5	n.d.
***Tanacetum vulgare*[6]**	**0.85**	**0.12**	**391.3**	n.d.
Ziziphora clinopodioides	5.12	0.79	66.9	15.3
Ascorbic acid	0.002	0.005	1899.5	85.1

[1] from leaves; [2] from root; [3] from flowers; [4] from stem; [5] from all part; [6] from Germany; n.a.- not active; n.d. - not determined

The DPPH and ABTS radical scavenging activity methods are widely used to evaluate antioxidant activities in a relatively short time compared with other methods. The time-dependent activity in DPPH and ABTS radical-scavenging of essential oils was observed as shown on Table 5.2 and 5.3. The results showed that the antioxidant activities of oils are increased rapidly in different intervals of time. It means that reaction between free radical and essential oils is proceed slowly. For example, in beginning of experiments, essential oils of *Ferula clematidifolia* and *Salvia sclarea* did not exhibit activity, but after time, they are become very active. Incubation time is critical issue for evaluation of essential oils by DPPH and ABTS assays.

Table 5.2. IC_{50} of essential oils measured at the different time of reaction with DPPH

Time, min	**30-45**	**80-95**	**130-145**	**280-300**	**1140-1200**
Species			**IC_{50}, mg/ml**		
Achillea filipendulina	4.83	3.09	3.22	2.72	1.13
Anethum graveolens	4.98	2.71	1.97	1.56	0.52
Artemisia absinthium	1.35	1	0.74	0.47	0.23
Artemisia rutifolia	7.91	4.88	2.62	2.72	1.15
Artemisia scoparia	2.55	1.88	0.83	0.55	0.25
***Ferula clematidifolia*[1]**	**15.7**	**8.95**	**6.98**	**6.5**	**0.88**
***Ferula clematidifolia*[2]**	**14.72**	**7.38**	**2.88**	**0.82**	**0.22**
Galagania fragrantissima[3]	8.34	6.14	4.9	3.43	2.41
Galagania fragrantissima[1]	8.21	6.01	5.23	3.29	2.19
Galagania fragrantissima[4]	8.13	6.28	4.48	3.5	2.27
Hypericum scabrum	3.71	2.27	2.06	0.88	0.19
Hyssopus seravschanicus	4.9	2.47	1.9	1.11	0.47
Mentha longifolia	2.31	1.24	0.98	0.81	0.43
Ocimum basilicum	5.94	5.08	4.81	4.91	4.07
Salvia sclarea	**12.5**	**7.53**	**4.84**	**2.23**	**0.99**
Tanacetum vulgare	7.69	4.38	2.31	1.85	1.05
Ziziphora clinopodioides	5.12	2.41	1.85	1.46	0.48
Ascorbic acid	0.002	0.002	0.002	0.002	0.002

[1] from leaves; [2] from root; [3] from flowers; [4] from stem

Table 5.3. IC_{50} of essential oils measured at the different time of reaction with ABTS

Time, min	30	60	180	240	400
Species			**IC_{50}, mg/ml**		
Achillea filipendulina	2.64	2.01	1.09	1.04	0.87
Anethum graveolens	6.92	4.12	1.56	0.86	0.99
Artemisia absinthium	**n.a.**	**n.a.**	**0.47**	**0.29**	**0.25**
Artemisia rutifolia	0.31	0.25	0.22	0.18	0.17
Artemisia scoparia	6.82	0.28	0.11	0.09	0.085
***Ferula clematidifolia*[1]**	**n.a.**	**5.55**	**1.13**	**0.98**	**0.62**
Ferula clematidifolia[2]	1.28	0.45	0.19	0.11	0.12
Galagania fragrantissima[3]	n.a.	n.a.	11.68	2.78	2.87
Galagania fragrantissima[1]	12.67	6.46	3.85	3.04	2.36
Galagania fragrantissima[4]	n.a.	n.a.	4.74	3.11	2.21
Hypericum scabrum	4.52	0.48	0.26	0.17	0.17
Hyssopus seravschanicus	2.67	1.39	0.61	0.47	0.45
Mentha longifolia	0.97	0.67	0.43	0.4	0.42
Ocimum basilicum	12.45	7.98	7.34	3.73	2.97
Salvia sclarea	**n.a.**	**5.03**	**1.14**	**1.05**	**0.84**
Tanacetum vulgare	n.a.	2.56	6.79	1.15	1.12
Ziziphora clinopodioides	1.05	0.79	0.35	0.43	0.21
Ascorbic acid	0.005	0.005	0.005	0.005	0.005

[1] from leaves; [2] from root; [3] from flowers; [4] from stem; n.a. - not active

5.2. Antioxidant activity of the essential oil components

Numerous essential oils have been investigated for their antioxidant and radical-scavenging properties, including such economically important ones as celery (*Apium graveolens*), sweet wormwood (*Artemisia annua*), black cumin (*Nigella sativa*), and thyme (*Thymus vulgaris*) (Amorati et al., 2013; Miguel, 2010). A number of different assays have been used to assess the radical-scavenging / antioxidant activity of plant extracts and essential oils (Antolovich et al., 2002; Apak et al., 2013). While these assays cannot be considered as proof of antioxidant activity, they have been used as preliminary assessments of it. We carried out three popular

assays, the DPPH radical scavenging, the ABTS radical scavenging, and the ferric-reducing antioxidant power (FRAP) assays, on several different essential oil components.

The antioxidant activities of eighteen different essential oil components are summarized in Table 5.4.

Table 5.4. Antioxidant activity of essential oil components (arranged by activity)

Compound	**DPPH** IC_{50} **(µg/mL)**[a]	**ABTS** IC_{50} **(µg/mL)**[b]	**FRAP** **µM Fe^{2+}/mg sample**[c]
Eugenol	0.5	1.2	2476.925
Thymol	0.5	1.7	2357.5
Carvacrol	4.7	2.1	2276.67
γ-Terpinene	219.8	5	915.83
Geranial	306.2	15.5	697.5
1,8-Cineole	912.9	3621	490
Thujone	1284	1166.4	392.5
Menthone	1834.7	12093.2	279.17
Linalool	1914.4	8821	283.33
Estragole	2368.1	625	883.33
p-Cymene	2639.9	30.4	655
β-Pinene	3116.3	2245	81.67
(*E*)-Anethole	4395.9	107.2	1310
Citronellal	5465.8	8401.9	610
Limonene	6158.6	5893.2	808.33
Citronellol	6828.8	27396.4	455.83
Camphor	9423.2	19729.4	367.5
Menthol	16805.8	142.8	227.5
Positive control	0.3[d]	7.2[d]	1904.7[e]

[a] IC_{50} after 20 h.; [b] IC_{50} after 21 h.; [c] FRAP value using 12.5 mg/mL compound recorded after 10 min.; [d] ascorbic acid; [e] caffeic acid.

The phenolic compounds, carvacrol, thymol and eugenol, showed the best antioxidant activities, while citronellol, camphor and menthol were the least active.

5.3. Total phenolic and flavonoid contents of the plant extracts

Medicinal plants are rich with phenolic compounds, including polyphenols, flavonoids, etc. It is fact that antioxidant activity of medicinal plant related with contents of phenolic compounds. In this connection, we evaluated the total phenolic and flavonoid contents of the plant extracts are displayed in Table 5.5 (from Tajikistan) and in Table 5.6 (from Germany).

Table 5.5. Total phenolic and flavonoid content of the plant extracts from Tajikistan

Species	**TP, mg CAE in 100 g DW**		**TF, mg QE in 100 g DW**	
	ME	**WE**	**ME**	**WE**
Galagania fragrantissima	2924.1	2368.3	733.3	258.7
Achillea filipendulina	2661.7	1713.9	46.5	114.6
Polychrysum tadshikorum	1831.5	731.1	640.6	143.7
Artemisia absinthium	1657	976.9	121.8	104.6
Artemisia rutifolia	1611.3	637.1	599.7	249.1
Origanum tyttanthum	1589.3	1353.9	59.2	78.3
Ziziphora clinopodioides	1508.4	822.8	825.2	108.8
Ferula clematidifolia	1443.4	699.3	533.6	60.5
Tanacetum parthenium	1413.5	1524.1	759.7	98.4
Tanacetum vulgare	1387.3	423.9	655	168.3
Anethum graveolens	846.8	369.3	117.9	6.5
Ocimum basilicum	794.2	1108	649.5	25.7
Hypericum scabrum	758.3	1530.1	371.4	159.4
Salvia sclarea	612.2	1478.6	18.2	399.8
Hypericum perforatum	559.5	800.6	462.3	98
Artemisia scoparia	355.3	408.2	312.2	36.5
Mentha longifolia	274.5	761.6	231.2	16.7
Melissa officinalis	129.1	70.4	177.1	3.5

The methanol and water extracts of *G. fragrantissima, A. filipendulina, P. tadshikorum, A.rutifolia and O. tyttanthum* showed the high value of phenolic and flavonoid contents, while *Melissa officinalis* and *Mentha longifolia* extracts showed the low value of phenolic and flavonoid contents.

Table 5.6. Total phenol and flavonoid content of the plant extracts from Germany

Species	Anatomical part	Total phenol content, mg CAE/g extract	Total flavonoid content, mg QE/g extract
Geranium macrorrhizum	**root**	**993.5**	**4.5**
Geranium macrorrhizum	leaf	753	48.95
Tanacetum vulgare	**whole plant parts**	**529.5**	**43.75**
Galega x hartlandii	fruit	382	67.5
Philadelphus purpureomaculatus	stem	378.5	12.5
Salvia sclarea	whole plant parts	377	20.3
Salvia discolor	whole plant parts	354.1	36.25
Galega x hartlandii	leaf	310.5	30.35
Papaver somniferum	flower	261.4	17.87
Salvia sclarea	root	253.2	5
Salvia officinalis	whole plant parts	242.5	23.75
Papaver somniferum	whole plant parts	240.5	33.37
Papaver somniferum	leaf	224.25	38.87
Philadelphus purpureomaculatus	leaf	212	13.25
Papaver somniferum	root	149.5	3.5
Papaver somniferum	cupsuls bark	148	17
Papaver somniferum	seed	125.9	4.3
Papaver somniferum	stem	113.25	7.7
Ficus carica	leaf	76.87	0.26
Pastinaca sativa	whole plant parts	64.5	56.25
Galega x hartlandii	stem	50	21
Ficus carica	fruit	15.85	2.01

The highest phenolic and flavonoid content were determined in the methanol extracts of *G. macrorrhizum and T. vulgare* and the lowest content observed in *Galega x hartlandii* and *Ficus carica* extracts.

In Figure 5.2. is represented the linear correlation between total phenol and flavonoid contents for the 58 sample of methanol and water extracts. Results are indicated that there is correlation between phenol and flavonoids in medicinal plants.

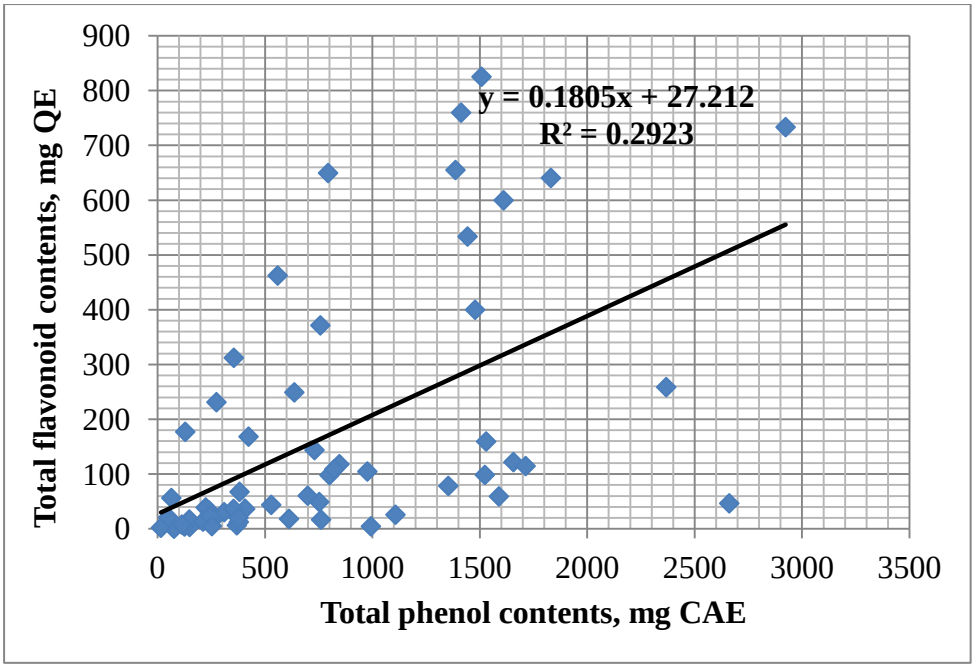


Figure 5.2. Linear correlation for the extracts (58 samples) between total phenol and flavonoid contents

5.4. Antioxidant activity of the plant extracts

The antioxidant activity of the plant extracts are summarized in Table 5.7 (from Tajikistan) and in Table 5.8 (from Germany). The extract of *G. fragrantissima, O. tyttanthum, A. graveolens* (from Tajikistan) and *G. macrorrhizum, T. vulgare* (from Germany) exhibited high antioxidant activity. *Melissa officinalis, Mentha longifolia* extracts (from Tajikistan) and *Galega x hartlandii, Pastinaca sativa* (from Germany) exhibited low antioxidant activity. Also, alkaloid fraction of *P. somniferum* exhibits 5-30 times more antioxidant activity that methanol extracts from different parts. Results show that the antioxidant activities of methanol extracts are mostly higher than water extracts.

It is fact that the antioxidant activities of plant extracts are influenced by thier phenolic composition. In order to investigate the contribution of phenolic constituents to the antioxidant activity in plant extracts, a linear regression was obtained between the total phenol contents (TP) and DPPH, ABTS, FRAP values. Higher correlations were found between TP content and ABTS (R = 0.3722) when compared with TP content and FRAP (R = 0.3598) and with TP and DPPH (R = 0.2327). Linear correlations for the extracts between total phenol contents and DPPH, ABTS, FRAP represented in Figure 5.3-5.5.

Table 5.7. Antioxidant activity of the methanol and water extract of plants from Tajikistan

Species	DPPH, µg/ml		ABTS, µg/ml		FRAP, mM $FeSO_4$/1 gm extract	
	ME	**WE**	**ME**	**WE**	**ME**	**WE**
Galagania fragrantissima	**4.1**	**24.8**	**4**	**16.6**	**1217.9**	**1082**
Anethum graveolens	**30.2**	**132.6**	**40.3**	**52.5**	**1119.6**	**579**
Ferula clematidifolia	10.2	55.4	3.8	64.6	976.1	550
Hypericum perforatum	52.7	75.6	62.5	71.3	783.9	577.7
Hypericum scabrum	26.1	25.8	74.9	62.5	768.9	650.5
Origanum tyttanthum	**9.5**	**31. 3**	**23.6**	**34.8**	**744.5**	**565.5**
Achillea filipendulina	15.6	4.2	23.6	8.8	627.2	708.3
Ziziphora clinopodioides	20.5	354.6	36.2	63	611.6	520.8
Artemisia rutifolia	12.7	21.5	16.6	15.8	580.4	564.1
Tanacetum parthenium	18.9	34.8	16	32.7	569.3	558.7
Ocimum basilicum	100.6	63.1	123.4	56.1	542.9	475.9
Salvia sclarea	39.2	23.4	84.8	15. 8	542	584.5
Tanacetum vulgare	23.8	73.6	31.3	62.7	514.1	417.7
Polychrysum tadshikorum	12.7	44.5	32.6	41.4	485	505.8
Artemisia absinthium	20.1	45.8	34.1	34	460.2	541.8
Artemisia scoparia	100.5	143.3	87.4	212.3	380.1	380
Mentha longifolia	285.6	134.7	n.a.[1]	54.9	250.7	268.2
Melissa officinalis	212.3	n.a.	n.a.	250.3	245.2	73.2
Caffeic acid	1.66		2.01		2393.2	

[1]n.a. - not active

Table 5.8. Antioxidant activity of methanol extracts from Germany

Species	Anatomical part	DPPH, IC_{50} (µg/mL)	ABTS, IC_{50} (µg/mL)	µM Fe^{2+}/mg sample
Geranium macrorrhizum	**root**	**5.5**	**4.7**	**286.6**
Papaver somniferum	**all part (alk. fr.)**	**7.4**	**8.1**	**750.1**
Geranium macrorrhizum	leaf	14.1	21.2	261.4
Ficus carica	leaf	17.7	32.5	163.3
Salvia sclarea	whole plant parts	21.6	71.1	n.d.[1]
Salvia officinalis	whole plant parts	23.8	68.3	n.d.
Papaver somniferum	flower	35.1	142.4	787.3
Papaver somniferum	**seed**	**42.6**	**138.5**	**835.3**
Salvia sclarea	root	65.9	141.6	50.6
Tanacetum vulgare	whole plant parts	65.9	n.d.	249.9
Papaver somniferum	whole plant parts	72.5	217	828.1
Ficus carica	fruit	73.4	100.8	727.5
Papaver somniferum	**leaf**	**75**	**230.4**	**1348.7**
Papaver somniferum	cupsuls bark	76.7	142.2	825.4
Galega x hartlandii	fruit	87.6	n.d.	236.4
Philadelphus purpureomaculatus	stem	89.4	50.1	236.4
Philadelphus purpureomaculatus	leaf	125.3	n.d.	230.1
Salvia discolor	whole plant parts	135.5	53	n.d.
Papaver somniferum	stem	156.7	306.3	487.2
Papaver somniferum	root	157.6	289.9	459.7
Pastinaca sativa	whole plant parts	229.4	198.7	n.d.
Galega x hartlandii	leaf	240.8	n.d.	n.d.
Galega x hartlandii	stem	600	n.d.	165.9
Caffeic acid		**1.66**	**2.01**	**2393.2**

[1]n.d. - not determined

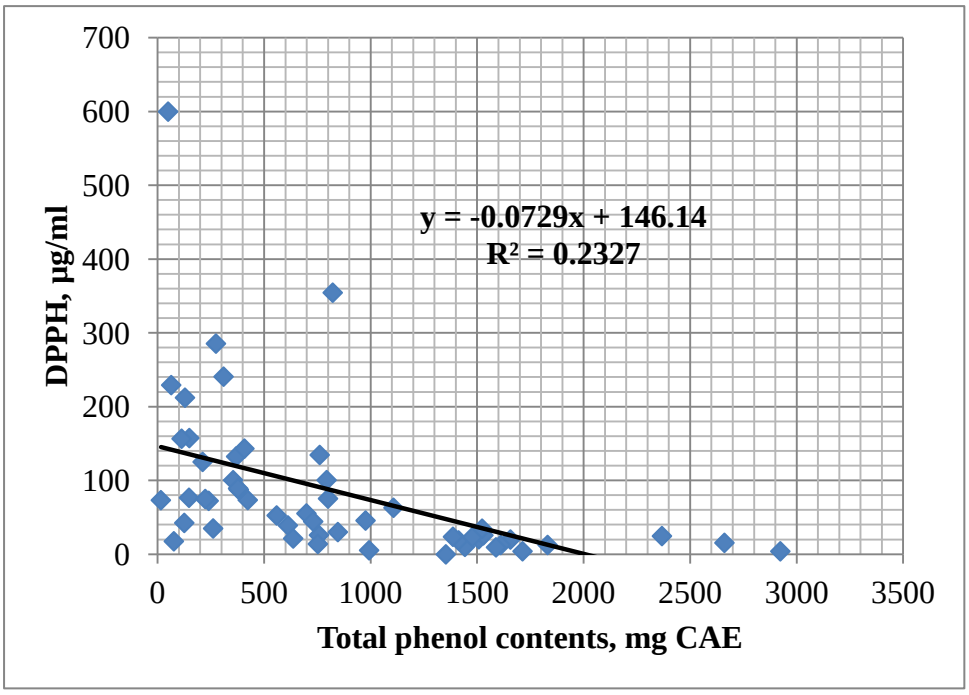


Figure 5.3. Linear correlation for the extracts between total phenol contents and DPPH data

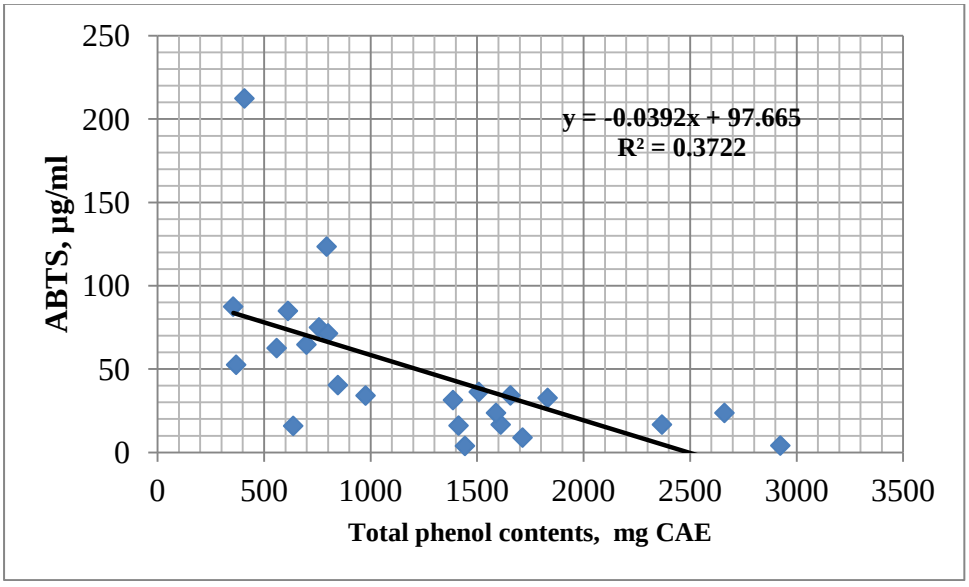


Figure 5.4. Linear correlation for the extracts between total phenol contents and ABTS data

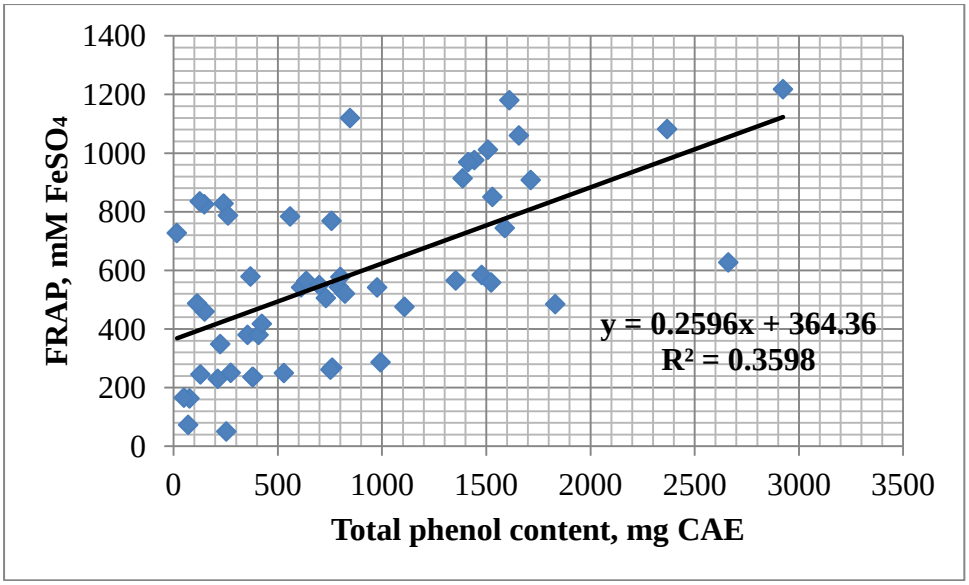


Figure 5.5. Linear correlation for the extracts between total phenol contents and ABTS data

Conclusions

Extracts (including essential oils) of *G. fragrantissima, O. tyttanthum, A. graveolens, G. macrorrhizum, T. vulgare* exhibit higher antioxidant activity. They are interesting candidates as antioxidant. Time of incubation is critical issue for evaluation antioxidant activity of the essential oils. In final conclusion, our assumption was confirmed that the antioxidant activities of medicinal plants are directly related to their phenolic contents.

Chapter 6. Cytotoxic activity against cancer cell lines

6.1. Medicinal plants as sources of anticancer secondary metabolites

Humans have used medicinal plants for several thousands of years to treat illness and health disorders (van Wyk and Wink, 2004). Medicinal plants are continued to be sources of new drugs. Plant secondary metabolites play a highly significant role in the drug discovery and development process (Wink, 2012a). Over the half of all anticancer prescription drugs which approved between 1981-2010 were natural product or their derivatives (Newman and Cragg, 2012).

Quite a large number of plant derivatives have been tested against different cancer cell lines which show significant anticancer activity. For example triptolide, a diterpene in *Tripterygium wilfordii* Hook F (thunder god vine), could be considered as a promising antiproliferative and immunosuppressive. For the long time, extracts of this plant been used in traditional Chinese medicine for treating a wide variety of diseases such as inflammation and arthritis. Triptolide contains three epoxide groups next to each other (Figure 6.1).

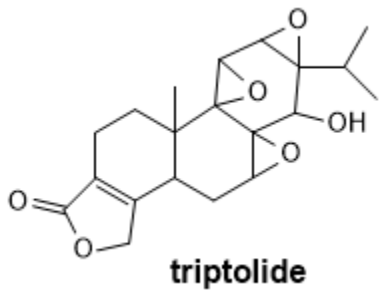


Figure 6.1. Triptolide stusture

At the cellular level, triptolide shows strong antiproliferative activity, inhibiting the proliferation of all 60 US National Cancer Institute cancer cell lines with an IC_{50} values in the low nanomolar range (average 12 nM) (Titov et al., 2011).

6.1.1. Essential oils as sources of anticancer molecules

New anticancer molecules can be found in essential oils. They can efficiently be exploited in pharmaceutical preparations with more research and some of them are already in the different phases of clinical trials (Gautam et al., 2014).

A large numbers of scientific reports has recently focused on the potential of essential oils as anticancer treatment in the attempt to overcome the development of multidrug resistance and important side effects associated with the antitumor drugs currently used (Russo et al., 2015).

More than hundred essential oils have been tested on more than twenty types of cancers in last past ten years (Bayala et al., 2014). Summary cytotoxic activity essential oils are represented in Table 6.1. Results of literature data show that essential oil activity (IC_{50}) ranged between 0.26-29 000 μg/ml for HeLa cell line and 0.8 - 42 000 μg/ml for MCF-7 cell lines. We are not agreeing with some data with very low IC_{50} in the Table 6.1. One common mistake is conversion of μl/ml to μg/ml value in calculation IC_{50} for essential oils. Most beginners are considered that 1μl of essential oil is equal to 1μg. This is not true. If we consider the density of essential oils 0.8-0.98 mg/ml, so 1μl of essential oil is approximately equal to 800-980 μg/ml.

In this study and in continuation of our previous studies in the search of the essential oil with high bioactivities, we have investigated the cytotoxicity of essential oils from 24 plant species against five human tumour cell lines, including HeLa (human cervical cancer), Caco-2 (human colorectal adenocarcinoma), MCF-7 (human breast adenocarcinoma), CCRF-CEM (human T lymphoblast leukaemia) and multidrug resistance CEM/ADR5000 (a high P-gp expressive adriamycin resistant leukaemia) cell lines. Furthermore, synergistic and hemolytic activities and morphological changes observed.

Table 6.1. Summary of essential oils with cytotoxic activity

Cancer cell line	Plant species	EO/composition	IC_{50}, µg/ml	Reference
HeLa	*Curcuma amada*	-	125	(Vasundra Devi and Suja, 2014)
HeLa	*Ricinus communis*	α-thujone, 1,8-cineole, α-pinene, camphor and camphene	2630	(Zarai et al., 2012)
HeLa	*Mentha spicata*		1.1	(Sharafia et al., 2010)
HeLa	*Eucalyptus benthamii*	*α*-pinene (37%), globulol (20%), aromadendrene (12-16%), and *γ*-terpinene (4-5%)	84-120	(Doll-Boscardin et al., 2012)
HeLa, MCF-7	*Lippia gracilis*	thymol, carvacrol	31.2-125 and 62.5 (MCF-7)	(Melo et al., 2014)
HeLa, MCF-7	*Libanotis transcaucasica*	germacrene B (20%), isospathulenol (11%), germacrene D (9%) and kessane (6%)	360 and 587.5 (HeLa)	(Shahabipour et al., 2013)
HeLa, MCF-7	*Artemisia vulgaris*	germacrene D (25%), caryophyllene (20%), α-zingiberene (15%) and borneol (11%)	2.5 (HeLa) and 1.0 (MCF-7)	(Saleh et al., 2014)
HeLa, MCF-7	*Boswellia carterii* and *Commiphora pyracanthoides*	2-cyclohexen-1-one, 4-ethynyl-4-hydroxy-3,5,5-trimethyl- and n-octylacetate	20 and 40 (MCF-7), 34 and 54 (HeLa)	(Chen et al., 2013)
MCF-7	*Pulicaria jaubertii*	p-menth-6-en-2-one (99%), dimethoxydurene (38%), durenol (27%) and 2',4'-dimethoxy-3'-methylacetophenone (20.5%)	4-9	(Fawzy et al., 2013)

Table 6.1 Continued.

Cancer cell line	Plant species	EO/composition	IC_{50}, µg/ml	Reference
MCF-7	*Thymus linearis* and *Thymus serpyllum*	*T. linearis* thymol (37%), carvacrol (9%). *T. serpyllum* carvacrol (44%), *o*-cymene (14%)	81 - 96	(Hussain et al., 2013)
HeLa	*Porcelia macrocarpa*	germacrene D (47%) and bicyclogermacrene (37%)	100	(da Silva et al., 2013)
HeLa	*Achillea wilhelmsii*	ρ- cymene (23%), 1,8-cineole (21%), cihydrocarvone (19%), camphor (7%)	46	(Ahmadi-Jouibari et al., 2013)
MCF-7	*Cedrelopsis grevei*	(*E*)-*β*-farnesene (28%), *δ*-cadinene (14%), *α*-copaene (8%) and *β*-elemene (7%)	21.5	(Afoulous et al., 2013)
MCF-7	*Libanotis transcaucasica*			(Shahabipour et al., 2013)
MCF-7	*Melissa officinalis*	geranial (43%), neral (31%), (E)-anethole (12%)	62	(Sharopov et al., 2013)
HeLa	*Lycopus lucidus*	α-humulene (16%), β-caryophyllene (11%) and humulene epoxide II (10%)	150	(Yu et al., 2011)
MCF-7	*Boswellia sacra*	*alpha*-pinene		(Suhail et al., 2011)
MCF-7, HeLa	*Murraya koenigii*	β-caryophyllene (19%) and α-humulene (15%)	2	(Nagappan et al., 2011)
MCF-7	*Salvia officinalis*	α-pinene (19%), trans-caryophyllene (18%), camphene (15%) and α-humulene (12%)	125.7	(El Hadri et al., 2010)
MCF-7	*Laurus nobilis, Origanum syriacum, and Salvia triloba*	*L. nobilis* and *S. triloba*- 1,8-cineol 41% and 45%, respectively *O. syriacum* - carvacrol (47%)	6-25	(Al-Kalaldeh et al., 2010)
MCF-7	*Schinus molle* L. and *Schinus terebinthifolius*	α-phellandrene (46% and 34%), β-phellandrene (21% and 11%), α-terpineol (8% and 6%)	*47-54*	(Bendaoud et al., 2010)
MCF-7	*Rosmarinus officinalis*	1,8-cineol (38%), camphor (17%)	190	(Hussain et al., 2010)

Table 6.1 Continued.

Cancer cell line	Plant species	EO/composition	IC_{50}, µg/ml	Reference
HeLa	*Amomum tsao-ko*	1,8-cineole (45%), ρ-propylbenzaldehyde (6%), geraniol (5%), geranial (5%)	66.5	(Yang et al., 2010)
HeLa	*Citrus limon*	octyl ester (16%), decanoic anhydride (13%), benzene, 2,4-difluoro-1-isocyanato (12%)	60	(Yan et al., 2010)
MCF-7	*Schefflera heptaphylla*	*β*-pinene (22%)	7.3	(Li et al., 2009)
MCF-7	*Eucalyptus sideroxylon, Eucalyptus torquata*			(Ashour, 2008)
HeLa	*Casearia sylvestris*	bicyclogermacrene (44%), β-caryophyllene (18%), spathulenol (16%)	63.3	(da Silva et al., 2008)
MCF-7	*Dictamnus dasycarpus*	*syn*-7-hydroxy-7-anisylnorbornene (29%), pregeijerene (15%) and geijerene (11%)	45	(Lei et al., 2008)
MCF-7	*Juniperus phoenicea*	α-pinene (38-39%), sabinene (24%), α-cedrol (31%)	0.8 - 1	(El-Sawi et al., 2007)
HeLa	*Zanthoxylum rhoifolium*	β-caryophyllene, α-humulene, α-pinene, myrcene and linalool	91	(da Silva et al., 2007)
HeLa	*Photinia serrulata*	10-epi-γ-eudesmol (13%), pinene (7%), sabinene (6%), α-humulene (6%) and α-thujene (5%)	43	(Hou et al., 2007)
MCF-7, HeLa	*Citrus limon* (L.), *C. medica* (L.), *C. sinsensis* (L.)	β-pinene (16%), α-terpineol (11%), γ-terpinene (4%), and trans- α-bergamotene (3%)	500-10000 (MCF-7) and 1000- 17000 (HeLa)	(Monajemi et al., 2010)
MCF-7	*Abies balsamea*	monoterpenes	760 - 1700	(Legault et al., 2003)
HeLa (Bel-7402)	*Rosmarinus officinalis*	1,8-cineole (27%), α-pinene (19%) and β-pinene (7%)	IC_{50} 0.1% (v/v)	(Wang et al., 2012)

Table 6.1 Continued.

Cancer cell line	Plant species	EO/composition	IC_{50}, µg/ml	Reference
Caco-2	*Myristica fragrans*	myristicin (33%), sabinene (16%), α-pinene (10%), β-pinene (9%)	155-167	(Piras et al., 2012)
MCF-7	*Foeniculum vulgare*	L-limonene	50	(Mohamad et al., 2011)
HeLa	*Ocimum basilicum*	methyl cinnamate (70%), linalool (17%)	90 - 96	(Kathirvel and Ravi, 2011)
MCF-7	*Satureja sahendica*	thymol (40%), γ-terpinene (28%), and ρ-cymene (22%)	16	(Yousefzadi et al., 2012)
MCF-7	*Satureja khuzistanica*	carvacrol (93%) and limonene (1%)	125	(Yousefzadi et al., 2014)
HeLa	*Casearia sylvestris*	bicyclogermacrene (44%), β-caryophyllene (18%), α-humulene (5%), spathulenol (16%)	63	(Silva et al., 2008)
HeLa, MCF-7	*Casearia sylvestris*	α -zingiberene (50%)	29 000 (HeLa) and 42200 (MCF-7)	(Bou et al., 2013)
HeLa	*Anemopsis californica*	elemicin, methyleugenol, piperitone and thymol	0.05% (v/v)	(Medina-Holguin et al., 2008)
HeLa	*Croton matourensis*	fenchyl acetate (20%), methyleugenol (14%), isoelemicine (11%), elemicine (8%), spathulenol (7%) and valencene (6%)	88	(Compagnone et al., 2010)
MCF-7	*Helichrysum gymnocephalum*	1,8-cineole (47%), bicyclosesquiphellandrene (6%), γ-curcumene (6%), α-amorphene (5%)	16	(Afoulous et al., 2011)
HeLa	*Marrubium vulgare*	g-eudesmol (12%), β-citronellol (10%), citronellyl formate (10%), germacrene-D (10%)	0.26	(Zarai et al., 2011)
MCF-7	*Dorema ammoniacum*	(Z)- ocimenone (22%), (E)-ocimenone (18%) and β-cyclocitral (10%)	312	(Yousefzadi et al., 2011)

Table 6.1 Continued.

Cancer cell line	Plant species	EO/composition	IC_{50}, µg/ml	Reference
CEM, MCF-7	*Thymus broussonettii*	borneol (34%), thymol (37%), carvacrol (5%)	0.003-0.004 (CEM) and 0.01-0.015 (MCF-7) (in % v/v)	(Jaafari et al., 2009)
MCF-7	*Solanium spirale* Roxb.	(E)-phytol (48%), n-hexadecanoic acid (7%), β-selinene (4%)	20	(Keawsa-ard et al., 2012)
MCF-7	*Pituranthos tortuosus*	β-myrcene, sabinene, trans-iso-elemicin, terpinen-4-ol	3	(Abdallah and Ezzat, 2011)
MCF-7	*Melaleuca armillaris*	1,8-cineole (86%), camphene (5%), and α-pinene (2%)	12	(Chabir et al., 2011)
MCF-7	*Erigeron acris*	-	14.5	(Nazaruk et al., 2010)
HeLa, MCF-7	*Aristolochia mollissima*	2,2,7,7-tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one	51 (HeLa) and 21 (MCF-7)	(Yu et al., 2007)
HeLa	*Liquidambar styraciflua*	d-limonene, α-pinene and β-pinene, germacrine D, α-cadinol	120-136	(El-Readi et al., 2013)
Caco-2	*Artemisia indica*	artemisia ketone (42%), germacrene B (9%), borneol (6%) and cis-chrysanthenyl acetate (5%)	19.5	(Rashid et al., 2013)
Caco-2, CCRF-CEM, HeLa, MCF-7	*Bupleurum marginatum*	tridecane (13%), unidecane (10%), pentadecane (9%), β-caryophyllene (5%) and β-caryophyllene oxide (5%)	170 (Caco-2), 19 (CCRF-CEM), 185 (HeLa), 106 (MCF-7)	(Ashour et al., 2009)

6.2. Cytotoxicity of the essential oils

Results of the cytotoxicity of essential oils from 20 plant species against five human tumour cell lines (HeLa, CaCo-2, MCF-7, and CCRF-CEM) are presented in Table 6.2.

Table 6.2. Cytotoxicity of essential oils (arranged by activity)

Species	HeLa	Caco-2	MCF-7	CCRF-CEM	CEM/ADR 5000
	IC_{50}, µg /ml				
Origanum tyttanthum	43.3	78.1	54.6	7.46	n.d.
Galagania fragrantissima	48.3	73.3	51.6	9.26	75.5
Mentha longifolia	42.3	62.8	58	12.1	25.4
Hypericum perforatum	64.2	65.9	55.9	41.9	124.8
Anethum graveolens	93.2	215.8	65.6	16.3	27.5
Artemisia absinthium	95.6	190.2	125.9	60.34	85.2
Tanacetum vulgare	n.d.	n.d.	n.d.	22.5	92.5
Achillea filipendulina	209.9	551.5	223.7	29.9	45.1
Foeniculum vulgare	206.7	74.8	58.6	32.3	165.5
Ferula clematidifolia	n.d.	n.d.	n.d	56.3	142.5
Salvia officinalis	n.d.	n.d.	n.d.	65.8	70.7
Ziziphora clinopodioides	164.4	407.2	134.9	70.95	73.6
Polychrysum tadshikorum	n.d.	n.d.	n.d.	77.4	137.9
Tanacetum parthenium	n.d.	285.7	n.d.	82.6	163.2
Hyssopus seravschanicus	218.1	423.0	206.5	110.8	127.7
Artemisia scoparia	78.3	179.1	73.2	151.7	n.d.
Hypericum scabrum	n.d.	n.d.	n.d.	158.9	280.9
Salvia sclarea	176.6	210.8	146.4	354	n.d
Artemisia rutifolia	295.2	478.6	456.2	387.2	560.6
Ocimum basilicum	n.d.	813.9	n.d.	798.4	1038.9
Doxorubicin	4.5	8.1	3.3	2.3	5.2

n.d. - not determined

Essential oils from *Origanum tyttanthum, Galagania fragrantissima, Mentha longifolia, Anethum graveolens, Artemisia absinthium* and *Hypericum perforatum* are exhibited a high cytotoxic effect. In contrary, *Ocimum basilicum, Artemisia rutifolia, Salvia sclarea* essential oils exhibited a low cytotoxic effect. The cytotoxic activity of essential oils is based on their individual components. In previous chapters, we reported that the major components of the essential oils from *Artemisia absinthium, Galagania fragrantissima, Hypericum perforatum, Mentha longifolia*, and *Origanum tyttanthum* were myrcene, (2*E*)-dodecenal, germacrene D, *cis*-piperitone epoxide, and carvacrol, respectively. The major components of the *Artemisia rutifolia, Ocimum basilicum, Salvia sclarea* essential oils were thujone, linalool and linalyl acetate, respectively. However, each essential oil consist at least 20 components.

6.3. Synergism of combinations of doxorubicin with essential oils

Previously, our group reported that combinations of secondary metabolites (in non-toxic concentrations) with chemotherapeutical agents could decrease the corresponding IC_{50} values and enhance cytotoxicity in cancer cells (Eid et al., 2012; Hamoud et al., 2014; Ma and Wink, 2008; Möller et al., 2006; Sun and Wink, 2014; Wink, 2012b). Also, it is fact that combinations of drugs with different modes of action can decrease the toxicity therapeutic drug because of the decreasing their concentrations (Chou, 2006). The current study investigated the combination of the intercalating agent doxorubicin and essential oils in leukemia cells (CCRF- CEM).

Results of the synergistic activity of the combinations of doxorubicin with essential oils of *Mentha longifolia, Anethum graveolens, Origanum tyttanthum, Galagania fragrantissima* and *Artemisia absinthium* are illustrated in Table 6.3. All examined essential oil have shown synergistic activity. The ratio of IC_{50} values of doxorubicin in dual combinations and IC_{50} value of doxorubicin alone were 3-15 folds.

This synergy is probably due to the lipophilic properties of essential oil, which mainly influence the permeability of the cell membrane. Moreover, the lipophilic core of proteins is considered as another target of essential oils. When essential oil molecules accumulate inside a protein or membranes, a disturbance of the fundamental interaction of membrane proteins with membrane lipids occurs, leading to changes of the 3D conformation of the protein (Wink, 2008).

Table 6.3. The cytotoxicity of doxorubicin alone or in dual combinations with essential oils

Sample	IC_{50}, µg/ml	$IC_{50\ Dox.}/IC_{50\ Dox.+essential\ oil}$ (Times)
Doxorubicin	2.34	-
Doxorubicin + *Mentha longifolia*	0.39	6
Doxorubicin + *Anethum graveolens*	0.89	2.6
Doxorubicin + *Origanum tyttanthum*	0.29	8.1
Doxorubicin + *Galagania fragrantissima*	0.155	15.1
Doxorubicin + *Artemisia absinthium*	0.17	13.8

6.4. Cytotoxicity of methanol extracts

The cytotoxicity of methanol extracts from different parts of 23 samples of 10 plant species from Heidelberg were evaluated against five human tumor cell lines (HeLa, CaCo-2, MCF-7, CCRF-CEM and CEM/ADR 5000) by the MTT assay. Furthermore, cytotoxic activity of the methanol extracts of 18 plant species from Tajikistan were evaluated against two human tumour cell lines (CCRF-CEM and CEM/ADR 5000). Results of the cytotoxicity of methanol extracts from Heidelberg and Tajikistan are presented in Table 6.4 and Table 6.5, respectively. The methanol extracts exhibited a pronounced cytotoxicity against most of cancer cell lines, especially those with a low expression of ABC transporters.

Results of cytotoxicity methanol extracts from Heidelberg show that *Tanacetum vulgare, Geranium macrorrhizum, Salvia officinalis* are exhibited a high cytotoxic effect. In contrary, *Galega x hartlandii* (from stem) and *Ficus carica* (from fruit) extracts were exhibited a low cytotoxic effect.

Alkaloid fraction of *Papaver somniferum* is exhibited a high cytotoxic effect. *Papaver somniferum* activity is attributed from high active alkaloids. Morphine is considered the "gold standard" for relieving pain and is currently one of the most effective drugs available clinically to relieve severe pain associated with cancer (Gach et al., 2011). Additionally, morphine has been discussed as a regulator of tumour growth (Bimonte et al., 2013).

Table 6.4. Cytotoxicity of methanol extracts of plants from Heidelberg

Species	Anatom. part	HeLa	Caco-2	MCF-7	CCRF-CEM	CEM/ADR 5000
		IC_{50}, µg /ml				
Papaver somniferum	**all part (alk. fr.)**	**75.4**	**15.25**	**198.4**	**22.1**	**15.7**
Tanacetum vulgare	whole plant parts	[1]n.d.	n.d.	n.d.	22.4	86.4
Geranium macrorrhizum	leaf	n.d.	n.d.	n.d.	22.4	112.3
Salvia officinalis	whole plant parts	257.8	n.d.	118.2	25.3	36.1
Galega x hartlandii	fruit	n.d.	n.d.	n.d.	24.9	65.7
Salvia discolor	whole plant parts	301	n.d.	208.8	26.2	34.5
Papaver somniferum	seed	223.1	148.5	545.6	19.5	120.4
Papaver somniferum	flower	206.55	> 1000	296.1	26.4	167.5
Salvia sclarea	root	273.3	n.d.	150	27.4	49.8
Papaver somniferum	whole plant parts	41.9	262.25	109.7	37.45	104.7
Philadelphus x purpureomaculatus	stem	n.d.	n.d.	n.d.	38.5	79.8
Pastinaca sativa	whole plant parts	n.d.	n.d.	n.d.	61.3	142.5
Salvia sclarea	whole plant parts	615.1	n.d.	202.5	65	89.8
Papaver somniferum	cupsuls bark	211.9	268	467.5	65.6	146.4
Geranium macrorrhizum	root	n.d.	n.d.	n.d.	98.3	154.2
Philadelphus x purpureomaculatus	leaf	n.d.	n.d.	n.d.	103.7	178.4
Galega x hartlandii	leaf	n.d.	n.d.	n.d.	106.3	174.5
Papaver somniferum	leaf	82.2	182.3	278.5	112.4	150.1
Papaver somniferum	root	295.2	189.15	442.1	158	197.2
Ficus carica	leaf	n.d.	n.d.	n.d.	162.4	251.8
Papaver somniferum	stem	210.2	> 1000	345.6	165.9	230.7
Ficus carica	fruit	n.d.	n.d.	n.d.	214.2	342.5
Galega x hartlandii	stem	n.d.	n.d.	n.d.	216.8	207.4
Doxorubicin		4.5	8.1	3.3	2.3	5.2

[1]n.d. - not determined

Results of cytotoxicity methanol extracts from Tajikistan indicate that *Tanacetum parthenium* and *Polychrysum tadshikorum* extracts are exhibited a high cytotoxic effect. In contrary, *Anethum graveolens* and *Artemisia scoparia* extracts were exhibited a low cytotoxic effect.

There is no data about composition of methanol extract of *Polychrysum tadshikorum* and future investigation of the plant composition will be required. Parthenolide is one the principal sesquiterpene lactone with the most responsible for biologically active of *Tanacetum parthenium* (Bohlmann and Zdero, 1982). Cytotoxicity of extracts of *Tanacetum parthenium* also may be depends to the parthenolide.

Table 6.5. Cytotoxicity of the methanol plant extracts from Tajikistan

Species	**IC_{50} µg/ml**	
	CCRF CEM	**ADR -5000**
Tanacetum parthenium	7.3	n.d.[1]
Polychrysum tadshikorum	10.2	32.5
Achillea filipendulina	35.5	25.8
Tanacetum vulgare	36	107.3
Melissa officinalis	41.8	120.8
Artemisia absinthium	52.3	133.1
Hypericum scabrum	57.7	67.5
Hypericum perforatum	62.5	225.4
Ocimum basilicum	62.8	310.1
Ferula clematidifolia	73.85	99.6
Salvia sclarea	80.3	148.7
Artemisia rutifolia	86.3	131.6
Mentha longifolia	94.0	205.24
Galagania fragrantissima	144.3	125.4
Ziziphora clinopodioides	151.2	175.9
Origanum tyttanthum	158.4	167.9
Anethum graveolens	205.3	201.4
Artemisia scoparia	215.6	134.5
Doxorubicin	2.3	5.2

n.d.[1] - not determined

Our experiments show that CCRF-CEM cells which do not express ABC transporters were more sensitive for the total extracts than the derived CEM/ADR5000 (multidrug resistant) cells indicating that some components of the extracts are substrates of ABC transporters (Eid, 2012; Wink, 2008). In Figure 6.2. is represented the linear correlation between CCRF-CEM and CEM/ADR 5000 for the 40 sample of methanol extracts from Heidelberg and Tajikistan plants. Results, indicated that a strong correlation exists between the cytotoxicity in CCRF-CEM and CEM/ADR 5000 cell lines (R = 0.4435). It is remarkable that some extracts (for example, the alkaloid extract from *Papaver somniferum*) exhibits cytotoxicity in all cancer cells which is apparently independent of the expression of ABC transporters.

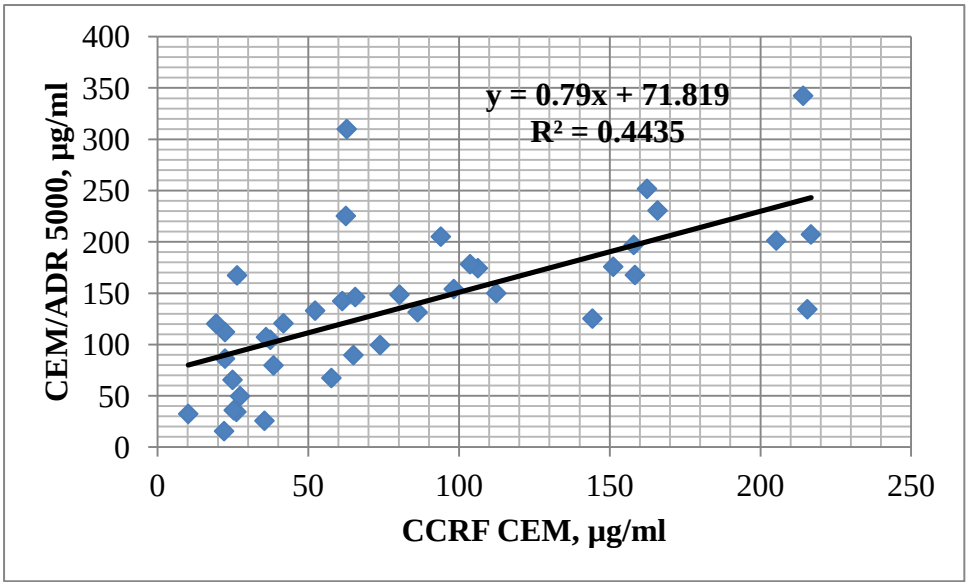


Figure 6.2. Linear correlation for the extracts (40 samples) between CCRF-CEM and CEM/ADR 5000 cell lines.

Conclusions

The cytotoxicity of essential oils from 20 plant species and methanol extract from 28 plant species were evaluated by the MTT assay in five human tumour cell lines (HeLa, CaCo-2, MCF-7, CCRF-CEM and CEM/ADR 5000). Essential oils from *Origanum tyttanthum*, *Galagania fragrantissima*, *Artemisia absinthium*, *Mentha longifolia*, *Hypericum perforatum* exhibited the high cytotoxic activity. A synergistic effect of essential oils seems promising area for future research. Methanol extracts from *Polychrysum tadshikorum*, *Tanacetum parthenium* and alkaloid fraction of *Papaver somniferum* were most active.

Chapter 7. Antibacterial and anti-inflammatory activities of essential oils

7.1. Essential oils as candidate of antimicrobial agents

A great numbers of antibiotics are used in control infections and diseases of humans. This is related to antibiotic resistance of human pathogens (Sokovic et al., 2010). In addition, due to spoiling of food are gradually increasing consumption of food additives. In this case, it is necessary to search alternative natural and safe agents for controlling bacterial and infections. Because of the high activity and low or non-toxicity essential oils are good candidate for prevention and treatment of diseases caused by pathogenic bacterial species (Sokovic et al., 2010). Essential oils derived from aromatic medicinal plants have been reported to exhibit exceptionally good antimicrobial effects against bacteria, yeasts, filamentous fungi, and viruses (Reichling et al., 2009).

The goal of the present study was to evaluate antibacterial activity essential oils against gram negative and gram positive bacteria.

7.2. Structure of gram positive and gram negative bacteria

Gram negative bacteria are more resistant to essential oils than gram positive bacteria (Trombetta et al., 2005). Approximately 90%–95% of the cell wall of gram positive bacteria consists of peptidoglycan, to which other molecules, such as teicoic acid and proteins, are linked (Nazzaro et al., 2013). The cell wall of gram negative bacteria is more complex. It has a peptidoglycan layer that is 2–3 nm thick, which is thinner than in the cell wall of gram positive bacteria, and composes approximately 20% of the dry weight of the cell. An outer membrane lies outside of the thin peptidoglycan layer.

Essential oils and their components have activity against a variety of targets, particularly the membrane and cytoplasm, and in some cases, they completely change the morphology of the cells (Nazzaro et al., 2013).

7.3. Antimicrobial activity of essential oils

Antimicrobial activity of 16 essential oils has been evaluated against both gram positive bacteria (*Staphylococcus aureus*) and gram negative bacteria (*Klebsiella pneumoniae* and *Escherichia coli*). For screening of antimicrobial susceptibility in each investigated sample, both positive and negative controls were set to determine MIC (minimum inhibitory concentration), MBC values (minimum bactericidal concentration) and growth inhibition zone diameters. *Origanum tyttanthum* and *Galagania fragrantissima* essential oils were

found to be highly bactericidal, as it has shown lowest MIC and MBC values. Results are presented in Table 7.1.

Table 7.1. Antimicrobial activity of essential oils

Species	***E. coli* ATCC 25922**		**MRSA NTCT 10442**	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Achillea filipendulina	5	10	5	5
Artemisia absinthium	>20	>20	>20	>20
Artemisia rutifolia	10	20	5	20
Artemisia scoparia	2.5	5	1.25	2.5
Ferula clematidifolia	>20	>20	>20	>20
Ferula foetida	>20	>20	>20	>20
Galagania fragrantissima	**>20**	**>20**	**0.04**	**0.08**
Hypericum perforatum	5	5	1.25	2.5
Hypericum scabrum	>20	>20	>20	>20
Hyssopus seravschanicus	10	10	5	10
Mentha longifolia	5	10	10	20
Ocimum basilicum	>20	>20	>20	>20
Origanum tyttanthum	**0.31**	**0.31**	**0.62**	**1.25**
Salvia sclarea	>20	>20	>20	>20
Tanacetum vulgare	>20	>20	20	20
Ziziphora clinopodioides	5	5	10	10
Ampicillin	0.003	0.006	0.008	0.016

Origanum tyttanthum essential oil was active for both of gram positive and gram negative bacteria and *Galagania fragrantissima* essential oil was very active only against gram positive bacteria.

Carvacrol and thymol are the major components of the essential oil of *Origanum tyttanthum*. We suppose that the antimicrobial activity of *Origanum* oil is also related to phenol components. Thymol and carvacrol exhibited the highest antibacterial activity, they are known for their membrane-disturbing activities, as well as cell lysis (Reichling, 2010). Thymol and carvacrol interfere with the activity of cell wall enzymes like chitin synthase/chitinase as well as α- and β- glucanases (Sokovic et al., 2010).

The main constituent of the essential oil of *Galagania fragrantissima* was aliphatic aldehyde, 2*E*-dodecenal (83.6%). Advantage of 2*E*-dodecenal is that it has hydrophobic alkyl (tail) chain and hydrophilic aldehyde group (head). Probably this molecule can pass a peptidoglycan layer of the cell wall of gram positive bacteria. Authors suppose that the combination of the carbonyl oxygen atom at the C_1 position with the conjugated double bond at the C_2 position as well as the length of the carbon chain might be important for the activity (Kano et al., 2012). Antibacterial activity of 2*E*-dodecenal is corrected with physico-chemical damage to the cells, such as the disruption of the membrane and probably interference with proteins (Cespedes, 2013) and nucleic acids (Reichling, 2010). 2*E*-dodecenal inhibited *Salmonella choleraesuis* (gram negative bacteria) with IC_{50} 6.25 µg/ml (Cespedes, 2013).

7.4. Antimicrobial activity of phenols

In chapter 5, we are discussed antioxidant activity of phenols. Phenols are most active compound in the essential oils. In continuing our investigation, antimicrobial activity four pure phenolic compounds (phenol, carvacrol, thymol, eugenol) have been evaluated against two bacteria species (*E. coli* and *A. baumanii*). Results of are presented Table 7.2. Results indicated that all investigated phenols have shown antimicrobial activity both of gram positive and gram negative bacteria. Carvacrol and thymol exhibit more activity than phenol and eugenol.

Table 7.2. Antimicrobial activity of phenols

Sample	*E. coli* ATCC 25922		*A.baumannii* KL 301347	
	MIC (mg/mL)	**MBC (mg/mL)**	**MIC (mg/mL)**	**MBC (mg/mL)**
Carvacrol	0.188	0.25	0.188	0.25
Eugenol	0.75	0.75	0.375	0.75
Thymol	0.25	0.25	0.125	0.25
Phenol	0.75	> 1	0.375	>1
Ampicillin	0.003	0.008	0.016	0.024

7.5. Anti-inflammatory activity of essential oils

The overproduction of prostaglandins and leukotrienes are initiated and maintained inflammation in injured cells (Mogana et al., 2013). Prostaglandins and leukotrienes are produced by the cyclooxygenase (COX) and lipoxygenase (LOX) enzymatic pathways, respectively. Enzymatic oxidation of arachidonic acid by 5-lipoxygenase produces 5(S)-hydroxyperoxyeicosatetraenoic acid (5-HETE). Dehydration of these products produces leukotrieneA4 (LTA4). Catalysing the hydrolysis of LTA4 and reaction of LTA4 with other substances, leads to the formation of inflammatory mediators (Ford-Hutchinson et al., 1994). Leukotrienes have been identified as mediators of a number of inflammatory and allergic reactions including rheumatoid arthritis, inflammatory bowel disease, atopic dermatitis, psoriasis, chronic urticaria, asthma (Claesson and Dahlen, 1999). Furthermore, oxidative and inflammatory processes are among the pathological features associated with the central nervous system (Houghton et al., 2007).

In continuation of previous studies on the bioactivity of essential oils, we are evaluated the anti-inflammatory activity of 12 essential oils by the inhibition of soybean 5-LOX. IC_{50} values of 5-LOX inhibition ranged between 7.34 and 221 µg/ml. Results are presented in Table 7.3.

Table 7.3. Anti-inflammatory activity of essential oils

Species	5-Lipoxygenase Inhibition, IC 50, µg/ml
Achillea filipendulina	221.3
Anethum graveolens	33.47
Artemisia absinthium	56.6
Artemisia rutifolia	75.6
Artemisia scoparia	184.3
Galagania fragrantissima	**7.34**
Hyssopus seravschanicus	100.7
Mentha longifolia	28.14
Origanum tyttanthum	14.78
Salvia sclarea	not active
Tanacetum parthenium	21.6
Ziziphora clinopodioides	33.12
Sodium linoleate (negative control)	-

The essential oils of *Origanum tyttanthum* and *Tanacetum parthenium* are exhibited high anti-inflammatory activity. However the essential oil of *Galagania fragrantissima* is exhibited potent anti-inflammatory activity. An unsaturated aldehyde ((2*E*)-dodecenal) of the *Galagania fragrantissima* oil may be responsible for anti-inflammatory activity. It is very electrophilic and can react with a variety of nucleophiles, such as amino groups either from proteins. Also, due to the structural similarities to fatty acids, aliphatic aldehydes (*trans*-2-decenal, dodecanal and decanal) have strong 5-lipoxygenase inhibitory activity (Baylac and Racine, 2003; Yakov, 2006).

Conclusions

The antimicrobial activity of 16 essential oils and 4 pure phenols were evaluated against both gram positive and gram negative bacteria. Essential oils of *Origanum tyttanthum, Galagania fragrantissima* and phenols (thymol and carvacrol) are exhibited high antimicrobial activity.
The anti-inflammatory activity of 12 essential oils is evaluated by the inhibition of soybean 5-LOX. *Galagania fragrantissima* oil is exhibited potent activity.
In general, essential oils of *Origanum tyttanthum* and *Galagania fragrantissima* are interesting candidates for a use in phytotherapy.

Chapter 8. Mode of action of some essential oils

8.1. Mechanism of action and target sites of the essential oils

A majority of secondary metabolites including essential oils, can interfere with several targets (multitarget) in a pleiotropic fashion (Wink, 2008). The cytotoxic activity of essential oils is based on their individual components (Reichling et al., 2009). In general, essential oil constituents due to their lipophilic prosperity and low molecular weights can cross cell membranes altering the phospholipid layers, increasing membrane fluidity, and leading to leakage of ions and of cytoplasmic content (Figure 8.1.) (Russo et al., 2015).

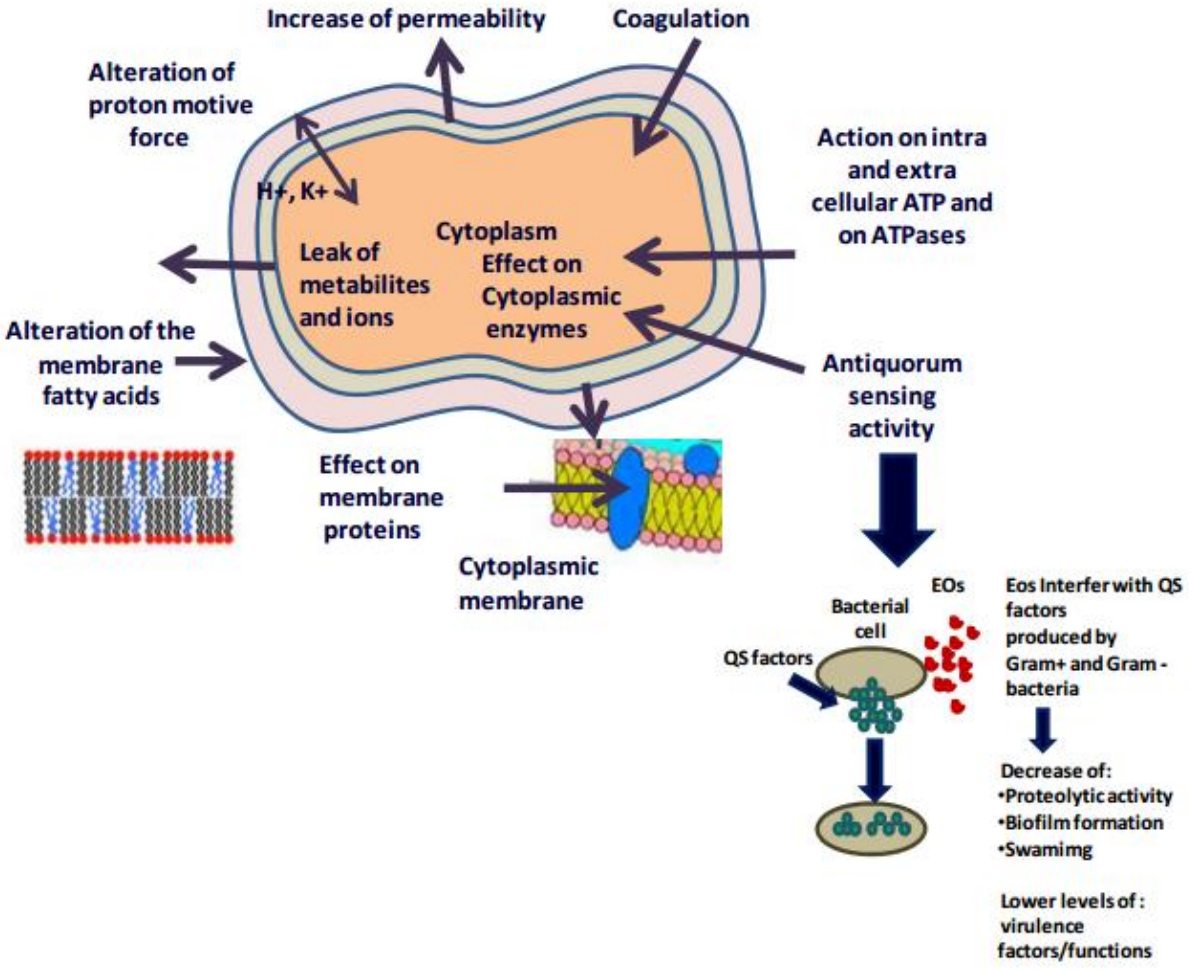


Figure 8.1. Mechanism of action and target sites of the essential oils on microbial cells (Nazzaro et al., 2013).

In addition, as result of disturbed cellular membranes will be reduce ATP production, alteration of pH gradient, and loss of mitochondrial potential (Russo et al., 2015). Essential oils and their constituents can effect on tumour suppressor proteins (p53 and Akt),

transcription factors (NF-κB and AP-1), MAPK-pathway, and detoxification enzymes like SOD, catalase, glutathione peroxidase, and glutathione reductase (Gautam et al., 2014).

Terpenes, as the main part of essential oils, are very hydrophobic compounds, which can bind to biomembranes. At higher doses, they are able to unselectively disturb membrane fluidity and permeability. Terpenes can stimulating death receptors, induce apoptosis, and modulating proteins in the membrane (Wink and Van Wyk, 2008).

For better understanding the mechanism of action in essential oil, we have investigated their hemolytic activity and their effect on the cell membrane. Also we investigate the interaction of essential oil of *Galagania fragrantissima* with number of amino acids containing different functional groups.

8.2. Hemolytic activity of essential oils

The results of hemolytic activity of essential oils in red blood cells are presented in Table 8.1.

Table 8.1. The hemolytic activity of essential oils

Species	**Hemolysis, IC_{50} mg/ml**
Achillea filipendulina	n.a.[1]
Anethum graveolens	0.9
Artemisia absinthium	0.41
Artemisia rutifolia	**n.a.**
Coriandrum sativum	2.3
Ferula clematidifolia	1.2
Galagania fragrantissima	0.4
Hypericum perforatum	1.7
Hyssopus seravschanicus	**n.a.**
Mentha longifolia	**n.a.**
Ocimum basilicum	**n.a.**
Origanum tyttanthum	1.8
Polychrysum tadshikorum	2.0
Salvia officinalis	13.9
Salvia sclarea	**n.a.**
Ziziphora clinopodioides	n.a.

[1]n.a.- not active

Essential oils of the *Galagania fragrantissima and Artemisia absinthium* are exhibited high activity. Six essential oils: *Artemisia rutifolia, Hyssopus seravschanicus, Mentha longifolia,*

Ocimum basilicum, Salvia sclarea, and *Ziziphora clinopodioides* have not shown hemolytic activity.

In order to investigate the contribution of hemolysis to the cytotoxicity and antimicrobial activity in essential oils, a linear regression was obtained between hemolytic activity and cytotoxicity; hemolytic activity and synergisms; hemolytic and antimicrobial activities of essential oils against gram negative bacteria. Higher correlations were found between hemolytic and synergistic activities (R = 0.4223) when compared with antimicrobial and hemolytic activities of essential oils against gram-positive bacteria (R = 0.2327) and with cytotoxicity and hemolytic activity of essential oils (R = 0.2056). Lower correlations were found between antimicrobial and hemolytic activities of essential oils against gram negative bacteria**.** This is in agreement that gram negative bacteria are more resistant to essential oils than gram-positive bacteria (Trombetta et al., 2005). Linear correlations between hemolytic activity and cytotoxicity; hemolytic activity and synergisms; hemolytic and antimicrobial activities of essential oils against gram negative are represented in Figure 8.2-8.5.

In conclusion, these results are indicating that there is correlation between hemolytic activity and antimicrobial, cytotoxic activities in essential oils.

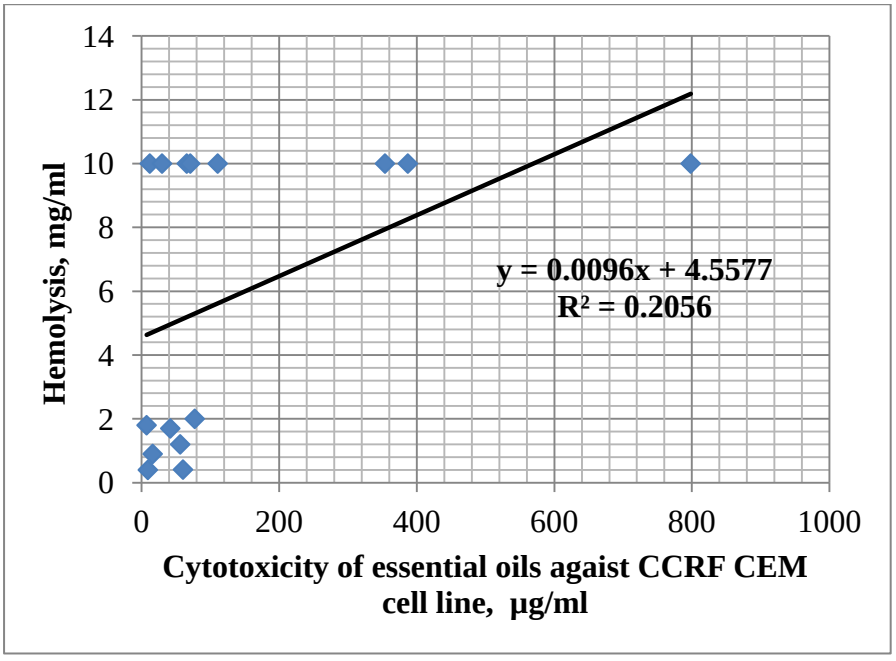


Fig.8.2. Correlation between cytotoxicity and hemolysis of essential oils

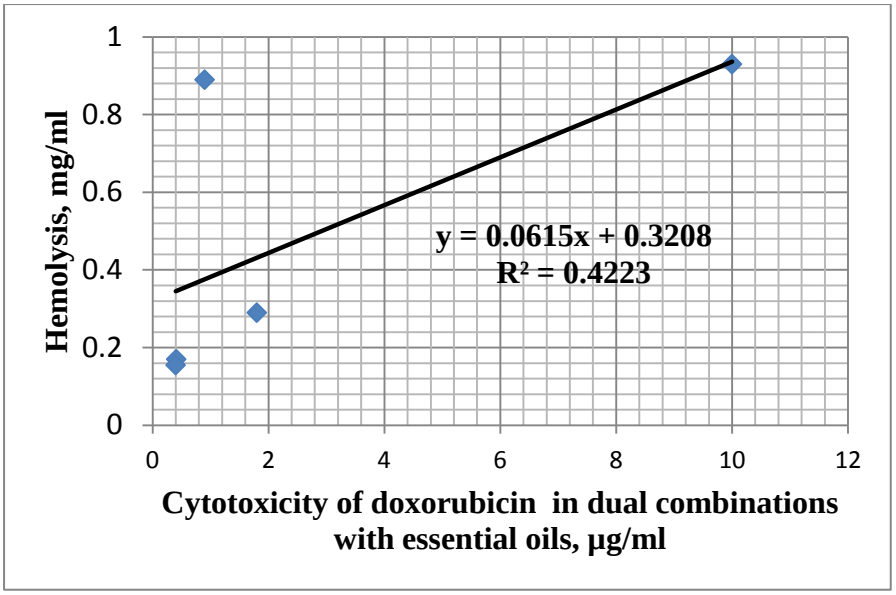


Fig.8.3. Correlation between synergistic and hemolytic activities of essential oils

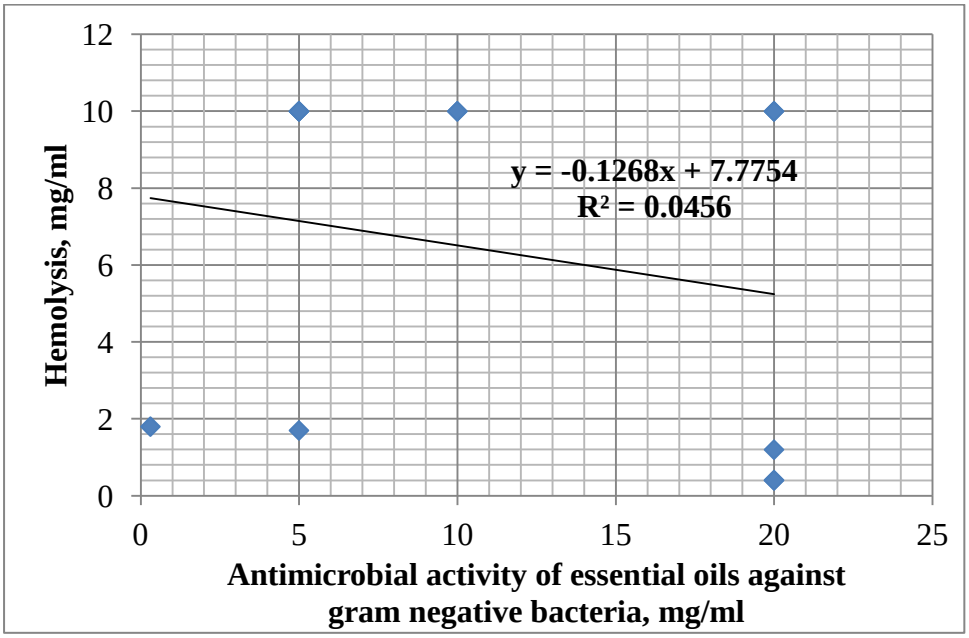


Fig.8.4. Correlation between antimicrobial and hemolytic activities of essential oils against gram negative bacteria

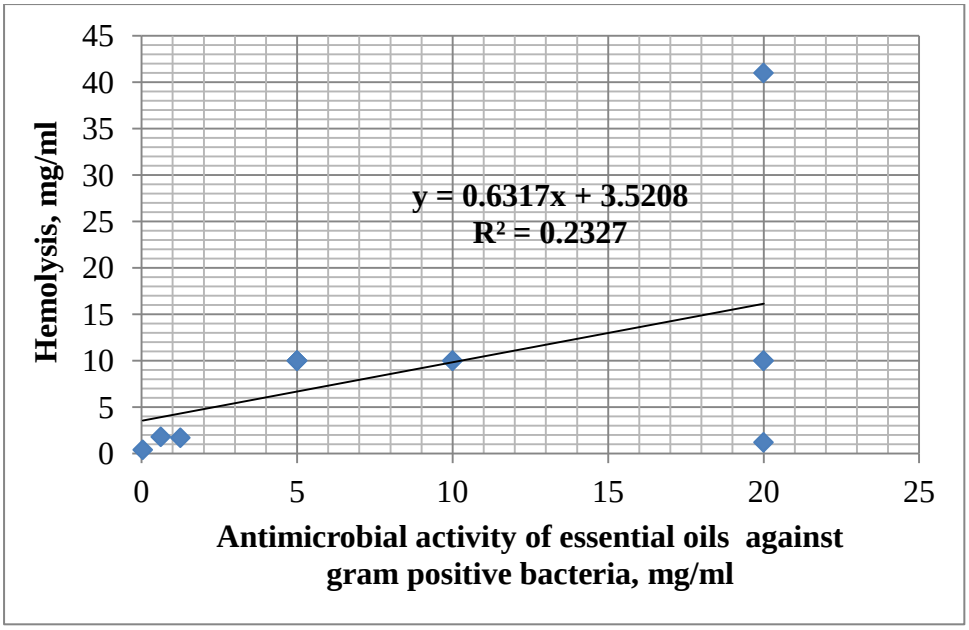


Fig.8.5. Correlation between antimicrobial and hemolytic activities of essential oils against gram positive bacteria

8.3. Fluorescence microscopic investigation

Authors have been studied morphological variation in CCRF-CEM after long-term culture and exposure to chemotherapeutic agents by electron microscope (Uzman et al., 1966). They reported that the most striking change being the appearance and persistence of very dense granules in the pars amorpha of the nucleoli (Uzman et al., 1966). The normal lymphoblast was significantly larger than the leukemic lymphoblast in total cell area, total cell length, nuclear length, and cytoplasmic area (Schumacher et al., 1973).

In order to investigate the effect of essential oils on the cell morphology, the images of untreated and treated CCRF cells with different essential oil were captured.

The images of untreated and treated CCRF cells with essential oil of *Mentha longifolia*, *Anethum graveolens*, *Origanum tyttanthum*, *Galagania fragrantissima* and *Artemisia absinthium* are illustrated in Figure 8.6-8.11. Results indicated that untreated control cells have a smooth surface, while cells treated with essential oils have the damage on the surface of cells.

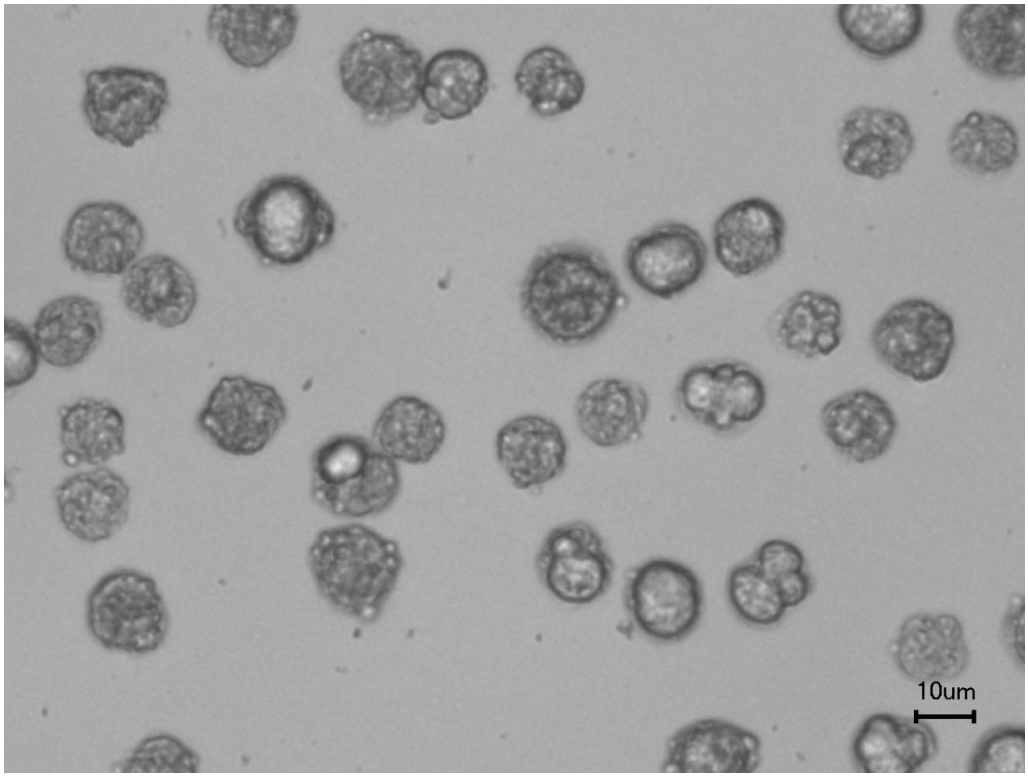


Figure 8.6. Treated CCRF cell with essential oil of *Galagania fragrantissima*

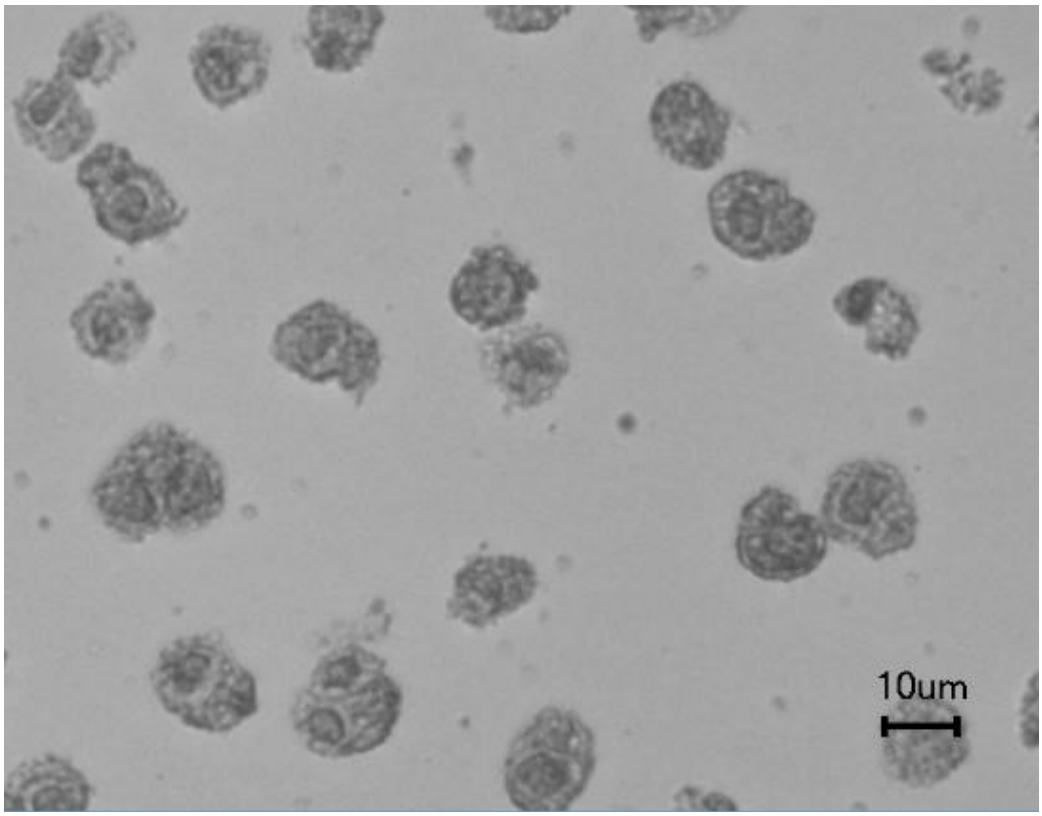


Figure 8.7. Treated CCRF cell with essential oil of *Anethum graveolens*

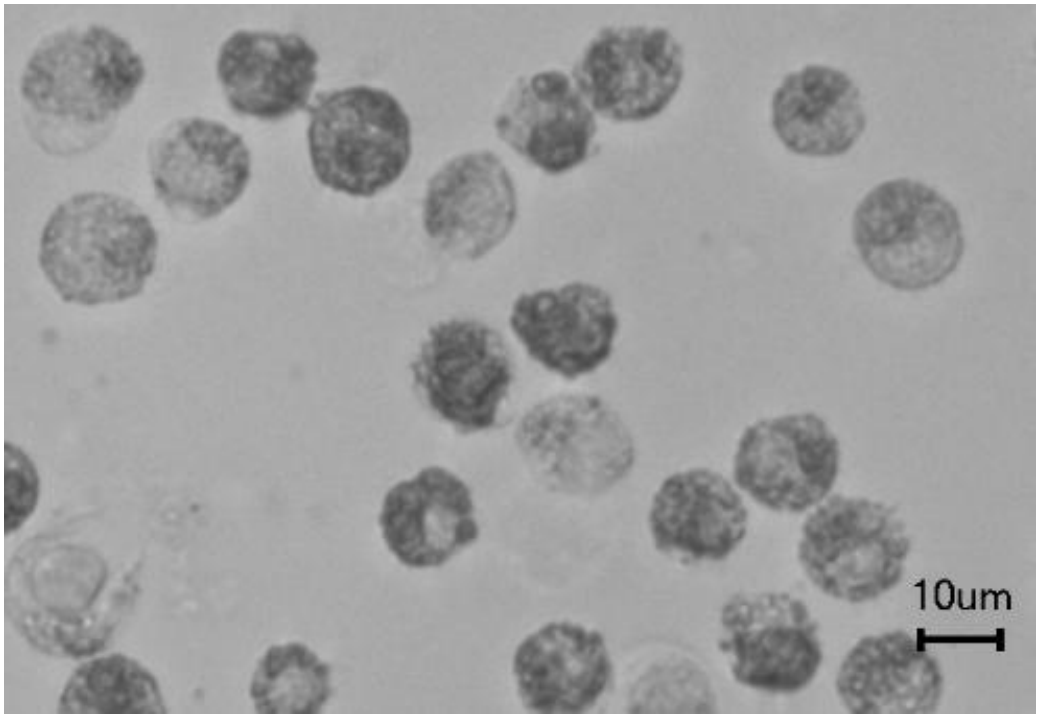


Figure 8.8. Treated CCRF cell with essential oil of *Mentha longifolia*

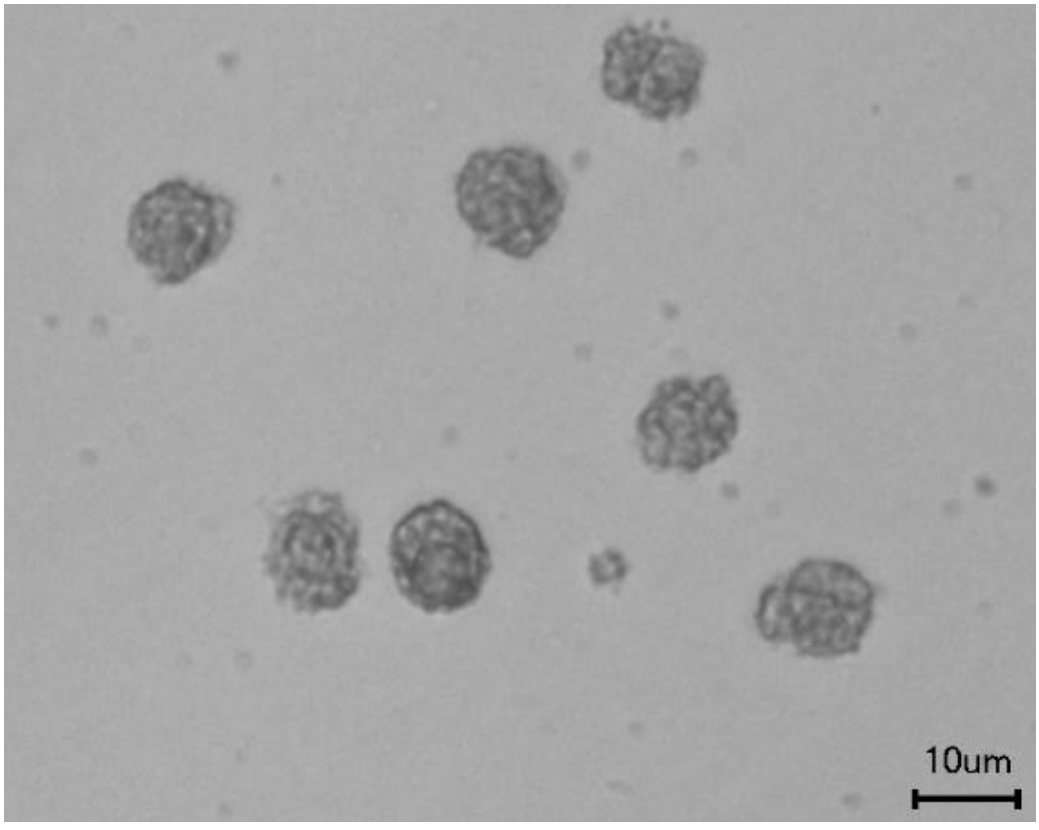


Figure 8.9. Treated CCRF cell with essential oil of *Origanum tyttanthum*

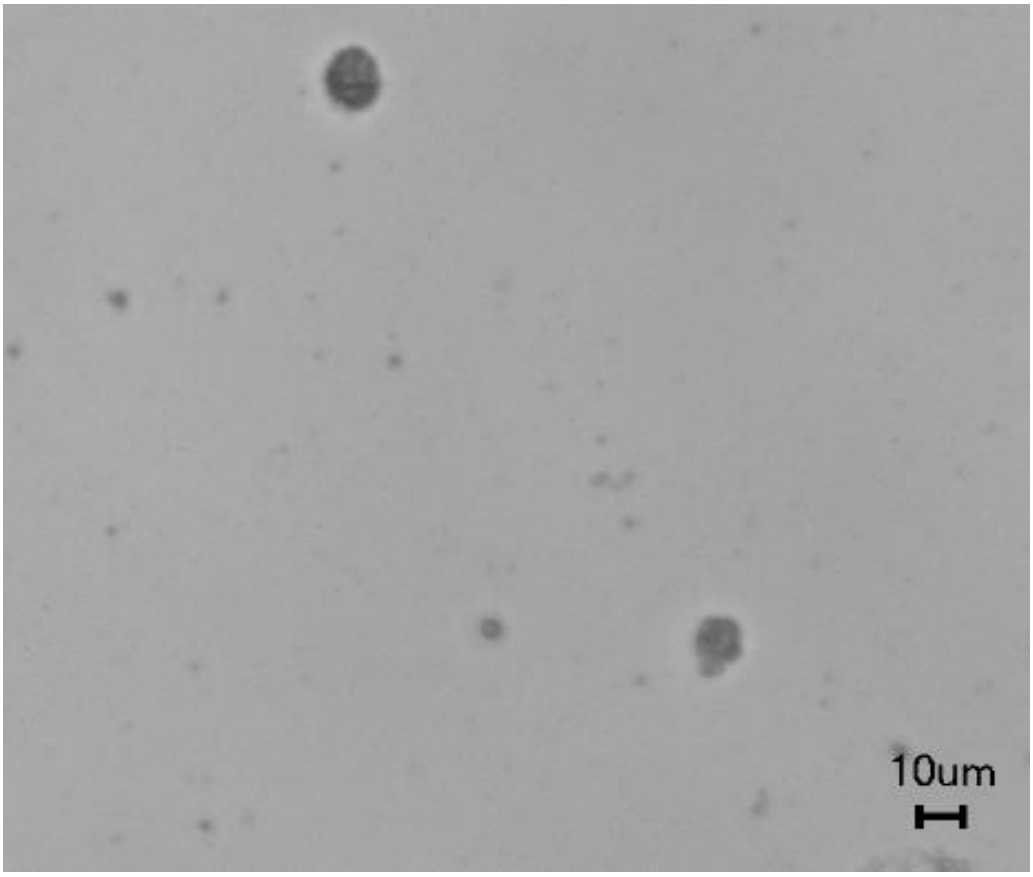


Figure 8.10. Treated CCRF cell with essential oil of *Artemisia absinthium*

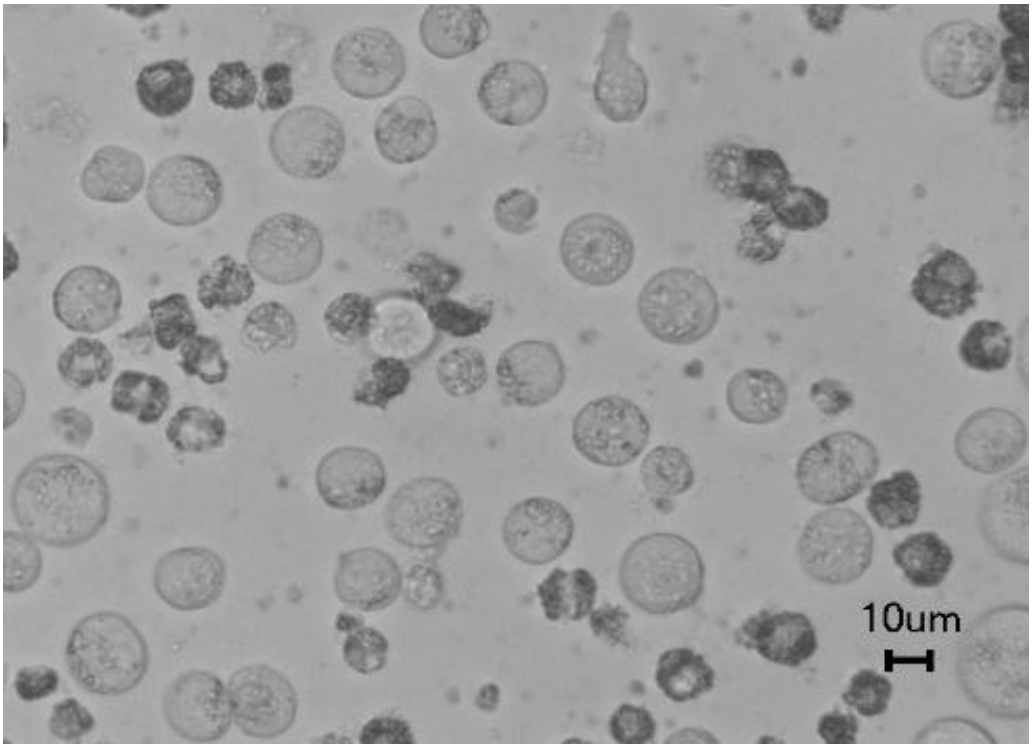


Figure 8.11. An untreated CCRF cell

Results of bioactivity, including cytotoxicity, antimicrobial and anti-inflammatory activities of essential oils have shown that essential oil of *Galagania fragrantissima* is most active oil among the investigated essential oils. Activity oil is mainly related to the 2*E*-dodecenal.

(*E*)-2-alkenals (C10-C16) that were isolated from leaves of coriander (*Coriandrum sativum* L.), have anti-deforming activity of Raji cells carrying the genome of Epstein-Barr virus (EBV) early antigens (Kano et al., 2012). Furthermore, analysis of (*E*)-2-decenal and its derivatives having the same carbon length showed that (*E*)-2-decenal is one of the strongest chemicals for the activity (Kano et al., 2012)

Conclusion

Essential oils are able to lysis the cell membrane. Bioactivity essential oils are related to their lysis ability. Microscopy investigation shows that essential oils can change morphology of cells. 2*E*-dodecenal the main component essential oil of G. *fragrantissima* binds covalently to proteins.

General conclusion

The study of "Phytochemistry and bioactivities of selected plant species with volatile secondary metabolites" resulted in a data base about large number of medicinal aromatic plants of Tajikistan and Germany. Our data base on the phytochemistry and various biological activity of plants might serve for practical applications in pharmaceutical, food, medicine, cosmetic, perfumery, agriculture and other industries. We have carried some basic characterisations such as investigating the chemical composition of plant secondary metabolites, their antioxidant, antimicrobial, anti-inflammatory, cytotoxic activities. Among investigated plants are many endemic plants of Tajikistan and Central Asia, which are of interest for local industries of this part of World.

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