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CICLO XXII

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PHYTOCHEMICAL ANALYSIS, ANTIPROLIFERATIVE AGAINST K562 HUMAM CHRONIC MYELOGENUS LEUKEMIA, ANTIVIRAL AND HYPOGLYCAEMIC ACTIVITIES OF CEDRUS SPECIES AND MEDICINAL PLANTS NATIVE FROM LIBANON

DOTTORANDO

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Abstract

Saab.A.M, 2011. Phytochemical analysis and antiproliferative against K562 human chronic myelogenus leukemia, antiviral and hypoglycaemic activities of Cedrus species and medicinal plants native from Lebanon.

A) Cedrus species.

There are four kinds of Cedar, three of them naturally present in the Mediterranean Sea region: *Cedrus libani*, in Lebanon, Syria and Turky, *Cedrus atlantica* in Algeria and Morocco, *Cedrus brevifolia* in Cyprus Island *While Cedrus deodara* in Himalaya Mountains and.

Cedrus libani are widely used as traditional medicine in Lebanon for treatment of different infection diseases. In the present study we reported the phytochemical composition analyzed by GC–MS of wood essential oil and cones and leaves ethanol extracts. The main components of wood essential oil were himachalol (22.50%), β-himachalene (21.90%), and α-himachalene (10.50%). Leaves ethanol extract was characterized by a high content of germacrene D (29.40%). The same extract obtained from cones essentially contained α-pinene (51.0%) and β-myrcene (13.0%).

The essential oils, obtained by hydro-distillation from leaves of *Cedrus libani* and *Cedrus deodara*, were analyzed by GC/MS system. Forty-nine components were identified. The experimental data demonstrated that Germacrene D and β-caryophyllene are the main components in *Cedrus libani*, while benzaldehyde,

myrcene and β-caryophylene are the principal components of *Cedrus deodara* essential oil.

The most abundant components characterizing *Cedrus libani* seeds chloroform extract are α-pinene, β-pinene, verbenol and abietadiene, while ethanol extract were characterized by oleic acid, neo-abeitol, abietadiene and methyl oleate. Both chloroform seeds and ethanol seeds extracts inhibited the proliferation of k 562 cell line with IC_{50} values of 40,57 and 69,20 µg/ml respectively. The Cedrus libani ethanol seeds extract showed a percentage of erythroid differentiation of 16 % at the concentration of 25 µg/ml while the chloroform seeds extract showed a percentage of erythroid differentiation of 12 % at the concentration of 50 µg/ml.

The wood essential oils of *C. libani, C .atalantica and C. deodara* inhibited the proliferation of k 562 cell line with IC_{50} Value of 23.38, 59.37 and 37.09 µg/ml respectively. Meanwhile *Cedrus libani* wood oils showed a percentage of erythroide differentiation of 15% at the concentration of 5µg/mlanother side *Cedrus deodara* wood oil was found a percentage of erythroide differentiation of 20% at the concentration 25µg/ml and *Cedrus atlantica* wood oils indicated a percentage of erythroide differentiation of 12% at concentration 10µg/ml.

The inhibitory activity of α-amylase of *Cedrus libani* essential oils obtained from wood, leaves and cones were investigated. The *C. libani* woods oil exhibit an IC_{50} value of 0.14 mg/ml, whereas the leaves and cones oils were devoid of any significant activity.

Moreover, we investigated extracts, essential oil, and identified compounds of *Cedrus libani* for their in vitro antiviral activities against herpes simplex virus type 1 (HSV-1). Cytotoxicity was evaluated by MTT assay in Vero cells. Cones and leaves ethanol extracts exhibited an interesting activity with IC_{50} of 0.50 and 0.66 mg/ml, respectively, at non-cytotoxic concentration.

B) Medicinal plants

The essential oils obtained from different officinal plants of Lebanon, belonging to the Magnoliophyta division, have been tested for their antiproliferative activity on humanerythroleukemic K562 cells. *Satureja montana* showed the most interesting biological activity in inhibiting the cell growth and inducing erythroid differentiation of K562 cells The essential oil of *Satureja montana* was therefore analyzed using a GC/MS (gas chromatography/mass spectrometry) system in order to identify the major constituents and compare them with analysis performed on *Satureja hortensis*. The major constituent of *Satureja hortensis* being carvacrol (50.61%) and that of *Satureja montana* being α-terpineol (12.66%). *Satureja Montana* essential oil displayed different natural derivatives characterized by higher activity than those present in *Satureja hortensis*. The common active principles are α-pinene, γ-terpinene, 4-terpineol, α-terpineol, τ-cadinene, τ-cadinol and caryophyllene. Both caryophyllene and α-terpineol showed important antiproliferative effects on K562 cells.

The essential oil from *Laurus nobilis* (Lauraceae) leaves and seeds collected in Lebanon have been tested for their antiproliferative activity on human erythroleukemic K562 cells in relation to their chemical composition. The most abundant components that characterized the leaves essential oil were 1,8-cineole, 1-*p*-menthen-8-ethyl acetate, linalool and sabinene while the seeds oil was characterized by β-ocimene, 1,8-cineole, α-pinene and β-pinene as main components. Both leaves and seeds essential oils inhibited the proliferation of K562 tumor cell line with IC_{50} values of 95 and 75µg/ml, respectively. The leaves oil showed a percentage of erythroide differentiation of 15% at the concentration of 10µg/ml. A value of 12% was found for the seeds essential oil at the concentration of 50µg/ml. When the oils were added to suboptimal concentration of the commercial drug cytosine arabinoside, a clear synergic effect was observed.

In the present investigation we report the inhibitory activity against digestive enzymes related to diabetes and angiotensin converting enzyme (ACE) of extracts of nine plant species collected in Lebanon, where they have a traditional use against diabetes. We decided to perform different extractions with methanol, *n*-hexane and chloroform of *Calamintha origanifolia*, *Satureja thymbra*, *Prangos asperula*, *Sideritis perfoliata, Asperula glomerata*, *Hyssopus officinalis*, *Erythraea centaurium*, *Marrubium radiatum* and *Salvia acetabulosa* and test each of them.

Marrubium radiatum and *Salvia acetabulosa* methanol extracts exerted the highest activity against α-amylase (IC_{50} 61.1 and 91.2µg/ml, respectively) and α-glucosidase (IC_{50} 68.8 and 76.9µg/ml, respectively). The same extracts exhibited a strong inhibitory activity against ACE with IC_{50} of 72.7 and 52.7µg/ml, respectively.

The chemical composition of the essential oils of Laurus nobilis, Juniperus oxycedrus ssp. oxycedrus, Thuja orientalis, Cupressus sempervirens ssp. pyramidalis, Pistacia palaestina, Salvia officinalis, and Satureja thymbra was determined by GC/MS analysis. Essential oils have been evaluated for their inhibitory activity against SARS-CoV and HSV-1 replication in vitro by visually scoring of the virusinduced cytopathogenic effect post-infection. L. nobilis oil exerted an interesting activity against SARSCoV with an IC_{50} value of 120 mg/ml and a selectivity index (SI) of 4.16. This oil was characterized by the presence of β-ocimene, 1,8-cineole, α-pinene, and β-pinene as the main constituents. J. oxycedrus ssp. oxycedrus oil, in which α-pinene and β-myrcene were the major constituents, revealed antiviral activity against HSV-1 with an IC_{50} value of 200 mg/ml and a SI of 5.

In this study we investigate the in vitro antiviral activity of eight methanol medicinal plant extracts against herpes simplex virus (HSV-1) infection on

monkey kidney cell The inhibitory concentration (IC_{50}) were determined at 233µg/ml, 220µg/ml, 660µg/ml, 410µg/ml, 690µg/ml, 373µg/ml, 290µg/ml and 270µg/ml for the following eight medicinal plants extract of Calamintha origanifolia, Satureja thymbra, Prangos aspurela, Sidiritis Perfoliata, Aspurela glomerata, Erythreae Centaurium, Hyssopus officinalis and Salvia accetabulosa. Cytotoxicity was evaluated by MTT assay in vero cells. The selective index (SI) of these eight medicinal plant extract ranged from 1,45 to 4,55.

Keywords: Cedrus libani, Cedrus atlantica, Cedrus deodara, Laurus nobilis, medicinal plants, antiproliferative activity, antiviral activity and hypoglycaemic activity

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To the memory of my father

Who should have lived

to see my thesis

The original publication papers

This thesis is based on the following original publications referred by Roman numerals.

I. **Antoine M. Saab**, Fouzi Yousef Harb, Weilfred A. Koenig. Essential oils components in heart wood of *Cedrus Libani* and cedrus Atlantica from Lebanon.

Minerva biot., 2005 ;17;159-161.

II. Ilaria Lampronti, **Antoine M. Saab**, Roberto Gambari. Effects of essential oils from Pistacia palestina on Proliferation and erythroid differentiation of human leukemic k 562 cells

Minerva. Biot. 2005,17;153-8

III. Ilaria Lampronti, **Antoine M. Saab**, Roberto Gambari. Antiproliferative activity of essential oils derived from plants belonging to the Magnioliphyta division.

Int. J. of oncology. 11,2006. 29, 989-995.

IV. Monica Rosa Loizzo, **Antoine M Saab**, Giancarlo A. Statti and Francesco Menichini. phytochemical composition and in vitro

α-Amylase inhibitory effect of essential oils from Cedrus libani.

Fitoterapia 2007,78,323-326.

V. Monica Rosa Loizzo, **Antoine M Saab**, Rosa Tundis, Giancarlo A Sttati, Ilaria Lampronti, Francesco Menichini, Roberto Gambari, Jindrich Cinatl, Hans Wilhelm Doerr. Phytochemical analysis and in vitro evaluation of

the biological activity against Herpes simplex virus type-1 of Cedrus Libani essential oils .

Phytomedicine, 2008,15,79-83 .

VI. Monica R Loizzo, **Antoine M Saab**, Rosa Tundis, Giancarlo A Statti, Francesco Menichini, Ilaria Lampronti, Roberto Gambari, Jindrich Cinatl, Wilhelm Doerr. Phytochemical analysis and in vitro antiviral activities of essential oils of seven Lebanon species

Chemistry and biodiversity.2008,5(3), 461-470 .

VII. Monica Rosa Loizzo, **Antoine M Saab**, Rosa Tundis, Federica Menichini, Marco Bonesi[1], Vitaliano Piccolo, Giancarlo A Statti, and Bruno de Cindio, Peter J Houghton and Francesco Menichini. *In vitro* inhibitory activities of plants used in Lebanon traditional medicine against angiotensin converting enzyme (ACE) and digestive enzymes related to diabetes. J.Ethnopharmacology 2008, 119, 109-116.

VIII. **Antoine M. Saab**, Faouzi Harb and Weilfred A. Koenig..Essential oils components in the leaves of Cedrus libani and Cedrus deodara.Minerva biotecnologica December2009,21(4) 201-205.

IX. **Antoine M. Saab** , Monica R. Loizzo[b], Rosa Tundis, Ilaria Lampronti, Monica Borgatti[a] Roberto Gambari, Federica Menichini, Fadi Esseily and Francesco Menichini. Antioxidant and antiproliferative activity against K562 human chronic myelogenous leukemia cells of *Laurus nobilis* L. (Lauraceae) seeds and leaves essential oils.

Natural product research (submitted).

X. **Antoine M Saab**; Ilaria Lampronti;Roberto Gambari; Monica Borgatti; Jindrich Cinatl; and Hans Wilhelm Doerr. Evaluation of the biological

activity of Lebanese medicinal plants extracts against herpes simplex virus type 1 in vitro.(Chemistry & Biodiversity).

XI. **Antoine M Saab**, Ilaria Lampronti,Alessandro Grandini Gianni sacchetti, Monica Borgatti, Alessia Finotti.Roberto Gambari and Alessandra Gerrini.

Phytochemical analysis and anti proliferative activity of Cedrus libani seeds extracts against k562 human chronic myelogenus Leukemia cells.

Pharmaceutical biology (submitted).

XII. **Antoine M. Saab**, Ilaria Lampronti, Monica Borgatti, Roberto Gambari and Alessia Finotti. Evaluation of anti proliferative activity of three Cedrus species (Cupressaceae) essential oils against k562 human chronic myelogenus Leukemia cells.

Pharmaceutical biology (submitted).

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1 Introduction

1.1 Definition, distribution and morphology of Cedrus species.

Lebanese Cedar (*Cedrus libani* A. Rich.) is a species of cedar native to the mountains of the Mediterranean region that spontaneously grows in the coastal mountains of Lebanon (at altitudes of 1050-1925 m), in the Alaouite mountains in Syria (1200-1850 m) and in Toros mountains in Turkey (530-2000 m), with varieties of it in southwest Turkey, Cyprus and the Atlas Mountains in Algeria and Morocco. In Lebanon, *Cedrus libani* covers 2000 ha, in Syria it is widely scattered over 20,000 ha. Turkey has the largest area of *C. libani* with 99,000 ha, of which 31,000 ha are degraded stands values (Ducrey et al., 2008).

There are only four kinds of cedar, classified by their Morphological diversity, three of them naturally present in the Mediterranean Sea region.

Cedrus libani, in Lebanon, Syria and Turkey.

Cedrus atlantica in Algeria and Morocco.

Cedrus deodara in Himalaya Mountains.

Cedrus brevifolia, in Cyprus Island.

Cedrus libani takes various forms such as the symbolical and the woody forms. The Symbolical form is a perennial tree having a short and wide trunk, thick branches extended horizontally and making up a broad corona with a pyramidal shape, present in the Lebanese flag. We may find this form in Bcharri forest and it derives from the cut off of the final branch or its premature death. The woody form is present in the forests of Barouk Mountain and the color of leaves becomes bluish green. The lower lateral branches grow in a more active way

than the upper branches up to 20 m; for this reason, lower lateral branches normally are cut giving the typical “umbrella like” appearance. Leaves of conical form are longer and sharper than those of the symbolic form. Whereas the natural homeland of *Cedrus atlantica* is the mountains of Algeria and Morocco, somewhere about the year 1840 it was introduced during the forestation and tree planting operation in this area. It is a tree having a beautiful look particularly during its oldness. Many small Atlantic Cedar trees are usually easy to break and have thin ramified branches. Atlantic Cedar is distinguished from Lebanese Cedar by its light green leaves while the Lebanese Cedar leaves are dark green.

The Atlantic “Glauga” form is called so because the color of its leaves is bluish. It is planted on a large scale in Europe parks for the beauty of its color and its form. Furthermore, *Cedrus deodara* is native to the western Himalayas in eastern Afghanistan, northern Pakistan, north-central India, southwestern Tibet and western Nepal, at an altitude of 1500-3200 m. It is a large evergreen coniferous tree reaching 40 to 50 m, exceptionally up to 60 m, with a trunk up to 3 m in diameter. It has a conic crown with level branches and drooping branchlets. It is usually planted in public parks with moderate climate . It was also introduced in the Cedar tree planting and forestation operation in USA before 1831. It differs from Lebanese Cedar and Atlantic Cedar by the length of its leaves and trunk since the segment of its leaves is one and a half inch while the length of the Atlantic Cedar tree leaves is one inch or less.

Finally, *Cedrus brevifolia* tree is native to Cyprus. It is a rare cedar found locally and reaching a height of almost 40 m; the distinguishing features of this species are its short (1cm) needles borne on short shoots. The needles are dark green and the twigs tend to be glabrous. The female cones are big, round and borne upright on branches while the male cones are long (chancy et al, 1993).

Lebanon is rich of medicinal plants, despite its small size, it considers as a rich floristically country with an estimated 2600 plant species. More than hundred plants are endemics and reported to have biological activities.

1.2 Definition of k562 human myelogenus leukemia, induction of erythroid differentiation, Herpes Simplex Virus type 1(HSV-1), The sever acute syndrome (SARS).

Chronic myelogenous leukemia is listed as a "rare disease" by the Office of Rare Diseases (ORD) of the National Institutes of Health (NIH). This means that chronic myelogenous leukemia, or a subtype of this cancer, affects less than 200,000 people in the US population. Although the cause of chronic myelogenous leukemia is the translocation between chromosome 9/22 (c-abl) (Zhang et al., 1993) . Differentiation of k562 cells is associated with an increase of expression of embryo-fetal globin genes, such as ζ- ε and γ-globin genes (Bianchi et al., 2003). The disease doesn't seem to be associated with radiation exposure, carcinogenic chemicals or viruses. Approximately 2 out of every 100,000 people develop chronic myelogenous leukemia annually, with 90% of cases found in people who are older than age 50. Many cases go undetected by routine blood tests in people who are asymptomatic. The disease is common in Jewish people of Russian or Eastern European descent, and is uncommon in Asia. Systemic chemotherapy includes alkylating agents usually chlorambucil, cyclophosphamide, vincristine, or fludarabine (singly or in combination), and steroids (prednisone) when autoimmune hemolytic anemia or thrombocytopenia occurs (Rathore and Elfenbein, 2003). Moreover, Gleevec (Imatinib) drug has led to an overall survival rate of 95% for Chronic myelogenus Leukemia (CML) patient who took the tyrosine kinase inhibitor daily for 60 months (Chen et al., 2009).

Herpes simplex virus type 1 (HSV-1) is a common human pathogen that causes localized skin infections of the mucosal epithelia of the oral cavity, the pharynx,

the oesophagus, and the eyes. The virus may establish an acute primary infection, followed by the development of a latent, lifelong infection (Coen et al., 1996) . Presently, the only aspect of the HSV life-cycle for which antiviral therapy has been successfully developed is the process of DNA replication, which is targeted by a small group of nucleoside analogues that include acyclovir (ACV), valaciclovir, and famciclovir. However, ACV-resistant strains of HSV and drug toxicity have been recently reported (Ernst et a., 1998).

The **severe acute respiratory syndrome** (SARS) is a febrile respiratory illness primarily transmitted by respiratory droplets or close personal contact. The causative organism has been identified as a novel coronavirus, i.e., SARS-CoV (peiris et al., 2004). The verriding clinical feature of SARS is the rapidity with which many patients develop symptoms of acute respiratory distress syndrome (ARDS). Currently, there are no approved or universally recommended therapies for SARS. Treatment for the disease is mainly supportive.

1.3 Uses in traditional medicines and rapport activities of Cedrus species and medicinal plants.

Essential oils from the Cedrus species are an important natural source of crude materials for the production of fragrance compounds. For this reason the chemical composition of the essential oils derived from different cedar species has been subject of different studies in recent years. Terpenoid (isoprenoids) are a subclass of the phenyllipids (terpenes, phenylquinones and sterols) that represent the oldest group of small molecules synthesized by plants as secondary metabolites excreted in the external environment, influencing its quality and characteristic. Numerous types of biological activities have been attributed to *Cedrus* species. The liquid tar obtained by destructive distillation of the *Cedrus atlantica* is considered the best oil of the cade and its recognition in the French codex was recommended. The *Cedrus* genus in Turkey is represented by one species only (*Cedrus libani*): there, a kind of tar is obtained

from its resinous roots and steam wood. This tar is usually employed for treating skin complaints of animals and for killing parasites, e.g. aphids and insects (Metin et al,. 1999). It was recently described that cones and leaves of *Cedrus libani* possess antimicrobial activity. Furthermore, the cones of *Cedrus libani* possess anti-ulcerogenic remedies, showing an anti-*Helicobacter pylori* activity (yesilada et al,. 1999).The antimicrobial activity of the ethanol extract of resins obtained from roots and stems of *Cedrus libani* was also investigated (kizil et al, 2002). Published data demonstrate that crude extract of resins of *Cedrus libani* are highly effective against micro-organism by preventing their growth to a greater extent. Essential oils and ethanol extracts derived from cones, leaves and wood of *Cedrus libani*, that posses antiviral activity against Herpes Simples Virus type-I were also investigated (Loizzo et al.,2008). Moreover, research showed that essential oils and same ethanol extracts derived from cones and wood of *Cedrus libani* possess anti-diabetic activity (loizzo et al, 2007). Regarding the biological activity of *Cedrus deodara*, an isolate ”CD lignan mixture”, comprising lignans from stem wood and consisted of (-)-wikstromal (75-79%), (-)-matairesinol (9-13%) and benzylbutyrolactol (7-11%), showed cytotoxic and apoptotic activity against human cancer cell lines: it possess the ability to induce tumor regression in vitro and in vivo (shingh et al.,2007). In addition, the volatile fraction of *Cedrus deodara* possess anti-inflammatory (shinde et al., 1999) and antioxidant activities (Tiway et al., 2001). three compounds with potent free radical scavenging activity were isolated in significant yields and identified: (-)-matairesinol, (-)-nortrachelogenin and Dibenzylbutyrolactollignan Both of Laurus nobilis essential oils (Leaves and seeds) are used for food, cosmetic and medical purpose. In Iranian traditional medicine the leaves had been used for reduce rheumatic pains. Moreover, *L. nobilis* essential oils were used for preparation of hair lotion for its antidandruff activity and for external treatment of psoriasis (Hafizoğlu and Reunanen, 1993). It was proved that *L. nobilis* ethanol extract as

well as fraction of oils seeds possess antiulcerogenic activity (Afifi et al., 1997). Previously, (Sayyah et al. 2003) demonstrated that the essential oils obtained from the *L. nobilis* leaves posses antinociceptive and anti-inflammatory activities *in vivo*. Moreover, *L. nobilis* seed oil exerted antiproliferative activity against C32 and ACHN with IC_{50} values of 75.45 and 78.24 µg/ml, respectively (Loizzo et al., 2007).

Laurel essential oil was reported to be used in the preparation of hair lotion for its antidandruff activity and for the external treatment of psoriasis (Hafizolu et al., 1993).Thuja oriontalis L. tree was used in various herbal remedies and aromatherapy preparation. Intraditional Chinese medicine, the leaves and stems of T. orientalis are used to treatnervous disorders, insomnia, and heart palpitations, as well as to stop hemorrhages andreduce fever. Recently, the polysaccharide fraction isolated from T. occidentalis was reported to demonstrate an anti-human immunodeficiency virus (HIV) activity (Hassan et al., 1996). Juniperus oxycedrus L. ssp. oxycedrus was used in folk medicine for the treatment ofvarious infection diseases (Karaman et al., 2003). Salvia officinalis L. is a medicinal plant well-known for its reputation of being a panacea. The inhibitory activity against HSV-1, HSV-2 of an aqueous extract of S. officinalis was recently reported (Nolkemper et al., 2006). The most common Satureja specimen is S. thymbra L., which is known as an herbal home remedy, due to its antimicrobial, gastrosedative, and diureticproperties (Chorianopoulus et al., 2004). *Pistacia palaestina Boiss.* and *Cupressus sempervirens ssp. Pyramidalis L*. essential oils have not been investigated so far for their chemical composition and biological activity.

The interest in medicinal plants and their biologically active derivatives has increased in recent years, in relation to the possible development of novel potential drugs for several pathologies of relevant social impact (Hedberg et al., 1993; Heinrich et al., 2001). In fact, it is well known that medicinal plants are

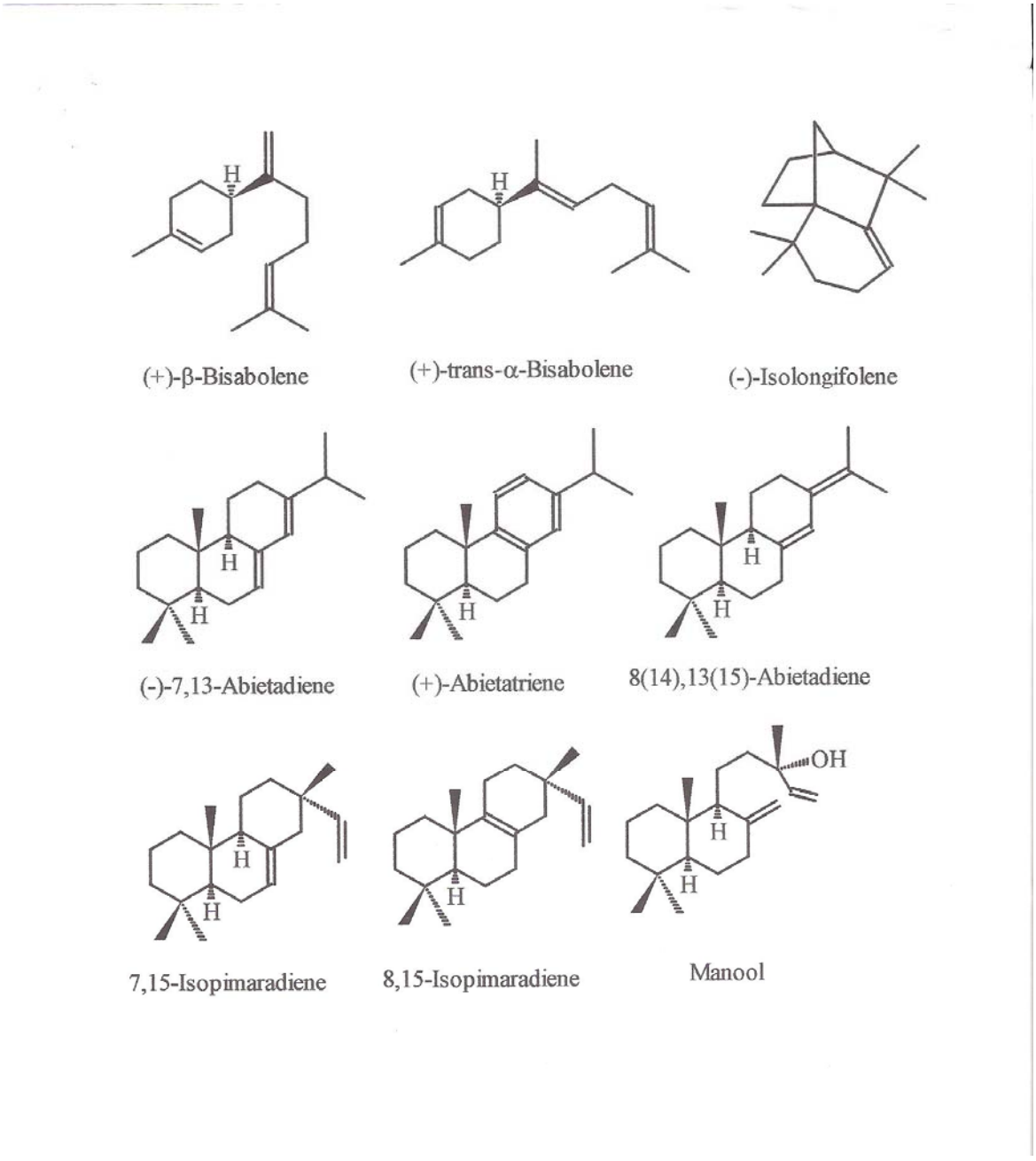
described and used in prenatal care, in obstetrics, in gynaecology, in respiratory disorders, in skin disorders, in cardiac diseases, in nervous and muscular disorders and in mental health (Ahmad et al., 1998; Pinn et al., 2001). With respect to potential anti-tumor activity, possible applications of medicines for cancer prevention have been recently described (Chang et al., 2002), (Ruffa et all., 2002). *Aegle marmelos* and *Emblica officinalis*, both medicinal plants derived from Bangladesh and studied by our research group, demonstrated remarkable antiproliferative activity on different human tumor cell lines (Jose et al., 2001). In order to further identify plants exhibiting antiproliferative activity, we analyzed a variety of officinal plants from Lebanon and belonging to the Magnoliophyta division, including *Satureja hortensis, Satureja montana, Salvia officinalis, Lavandula officinalis, Thymus vulgaris, Calamintha origanifolia, Foeniculum vulgare* and *Mentha arvensis*. From the Mediterranean area, several medicinal plants from Lebanon have been described (Bruno et al.,2002).. For instance, (Barbour et al., 2004*)* tested the *in vitro* antimicrobial efficacy of extracts derived from different parts of 27 indigenous wild plant species that have been commonly used in Lebanese folk medicine.

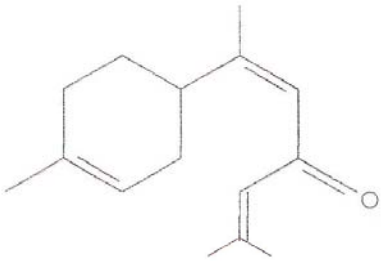
However, despite these interesting findings, few biological data are available for most medicinal plants from Lebanon. All obtained essentials oils and related available pure compounds were analyzed for their antiproliferative activity on human K562 cells. Also, their ability to induce erythroid differentiation was determined.

Calamintha origanifolia Vis is grown on the slope of Sanin Mountain, at the altitude of 2000 m above the sea in Lebanon, and belongs to Lamiaceae family. It is rich in essential oils and extract and also reduces blood pressure, possesses anti-microbial activity and anti-proliferative activity on human erythroleukemic k562 cells (lampronti et al., 2006). *Saturea thymbra L* is grown in several regions in Lebanon. It belongs to lamiaceae family. Known to be rich in

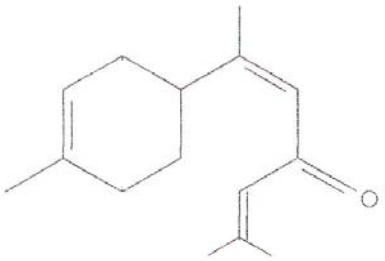
essential oils and extract, reduces blood pressure and pain in joints and possesses anti-microbial activity. *Prangos aspurela boiss* is quite an interesting medicinal plant, for agriculture and pharmaceutical institutes, due to their higher oils yield production and the valuable use of traditional medicine. It grows in several slopes of "Mont-Liban", and belongs to the Apiaceae family. It reduces blood pressure, cures skin disease, digestive disorder and hemorrhoids. Moreover, essential oils of *p. asperula* possesses anti proliferative activity in human renal adenocarcinoma and amelanotic melanoma cells. *Sideritis perfoliata L*. is grown in several sites in Lebanon, especially near 'Kfrakaab town', Mont-Liban. It belongs to the Lamiaceae family. It is rich in essential oils, reduces blood pressure, and pain in joints. In addition, it possess anti inflammatory and anti microbial activities, decongestant of the respiratory tract and astringent property. *Asperula glomerata (M. Bieb) Griseb* is grown on the highest slopes of 'Sanin' mountain at the altitude of 2000m above the sea, belongs to the Rubiaceae family. It is also recognized for its reduction in blood pressure, inflammation and edema. *Hyssopus officinalis L* is grown in several sites of Lebanon and belongs to the Labiateae family. It is rich in essential oils and extract. Known to reduce blood sugar, blood pressure, spasm in the smooth muscle of the gastrointestinal tract and possess anti microbial activity. *Centaurium erythreae L* is quite an interesting plant for inflammatory therapeutic use in folk medicine. Found near "Kfarkaab" town in Mont-Liban. It belongs to the Gentianaceae family. It reduces spasm in the gastrointestinal tract, fever, inflammation and edema. Also cures digestive and stomach disorder. *Salvia acetabulosa L,* is an aromatic plant, grown in several sites in Lebanon and especially in "Dair Al Ahmar" village. It belongs to Lamiaceae family. It reduces blood sugar, blood pressure. Possess antimicrobial, eupeptic, antihydrotic effects and also known to reduce pain in joints, anxiety and depression (Loizo et al.,2008).

1.4 Molecular structures belonging to Cedrus species and medicinal plants.

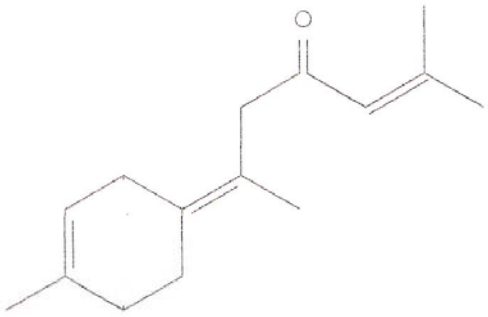




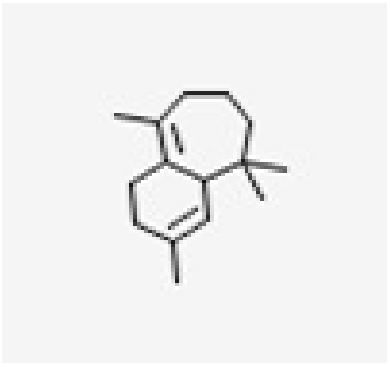
E - α – atlantone



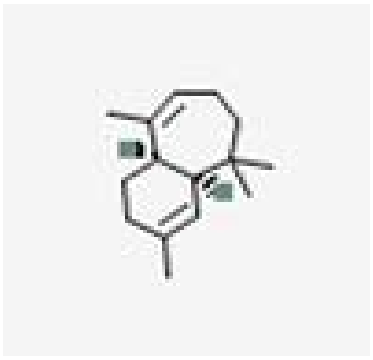
Z – α – atlantone



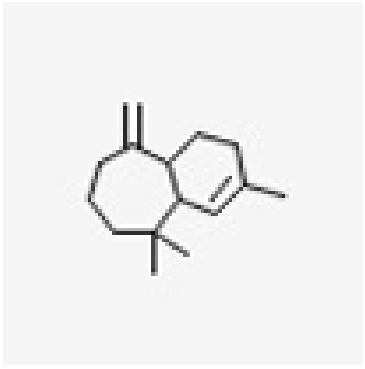
Z – γ - atlantone



γ- Himachalene



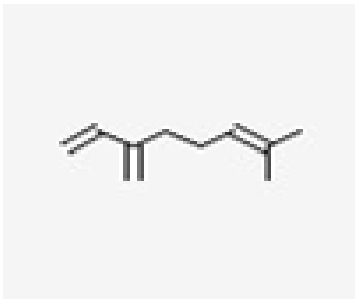
β-Hymachalene



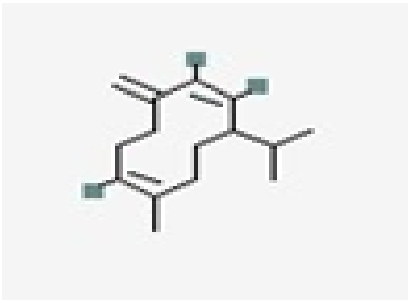
α- Hymachalene

α-Pinene

β- Pinene

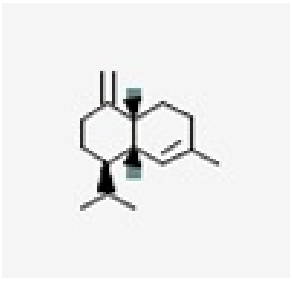


Myrcene

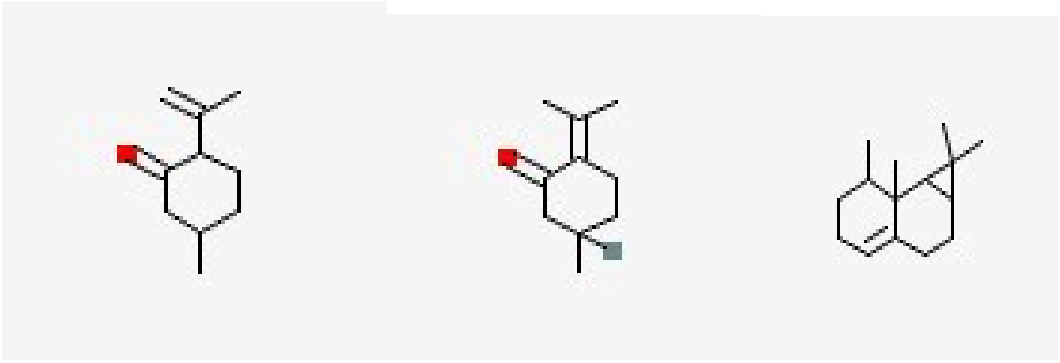


Germacrene D

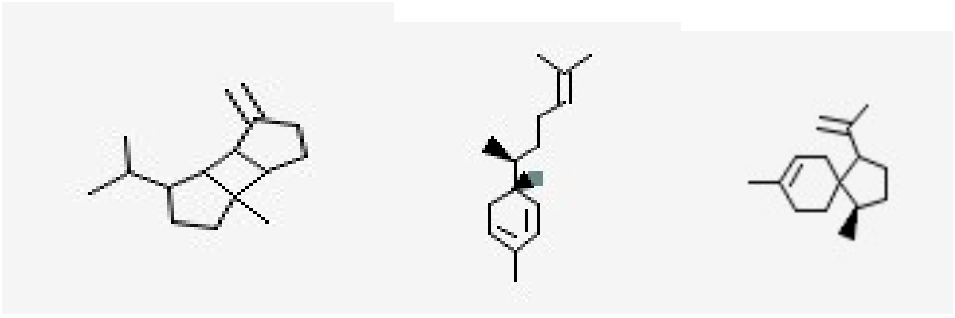
Csriophylene



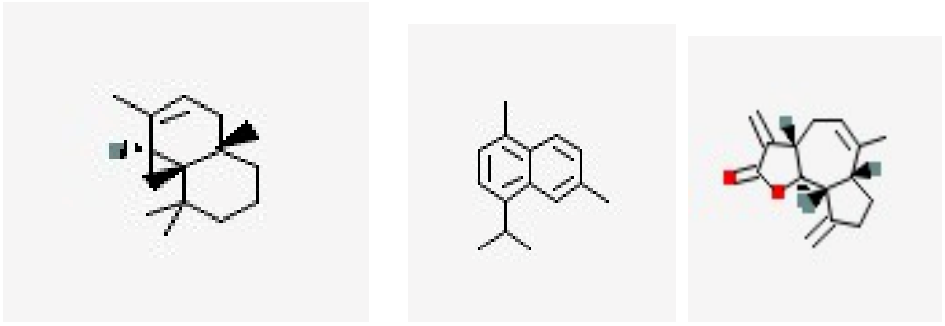
γ- Muurolene



Isopulegone **Pulegone** **Calarene**



β-Bourbonene **Zingiberene** **β-acoradiene**



Widdrene **Cadalene** **Eremanthin**

2 Part-A : Cedrus species

2.1 Phytochemical analysis of heart wood of cedrus libani and cedrus Atlantica

2.1.1 Material and Methods. Plant material. Essential oils extraction . Analysis conditions.

Material and Methods

Collection of plant material

Wood of *Cedrus libani* and *atlantica* were collected on Hadath eljubi-Tanourine reserve, in Lebanon at the altitude of 1800 m. The plant material is aromatic and the derived essential oils might be prepared by hydro-distillation.

Preparation of essential oils

The collected *Cedrus* species were authenticated according to the conventional protocol. Starting from 200 g of fresh wood from each species, all the material was submitted to hydro-distillation for two hours in two liters using a Clevenger type apparatus (Clevenger 1928). The essential oils were dried over anhydrous sodium sulphate to remove trace of moisture and to give yield 0.75 % w/w for wood (stored at 4-8°C).

Gas-Chromatography/Mass Spectrometry (GC/MS) analysis of the essential oils from Lebanon

The essential oils of *Cedrus libani* and *C.atlantica* were analyzed by Electron impact (70 eV) GC/MS system, utilizing Hewlett–Packard HP 5890 gas chromatograph (25 m fused silica capillary with polydimethylsiloxane CPSil-5) coupled to a VG Analytical 70-250S mass spectrometer (ion source temp. 250 °C). Mass spectra and retention indices were compared with libraries of data derived from authentic compounds, established under identical

experimental conditions (Lu et al.,2003).(table 1)

Table 1: List of identified compounds following GC/MS of wood from Cedrus species

Compound	**R.T.**	**%*Cedrus Libani*(A)**	**% *Cedrus Libani*(B)***	**% Cedrus Atlantica(A)**	**%Cedrus Atlantica(A)***
Isolongifolene	30.23	0.48	-----	0.33	----
α-Himachalene	31.50	7.12	10.50	7.03	6.60
γ-Himachalene	32.75	11.90	21.90	13.95	15.80
β-Himachalene	33.77	7	9.10	7.03	9.60
α-Himachalene epoxide	36.39	0.10	----	0.86	----
Longiborneol	39.05	0.80	----	1.40	-----
Himachalol	39.42	43.11	22.50	46.32	42.20
Allohimachallol	39.93	3.92	3.20	4.97	4.70
(z)-γ-Atlantone	40.91	----	1.72	-----	0.6
(E)-γ-Atlantone	41.41	----	1.73	----	0.9
(Z)-α-Atlantone	41.83	----	2.10	----	----
(E)-α-Atlantone	44.16	0.89	8.20	1.47	2.30
Manool	54.44	****	1.20	****	1.70

***Non identified. ----- 0 %

2.2 Phytochemical analysis of wood, leaves and cones of Cedrus Libani.

2.2.1 Material and methods. Plant material. Essential oils and extracts. Gc-MS. Analysis condition.

Material and methods

Plant material

Cones, leaves and wood of C. libani A. Rich. Were collected on Hadath eljubi-Tanourine Reserve (Lebanon), in November 2003. Voucher specimens wereauthenticated botanically by Prof. S. Safi, BiologyDepartment, Faculty of Sciences II, Lebanese University and deposited in the Chemistry Department Herbarium, Faculty of Sciences II, Lebanese University.

Essential oil and extracts

Fresh parts of plant were air dried for 7 days at room temperature. A total of 200 g of wood was submitted to hydrodistillation for 3 h respectively using a Clevenger-type apparatus (Clevenger, 1928). The essential oil was dried over anhydrous sodium sulfate to remove trace of moisture (2% w/w). The dreid leaves (100g) and dreid cone (100g) were finely crumbed into small paricules and soaked in 95% ethanol for two weeks (3.500ml) and concentrated under reduced pressure to give a yield of 5 % (w/w) and 4% (w/w), repectively.

Gas-Chromatography/Mass Spectrometry (GC/MS) analysis of the essential oils from Lebanon.

The essential oils of *Cedrus libani*, wood, leaves and cone were analyzed by Electron impact (70 eV) GC/MS system, utilizing Hewlett–Packard HP 5890 gas chromatograph (25 m fused silica capillary with polydimethylsiloxane CPSil-5) coupled to a VG Analytical 70-250S mass spectrometer (ion source

temp. 250 °C). Mass spectra and retention indices were compared with libraries of data derived from authentic compounds, established under identical experimental conditions (Lu et al.,2003).(table2)

Table 2: Compounds present within Cedrus libani wood oil,leaves, and cones identified by GC/MS

Compounds	RT	Leaves%	Cones%	Wood%
α-pinene	7.79	2.20	51	------
Camphene	8.16	------	2.15	------
β-pinene	9.33	0.54	------	------
Myrcene	9.97	0.73	13	------
β-phellandrene	10.34	------	0.67	------
Δ-3-carene	10.74	------	0.72	------
α-terpinene	10.94	------	0.55	------
ρ-cimene	11.07	------	0.68	------
α-limonene	11.41	0.44	2.25	------
γ-terpinene	12.32	trace	0.61	------
Terpinolene	12.82	trace	3.10	------
α-terpineol	14.23	------	0.74	------
trans-pinocarveol	14.40	trace	------	------
Cis-verbenol	15.60	trace	------	------
α-terpineol	18.72	------	0.35	------
Terpinen-4-ol	22.13	trace	------	------
Bormyacetate	26.72	0.26	0.35	------
α-Cubebene	27.37	0.46	------	------
α-Ylangene	28.34	0.26	------	------
α-Copaene	28.55	0.73	------	------

β-Bourbounene	28.87	0.79	------	------
Longifolene	30.23	------	0.15	------
β-Caryophylene	30.37	5.6	-----	------
β-Copaene	30.81	1.01	-----	------
E-β-Fernesene	30.93	------	0.58	------
α-Himachalene	31.50	1.74	------	10.50
4β-10β-1(5),6Guiadiene	31.63	0.56	-----	------
α-Humulene	31.72	1.30	------	------
Muurola-4(5),5-diene	32.13	0.26	------	------
γ-Himachalene	32.75	-------	------	9.10
γ-Muurolene	32.85	4.83	-----	------
Germacrene D	33.02	29.40	-----	------
γ-Amorphene	33.39	0.53	------	------
α-Muurolene	33.54	1.17	------	------
β-Himachalene	33.77	0.29	-----	21.90
γ-Cadinene	34.02	2.87	------	-----
δ-Cadinene	34.37	5.60	-------	------
Cadina-1,4-diene	34.79	0.44	-------	-------
Trans-α-Bisabolene	35.69	5.90	1.42	-------
γ—Dehydro-ar-himachalene	36.39	-----	------	0.40
Dodecanoic acid	36.91	1.72	------	-------
4(14) Salvialene -1-one	37.21	4.00	-----	-----
ar-hymachalene	38.56	-----	-----	Trace
Longiborneol	39.05	------	-----	0.80
Himachalol	39.42	------	------	22.50
Allohimachalol	39.93	------	-------	3.20

1-epi-Cubenol	40.51	6.30	-----	------
z-γ-Atlantone	40.91	-----	-----	1.72
E-γ-Atlantone	41.41	-----	-----	1.73
z-α-Atlantone	41.83	-----	-----	2.10
E-α-Atlantone	44.16	-----	-----	0,82
Trans-Cadinol	44.50	3.40	-----	------
7,15- Isopimaradiene	53.28	trace	-----	-----
8,15- Isopimaradiene	53.63	trace	-----	-----
Manool	54.44	trace	----	1.70
Abietatriene	54.60	-----	1.00	-----
7,13-Abietadiene	55.46	------	3.20	-----
8(14),13(15)-Abietadiene	58.81	-----	0.27	------

2.3 Phytochemical analysis of Cedrus libani leaves and Cedrus deodara leaves.

2.3.1 Material and methods. plant material, Essential oils extraction. Gc-MS. Analysis conditions.

Material and Methods

Collection of plant material

Leaves of *Cedrus Libani* and *Deodara* were collected on Hadath eljubi-Tanourine reserve, in Lebanon at the altitude of 1800 m. The plant material is aromatic and the derived essential oils might be prepared by hydro-distillation.

Preparation of essential oils

The collected *Cedrus* species were authenticated according to the conventional protocol. Starting from 200 g of fresh leaves from each species, all the material was submitted to hydro-distillation for two hours in two liters using a Clevenger type apparatus (Clevenger 1928). The essential oils were dried over anhydrous sodium sulphate to remove trace of moisture and to give yield 2% w/w for leaves (stored at 4-8°C).

Gas-Chromatography/Mass Spectrometry (GC/MS) analysis of the essential oils from Lebanon

The essential oils of *Cedrus libani* and *C. deodara* were analyzed by Electron impact (70 eV) GC/MS system, utilizing Hewlett–Packard HP 5890 gas chromatograph (25 m fused silica capillary with polydimethylsiloxane CPSil-5) coupled to a VG Analytical 70-250S mass spectrometer (ion source temp. 250 °C). Mass spectra and retention indices were compared with libraries of data derived from authentic compounds, established under identical experimental conditions (Lu et al., 2003). (table3)

Table 3: List of identified compounds following GC/MS of leaves from Cedrus species

Compound	**R.T.**	**%*Cedrus Libani*(A)**	**% *Cedrus Libani*(B)***	**% *Cedrus Deodara***
Benzaldehyde	7.71	--	--	19.40
α-Pinene	7.79	2.20	0.90	5.65
β-Pinene	9.33	0.54	0,40	5.85
Myrcene	9.97	0.73	0.60	10.70
δ-3-Carene	10.20	--	--	Trace
α-Terpine	10.70	--	--	Trace
ρ-Cimene	11.20	--	--	Trace
Limonene	11.62	0.44	0.40	2.70
Terpinolene	12.82	Trace	--	0.85
Benzoic acid	14.10	--	--	Trace
Trans-Pinocarveol	14.40	Trace	--	--
Cis-Verbenol	15.50	Trace	--	--
Terpineol	18.80	0.71	--	--
Terpinen-4-ol	22.13	Trace	--	--
2-undecanone	26.50	___	--	0.28
Bornylacetate	26.72	0.26	0.30	--
α-Cubebene	27.36	0.46	0.50	--
α-Ylangene	28.34	0.26	--	--
α-Longipinene	28.40	--	Trace	___
α-Copaene	28.55	0.73	1.20	0.70
β-Bourbounene	28.87	0.79	0.70	0.50
Longifolene	29.66	--	--	Trace
α-Cedrene	29.97	--	--	0.60

β-Caryophylene	30.37	5.60	8.30	10.90
β-Copaene	30.81	1.01	1.20	0.20
4β-10β-1(5),6-Guaiadiene	31.56	0.56	--	--
α-Himachalene	31.63	1.74	5.40	1.40
β-Humulene	31.98	1.30	1.80	1.90
Muurola-4(15),5-diene	32.50	0.26	--	--
γ-Muurolene	32.85	4.83	6.50	1.75
Germacrene D	33.02	29.40	26.60	9.35
γ-Amprhene	33.39	0.53	1.10	0.45
α-Muurolene	33.54	1.17	1.10	0.39
β-Himachalene	34.02	0.29	5.40	1.90
γ-Cadinene	34.37	2.87	2.10	0.38
δ-Cadinene	34..79	5.60	5.80	1.90
Cadina-1,4-diene	35.69	0.44	0.50	--
Trans-α-Bisabolene	35.69	5.90	3.90	--
Spathulenol	36.50	--	2.20	--
Dodecanoic acid	36.91	1.72	--	--
4(14) Salvialen-1-one	37.21	4.00	0.50	--
1-epi-Cubenol	40.51	6.30	0.50	Trace
T-Muurolol	40.60	--	1.50	--
α-Cadinol	40.75	--	1.60	--
1(10),4 cadinadien-8-α-ol	40.85	--	1.70	--
T-Cadinol	44.59	3.40	--	--
7-15-isopimaradiene	53.50	trace	--	--
8-15-isopimaradiene	53.63	trace	--	--
Manool	53.84	trace	--	--

This work is presented in the memory of Prof. Wilfried A. Koenig (University of Hamburg).

2.4 Phytochemical analysis of Cedrus libani seeds extracts.

2.4.1 Material and methods, plant material, Gc-MS, Analysis conditions.

Experimental

Plant material and chemicals

C. libani seeds were collected from Tanourine reserve (Lebanon) in November 2009 at the altitude 1700 m above the sea. Voucher specimen was authenticated botanically by Prof. S. Safi, Biology Department, Faculty of Sciences II and deposited in the Chemistry Department Herbarium, Faculty of Sciences II of Lebanese University.

Chemicals and reagents used for the study of the antiproliferative activities were purchased from Sigma-Aldrich Co. while other chemicals, solvents and reagents were purchased from VWR (Milan, Italy). The fetal bovine serum was obtained from CELBIO (Milano, Italy).

Preparation of plant extracts

The dried and ground sample (0.9 g) of *C. libani* seeds was extracted with 15 ml of chloroform by maceration using vortexing for 30 seconds and sonication for 20 minutes. The material was centrifuged at 3000 rpm for 20 minutes; the supernatant was transferred into a 50 ml round bottom flask, then taken to dryness with a rotary vacuum evaporator. The extraction was performed three times (choi et al, 2004). In order to obtained extracts with different polarity, the dried and ground sample (0.9 g) of *C. libani* seeds was also extracted with 15 ml of ethanol by maceration using the same procedure described above. The

extraction was performed three times. The yield (w/w) was 13.8±1.2% for chloroform and 35.8±2.8% for ethanol *C. libani* seeds extract respectively.

Gas-chromatography/mass spectrometry (GC/MS)

The chloroform and ethanol extracts were analyzed by a Varian GC-3800 gas chromatograph equipped with a Varian MS-4000 mass spectrometer using electron impact and hooked to NIST library. A Varian FactorFour VF-5ms poly-5% phenyl-95%-dimethyl-siloxane bonded phase column (i.d., 0.25 mm; length, 30 m; film thickness, 0.15 μm) was used.

Operating conditions were as follows: injector temperature 300°C; FID temperature 300°C, Carrier (Helium) flow rate 1 ml/min and split ratio 1:50. Oven temperature was initially 55°C and then raised to 100°C at a rate of 1°C/min, then raised to 250°C at a rate of 5°C/min and finally held at that temperature for 15 minutes. One μl of each sample dissolved in CH2Cl2 as injected. The MS conditions were as follows: ionization voltage, 70 eV; emission current, 10 μAmp; scan rate, 1 scan/s; mass range, 29-400 Da; trap temperature, 150°C, transfer line temperature, 300°C.

The constituents of the volatile oils were identified by comparing their relative ritention time, KI and the MS fragmentation pattern with those of other essential oils of known composition, with pure compounds and by matching the MS fragmentation patterns with the above mentioned mass spectra libraries and with those in the literature (Adam, 2001). In order to determine the Kovats index of the components, a mixture of alkenes (C8–C24) was added to the essential oil before injecting in the GC–MS equipment and analyzed under the same conditions as above.(Table 4).

Table 4: Cedrus Libani seeds extract chemical profile (%)

		***Cedrus libani* seeds**		**ID method[a]**
Constituents	**t_R (min)[b]**	**Chloroform extract (%)[c]**	**Ethanol extract (%)[c]**	
α-pinene	8,347	34.42±1.22	-	GC-MS, Co-GC
camphene	9,204	0.38±0.02	-	GC-MS
thuja-2,4(10)-diene	9,678	-	0.54±0.03	GC-MS
β-pinene	10,784	33.28±1.08	-	GC-MS, Co-GC
Myrcene	11,548	1.10±0.09	-	GC-MS
p-cymene	14,036	0.48±0.04	-	GC-MS
limonene	14,325	1.18±0.07	-	GC-MS, Co-GC
β-phellandrene	14,453	1.98±0.11	-	GC-MS
cis-limonene oxide	21,460	0.46±0.03	-	GC-MS
6-camphenol	23,297	0.48±0.02	.	GC-MS
trans-pinocarveol	24,524	2.82±0.15	0.71±0.04	GC-MS
verbenol	25,229	3.16±0.89	-	GC-MS
pinocarvone	26,886	0.53±0.04	-	GC-MS
Borneol	27,856	0.34±0.02	-	GC-MS, Co-GC
4-terpineol	28,884	0.37±0.01	-	GC-MS, Co-GC
p-cymen-8-ol	29,920	0.43±0.03	-	GC-MS
myrtenal	30,560	0.89±0.07	0.29±0.01	GC-MS
myrtenol	30,739	1.99±0.12	-	GC-MS
verbenone	31,973	1.15±0.06	0.46±0.02	GC-MS
(E)-carveol	33,555	0.54±0.04	-	GC-MS
bornyl acetate	40,928	0.75±0.06	0.34±0.02	GC-MS, Co-GC
β-farnesene	53,547	1.89±0.15	0.41±0.02	GC-MS, Co-GC
α-bisabolene	56,703	1.16±0.09	0.57±0.03	GC-MS
methyl palmitate	65,146	-	0.46±0.01	GC-MS
palmitic acid	66,990	-	0.53±0.03	GC-MS, Co-GC
Manoyl oxide	67,566	-	0.35±0.01	GC-MS
abietatriene	71,234	1.50±0.12	2.05±0.13	GC-MS

methyl linoleate	71,997	-	1.43±0.12	GC-MS
abietadiene	72,223	2.58±0.22	7.99±0.46	GC-MS
methyl oleate	72,445	-	7.77±0.42	GC-MS
methyl stearate	72,898	-	0.54±0.04	GC-MS
oleic acid	73.231	-	17.26±1.34	GC-MS, Co-GC
abieta-8(14),13(15)-diene	73.456	-	7.92±0.66	GC-MS
ethyl oleate	73.682	-	5.27±0.48	GC-MS
dehydro abietal	75.560	-	1.07±0.09	GC-MS
Abietal	76.244	-	2.75±0.21	GC-MS
Abietol	77.980	-	6.04±0.55	GC-MS
neo-abietol	78.554	-	11.81±0.98	GC-MS
total peak identified		**93.85**	**77.03**	

[a]*GC-MS: gas-chromatography-mass spectrum, Co-GC:co injection with autenthic compound*

[b]*Retention time*

[c]*relative peak area identified by GC-FID*

2.5 Anti proliferative activity of cedrus libani seeds extracts against K 562 human chronic leukemia cell line.

2.5.1 Cell line, culture condition and in vitro antiproliferative activity assay. In vitro induction of erythroid differentiation.

Cell lines, culture conditions and in vitro antiproliferative activity assays

Human chronic myelogenous leukemia K562 cells (Lampronti *et al.*, 2003a) were cultured in a humidified atmosphere at 5% CO_2 in RPMI-1640 (Flow laboratories, Irvine, UK) supplemented with 10% fetal bovine serum. 100 units/ml penicillin and 100 mg ml^{-1}streptomycin. The *in vitro* antiproliferative activity of *C. libani* seed extracts were assayed as follows. Cell number ml^{-1}

was determined by using a model ZBI coulter Counter (coulter Electronics, Hialeah, FL). Cells were plated at an initial concentration of 3×10^4 cells ml^{-1} and the cell number ml^{-1} was determined after 4 days, when untreated cells are in the log phase of log phase of cell growth.

In vitro induction of erythroid differentiation

Erythroid differentiation of *C. Libani seeds* extracts was evaluated by counting benzidine positives cells after suspending the cells in a solution containing 0.2% benzidine in 0.5 M glacial acetic acid, 10% H_2O_2, as elsewhere describe (Bianchi *et al.*, 2001; Gambari, 2003). Induction of differentiation was compared with that obtained using well established inducers of differentiation of K562 cells, such us cytosine arabinose, mitramicine, angelicin, hydroxyurea and butyric acid (Hoffman *et al.*, 1979; Cortesi *et al.*, 1998; Lampronti *et al.*, 2003b; Iyamu et al., 2003). (table 5).

Statistical analysis

All experiments were carried out in triplicate. Data were expressed as means ± S.D. Differences were evaluated by one-way analysis of variance (ANOVA) test completed by Dunnett's test. Differences were considered significant at $^{**}p < 0.01$. The 50% inhibitory concentration (IC_{50}) was calculated by nonlinear regression curve with the use of Prism Graphpad Prism version 4.0 for Windows [GraphPad Software, San Diego, CA, USA]

Table 5: Effect of Cedrus Libani seeds extracts on K562 cell growth and differentiation (% of benzidine-positive cells after five days of culture at the indicated concentrations).

Seeds Extracts	IC_{50}	Differentiation max %
Ethanol seed	40,57±1,16 μg ml^{-1}	16,00±1,52 (10 μg ml^{-1})
Chlorofom seeds	69,20±1,69 μg ml^{-1}	12,00±1,25 (50 μg ml^{-1})
AraC	250,0±0,2 nM	92,00±2,70 (0.5 μM)

IC_{50} values are mean (n = 3). Differences within and between groups were evaluated by one way analysis of variance test *** $P < 0.0001$ (F=187.6 r^2= 0.98) followed by a multicomparison Dunnett's test: **$P < 0.01$ (reference to???) compared with the positive control Ara C

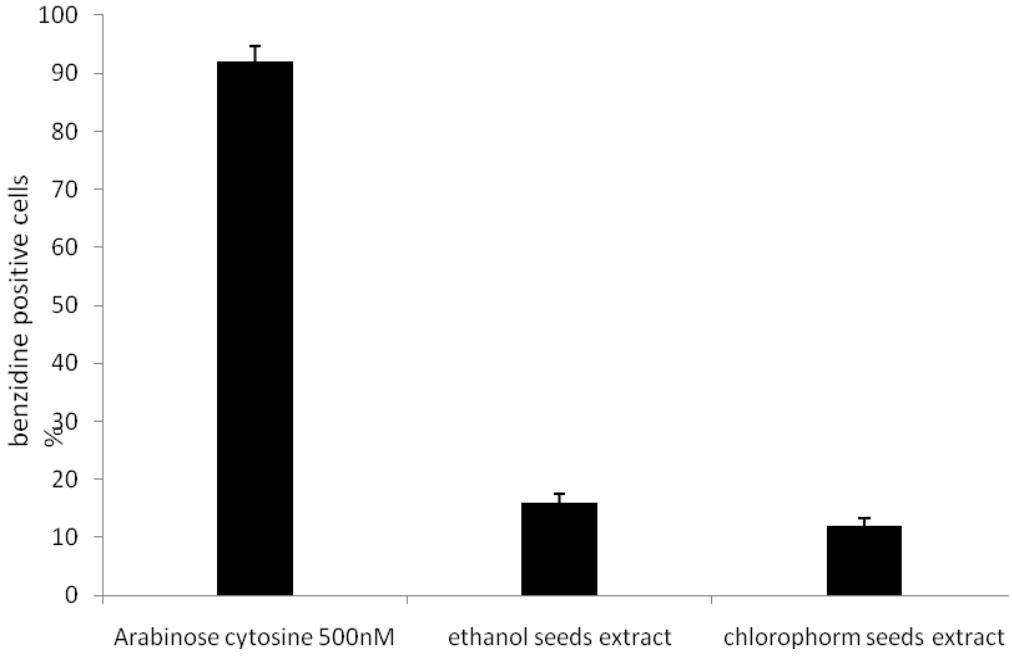


Figure 1: Concentration-dependent effect of C.Libani extracts after 5 days culture.

2.6 Evaluation of anti proliferative activities of essential oils Wood of cedrus species against k 562 human chronic Leukemia cell.

2.6.1 cell line, culture condition and in Vitro antiproliferative activity assay. In vitro induction of Erythroid differentiation.

Cell lines, culture conditions and in vitro antiproliferative activity assays.

Human chronic myelogenous leukemia K562 cells (Lampronti *et al.*, 2003a) were cultured in a humidified atmosphere at 5% CO_2 in RPMI-1640 (Flow laboratories, Irvine, UK) supplemented with 10% fetal bovine serum. 100 units/ml penicillin and 100 mg ml^{-1} streptomycin. The *in vitro* antiproliferative activity of essential oils from the three cedrus species wood oils were assayed as follows. Cell number ml^{-1} was determined by using a model ZBI coulter

Counter (coulter Electronics, Hialeah, FL). Cells were plated at an initial concentration of 3×10^4 cells ml^{-1} and the cell number ml^{-1} was determined after 4 days,when untreated cells are in the log phase of log phase of cell growth.

In vitro induction of erythroid differentiation

Erythroid differentiation of *The three cedrus species wood* oils was evaluated by counting benzidine positives cells after suspending the cells in a solution containing 0.2% benzidine in 0.5 M glacial acetic acid, 10% H_2O_2, as elsewhere describe (Bianchi *et al.*, 2001; Gambari, 2003). Induction of differentiation was compared with that obtained using well established inducers of differentiation of K562 cells, such us cytosine arabinose, mitramicine, angelicin, hydroxyurea and butyric acid (Hoffman et al.,1979, Cortesi et al.,1998,Lampronti et al.,2003,Iyama et al.,2003). (Table 6).

Statistical analysis

All experiments were carried out in triplicate. Data were expressed as means ± S.D. Differences were evaluated by one-way analysis of variance (ANOVA) test completed by Dunnett's test. Differences were considered significant at $^{**}p < 0.01$. The 50% inhibitory concentration (IC_{50}) was calculated by nonlinear regression curve with the use of Prism Graphpad Prism version 4.0 for Windows [GraphPad Software, San Diego, CA, USA (www.graphpad.com)].

Table 6: Effect the three cedrus Species on K562 cell growth and differentiation (% of benzidine-positive cells after five days of culture at the indicated concentrations).

Essential oils	IC_{50}	Differentiation max %
Cedrus Libani wood	23.38± 1.7(µg ml^{-1})	15±1.95 (5 µg ml^{-1})
Cedrus Atlantica wood	59.76 ± 2,6(µg ml^{-1})	12±2 (10 µg ml^{-1})
Cedrus deodara wood	37.79± 0.4(µg ml^{-1})	20±2.10(25µg.ml^{-1})
Manool	18,22±2.3(µg ml^{-1})	4±1,5(25 µg ml^{-1})
AraC	250±02(nM)	75±3(500nM)

IC_{50} values are mean (n = 3). Differences within and between groups were evaluated by one way analysis of variance test *** $P < 0.0001$ (F=187.6 r^2= 0.98) followed by a multicomparison Dunnett's test: **$P < 0.01$ compared with the positive control Ara C.

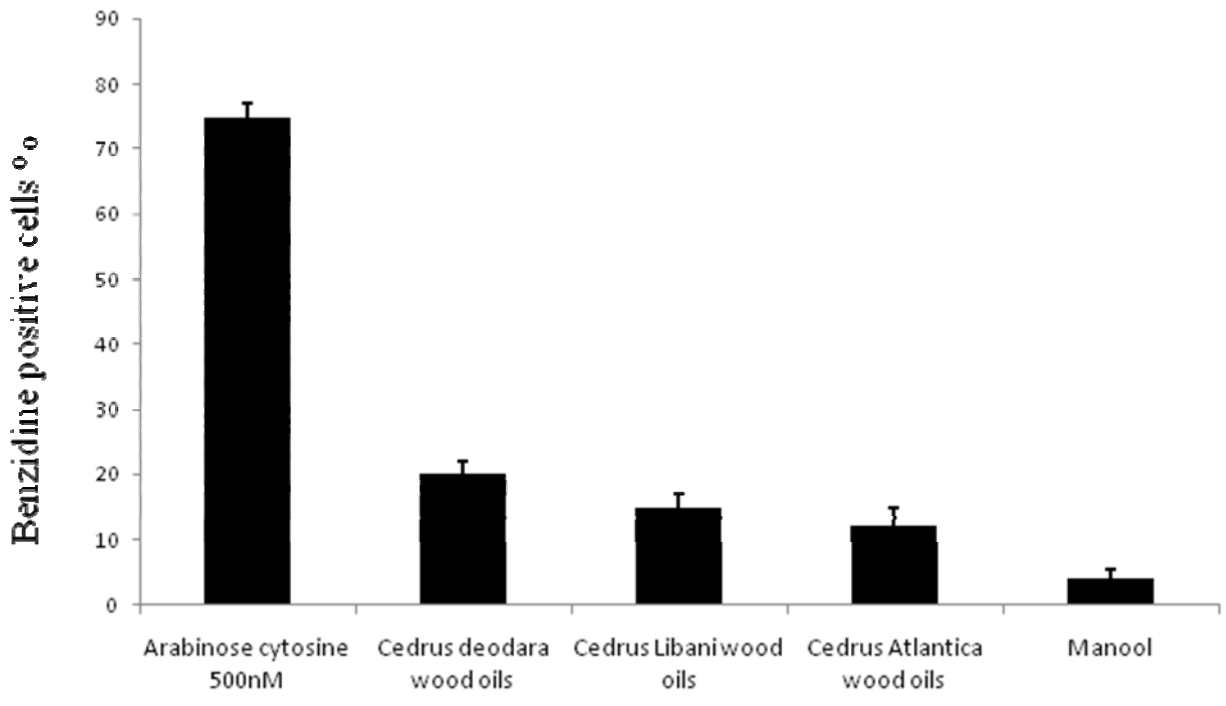


Figure 2: Concentration-dependent effect of *C. species* essential oils after 5 days culture

Cedrus brevefolia

Cedrus libani

Cedrus deodara

Cedrus atlantica

3 Part-B: Medicinal plants

3.1 Phytochemical analysis of Saturja Montana and Saturja Hortensis essential oils from Lebanon.

3.1.1 Material and Methods. Essential oils extraction. Analysis Condition.

Material and Methods

The fresh *Essential oils from Satureja hortensis and Satureja montana.* leaves of *S. Montana* and *S. hortensis* were collectedon Sannine Mountain in Lebanon, in July 2003 when fully flowering, at a 1800-m altitude. The collected species were authenticated according to conventional methods. Then, 200 gof fresh leaves and flowers of each species were submitted to hydro-distillation for 2 h. The essential oils were finally dried over anhydrous sodium sulfate to removetraces of moisture and give a yield of 0.85%. The obtained essential oils were stored at 4˚C. *Essential oils from Salvia officinalis, Lavandula officinalis, Thymus vulgaris, Calamintha origanifolia, Foeniculum vulgare and Mentha arvensis.* The fresh leaves (200-300 g) of *Salvia officinalis, Lavandula officinalis, Thymus vulgaris, Calamintha origanifolia* and *Mentha arvensis* and the leaves and seeds (200 g) of *Foeniculum vulgare* were collected in Lebanon in March 2003. All of the Lebanese plants were harvested in a flowering vegetative state, except *Salvia officinalis*, which was collected before the flowering season in the Ain-saadeh region (600 m). *Thymus vulgaris* and *Mentha arvensis* came from the Baskinta region (1400 m), *Lavandula officinalis* and *Foeniculum vulgare* were collected in the Kfarakaab and Ain-alkabou regions, respectively (1200 m); and *Calamintha origanifolia* was harvested at Bakish Mount, Lebanon (1800 m). All the collected species were authenticated according to conventional methods. Samples of each species were submitted to hydro-distillation for 2 h with 2 L of solvent.The essential oils were dried over anhydrous sodium sulfate.

Analysis condition

Gas chromatography/mass spectrometry (GC/MS) analysis of essential oils from Satureja montana and Satureja hortensis. A Fisons (Thermo Finnigan, San Jose, CA) model GC 8000 gas chromatograph interfaced to a Fisons model MD 800 quadrupole mass spectrometer was used for all measurements. The fused-silica gas chromatographic capillary column was a MEGA SE 54 (methyl phenyl polysiloxanes), 25 m x 0.25 mm I.D. and 0.25 μm film thickness (32). The head pressure of the carrier gas (helium, 99.99% purity) was 50 kPa (7.2 p.s.i.). One μl of sample dissolved into appropriate solvents was injected into the gas chromatograph. The injector and detector temperatures for the gas chromatograph were 250˚C and 300˚C, respectively. The column oven temperature was increased linearly from 40˚C (held for 4 min) to 200˚C (held for 10 min) at 10˚C/min. The mass spectrometer operated at source and interface temperatures of 250˚C. The ionization mode was Electron Impact (E.I.) (70 eV). The 'solven delay', the time gap of a given analysis in which the mass spectrometer is turned off, was 3 min. The GC/MS system was operated in 'full scan' mode. The software utilized was Excalibur (Fisons) with NIST library to recognize all the derivatives found in plant extract. GC/Ms results are summarizied in table 7 and 8.

Table 7: Composition of Saturja Montana essential oils

Compound	R.t.(min)	Composition %
α·-pinene	5.13	4.35
4(10)-thujene	7.00	6.27
p-mentha-1,8-dien-6-ol	7.43	1,00
eucalyptol (1,8-cineole)	7.53	8.87
γ-terpinen	8.08	1,03
ß-linalool	9.04	11.41
(E)-3(10)-caren-4-ol	9.48	1.15
(S)-cis-verbenol	9.65	0.18
4-terpineol	10.19	3.98
·α-terpineol (p-menth-1-en-8-ol)	10.53	12.66
2-pinen-4-one	10.72	0.35
p-allyl-anisole	11.85	0.13
nerol acetate	11.99	1.58
copaene	13.19	2.77
caryophyllene	13.81	6.66
germacrene D	14.61	1.70
t-cadinene	15.01	0.44
cadina-1(10),4-diene	15.16	6.05
4,5,9,10-dehydro-isolongifolene	15.53	0.87

±trans-nerolidol	15.68	2.57
caryophyllene oxide	15.92	4.70
ledol	16.16	3.00
t-cadinol	16.61	2.73
α·-cadinol	16.76	0.94
4-(2-acetyl-5,5'-dimethylcyclopent- 2-enylidene)butan-2-on	17.15	1.20
labda-8(20),13(16),14-triene	20.71	0.57
7-isopropyl-1,1,4·,9,10,10·-α octahydrophenantrene	21.19	0.48
p-(2,2,4-trimethyl-4- chromanyl)phenol	22.07	0.35
		Total :88.72

Table 8: Composition of Saturja hortensis essential oils

Compounds	R.t.(min)	Composition %
α·-pinene	5.13	2,19
1-octen-3-ol (amyl vinyl carbinol)	6.65	1,21
ß-pinene	6.85	2.34
3-octanol	6.99	2.03
m-cymene	7.49	9.07
γ-terpinene	8.21	15.26
ß-linalool	8.97	1.29
borneol	10.01	0.28
4-terpineol	10.23	1.19
α·-terpineol (p-menth-1-en-8-ol)	10.79	0.38

trans-geraniol	11.92	0.02
carvacrol	12,31	50.61
acethyl thymol	12.97	0.08
caryophyllene	13.87	3.85
α·-caryophyllene	14.29	0.33
t-cadinene	15.01	0.11
1-methyl-4-(methyl-1-methylene-4-hexenyl)-cyclohexene	15.04	1.49
caryophyllene oxide	15.95	0.43
t-cadinol	16.66	0.24
		Total:92.4

3.2 Phytochemical analysis of eight traditional plant extracts native from Lebanon.

3.2.1 Material and methods. Essential oils extraction. GC-MS Analysis condition.

Materials and methods

Plant material

Calamintha origanifolia Vis. (Lamiaceae), *Satureja thymbra* L.(Lamiaceae), *Prangos asperula* Boiss (Apiaceae), *Sideritis perfoliata* L. (Lamiaceae), *Asperula glomerata* (M. Bieb) Griseb (Rubiaceae), *Hyssopus officinalis* L. (Labiateae), *Erythraea centaurium* Rafn. (Gentianaceae), *Marrubium radiatum* Devile ex Benth (Lamiaceae) and *Salvia acetabulosa* L. (Lamiaceae) were selected from a list obtained from informal interviews with herbal shopkeepers in Beirut and other parts of Lebanon and collected in 2005 from various localities in Lebanon. The selected species were recommended by at least two

herbalists and authenticated by Dr. Katia Saadé, Biology Department, Faculty of Sciences II, Lebanese University. A voucher specimen of each species was deposited in the Herbarium, Faculty of Sciences II, LebaneseUniversity (Table 1). The dried plant materials were ground and stored in brown glass bottles until extraction at 25 ◦C.

Preparation of plant extracts

The air-dried and ground sample (1 g) was extracted with 15 ml ofchloroform and *n*-hexane by Maceration using vortexing for 30 s and sonication for 1 min. The material was then centrifuged at 3000rpm for 20 min. The supernatant was transferred into a 25ml round bottom flask and then taken to dryness with a rotary vacuum evaporator (Choi et al., 2004). The extraction was performed three times. Percent yields (w/w) ranged from 11.1 to 13.9 for methanol extracts, from 5.9 to 7.2 for chloroform extracts and from 12.5 to 14.6 for *n*-hexane extracts.

GC/MS analysis

The identification of the compounds from *n*-hexane and chloroform extracts was made using GC–MSanalysis on an Hewlett Packard 6890N gas chromatograph equipped with a non-polar capillary column (SE-30, 30m×0.25mm i.d.×0.25_m film thickness) and interfaced with a Hewlett Packard 5973N mass selective detector, operating in electron ionisation (EI) (70 eV). The carrier gas was helium and the "solvent delay", the time gap of a given analysis in which the mass spectrometer is turned off, was 3min. Extracts were diluted to a final volume of 1 ml with methanol (approximately 1 mg/ml). One microliter of sample was dissolved in the appropriate solvent and injected into the gas chromatograph.The injector and detector temperatureswere 250 and 280 ◦C, respectively. The analytical conditions worked with the following program: oven temperature was programmed from 60 to 280 ◦C at a rate of 16◦C/min; the

final temperature of 280 ◦C was held for 10 min. Identification of the compounds were based on the comparison of the mass spectral data on computer matching against Wiley 138 and Wiley 275 Library. GC/MS results are summarized in Tables 9 and 10.

Table 9: Constituents of n-hexane extracts determined by GC–MS

Compounds	**tR**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
Monoterpenes										
α-Thujene	6.97			x	x					x
α-Pinene	7.13		x	x	x	x				x
Sabinene	7,94		X				x	x	x	x
α-Phellandrene	8.49					x				
α-3-Carene	8.62							x		
α-Terpinene	8.71							x		x
1,8-Cineole	8.98		x			x			x	x
γ-Terpinene	9.45		x	x	x	x	x	x		x
p-Cresol	9.78			x						
Sesquiterpenes										
α-Copaene	13,71		x							
α-Humulene	14.43			x						x
γ-Cadinene	15.15		x	x						
δ-Cadinene	15.21		x							
Lepidozene	16.61			x						
Fatty acid esters and alkanes										
Methyl palmitate	18.44	x	x	x	x	x	x	x	x	x

Palmitic acid	18.73	x	x	x	x	x	x	x	x	x
Ethyl palmitate	18.95			x	x	x			x	
Methyl linoleate	19.72	x		x				x	x	x
Methyl linolenate	19.78	x		x					x	
Eicosane	19,84	x	x			x	x	x	x	
Oleic acid	20.09	x			x		x		x	
Ethyl linoleate	20.19	x		x	x		x	x	x	x
Ethyl oleate	20.23	x		x						x
Docosane	20.29	x	x			x				
Ethyl laurate	20.38				x					
Tricosane	21.08	x			x		x		x	x
Alloimperatorin	21,94	x		x			x			
Pentacosane	22.36				x	x		x		x
Heptacosane	23.87	x		x		x		x	x	
Nonacosane	25.92		x	x		x			x	
Steroids										
Stigmast-5-en-3-ol	34.54			x	x	x	x		x	x

x: presence of compound in (A) *Calamintha origanifolia*; (B) *Satureja thymbra*; (C) *Prangos asperula*; (D) *Sideritis perfoliata*; (E) *Asperula glomerata*; (F) *Hyssopus officinalis*; (G) *Erythraea centaurium*; (H) *Marrubium radiatum*; (I) *Salvia acetabulosa*.

a Compounds eluted from non-polar SE-30 MS column.

b Retention time (min).

Table 10: Constituents of chloroforme extracts determined by GC–MS

Compounds	tR	A	B	C	D	E	F	G	H	I
Monoterpenes										
α-Thujene	6.97		x							x
α-Pinene	7.13		x		x			x		x
Sabinene	7,94		X	x	x		x	x	x	x
α-Phellandrene	8.49					x		x		
α-Terpinene	8.66		x			x				
m-Cymene	8.87							x		
1,8-Cineole	9.07		x							
-γ-Terpinene	9.45		x			x	x	x	x	
2-Heptanoic acid, methyl ester	9.55							x		
Terpinen-4-ol	11,28		x							
Pulegone	12.07	x								
Sesquiterpenes										
α-Humulene	14,43									x
Spathulenol	15,73				x					
Fatty acids and alkanes										
Neophytadiene	17.72				x			x		
2-Heptadecanone	18.23						x	x		
Methyl palmitate	18.40				x	x	x	x		
Palmitic acid	18.73	x	x		x	x		x		
Ethyl palmitate	18.90									
Stearic acid	19.23	x	x					x		

8,11-Octadecadienoic acid, methyl ester	19.68							x		
8-Octadecenoic acid, methyl ester	19.72	x						x		
Methyl stearate	19.88	x						x		
Ethyl linoleate	20.15			x	x		x	x	x	x
Ethyl oleate	20.18	x						x		
Linolenic acid	20,57	x								
Triacontane	20.66	x						x		
Tricosane	21.04	x			x				x	x
Tetracosane	21.44	x						x		
Pentacosane	22.31				x					
Heptacosane	23.87	x	x		x			x		
Oleic acid, 2-hydroxyethyl ester	23.90							x		
Methyl lignocerate	24.09						x	x		
Eicosane	24.72		x			x				
Hexatriacontane	24.85	x						x		
Nonacosane	25.85		x		x			x		
Methyl cerotinate	26.72							x		
Octacosane	28.83									
Steroids and triterpenoids										
Stigmast-5-en-3-ol	34.32				x	x	x	x	x	x
β-Amyrin	35.33				x			x		
3-Keto-urs-12-ene	35.93							x		
α-Amyrin	36.78				x			x		

Stigmasta-3,5-dien-7-one	37.12							x		

x: presence of compound in (A) *Calamintha origanifolia*; (B) *Satureja thymbra*; (C) *Prangos asperula*; (D) *Sideritis perfoliata*; (E) *Asperula glomerata*; (F) *Hyssopus officinalis*; (G) *Erythraea centaurium*; (H) *Marrubium radiatum*; (I) *Salvia acetabulosa*.

a Compounds eluted from non-polar SE-30 MS column.

b Retention time (min).

3.3 Phytochemical analysis of leaves and seeds of Laurus Nobilis essential oils native from Lebanon.

3.3.1 Material and methods. essential oils extraction. GC-MS Analysis condition.

Plant materials

Laurus nobilis L. leaves and seeds were collected from Nahr-Ibrahim reserve (Lebanon) in November 2005 at the altitude 600 m above the sea. Voucher specimen was authenticated botanically by Prof. S. Safi, Biology Department, Faculty of Sciences II, Lebanese university and deposited in the Chemistry Department Herbarium, Faculty of sciences II, Lebanese University.

Isolation of the essential oils

L. nobilis seeds and leaves were air dried for 7 days at room temperature. The leaves (200 g) were submitted to hydrodistillation for 3 h using a Clevenger-type apparatus (Clevenger, 1928). The essential oil was dried over anhydrous sodium sulphate to remove trace of moisture to yield 1.5% w/w. The seeds (100 g) were boiled in water. The obtained oil was treated also with anhydrous sodium sulphate to remove trace of moisture to give 25% w/w. Both essential oils were stored at 4-8 $^{\circ}$C in brown bottle.

Gas chromatography-mass spectrometry

The essential oils from seeds and leaves of *L. nobilis* were analysed by Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS). GC analysis were performed on a Shimadzu GC17A gas chromatograph equipped with a flame ionization detector (FID) and controlled by Borwin Software. The samples were analysed on a fused silica 30 m SE-30 capillary column with an internal diameter of 0.25 mm and a film thickness of 0.25 µ. Nitrogen was used as the gas vector at a constant flow of 1.0 ml/min; split ratio 1:30. Injector and detector were maintained at 250 °C and 280 °C, respectively. The oven temperature programming was 50 °C during injection, and then increased from 50 to 280 °C at the rate of 13 °C/min. GC-MS analysis of the oils were carried out using a Hewlett-Packard 6890 gas chromatograph equipped with an methylsilicone SE-30 capillary column (30 m length, 0.25 mm i.d., 0.25 µm film thickness) and interfaced with a Hewlett Packard 5973 Mass Selective. Ionization of the sample components was performed in electron impact mode (EI, 70 eV). The carrier gas was helium (1 ml/min) and the analytical conditions worked with the following program: oven temperature was 3 min isothermal at 60 °C, then 60-280 °C at a rate of 16 °C/min; then held isothermal for 10 min. Injector and detector were maintained at 250 °C and 280 °C, respectively. The mass range from 50 to 550 amu was scanned at a rate of 2.9 scans/s. For analysis, oils were dissolved in dichloromethane (ca. 1 mg/ml) and aliquots (1 □l) were directly injected. The identification of the compounds was based comparing the mass spectral data with Wiley 138 and Wiley 275 libraries and by referring to compounds known in the literature data (Adams, 1995). Retention indices for all compounds were determined by co-injection of the sample with a solution containing a homologous series of C9–C31 *n*-alkanes (Tranchant, 1995). The quantitative determinations of each oil component

expressed in percentages, was carried out by peak area normalization measurements using an external standard method. (Table 11).

Table 11: L. nobilis leaves and seeds essential oils chemical profile (%).

		***L. nobilis* essential oil**		**ID method[a]**
Constituents	**t_R**	**Leaves (%)**	**Seed (%)**	
α-Thujene	6.97	0.44 ± 0.09	0.10 ± 0.01	MS
α-Pinene	7.13	5.72 ± 0.12	3.67 ± 0.03	MS, Co-GC
Camphene	7.37	0.14 ± 0.06	1.69 ± 0.04	MS
Sabinene	7.95	6.17 ± 0.14	1.64 ± 0.03	MS, Co-GC
β-Pinene	8.02	3.46 ± 0.08	2.14 ± 0.01	MS, Co-GC
β-Myrcene	8.22	0.45 ± 0.10	0.56 ± 0.01	MS
α-Phellandrene	8.47	0.12 ± 0.03	0.11 ± 0.07	MS
α-Terpinene	8.71	0.86 ± 0.05	0.15 ± 0.01	MS, Co-GC
p-Cymene	8.85	2.23 ± 0.11	0.12 ± 0.05	MS, Co-GC
Limonene	8.94	1.10 ± 0.08	0.10 ± 0.01	MS, Co-GC
1,8 Cineole	9.07	35.15 ± 0.36	9.43 ± 0.07	MS, Co-GC
β-Ocimene	9.09	0.10 ± 0.005	21.83 ± 0.18	MS
γ-Terpinene	9.47	1.50 ± 0.09	0.10 ± 0.01	MS
α-Terpinolene	9.93	0.49 ± 0.07	-	MS
Fenchone	9.96	-	0.12 ± 0.02	MS
Linalool	10.13	7.08 ± 0.14	-	MS, Co-GC
Camphor	10.83	-	0.35 ± 0.04	MS
Terpinen-4-ol	11.24	4.42 ± 0.07	-	MS
α-Terpineol	11.46	2.42 ± 0.04	0.40 ± 0.02	MS, Co-GC
Isoborneol	11.58	-	0.31 ± 0.01	MS

Bornyl acetate	12.59	-	0.23 ± 0.01	MS
α-Terpinyl acetate	12.62	4.43 ± 0.10	-	MS
1-*p*-Menthen-8-ethyl acetate	13.31	13.52 ± 0.25	-	MS
Eugenol	13.42	3.73 ± 0.16	-	MS
α-Ylangene	13.67	0.17 ± 0.06	0.23 ± 0.08	MS
α-Copaene	13.71	-	0.17 ± 0.01	MS, Co-GC
α-Bergamotene	13.75	-	0.10 ± 0.04	MS
β-Elemene	13.82	0.10 ± 0.08	1.0 ± 0.06	MS
Methyl eugenol	13.86	2.52 ± 0.04	-	MS
trans-Caryophyllene	14.20	0.38 ± 0.08	0.32 ± 0.04	MS, Co-GC
α-Humulene	14.43	-	0.10 ± 0.01	MS, Co-GC
trans-β-Farnesene	14.52	-	0.13 ± 0.01	MS
α-Guaiene	14.85	0.22 ± 0.03	-	MS
γ-Cadinene	15.15	-	0.36 ± 0.02	MS
δ-Cadinene	15.21	-	0.14 ± 0.01	MS
Spathulenol	15.76	0.31 ± 0.02	-	MS

t_R: Retention time (as min); -: mot detected. % identification: peak area identified relative to total peak area (%). [a] MS, mass spectrum; Co-GC: co injection with authentic compound.

3.4 Phytochemical analysis of seven medicinal plants: Essential oils growing in Lebanon.

Experimental Part

Plant Materials. Berries of Laurus nobilis L. (Lauraceae) and Juniperus oxycedrus L. ssp. Oxycedrus (Cupressaceae), fruits of Thuja orientalis L. (Cupressaceae), Cupressus sempervirens L. ssp. pyramidalis(Cupressaceae), and Pistacia palaestina Boiss. (Labiatae), Salvia officinalis L. (Labiatae), and leaves ofSatureja thymbra L. (Labiatae) were collected from June to November 2003 in Lebanon (Table 1). Avoucher specimen of each plant was authenticated botanically by Prof. S. Safi, Biology Department,

Faculty of Sciences II, Lebanese University, and deposited with the Herbarium of Faculty of Sciences II,Lebanese University.

Isolation of Essential Oils.

The fresh aerial parts (200 g of each of the above mentioned species) weresubmitted to hydrodistillation for 3 h using a Clevenger-type apparatus as described in (Loizzo et al, 2007), yields in percent (w/w%) ranged between 1.5 and 3 (table 12). The white-yellow essential oils were dried (anh. Na2SO4) to remove traces of moisture and stored at 4–88 in bottles covered with aluminium foil to prevent the negative effect of light.

GC/MS Analysis.

To determine the essential oils composition, analyses were carried out using a GC system (Hewlett-Packard Co., model 6890) with a fused cap. column (30 m length; 0.25 mm i.d.; 0.25-m

film thickness; static phase methylsilicone SE-30) directly coupled to a selective mass detector (Hewlett Packard 5973). Electron impact ionization was carried

out at an energy of 70 eV. He was used as carrier gas. Injector and detector were maintained at 2508 and 2808, resp. The anal. conditions were as follows: oven temp. was 5 min isothermal at 608, then 60–2808 at a rate of 168/min, then held isothermal for 10 min. The mass range from 50 to 550 amu was scanned at a rate of 2.9 scans/s. Identification of the components was based on the comparison of the MS data on computer matching against Wiley 138 and those described in literature (Table 12).

Table 12: Sources of the samples of the studied essential oils

Plants	**Location**	**Studied material**	**% W/W**
L.nobilis L	Nahr Ibrahim	Berry	3
J.oxycedrus L.Spp.oxycedrus	Baskinta	Berry	2.5
T. oriontalis L.	Ayoun Kourkouch	Fruits	3.5
C.sempervirens L.ssp. pyramidalis	Ajaltoun	Fruits	3
P. palaestina Boiss.	Ain-Saade´	Fruits	2
S. officinalis L.	Ain-Saade´	Leaves	2.25
S. thymbra L.	Ayoun-kourkoush	Leaves	1.5

Table 13: Composition [%] of Essential Oil

Composition [%] *of Essential Oils Obtained from* L. nobilis (*Ln*), J. oxycedrus *ssp.* oxycedrus (*Joo*), T. orientalis (*To*), C. sempervirens *ssp.* pyramidalis (*Csp*), P. palaestina (*Pp*), S. thymbra (*St*), *and* S. officinalis (*So*)

Compound[a])	t_R[b])	*Ln*[c])	*Joo*[c])	*To*[c])	*Csp*[c])	*Pp*[c])	*St*[c])	*So*[c])	Method[d])
Tricyclene	6.78	–	–	0.28±0.01	0.17±0.01	–	–	0.10±0.02	GC/MS
α-Thujene	6.97	0.10±0.01	–	0.25±0.02	0.18±0.01	0.92±0.05	0.89±0.11	0.16±0.01	GC/MS
α-Pinene	7.13	3.67±0.03	27.40±0.05	35.72±0.63	53.56±0.32	6.81±0.12	10.15±0.32	4.72±0.11	GC/MS
α-Fenchene	7.34	–	–	1.21±0.11	0.72±0.018	–	–	–	GC/MS
Camphene	7.38	1.69±0.04	0.10±0.02	0.19±0.04	0.24±0.01	0.39±0.01	0.08±0.009	2.55±0.08	GC/MS
m-Cymene	7.82	–	–	0.24±0.02	–	–	–	–	GC/MS
Sabinene	7.94	1.64±0.03	4.51±0.01	0.85±0.02	1.01±0.034	17.08±0.25	8.64±0.15	6.97±0.21	GC/MS
β-Pinene	7.96	2.14±0.01	0.40±0.05	–	1.78±0.076	6.48±0.09	2.90±0.18	3.01±0.14	GC/MS
β-Myrcene	8.21	0.56±0.01	18.90±0.07	1.64±0.07	–	–	0.68±0.03	tr	GC/MS
α-Phellandrene	8.49	0.11±0.07	7.10±0.002	0.17±0.01	–	1.13±0.03	tr	–	GC/MS
δ-3-Carene	8.62	–	–	9.48±0.12	–	tr	–	–	GC/MS
α-Terpinene	8.71	0.15±0.01	–	0.15±0.01	18.9±0.14	3.60±0.06	1.10±0.12	0.17±0.01	GC/MS
p-Cymene	8.86	0.12±0.05	0.51±0.08	1.23±0.02	0.82±0.02	6.01±0.09	10.76±0.53	1.08±0.04	GC/MS
Limonene	8.94	0.10±0.01	6.70±0.05	2.90±0.02	1.95±0.08	8.56±0.11	0.57±0.09	1.20±0.05	GC/MS, CoI
1,8-Cineole	8.98	9.43±0.07	–	–	–	–	0.28±0.06	43.62±0.44	GC/MS, CoI
β-Ocimene	9.09	21.83±0.18	–	0.18±0.03	–	–	–	–	GC/MS
α-Ocimene	9.24	–	–	0.12±0.01	–	–	–	–	GC/MS
γ-Terpinene	9.45	0.10±0.01	0.10±0.01	0.87±0.10	0.31±0.01	6.33±0.12	7.56±0.11	0.39±0.08	GC/MS
Terpinolene	9.94	–	0.22±0.04	2.94±0.22	3.15±0.13	2.86±0.06	0.62±0.15	tr	GC/MS
Fenchone	9.96	0.12±0.02	–	–	–	–		–	GC/MS
Linalool	10.08	–	0.40±0.02	–	–	–	2.81±0.11	–	GC/MS, CoI
α-Thujone	10.32	–	–	–	–	–	0.08±0.11	12.99±0.13	GC/MS
Fenchol	10.35	–	–	0.28±0.01	–	–	–	–	GC/MS
δ-Isothujone	10.39	–	–	–	–	–	–	1.48±0.02	GC/MS
cis-p-2-Menthen-1-ol	10.45	–	–	–	–	0.32±0.05	–	–	GC/MS
α-Campholene aldehyde	10.52	–	0.10±0.06	0.22±0.01	–	–	0.25±0.11	–	GC/MS
trans-Pinocarveol	10.74	–	0.10±0.03	0.57±0.07	–	–	–	–	GC/MS
Camphor	10.81	0.35±0.04	–	0.84±0.09	–	–	–	5.71±0.12	GC/MS
trans-Pinocamphone	11.03	–	–	0.36±0.01	–	–	–	–	GC/MS
Isopinocamphone	11.23	–	–	0.44±0.01	–	–	–	–	GC/MS

Compound[a])	t_R[b])	*Ln*[c])	*Joo*[c])	*To*[c])	*Csp*[c])	*Pp*[c])	*St*[c])	*So*[c])	Method[d])
Terpinen-4-ol	11.28	–	0.10 ± 0.05	–	–	–	–	–	GC/MS
α-Terpineol	11.42	0.40 ± 0.02	0.30 ± 0.08	0.20 ± 0.01	1.08 ± 0.02	2.43 ± 0.08	1.53 ± 0.16	3.18 ± 0.32	GC/MS
Myrtenol	11.52	–	–	0.16 ± 0.03	–	–	–	–	GC/MS, CoI
Isoborneol	11.58	0.31 ± 0.01	–	0.15 ± 0.01	–	–	–	–	GC/MS
cis-Piperitol	11.65	–	–	–	–	–	0.10 ± 0.06	–	GC/MS
Verbenone	11.69	–	0.10 ± 0.01	0.19 ± 0.01	–	–	–	–	GC/MS
Fenchol acetate	11.80	–	–	0.30 ± 0.02	–	–	–	–	GC/MS
Isopulegone (**1**)	11.82	–	–	–	–	–	0.10 ± 0.02	–	GC/MS
trans-Carveol	11.93	–	0.20 ± 0.03	–	–	–	–	–	GC/MS
Methyl thymyl ether	12.05	–	–	–	0.14 ± 0.01	0.38 ± 0.02	–	–	GC/MS
Pulegone (**2**)	12.07	–	–	–	–	–	tr	–	GC/MS
Citronellol	12.18	–	0.30 ± 0.05	–	–	–	–	–	GC/MS
Carvone	12.25	–	0.10 ± 0.09	–	–	–	–	–	GC/MS
Geraniol	12.32	–	0.10 ± 0.02	–	–	–	tr	–	GC/MS
Neryl acetate	12.57	–	–	–	–	–	0.26 ± 0.03	–	GC/MS
Bornyl acetate	12.59	0.23 ± 0.01	0.62 ± 0.04	0.86 ± 0.04	–	–	–	0.24 ± 0.06	GC/MS
α-Terpinyl acetate	12.62	–	0.10 ± 0.03	–	–	–	–	–	GC/MS
Thymol	12.63	–	–	–	3.84 ± 0.11	–	9.92 ± 0.07	–	GC/MS
α-Fenchyl acetate	12.65	–	–	–	–	2.05 ± 0.05	–	–	GC/MS
Carvacrol	12.90	–	–	–	–	–	4.98 ± 0.08	–	GC/MS
Bornylene	13.19	–	–	–	0.72 ± 0.03	–	–	–	GC/MS
1-p-Menthen-8-yl acetate	13.31	–	–	–	–	–	0.40 ± 0.08	–	GC/MS
α-Cubebene	13.35	–	0.53 ± 0.07	–	–	0.52 ± 0.03	–	tr	GC/MS
Eugenol	13.41	–	–	–	–	–	–	–	GC/MS
α-Ylangene	13.67	0.23 ± 0.08	0.40 ± 0.05	0.46 ± 0.02	–	0.22 ± 0.01	–	–	GC/MS
Calarene (**3**)	13.69	–	–	–	0.31 ± 0.01	–	–	–	GC/MS
α-Copaene	13.71	0.17 ± 0.01	0.30 ± 0.04	0.44 ± 0.01	–	0.20 ± 0.01	1.67 ± 0.13	–	GC/MS
α-Bergamotene	13.75	0.10 ± 0.04	–	0.10 ± 0.01	–	–	–	–	GC/MS
β-Bourbonene (**4**)	13.79		–	–	–	–	0.24 ± 0.05	–	GC/MS
β-Elemene	13.81	1.0 ± 0.06	–	–	–	0.10 ± 0.01	0.21 ± 0.01	–	GC/MS
Methyl eugenol	13.86	–	–	–	–	–	–	–	GC/MS
Zingiberene (**5**)	13.90	–	–	0.11 ± 0.01	–	0.48 ± 0.01	–	–	GC/MS

*

Compound[a])	t_R[b])	*Ln*[c])	*Joo*[c])	*To*[c])	*Csp*[c])	*Pp*[c])	*St*[c])	*So*[c])	Method[d])
Longifolene	13.97	–	0.20±0.02	–	–	–	–	–	GC/MS
Isolongifolene (**6**)	14.05	–	–	–	1.35±0.02	–	–	–	GC/MS
α-Gurjunene	14.06	–	–	–	–	–	0.51±0.06	–	GC/MS
trans-Caryophyllene	14.17	0.32±0.04	1.60±0.09	3.43±0.02	–	0.63±0.01	3.67±0.11	1.05±0.07	GC/MS, CoI
Widdrene (**7**)	14.24	–	–	0.81±0.01	–	–	–	–	GC/MS
Aromadendrene	14.30	–	–	–	–	3.99±0.01	–	0.99±0.04	GC/MS
cis-Thujopsene	14.32	–	0.30±0.02	–	–	–	–	–	GC/MS
β-Gurjunene	14.36	–	0.41±0.01	–	–	–	–	–	GC/MS
trans-β-Farnesene	14.38	0.13±0.01	0.32±0.01	0.71±0.02	–	–	–	–	GC/MS
α-Humulene	14.43	0.10±0.01	1.01±0.05	0.42±0.03	–	0.29±0.01	0.34±0.03	3.41±0.45	GC/MS
β-Acoradiene (**8**)	14.63	–	–	0.62±0.01	–	–	–	–	GC/MS
Epibicyclosesquiphellandrene	14.79	–	2.30±0.02	1.10±0.07	–	2.40±0.03	1.68±0.14	–	GC/MS
α-Guaiene	14.85	–	–	–	–	–	–	–	GC/MS
β-Bisabolene	14.95	–	–	2.19±0.12	–	–	–	–	GC/MS
β-Himachalene	14.98	–	–	0.89±0.06	–	–	–	–	GC/MS
β-Selinene	15.01	–	0.80±0.08	–	–	–	0.11±0.02	–	GC/MS
α-Muurolene	15.09	–	0.90±0.02	–	–	–	0.37±0.04	–	GC/MS
γ-Cadinene	15.15	0.36±0.02	0.61±0.07	–	–	–	–	tr	GC/MS
δ-Cadinene	15.21	0.14±0.01	2.20±0.08	2.86±0.02	0.22±0.01	1.51±0.07	3.11±0.12	0.1±0.01	GC/MS
Cadina-1,4-diene	15.38	–	0.10±0.04	–	–	–	–	–	GC/MS
Palustrol (**9**)	15.67	–	–	–	–	–	0.99±0.08	–	GC/MS
Spathulenol	15.73	–	–	–	–	–	0.61±0.07	–	GC/MS
γ-Gurjunene	15.88	–	–	–	–	0.33±0.11	–	1.16±0.05	GC/MS
Viridiflorol (**10**)	15.90	–	–		–	–	0.78±0.04	0.11±0.04	GC/MS
α-Cedrol	16.03	–	0.31±0.05	9.55±0.07	–	–	–	–	GC/MS
β-Guaiene	16.14	–		–	–	0.16±0.03		–	GC/MS
β-Maaliene	16.44	–	–	–	–	3.08±0.04	–	–	GC/MS
Eremophilene	16.52	–	0.20±0.03	–	–	–	–	–	GC/MS
Cadalene (**11**)	16.79	–	0.10±0.02	–	–	–	–	–	GC/MS
(*E*,*E*)-Farnesol	17.02	–	0.60±0.06	–	–	–	–	–	GC/MS

Compound[a])	t_R[b])	*Ln*[c])	*Joo*[c])	*To*[c])	*Csp*[c])	*Pp*[c])	*St*[c])	*So*[c])	Method[d])
4-Oxo-α-ylangene	17.37	–	–	–	–	–	0.15±0.07	–	GC/MS
Methyl palmitate	18.46	–	0.10±0.04	–	–	–	–	–	GC/MS
Biformene	18.64	–	–	–	–	0.56±0.01	0.10±0.05	–	GC/MS
Palmitic acid	18.74	–	0.21±0.08	–	–	–	–	–	GC/MS
Manoyl oxide	19.26	–	0.20±0.03	–	–	–	–	–	GC/MS
Eremanthin (**12**)	19.31	3.65±0.09			–	–	–	–	GC/MS
Rimuene	19.39	–	–	–	–	–	0.71±0.24	–	GC/MS
Ethyl palmitate	19.56	–	0.11±0.07	–	–	–	–	–	GC/MS
Methyl stearate	19.95	–	0.12±0.06	–	–	–	–	–	GC/MS
Ethyl linoleate	20.29	–	tr	–	–	–	–	–	GC/MS
Ethyl stearate	20.47	–	tr	–	–	–	–	–	GC/MS
Dehydrocostuslactone (**13**)	20.82	7.57±0.12	–	–	–	–	–	–	
Identified compounds		56.82	82.39	86.68	90.45	79.82	80.91	94.39	

[a]) Compounds listed in order of elution from *SE30 MS* nonpolar column. [b]) t_R: Retention time [min]. c) Relative area percentage (peak area relative to total peak area in %). tr: trace, *i.e.*, <0.05%. [d]) CoI: co-injection with authentic compound.

3.5 Anti proliferative activities of essential oils derived fromPlants belonging to Magnoliophyta division against k562 Human Leukemia cell line.

3.5.1 Material and methods, cell line, culture condition and in vitro antiproliferative activity assay. In vitro Induction of erythroid differentiation.

Cell lines, culture conditions and assays of in vitro antiproliferative activity. Human erythroleukemia K562 cells(Lampronti et al.,2008) were cultured in a humidified atmosphere at 5% CO2, in RPMI-1640 (Flow laboratories, Irvine, UK) supplemented with 10% fetal bovine serum (FBS; CELBIO, Milano, Italy),100 units/ml penicillin and 100 mg/ml streptomycin (Aldrich,St. Louis, MO, USA). The *in vitro* antiproliferative activityof essential oils and pure commercially available derivativesfrom *S. montana* and *S. hortensis* was assayed afollows. Cell number/ml was determined by using a model ZBI Coulter Counter (Coulter Electronics, Hialeah, FL). Cells were seeded at an initial concentration of 3x104 cells/ml and the cell number/ml was determined after 2, 3, 4 and 5 days of cell culture. The IC_{50} was usually determined after 4 days, when untreated cells are in the log phase of cell growth.

Assays of in vitro induction of erythroid differentiation.

Erythroid differentiation of essential oils from *S. Montana* and *S. hortensis* and their pure derivatives was determined by counting benzidine positive cells after suspending the cells in a solution containing 0.2% benzidine in 0.5 M glacial acetic acid, 10% H2O2, as elsewhere described (Bianchi et al2001). Induction of differentiation was compared with that obtained using well established inducers of differentiation of K562 cells, such as cytosine arabisonide (Cortesi et al 1998) , mithramycin (Bianchi et al 1999), angelicin (Lampronti et al.,2003), hydroxyurea (Iyamu et al 2003) and butyric acids (Hoffman et al.,1979). (Table 14 and15).

Table 14: Effects of essential oils from Lebanon on K562 cellgrowth (IC_{50}) and differentiation (% of benzidine-positivecells after 5 days of culture at the indicated concentrations

Essential oils	IC_{50} (µg/ml)	% Differentiation (µg/ml)
Satureja hortensis	85.4±2.4	16 (0.5-5)
Satureja Montana	56.15±6.15	30 (0.05-50)
Salvia officinalis	217.7±0.5	23 (0.5-50)
Lavandula officinalis (silice land)	256.1±0.3	18 (0.05-50)
Lavandula officinalis (argile land)	111.6±24.6	11 (0.05-5)
Thymus vulgaris	136.6±25	25 (0.05-0.5)
Calamintha origanifolia	>500	34 (5-250)
Foeniculum vulgare	190.8±0.5	32 (5-50)
Mentha arvensis	40.6±3.5	13 (0.5)

Table 15: Effects of pure compounds identified in Satureja montana and Satureja hortensis on K562 cell growth (IC50) and differentiation (% of benzidine-positive cells after 5 days of culture at the indicated concentrations).

Compounds	IC_{50} (µM)	*% differentiation (µM)*
α-pinene	117.3±14.4	5 (10)
ß-pinene	157.4±21.6	4 (50)
Eucalyptol	>400	3 (10)
γ-terpinene	329.9±0.5	3 (200)
Linalool	150.0±3.5	5 (100)
·α-terpineol	75.0±1.5	4 (100)
Copaene	136.5±3.2	4 (50)
trans-nerolidol	132.9±4.6	3 (50)
Caryophyllene	98.0±0.7	4 (50)
α·-caryophyllene	98.7±1.2	4 (5-50)
caryophyllene oxide	136.4±2.5	7 (10)
1-octen-3-ol	>400	4 (400)
3-octanol	>400	3 (50-200)
m-cymene	>400	4 (200-400)
Carvacrol	112.5±12.2	7 (400)

3.6 Anti proliferative activities of leaves and seeds Essential oils of Laurus nobulis against k562 human Leukemia cell.

Cell lines, culture conditions and in vitro antiproliferative activity assay

Human chronic myelogenous leukemia K562 cells (Lampronti et al., 2003a) were cultured in a humidified atmosphere at 5% CO_2 in RPMI-1640 (Flow laboratories, Irvine, UK) supplemented with 10% fetal bovine serum. 100 units/ml penicillin and 100 mg ml^{-1} streptomycin. The *in vitro* antiproliferative activity of essential oils from *L. nobilis* leaves and seeds was assayed as follows. Cell number/ml was determined by using a model ZBI coulter Counter (coulter Electronics, Hialeah, FL). Cells were plated at an initial concentration of 3×10^4 cells/ml and the cell number/ml was determined after 4 days, when untreated cells are in the log phase of log phase of cell growth

In vitro induction of erythroid differentiation

Erythroid differentiation of *L. nobilis* essential oils was evaluated by counting benzidine positives cells after suspending the cells in a solution containing 0.2% benzidine in 0.5 M glacial acetic acid, 10% H_2O_2, as elsewhere describe (Bianchi et al., 2001; Gambari, 2003). Induction of differentiation was compared with that obtained using well established inducers of differentiation of K562 cells, such us cytosine arabinose, mitramicine, angelicin, hydroxyurea and butyric acid (Hoffman *et al.*, 1979; Cortesi *et al.*, 1998; Lampronti *et al.*, 2003b; Iyamu et al., 2003). (Table 16 and 17)

Statistical analysis

All experiments were carried out in triplicate. Data were expressed as means ± S.D. Differences were evaluated by one-way analysis of variance (ANOVA) test completed by Dunnett's test. Differences were considered significant at $**p$

< 0.01. The 50% inhibitory concentration (IC_{50}) was calculated by nonlinear regression curve with the use of Prism Graphpad Prism version 4.0 for Windows [GraphPad Software, San Diego, CA, USA (www.graphpad.com)].

Table 16: Effect of L. nobilis essential oils on K562 cell growth and differentiation (% of benzidine-positive cells after five days of culture at the indicated concentrations).

Essential oils	IC_{50} (µg ml^{-1})	Differentiation max %
Leaves	95 ± 1.7** µg ml^{-1}	15±1.52 (10 µg/ml)
Seeds	75 ± 1.4** µg ml^{-1}	12±1.25 (50 µg/ml)
AraC	250±02 nM	92.0±2.51 (0.5 µM)

IC_{50} values are mean (n = 3). Differences within and between groups were evaluated by one-way analysis of variance test *** $P < 0.0001$ (F=187.6 r^2= 0.98) followed by a multicomparison Dunnett's test: **$P < 0.01$ compared with the positive control Ara C.

Table 17: Level of K 562 differentiation induced by known erythroid inducers and L. nobilis oils in synergic effect with sub-optimal concentration of commercial drug citosine arabinose (AraC).

Inducer	Differentiation max (%)
L. nobilis leaves oil + AraC	23.0±1.75 (1µg/ml oil + AraC 25 nM)
L. nobilis seeds oil + AraC	25.0±2.02 (0.1µg/ml oil + AraC 25 nM)
AraC	92±2.51.0 (0.5 µM)
Mitramicyn	86.0±2.20 (0.05 µM)
Butyric acid	32.5±3,4(2mM)

% values are mean (n = 3).

4 Results and discussions.

4.1 Comparison between Cedrus libani and Cedrus atlantica wood essential oils

Mono-,sesqui-, and diterpene are good markers for distinguishing conifer species from each other. Himachalol appears as a specific maker of Cedrus libani and Cedrus atlantica accordingly, we should add α-,β-,γ- Himachalene as markers of genus Cedrus (Table 1). We noted some genus of Cedrus libani and Cedrus atlantica are similar, particularly their cones, leaves, buds and their mono-, sesqui-, and diterpene. The concentration of compounds of these two species increase one year and decrease another year during the period of dormancy. Some of sesquiterpene, especially Himachalene is an important product used in perfume industry .In addition there are some oxygenate sesquiterpene, such as α-,β-,γ- atlantone which may the basis of anti-inflammatory pharmaceutics as well as anti-cancer additive.

4.2 Analysis of Cedrus libani and Cedrus deodara Leaves

The terpenoid substances are excellent makers for distinguishing *Cedrus* species for each other. Germacrene D and β-Cariophyllene in our study, appear as specific makers of *C. libani* and *C. deodara*. With the aim of investigating the role of the active principles in different biological systems and to correlate the activity of crude essential oils with that of single molecules responsible of the biological and pharmacological effects, we analyzed, by GC/MS, the composition of *C. libani* and *C. deodara*. In the present study, we were able to identify 49 active principles, present at different concentrations, in the analyzed cedar species. We utilized two different essential oils obtained from leaves of *C. libani*, in order to have an internal standard, and one essential oil derived from leaves of C deodara.

Table 3 shows the obtained results: the main components of C. libani (A) leaves, extracted by hydro-distillation, were Germacrene D (29.40%), 1-epi-Cubenol (6.30%), δ-cadinene (5.60%) and β-Cariophyllene (5.60%).Whereas, the major component of *C. deodara* is benzaldehyde (19.40%), β-Cariophyllene (10.90%), myrcene (10.70%) and Germacrene D (9.35%). Benzaldehyde is not present in oils from *C. libani*, while Germacrene D is more concentrated in *C. libani*, and, on the contrary, we reveled more quantity of β-Cariophyllene in *C. deodara*. Another important difference between these species is the presence of Myrcene: in *C. deodara* the relative concentration is 10.70%, while in *C. libani* is only 0.54%. Also α-, and β-pinene are more concentrated in *C. deodara* (5.65% and 5.85%, respectively) then in *C. libani* (2.20% and 0.40%, respectively). Finally, we observed 2.70% of limonene in *C. deodara* against 0.44% in *C. liban*.A. C . *libani* B, our internal standard, shows similar values of all the active principles present in *C. libani* A, with a minimal deviation in some identified compounds.

4.3 Analysis, Anti herpes type 1 Activity and anti diabetic of Cedrus Libani wood, leaves and cones.

4.3.1 Analysis

Since a long time, medicinal plants have been used for the treatment of many infections diseases including viral infections. Accordingly, large number of synthetic and plant-derived anti-HSV drugs have been described in several studies (Ferrea et al., 1993; Bourne et al., 1999; Ikeda et al., 2000).

In the present investigation we reported, for the first time, the antiviral activity of wood essential oil and cones and leaves ethanol extracts from C. libani supporting by phytochemical analysis. The GC/MS analysis of wood essential oil revealed the presence of 13 compounds, while 19 and 36 are identified for cones and leaves ethanol extract, respectively (Table 2). In ethanol extract of

leaves the main compound is germacrene D (29.40%), known in literature as antimicrobial sesquiterpene (Juliani et al., 2002). Other identified compounds were 1-epi-cubenol (6.30%), trans-α-bisabolene (5.90%), β-caryophylene (5.60%), δ-cadinene (5.60%), and γ-muurolene (4.83%). On the contrary ethanol extract obtained from cones was characterized by the presence of α-pinene (51.0%), β-myrcene (13.0%), 7,13-abietadiene (3.20%), terpinolene (3.10%), and limonene (2.25%). The himachalol (22.50%), β-himachalene (21.90%), and α-himachalene (10.50%) were the most characteristics compounds in the wood essential oil.

4.3.2 Anti herpes type1

Essential oils have been widely used in traditional medicine. Among others, antibacterial, antifungal, immunomodulatory, antinflammatory, and antirheumatic activities have been described (Saller et al., 1995; Reichling, 2001). Hitherto, there is only little information on the effects of essential oils on viruses or viral infections. Recently, the anti-herpes activity of several essential oils of different plant sources as well as of various constituents of essential oils was demonstrated (Bourne et al., 1999; Benencia and Courreges, 1999; De Logu et al., 2000; Schuhmacher et al., 2003). In our study C. libani essential oil and ethanol extracts were investigated against HSV-1 infected Vero cells. Results are summarized in Table 18. Assessment of cytotoxicity is clearly an important part of the evaluation of a potential antiviral agent because a useful oil, extract or compound should be selective for virus specific processes with no or only few effects on cellular metabolism. In Vero cells the TC50 of C. libani wood essential oil and leaves and cones ethanol extracts was between 0.87 and 1.92 mg/ml. The ethanol extracts from cones and leaves inhibited HSV-1 replication with IC50 values ranging from 0.50 to 0.66 mg/ml. In particular, extract obtained from leaves showed antiviral activity with a TI of 2.91. The phytochemical analysis demonstrates that the main constituents of C. libani

cones extract are two monoterpens: α-pinene and β-myrcene (Khan et al. 2005). reported that some monoterpenes such as isoborneol tested against HSV-1 inactivated very effectively virus by a mechanism which involved the inhibition of glycosylation of viral polypeptides. At the same time the 1,8-cineole and borneol (a stereoisomer of isoborneol) did not inhibit the glycosylation but completely inhibited viral replication, without affecting viral adsorbtion (Armaka et al., 1999). α-Pinene and β-myrcene evaluated at 0.1 mg/ml for antiviral activity did not exhibited antiviral activity. The significant activity of cones ethanol extract against HSV-1, in relation to the inactivity of the two most abundant compounds, suggest the existence of additional antiviral components in this extract. This result seems indicative of a synergetic effect induced by other components of cones ethanol extract. In conclusion, in the present investigation we reported for the first time the phytochemical composition of wood essential oil. The antiviral test demonstrated that HSV-1 growth was partly inhibited by C. libani. The exact mechanism of the antiviral activity of the C. libani is not known and further investigation should be done to identify other active compounds and the way of action.

Table 18: Antiviral drug testing (mg/ml) of Cedrus libani essential oil and ethanol extract

Cedrus Libani	IC50	TC50	TI
Wood essential oils	0.44	o.88	2
Leaves ethanol extract	0.66	1.92	2.91
Cones ethanol extract	0.50	0.87	1.74
α-Pinene	>1	>1	>1
β-Myrcene	>1	>1	-----
ACV	3.77µM	>100µM	>27

IC50 (inhibitory concentration 50%): concentration required to inhibit 50% of virus growth); TI ¼ therapeutic index (TC50/IC50).

4.3.3 Anti-diabetic

α-amylase is the main enzyme in humans responsible for the breakdown of starch and more plain sugar (dextrin, maltotriose, maltose and glucose) (Alexander et al.,1992). Although the activity of enzyme has not been directly involved in the aetiology of diabetes, α-amylase inhibitors have been thought to improve glucose tolerance in diabetic patients (Lebovit et al 1998). Extensive efforts have been made over the past decades to find a clinically effective α-amylase inhibitor with the aim of obtaining better control of diabetes (Jung et al., 1996). The results revealed that oil obtained by hydrodistillation from C. libani woods exhibits an interesting activity with IC50 of 0.14 mg/ml. While oils from cones exhibited a percentage of inhibition of 31% at 1 mg/ml (IC50>1 mg/ml) and no inhibition was found when oil from leaves was used. In C. libani wood oil, the main constituent is a sesquiterpene alcohol himachalol that was not found in leaves or cones oils. It could be possible that this compound,

known in literature for other type of biological activity is responsible of α-amylase inhibition (Cowduhry et al., 1997; Kar et al., 1975).

Table 19: Inhibitory effect of the C. libani cones, leaves, woods oils on α-amylase

Oils of Cedrus Libani	IC_{50}(mg/ml)
Cones	>1
Leaves	--
Wood	0,14

n=3; Positive control: acarbose IC50 50 μg/ml; –: NO inhibition.

4.3.4 Anti proliferative activity of *Cedrus libani* seeds extracts against k562 Cell Line, chemical composition, biological activity.

Cedrus libani plants and their different parts are widely used as traditional medicine in Lebanon for treatment of different diseases (Kurt, 2008). Cedrus libani plants are moreover a natural source of raw materials for the production of medicaments and cosmetics for treating skin and hair diseases (Safai, 1992). With reference to these evidences the chemical composition of the extracts and essential oils from different Cedar species has been the subject of many studies in recent years. In particular, the terpenoid substances are the secondary metabolites that seems to characterize C.libani derived products and their functional properties (Kizil, 2002; Yilmaz, 2005; Loizzo, 2008)

4.3.5 Chemical composition

On these bases in the present investigation it has been reported the chemical composition of *C.Libani* seeds chloroform and ethanol extracts, never studied before, and evaluation of their potential as antiproliferative agents against human chronic myelogenous leukemia cells. Ethanol and chloroform were

chosen as solvent to extract compounds with wide spectrum polarity strategy. Twenty two compounds, checked and identified by GC and GC-MS, characterized the ethanol seeds extracts (table 4). The most abundant components were fatty acids and esthers, typical components of seeds, as oleic acid (17,26%), methyloleate (7,77%), ethyloleate (5,27%) and diterpenes as neo-abietol (11,81%), abieta-7,13-diene (7,99%), 8(14),13(15)-abietadiene (7,92%), abietol (6,04%), abietal (2,75%), abieta-8,11,13-triene (2,05%). The ethanol extract was quite similar to the composition of cones ethanol extract for the presence of diterpenes abieta-7,13-diene (3,20%), 8(14),13(15)-abietadiene (0,27%), abieta-8,11,13-triene (1,00%) (Loizzo, 2008), but the most abudant and characteristic diterpene in our extract was neo-abietol.

GC-MS of chloroform seeds extract revealed instead the presence of twenty four components: the most interesting in terms of abudance were α-pinene (34,42%), β-pinene (33,28%), verbenol (3,16%), trans-pinocarveol (2,82%), abieta-7,13-diene (2,58%), myrtenol (1,99%), β-phellandrene (1,98%), β-farnesene (1,89%) and abieta-8,11,13-triene (1.50%). The composition of chloroform extract only partially reflected the results obtained with essential oil from female cones oleoresin of Cedrus libani grown in Turkey, for the presence of the α-pinene (24,78%), abieta-7,13-diene (16,67%), abieta-8,11,13-triene (6,85%) (Yilmaz, 2005), whereas the abundance of β-pinene was typical of leaf and wood volatile constituents (Fleisher, 2000).

4.3.5.1 Biological activity

In order to evaluate the efficacy of *C.libani* seed extracts in human chronic myelogenous leukemia, the antiproliferative activity on K562 cells were assayed. The *Cedrus libani* ethanol and chloroform seed extracts were able to exert antiproliferative activity against K562 cells with IC_{50} values of 40,57µg/ml and 69,20µg/mlrespectively (table 5). The C*edrus libani* ethanol

seeds extract showed a percentage of erythroid differentiation of 16 % at the concentration of 25µg.ml^{-1} while the chloroform seeds extract showed a percentage of erythroid differentiation of 12 % at the concentration50 µg.ml^{-1}. In this experiment we used as control the DNA binding compounds arabinose cytosine that is a potent inducer of erythroide differentiation in K562 cell line. The treatment of cell culture with 500 nM of AraC leads to 85% of benzidine-positive cells. Extracts obtained from Bangladeshi medicinal plant Aegle marmelos Correa have been previously tested for their antiproliferative activity on the same tumor cell line CML (Lampronti et al, 2003). Monoterpenes have been shown to exert chemopreventive as well as chemotherapeutic activities also in mammary tumor models and thus may represent a new class of therapeutic agents. The mechanism of action of monoterpenes is based on two main approaches, chemoprevention and chemotherapy (Morse and Stoner, 1993). Our evidences point out that seeds can be useful as sources of antiproliferative chemicals. β-pinene exibited antiproliferative activity against the cancer cells MCF-7, A375, Hep G2 (Li, 2009), α-pinene and β-pinene against human leukemic K562 cells (Lampronti, 2006): so they could be the possible active compounds in chloroform extract. In other hand, palmitic acid and methyl linoleate exhibited antiproliferation activity against human leukemic k562 cells (lampronti et al, 2003) So they are active compounds in ethanol extract, it comes to our attention that Chloroform and ethanol seeds extracts possess anti proliferative compounds against k562 cell line, we conclude that seeds of Cedrus libani could be useful as nutrient source for antiproliferative activity. Moreover, palmitic acid possess anti bacterial activity (Yff et al, 2002). Five abietane compounds were isolated from the neutral part of the hexane extract of the cone of Cedrus atlantica collected in Marocco (Barero et al, 2005). Ether extract from cones of Cedrus species C.Atlantica and C.libani have yield resin acid of abietane and related diterpenoide (Norin et al,1971).Abietane diterpenoid compounds were evaluated for there in vitro

antiproliferative activity against five human cancer cell lines MCF-7, NCI-H460, SF-268, TK-10, and AUCC-62, exhibited the strongest effect antiproliferative (Marques et al,2002). In conclusion, we reported in the present investigation the phytochemical composition and the antiproliferative activity of C.libani seed extracts against k 562 human chronic myolegenus leukemia cells. And further inverstigation should be done to evaluate other active compounds. Informations about antiproliferative activity of monoterpenes and diterpenes found in both extracts were not described in literature. Further investigations should be done to identify active compounds in ethanol extract.

4.3.6 Anti proliferative activity of three *Cedrus* species essential oils against k562,Biological activity, conclusion.

4.3.6.1 Biological activity.

In order to evaluate *C. liban, C. atlantica, C. deodara* essential oils in human chronic myelogenous leukemia, K562 cells were seeded at the initial cells concentration of 30 000 cells/ml and then cultured for 5 days in presence or absence of essential oils at concentrations ranging from 0.1 µg ml^{-1} to 100 µg/ ml. The essential oils of three cedrus species are able to exert antiproliferative activity against K562 cells with IC_{50} values of 23.38±1.7 µg /ml, 59.37±2.6 µg/ ml and 37.09±1.4 µg/ml. Meanwhile *Cedrus Libani* wood oils showed a percentage of erythroide differentiation of 15±2% at the concentration of 5µg ml^{-1}, another side *Cedrus Deodara* wood oil was found a percentage of erythroide differentiation of 20±2% at the concentration 25µg/ ml and *Cedrus Atlantica* wood oils indicated a percentage of erythroide differentiation of 12±1.8 % at the concentration 10µg/ml. In other hand, Manool are able to exert antiproliferative activity against K562 cell with IC_{50} value of 18.20±2.3 µg/ ml with proved a percentage of erythroide differentiation of 4±1.5% at the concentration 25 µg ml^{-1}(table6). Meanwhile, Manool was identified in our previous analysis essential oils wood of *Cedrus Libani* (Loizzo et al, 2008).

4.3.6.2 Conclusions

The essential oils obtained from different Lebanon officinal plants have been previously tested for their antiproliferative activity on the same tumor cell line (Lampronti et al.,2006).. Interesting results were obtained with the essential oil from *Satureja hortensis*, *S. montana* and *Mentha arvensis* with IC_{50} values of 85.4, 56.1 and 40.6 μg /ml respectively.The results also indicated that linalool was active in a similar manner against K562 cell line with an IC_{50} of 150.0 μg/ ml. Interestingly,in our previous study linalool exerted cytotoxic activity on amelanotic melanoma and renal cell adenocarcinoma with IC_{50} values of 23.16 μg ml^{-1} and 23.77 μg /ml, respectively (Loizzo et al., 2007). Moreover, the liquid tar obtains by destructive distillation of the *Cedrus Atlantica* was stated to be the best oil of the cade and its recognition in the French codex was recommended. The *Cedrus* genus in Turkey is represented by one species only (*Cedrus Libani*). A kind of tar is obtained from its resinous root and steam wood. This tar is usually employed for treating skin complaints of animals and for killing parasites, e.g. aphids and insects (Metin et al., 1999). Furthermore, the cones of *Cedrus Libani* possess anti-ulcerogenic remedies for anti-*Helicobacter pylori* activity (Yesilada et al., 1999). The antimicrobial activity of the ethanol extract of resins obtained from the roots and stems of *Cedrus Libani* was also investigated. Result revealed that crude extract of the resins of *Cedrus Libani* are highly effective against micro-organism by preventing their growth to a greater extent (Kizil et al., 2002). We investigated the essential oils and ethanol extracts derived from cones, leaves and wood of *Cedrus Libani* possess antiviral activity against Herpes Simples Virus type-I (Loizzo et al.,2008). Moreover we proved the essential oils and ethanol extracts derived from cones and wood of *Cedrus libani* possess anti-diabetic activity (Loizzo et al., 2007). An isolate CD lignan mixture comprising lignans from wood of *Cedrus Deodora* has cytotoxic potential against human cancer cell lines. It has

the ability to induce tumour regression in vitro .The volatile oil of *Cedrus Deodara* possess anti-inflammatory activity and it also inhibited heat-as well as solution induced haemolysis of erythrocytes in vitro (Shinde et al., 1999, Singh et al., 2007). Beside these previously biological activities of *Cedrus* species, we can confirm the results in this present study support the use of essential wood oils of the three *Cedrus* species as additive in traditional remedies for treatment of k562 human chronic myelogenous leukemia cells.

4.3.7 Gas chromatography/mass spectrometry analysis of Satureja montana and Satureja hortensis.

In order to identify putative active compounds present within *Satureja montana* essential oil, we employed a gas chromatography/mass spectrometry (GC/MS) system. We decided to analyze and compare *Satureja montana* and *Satureja hortensis. S. montana* and *S. hortensis* essential oils were dissolved in acetonitrile (2% V/V) and 1 µl of each solution was injected into the gas chromatograph using an appropriate microsyringe, chromatographed using a fused-silica capillary column and analyzed with a quadrupole mass spectrometric detector. (*Satureja montana; Satureja hortensis*) indicate that, in both assayed essential oils, seven derivatives are present, corresponding to α-pinene, γ-terpinen, 4-terpineol, α-terpineol, caryophyllene, τ-cadinene and τ-cadinol. The other identified molecules differ between the analyzed oils. All the compounds corresponding to the major peaks identified are shown in Tables 7 and 8 (*Satureja montana* and *Satureja hortensis,* respectively). The commercially available derivatives (α-pinene, ß-pinene, 1- octen-3-ol, 3-octanol, γ-terpinen, ß-linalool, borneol, 4-terpineol, carvacrol, caryophyllene, α-caryophyllene, caryophyllene oxide, eucalyptol, copaene and ±trans-nerolidol) were tested for their antiproliferative and differentiating activities on K562 cells.

4.3.7.1 Effects of essential oils on in vitro proliferation of human leukemic K562 cells.

The effects of increasing amounts of essential oils derived from *Satureja hortensis, Satureja montana, Salvia officinalis, Lavandula officinalis, Thymus vulgaris, Calamintha origanifolia, Foeniculum vulgare* and *Mentha arvensis* on cell proliferation of K562 cells. We analyzed two different essential oils derived from*L. officinalis* cultivated in silice and argile land. K562 cells were seeded at an initial concentration of 30000 cells/ml and then cultured for 7 days in the absence or presence of 0.05-500 μg/ml of all of the hydro-distillate essential oils. The obtained data show that the IC50 of *S. hortensis, S. Montana* and *M. arvensis* essential oils were 85.4± 2.4, 56.15± 6.15 and 40.6± 3.5 μg/ml, respectively. The essential oil extracted from *C. origanifolia* was completely unable to inhibit K562 cell growth at the used concentrations (IC50 >500 μg/ml), and the remaining extracts demonstrated an intermediate activity.

4.3.7.2 Effects on K562 erythroid differentiation.

Since it is well known that many antiproliferative agents are able to exert their anti-tumor effects by the activation of terminal programs of differentiation of targeted tumor cells, we tested the ability of all essential oils to induce the erythroid differentiation of K562 cells (33). K562 erythroid differentiation was analyzed as reported elsewhere by the benzidine-staining procedure (Bianchi et al.,2000; Gambari et al 2003). *C. origanifolia, F. vulgare* and *S. montana* induced differentiation (30-34%) at concentrations lower than that causing 50% inhibition of K562 cell growth. Also, *Thymus vulgaris* and *Salvia officinalis* induced intermediate levels of differentiation (25%), whereas the percentage of differentiation decreased (13-18%) when *Lavandula officinalis, Mentha arvensis* and *Satureja hortensis* were employed (Table 14). In our opinion,

Satureja montana differs from the other essential oils because its high antiproliferative effect is associated to the interesting ability to induce erythroid differentiation of K562 cells. Accordingly, *Satureja Montana* extracts were further analyzed to identify putative bioactive compounds.

4.3.7.3 Biological activities of pure compounds identified within essential oils from *Satureja montana and Satureja hortensis.*

The commercially available compounds present in *Satureja montana* and *Satureja hortensis* were analyzed for their antiproliferative activity and also for their possible effect on K562 differentiation, in order to identify putative active derivatives. We tested 15 pure compounds, identifying three derivatives present within the plant extracts very active in inhibiting K562 cell growth, including α-terpineol (IC50 =75.0 μM), caryophyllene (IC50 = 98.0 μM) and α-caryophyllene (IC50 = 98.7 μM). Caryophyllene and α-terpineol are present in both essential oils, but at a different concentration; caryophyllene represents 6.66% of relative composition in *Satureja montana* (Table 7) and 3.85% in *Satureja hortensis* (Table8), whereas α-terpineol represents 12.66% in *S. montana* and 0.38% in *S. hortensis*. In Table 15, the IC50 is reported for all compounds tested with respect to the ability to inhibit K562 cell growth. From the data shown in Table 15, it is clearly evident that some compounds exhibit no antiproliferative activity, e.g. eucaliptol, 1-octen-3-ol, 3-octanol and m-cymene (IC50 >400 μM). Three molecules found in both essential oils (α-pinene, α-terpineol and caryophyllene) exhibit high antiproliferative activity, even if at different concentrations (IC50 = 117.3 μM, 75.0 μM and 98.7 μM, respectively). Interestingly, all of these active compounds are more concentrated in *Satureja montana*, which is more active as crude extract than *Satureja hortensis* (IC50 = 56.15±6.15 μg/ml and 85.4±2.4 μg/ ml, respectively). Since *Satureja montana* induce erythroid differentiation of K562

cells (30% of induction with concentrations ranging between 0.05 and 50 μg/ml), we also analyzed the effects of the identified bioactive components utilizing the benzidinestaining procedure. No compound showed any detectable activity in inducing erythroid differentiation of K562 cells (Table 15). The obtained results suggest that the antiproliferative effects found in several active compounds of S*atureja montana*, including α-pinene, γ-terpinen, ß-linalool, terpineol, carvacrol, caryophyllene, caryophyllene oxide, copaene and ±transnerolidol, are not associated to induction of differentiation. The ability of *S. montana* extracts to induce erythroid differentiation could be ascribed to other unidentified or not commercially available pure compound(s) and/or to a combination of singularly inactive molecule.

4.3.8 Laurus Nobil, Essential oils composition antiproliferative activity

4.3.8.1 Essential oils composition

Aromatic plants had been used since ancient times for their preservative and medicinal properties, and to impart aroma and flavor to food. The pharmaceutical properties of aromatic plants are partially attributed to essential oils. Essential oils are natural, complex, multi-component systems composed mainly of terpenes in addition to some other non-terpene components. The diverse therapeutic potential of essential oils has drawn the attention of researchers to test them for anticancer activity, taking advantage of the fact that their mechanism of action is dissimilar to that of the classic cytotoxic chemotherapeutic agents (Rajesh et al., 2003). Early reports had indicated that essential oil components, especially monoterpenes, have multiple pharmacological effects on mevalonate metabolism which could account for the terpene-tumor suppressive activity (Elson, 1995),

On the basis of these considerations and of perusal of literature, in the present investigation we reported the antioxidant and antiproliferative activity on

human chronic myelogenous leukemia cells of *L. nobilis* leaves and seeds essential oils supporting by phytochemical analysis. As shown in (Table 11), twenty-six compounds characterized the essential oil obtained from the *L. nobilis* leaves. The most abundant components were 1, 8-cineole (35.15%), 1-*p*-menthen-8-ethyl acetate (13.52%), linalool (7.08%), sabinene (6.17%), and α-pinene (5.72%). The GC-MS profile of *L. nobilis* seeds oil revealed the presence of twenty-seven compounds, among them β-ocimene (21.83%), 1,8-cineole (9.43%), and β-pinene (2,14%) are the main component There are many studies on chemical composition of the essential oil obtained from the leaves of Mediterranean and European *L. nobilis* (Riaz et al., 1989; Lin et al., 1990; Baghdadi et al., 1993; Putievsky et al., 1994; Fiorini et al., 1997).

In a recent study the variation in the essential oil composition of *L. nobilis* of different growth stages cultivated in Iran was evaluated (Verdian-rizi, 2009). The oils obtained from the different phenological stages were found to have similar compositions. The main compounds were 1,8-cineole, *trans*-sabinene hydrate, α-terpinyl acetate, methyl eugenol, sabinene, eugenol, α-pinene, and α-terpineol. Thus the time of harvesting of this plant does not have a major effect on chemical composition of the essential oil but it effects on the essential oil content of the plant and the flowering stage is the best time for harvesting the plant and obtaining the essential oil because at this time the plant contains highest percent of the essential oil.

4.3.9 Antiproliferative activity

In order to evaluate the *L. nobilis* leaves and seeds essential oils in human chronic myelogenous leukemia, K562 cells were seeded at the initial cells concentration of 30.000 cells/ml and then cultured for 5 days in presence or absence of essential oils at concentrations ranging from 0.1 to 100 µg/ml. Both essential oils are able to exert antiproliferative activity against K562 cells with IC_{50} values of 95 and 75 µg/ml for leaves and seeds, respectively (Table 3).

The essential oils obtained from different Lebanon officinal plants have been previously tested for their antiproliferative activity on the same tumor cell line (Lampronti et al., 2006). Interesting results were obtained with the essential oil from *Satureja hortensis*, *S. montana* and *Mentha arvensis* with IC_{50} values of 85.4, 56.1 and 40.6 µg/ml, respectively. The other essential oils tested showed IC_{50} values ranging from 111.6 µg/ml for *Lavandula officinalis* to > 500 µg/ml for *Calamintha origanifolia*, respectively.

Since it is known that many antiproliferative agents are able to exert their antitumor activity by the activation of terminal programs of differentiation of targeted tumor cells, we have tested the ability of the *L. nobilis* leaves and seeds essential oil to induce the erythroid differentiation of K562 cell line (Hoffman et al., 1979; Bianchi et al., 1999; Chirabelli et al., 2003). The leaves essential oil showed a percentage of erythroide differentiation of 15% at the concentration of 10 µg/ml. A percentage of 12% was found for the seeds essential oil (at the concentration of 50 µg/ml) (Table 17).

In these experiment we used as control the DNA binding compounds arabinose cytosine (25 nM) that is a potent inducer of erythroide differentiation in K562 cell line. The obtained results suggested that the antiproliferative activity of *L. nobilis* is not associated to the induction of differentiation. However, when the essential oils of *L. nobilis* leaves and seeds are used with sub-optimal concentration of arabinose cytosine (AraC), a clear synergic effect was observed. The treatment of cell culture with 500 nM of AraC leads to 85% of benzidine-positive cells. When 25 nM of AraC were added with 10 µg/ml of leaves essential oil, the combination determined a significant increase of the proportion of benzidine-positive cells from 15% to 23%. A similar observation could be done with the association AraC (25 nM) *plus* 50 µg/ml of seeds oil. In fact, the increase of the proportion of erythroid differentiation rises from 12% to 25% (Table 4). Interestingly, the value of differentiation of the combination oils

and AraC is lower than mitramicyn relating to proportion of erythroid differentiation.

The commercially available compounds identified in the *L. nobilis* essential oils were previously tested for their antiproliferative activity and for their possible effect on K562 differentiation (Lampronti et al., 2006). α-Terpineol exhibited the highest cytotoxic activity with an IC_{50} value of 75.0 µg/ml. Other interesting results were obtained with α-pinene and α-copaene (IC_{50} values of 117.3 and 136.5 µg/ml, respectively).

The results also indicated that linalool was active in a similar manner against K562 cell line with an IC_{50} of 150.0 µg/ml. Interestingly, in our previous study linalool exerted cytotoxic activity on amelanotic melanoma and renal cell adenocarcinoma with IC_{50} values of 23.16 and 23.77 µg/ml, respectively (Loizzo et al., 2007).

The other commercially available compound tested was 1,8-cineole, that was the most abundant constituent in *L. nobilis* leaf oil. Interestingly, the cytotoxic activity of this oil could not be explained in terms of the 1,8-cineole content. In fact, this monoterpene was inactive against the cell line used in this study.

Monoterpenes have been shown to exert chemopreventive as well as chemotherapeutic activities also in mammary tumor models and thus may represent a new class of therapeutic agents. The mechanism of action of monoterpenes is based on two main approaches, chemoprevention and chemotherapy. Chemoprevention occurs during the initiation phase of carcinogenesis to prevent the interaction of chemical carcinogens with DNA, by induction of phase I and phase II enzymes to detoxify the carcinogen (Wattenberg, 1992). Chemotherapy works during the promotion phase, in which inhibition of tumor cell proliferation, acceleration of the rate of tumor

cell death and/or induction of tumor cell differentiation may occur (Morse and Stoner, 1993).

4.3.10 In Vitro hypoglycaemic and ACE inhibitor activities of traditional medicinal plants from Lebanon

4.3.10.1 Purpose

Marrubium radiatum ,Calamintha origanifolia, Saturja thymbra,Erythraea centaurium,Asperula glomerata,Hyssopus officinalis and *Salvia acetabulosa*-- were used for treatment of hypertension and diabetes in Lebanon traditional medicine. In order to find a scientific validation of their traditional use, *in vitro* assays for α-amylase and α-glucosidase inhibition were performed while the antihypertensive activity was analysed by the inhibition of Angiotensin Converting Enzyme (ACE).

4.3.10.2 Hypogliacemic activity

Monosaccaride glucose can be readily absorbed from gastro-intestinal tract into the blood stream after the hydrolysis of glycosidic bonds in digestible carbohydrate foods containing starch by the enzyme a-amylase and α-glucosidase. Although the activity of both enzymes have not been directly involved in the ethiology of diabetes, inhibitors have been thought to improve glucose tolerance in diabetic patients (Lebovit et al., 1998). The α-amylase and α-glucosidase bioassay method were adopted and modified from Sigma-Aldrich (Conforti et al., 2005).

4.3.10.3 Antihypertensive activity

It is well recognized that the Renin–Angiotensin System (RAS) has an important role in cardiovascular physiology, water-electrolyte balance, and cell function. Excessive activation of this system has been considered to be a main cause of hypertension. ACE is the most important regulatory site of RAS. The

in vitro ACE inhibitory activity was measured trough the cleavage of the chromophore-fluorophore labelled substrate dansyltriglycine by ACE into dansylglycine, which is quantitatively measured by HPLC (Loizzo et al., 2006).

4.3.10.4 Results and conclusion

Both *M. radiatum* and *S. acetabulosa* are able to inhibit a-amylase, in particular the MeOH extract exert highest activity with IC_{50} values of 61.12 and 91.16 mg/mL, respectively. *M. radiatum and S. acetabulosa n*-hexane extracts exhibited IC_{50} values of 438.81 and 205.50 mg/mL, respectively. Both species demonstrated a certain activity on α-glucosidase. In particular *M. radiatum* exerted the highest activity with IC_{50} of 68.82 and 114.64 mg/ml for MeOH and *n*-hexane, respectively). A less activity was found when *S. acetabulosa* was used (IC_{50} of 76.87 and 212.05 mg/ml for MeOH and *n*-hexane, respectively) The GC/MS analysis of both *n*-hexane extracts revealed the presence of sterols and fatty acids. In *S. acetabulosa* the cumarin alloimperatorin was also found in high percentage.In ACE-inhibition assay *M. radiatum* showed IC_{50} of 72.79 and 75.42 mg/mL for MeOH and *n*-hexane, respectively, while *S. acetabulosa* exhibited IC_{50} of 52.71 and 105.22 mg/mL.

Among the other species analysed in this work, the *n*-hexane and chloroform extracts of *Calamintha origanifolia* exerted the highest α-glucosidase inhibition activity (IC50 of 63.5 and 102.1µg/ml, respectively). Both extracts also inhibited α-amylase with IC50 values of 94.1 and 91.6 µg/ml, respectively. Fatty acids, their methyl esters and alkanes were detected in both extracts (Tables 9and 10)

It is of interest that the *Calamintha origanifolia* chloroform extract exhibited an interesting activity against ACE with IC50 of 106.2µg/ml. The *Erythraea centaurium* chloroform extract exhibited a good inhibitory activity on both digestive enzymes with an IC50 of 64.9 and 74.9µg/ml for α-amylase and α-

glucosidase, respectively and also against ACE but its methanol and *n*-hexane extracts exhibited no inhibition. The *Erythraea centaurium* chloroform extract revealed the presence of 29 compounds, mainly terpenes and fatty acids (Table 10).

Hyssopus officinalis extracts were active only on the α-glucosidase enzyme with IC50 values ranging from 127.3 to 908.4µg/ml. At the same time *Hyssopus officinalis n*-hexane extract exhibited a strong inhibitory potency against ACE (IC50 values of 52.0µg/ml). *Asperula glomerata* methanol extract inhibited both α-glucosidase and ACE (IC50 128.5 and 165.6µg/ml, respectively) with weaker effects against α-amylase (IC50 of 209.7µg/ml). *Satureja thymbra* chloroform extract exhibited a weak activity of against both digestive enzyme, α-amylase and α-glucosidase withIC50 of 351.6 and 289.8µg/ml, respectively, and against ACE. In conclusion, this study supports the traditional use of this species and further work is necessary in order to identify active principles responsible for the found activities.

4.3.11 In vitro Antiviral Activities of the Essential Oils of Seven Lebanon Species.

4.3.11.1 Chemical composition

The yields of essential oils ranged from 1.5 to 3.5% (Table 12). To identify putative active compounds present within the essential oils, gas-chromatography (GC) systems were employed. The chemical composition of the oils was reported in (Table 13). L. nobilis berry oil was characterized by the presence of β-ocimene (21.83%), 1,8-cineole (9.43%), α-pinene (3.67%), and β-pinene (2.14%) as major constituents. Two interesting sesquiterpenes, i.e., eremanthin (3.65%) and dehydrocostuslactone (7.57%), were also identified. T.orientalis oil was characterized by 43 constituents (86.68% of the total oil) in which the main components were α-pinene (35.72%), δ-3-carene (9.48%), and α-cedrol (9.55%). A total of 48compounds (82.39% of the total oil) were

identified in J. oxycedrus ssp. oxycedrus berry oil. α-pinene (27.4%) and β-myrcene (18.9%) were the major constituents. Other identified compounds were α-phellandrene (7.1%), limonene (6.7%), epibicyclosesquiphellandrene (2.3%), and δ-cadinene (2.2%). Forty-one components, representing 80.91% of the total, were identified in S. thymbra oil, in which *p*-cymene (10.76%), α-pinene (10.15%), thymol (9.92%), sabinene (8.64%), γ-terpinene (7.56%), carvacrol (4.98%), trans-caryophyllene (3.67%), β-pinene (2.90%), and linalool (2.81%) were the main abundant compounds. C. sempervirens ssp. pyramidalis oil was characterized by 19 components, representing 90.45% of the total oil. The main components were α-pinene (53.56%), α-terpinene (18.90%), thymol (3.84%), and terpinolene (3.15%). Twenty-six compounds were identified in S. officinalis (94.39% of the total oil) in which 1, 8-cineole (43.62%), α-thujone (12.99%), sabinene (6.97%), camphor (5.71%), α-pinene (4.72%), α-humulene (3.41%), α-terpineol (3.18%), and β-pinene (3.01%) were the major components. P. palaestina oil was characterized by 29 components, representing 79.82% of the total oil. The main components were sabinene (17.08%), limonene (8.56%), β-pinene (6.48%), γ-terpinene (6.33%), *p*-cymene (6.01%), and aromadendrene (3.99%).

4.3.11.2 Antiviral activities

In this study, we report the antiviral activity of seven essential oils obtained from berry, fruits, and leaves of different species collected in Lebanon. Results are summarized in Table 20. Our results demonstrated how L. nobilis berries oil exhibited an IC50 value of 120 mg/ml against SARS-CoV with a selectivity index (SI; TC50/IC50) of 4.2. An interesting activity with an IC50 value of 60 mg/ml was found when L. nobilis berry oil was incubated with HSV-1 virus. (Armaka et al.1999). reported the ability of isoborneol to completely inhibit HSV-1 replication, without affecting viral adsorption (Armaka et al., 1999). The content of this monoterpene in L. nobilis berry oil was found in low

percentage, and probably, therefore, it is not able to exert antiviral activity against HSV-1.

A certain activity against SARS-CoV was found for T. orientalis and J. oxycedrus ssp. oxycedrus oils with IC50 values of 130 and 270 mg/ml, and a SI of 3.8and 3.7, respectively. Interestingly, J. oxycedrus ssp. oxycedrus oil exhibited the highest activity against HSV-1 with a IC50 value of 200 mg/ml and a SI of 5. On the other hand, T. orientalis oil did not show any antiviral activity against HSV-1 when it was incubated under the same conditions. HSV-1 Growth was inhibited also when S. thymbra oil was used (IC50 of 220 mg/ml and SI of 4.6). The C. sempervirens oil did not exhibit any activity against HSV-1 (IC50>1000 mg/ml). These results may be related to the inactivity of the main component a-pinene as we have previously demonstrated (Loizo et al., 2007). A weak activity against SARS-CoV was found when C. sempervirens ssp. pyramidalis and S. officinalis essential oils were applied in virus culture (IC50 700 and 870 mg/ml, resp.). P. palaestina essential oil was inactive against SARS-CoV (IC50>1000 mg/ml) and less active against HSV-1 (IC50 500 mg/ml). Interestingly, L. nobilis, T. orientalis, and J. oxycedrus ssp. oxycedrus oils exhibited higher potencies to inhibit SARS-CoV and a great margin of safety compared to the positive control glycyrrhizin (IC50 641 mg/ml; SI 1.2).
Conclusions. – Severe acute respiratory syndrome (SARS) is an emerging diseaset hat created international anxiety because of its relatively high infectious, rapid progression and relatively high death rate. The fact that no conventional medicine was used for the treatment of SARS was based on the evidence that natural products from plants exhibited antiviral activity to other coronaviruses although the mechanism of action of these herbal products is mainly through inhibition of viral replication (McCutcheon et al.,2005). In this paper, we presented the first evidence for a strong antiviral activity of L. nobilis oil against SARS-CoV, and we also reported the interesting anti-herpetic

activity of J. oxycedrus ssp. oxycedrus and S. thymbra oils providing a potential use of these oils for treatment of viral infectious diseases.

Table 20: Anti viral activities of Lebanon essential oils

Essential oils	**Vero cells**	**HSV-1**	**HSV-1**	**SARS-COV**	**SARS-COV**
	TC50 µg/ml	**IC50 µg/ml**	**SI**	**IC50 µg/ml**	**SI**
L. nobilis	500±1.02	60±	8.3	120±1.2	4.2
T. orientalis	>1000	>1000	> 1	130±0.4	3.8
J. oxycedrus	1000±1.7	200±1.2	5	270±1.5	3.7
C.sempervirens pyramidalis	>1000	>1000	>1	700±2.3	1.5
P. palestina	500±0.8	500±2.2	>1	>1000	>1
S.officinalis	>1000	>1000	>1	870±1.5	>1
S. thymbra	>1000	220±1.6	4.5	-----	-----
Acyclovir	>22.5(100µM)	0.85(3,77µM)	26.5	-----	-----
Glycyrrhirin	783.4(952µM)	----	-----	641.0(779µM)	1.2

a) Results are shown as mean_SD, n=3. IC50 : concentration required to inhibit 50% of virus growth: TC50 : drug concentration that reduces the cell growth by 50% (cellular toxicity); SI=selectivity index (TC50/IC50); --- : not tested

4.3.12 Evaluation of the anti viral activity of Lebanese medicinal plants extracts against herpes simplex virus type 1 in vitro.

4.3.12.1 Anti viral activity.

Medicinal plants have been used since long time ago for treatment of many infectious diseases including bacterial and viral infection. The utilization of medicinal plants extract as drugs depends on their biological activities which

can provide our human-system health care services. During the last years, scientists try to increase the number of anti-viral drugs. Most of these substances belong to the class of nucleoside .e.g acyclovir and ribavirin (Fyfe et al., 1978). In our present investigation we report the anti-viral activity of eight medicinal plants which we are extracting with methanol solvent by maceration. Our results proved that medicinal plant extract of Calamintha origanifolia Vis exhibited (IC_{50}) value of 233µg/ml with selective index (SI) superior of 4,29.In other hand Satureja thymbra L exhibited (IC_{50}) of 250µg/ml with selective index (SI) of 4,55. More over Salvia accetabulosa L exhibited (IC_{50}) of 270µg/ml with selective index (SI) superior of 3,7. In other side, Centaurium erythreae L exhibited (IC_{50}) of 290 µg/ml with selective index (SI) superior of 3,45. Hyssopus officinalis L exhibit (IC_{50}) of 373µg/ml with selective index (SI) superior of 2,68. Sidiritis perforiata L exhibit (IC_{50}) of 410 µg/ml with selective index (SI) of 2,44. Asperula glomerata exhibit (IC_{50}) of 690 µg/ml with selective index (SI) of 1,45. Finally, Prangos aspurela Boiss exhibit (IC_{50}) of 660µg/ml with selective index (SI) superior of 1,5 (table21).

In conclusion, the eight methanol plant extracts possess anti viral activity which their selective index (SI) range between 1,5 and 4,5. This study merits other investigation on anti-viral activity by isolation and purification pure compound for these medicinal plant extracts witch possess anti-viral drugs in low concentration.

Table 21: Anti viral activity of Lebanese plant extracts against herpes simplex virus type 1 infection in vitro.

Plant extracts	TC_{50} (µg/ml)	IC_{50}(µg/ml)	SI
C.origanifolia	>1000	233 ± 1.1	4.29
S.thymbra	1000 ± 1.7	220 ± 0.8	4.55
P. aspurela	> 1000	660 ± 2.3	>1.5

S. Perforiata	1000 ± 1.9	410 ± 2.1	2,44
A.glomerata	>1000	690 ± 2.5	>1.45
H. officinalis	>1000	373 ± 1.7	2.68
E.Centaurium	>1000	290 ± 1.4	3,45
S. acetabulosa	1000 ± 2.2	270 ± 1.2	3.7
Acyclovir	>22,5(100µM)	0.85(3,77µM)	26.5

IC_5 : concentration required to inhibit 50% of virus growth; TC_{50}: drug concentration that reduces the cell growth 50% (cellular toxicity); SI: Selective index (TC_{50}/IC_{50})

Satureja thymbra

Salvia accetabulosa

Sidiritis perfoliata

Prangos aspurela

Erythreae centaurium

Hyssopus officinalis

Maribium Radiatum

Salvia officinalis

Juniperus oxycedrus

Laurus nobilis

Thuya oriontalis

Pistacia palestina

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6 References

Abo KA, Adeyemi AA and Adeite DA(2000). Ethnobotanical survey of plants used in the treatment of infertility and sexually transmitted diseases in southwest Nigeria. Afr J Med Med Sci 29: 325-327

Adams P (2007). Identification of essential oil components by Gas Chromatography/Mass Spectroscopy. Allured Publishing Co., Carol Stream, IL, USA.

Afifi FU, Khalil E, Tamimi SO, Disi A (1997). Evaluation of the gastroprotective effect of *Laurus nobilis* seeds on ethanol induced gastric ulcer in rats. J. Ethnopharmacol. 58, 9-14.

Ahmad R, Tripathi AK, Tripathi PA, Singh R, Singh S, Singh R.K(2008). Oxidative stress and antioxidant status in patients with chronic myeloid leukemia. Indian J. Clin. Biochem. 23, 328-333

Ahmad I, Mehmood Z and Mohammad F (1998). Screening of some Indian medicinal plants for their antimicrobial properties. J Ethnopharmacol 62: 183-193,

Alexander R. Maltodextrins (1992). production, properties and applications. In: Shenk F, Hebeda R, editors. Starch hydrolysis products; world wide technology: production and applications. New York: VCH Publishers; 1992. p. 62.

Armaka M, Papanikolau E, Sivropoulou A, Arsenakis M (1999). Antiviral properties of isoborneol, a potent inhibitor of herpes simplex virus type 1. Antiviral Res. 43, 79–92.

Angelini LG, Carpanese G, Cioni PL, Morelli I, Macchia M and Flamini G (2003). Essential oils from Mediterranean lamiaceae as weedgermination inhibitors. J Agric Food Chem 51: 6158-6164,

Ankli A, Heinrich M, Bork P, Wolfram L, Bauerfeind P, Brun R, Schmid C, Weiss C, Bruggisser R, Gertsch J, Wasescha M and Sticher O: Yucatec Mayan(2002). medicinal plants: evaluation based on indigenous uses. J Ethnopharmacol 79: 43-52.

Anonymous. (1978) *Sigma Tech. Bull No. 510 6/76* Sigma Chemical Company,

St. Louis, MO, USA. 63178

Assimopoulou AN and Papageorgiou VP (2005). GC-MS analysis of penta- and tetra-cyclic triterpenes from resins of Pistacia species. Part I. *Pistacia lentiscus* var. Chia. Biomed Chromatogr 19: 285-311

Baghdadi HH, Ahmad SS, Fournier G, and Refaat A M (1993). On the essential oil of *Laurus nobilis* grown in Egypt. Egypt. J. Horticul. 19, 93-97.

Banerjee A, Dasgupta N (2005). *In vitro* study of antioxidant activity of *Syzygium cumini* fruit. Food Chem. 90, 727-733.

Barbour EK, Al sharif M,Sagherian Vatche K, Habre Ak,Talhouk RS, Talhouk SN (2004). Screening of selected plants of Lebanon for antibacterial activity. Ethnopharmacology, Volume93, issue1, page 1-7.

Baytop T. Türkiyé de bitkiler ile tedavi gecmiste ve bugun. Istanbul Universitesi Yayinlan Yayin no 3255, Ecz. Fak. o:40,sayfa203.Istanbul.Türkiyé.

Bean, B, (1992). Antiviral therapy: current concept and practices. Clin. Microbiol. Rev. 5, 146-182

Benencia F, Courreges MC (1999). Antiviral activity of sandalwood oil against herpes simplex viruses-1 and -2. Phytomedicine 6, 119–123.

Bourne, KZ, Bourne N, Reising SF, Stanberry L.R,(1999). Plant products as topical microbicide candidates: assessment of in vitro and in vivo activity against herpes simplex virus type 2. Antiviral Res. 42, 219–226.

Bianchi N, Ongaro F, Chiarabelli C, Gualandi L, Mischiati C, Bergamini P and Gambari R (2000). Induction of erythroid differentiation of human K562 cells by cisplatin analogs. Biochem Pharmacol 60: 31-40

Bianchi N, Osti F, Rutigliano C, Corradini FG, Borsetti E, Tomassetti M, Mischiati C, Feriotto G, Gambari R, (1999). The DNA-binding drugs mithramycin and chromomycin are powerful inducers of erythroid differentiation of human K562 cells. Br. J. Haematol. 104, 258-265.

Bianchi N, Chiarabelli C, Borgatti M, Mischiati C, Fibach E, Gambari R(2001). Accumulation of gamma-globin mRNA and induction of erythroid differentiation after treatment of human leukaemic K562 cells with tallimustine. Br. J. Haematol. 113, 951-961.

Bianchi Nicoletta, Chiarabelli christiano, Borgatti Monica, Mischati carlo Fibach ethian and Gambari Roberto (2001). Accumulation of γ-globin mRNA and induction of erythroid differentiation after treatment of human leukaemic k562 cells with tallimustine Brith journal of haematelogy 113, 951-96

Bruno M, Bondi ML, Rosselli S, Maggio A, Piozzi F and Arnold NA (2002) Neoclerodane diterpenoids from *Teucrium montbretii* subsp. libanoticum and their absolute configuration. J Nat Prod 65: 142-146

Buhajar JA, Podesta MT, Wilson AP, Micallef M.J, Ali S(1999). The induction of apoptosis in human melanoma, breast and ovarian cancer cell lines using an essential oil extract from the conifer *Tetraclinis articulata*. Anticancer Res. 6B, 5435-5443.

Cassady KA, Whitley RJ (1997). New therapeutic approaches to the alphaherpesvirus infections. J. Antimicrob. Chemother. 39, 119–128.

Chancy R, William J(1993). Cedrus Libani. J. Tree of the month: 13,26-27.

Chang FR, Hsieh TJ, Huang TL, Chen CY, Kuo RY, Chang YC, Chiu HF and Wu YC(2002) Cytotoxic constituents of the stem bark of Neolitsea acuminatissima. J Nat Prod 65: 255-258,

Chen Z, Wang C, Xu X, Feng W (2009). Cost-effectiveness study comparing imatinib with interferon-alpha for patients with newly diagnosed chronic-phase (CP) chronic myeloid leukemia (CML) from the Chinese public health-care system perspective (CPHSP). J.Value Health ,12,85-8

Chirabelli G, Bianchi N, Borgatti M, Prus E, Fibach E, Gambari R (2003). Induction of gamma-globin gene expression by tallimustine analogs in human erythroid cells. Hematologica 88, 826-827.

Choi HK, ChoiYH, Verbeme M, Lefeber AW, M, Erkelens C, Verpoorte R (2004). Metabolic fingerprinting of wild type and transgenic tobacco plants by $_1$HNMR and multivariate analysis technique. Phytochemistry 65, 857-864.

Chowduhry L, Khan ZK, Kulshrestha DK (1997). Indian J Exp Biol ,35:727

Cinatl Jr., J., Cinatl, J., Rabenau, H., Kornhuber, B., Doerr, H.W (1992). HeLa cells grown continuously in protein-free medium: a novel model for the study of virus replication. Intervirology 33, 41–48.

Clevenger J.F (1928). Apparatus for the determination of volatile oils. J. Am. Pharm. Assoc. 17, 341-346.

Conforti F, Statti G, Loizzo MR, Sacchetti G, Poli F, Menichini F. Biol Pharm Bull (2005) , 28:1098.

Conforti F., Statti G., Uzunov D., Menichini F (2006). Comparative chemical composition and antioxidant activities of wild and cultivated Laurus nobilis L. leaves and *Foeniculum vulgare* subsp. *piperitum* (Ucria) coutinho seeds. Biol. Pharm. Bull. 29, 2056-2064.

Cortesi R., Gui V., Osti F., Nastruzzi C., Gambari R (1998). Human leukemic K562 cells treated with cytosine arabinoside: enhancement of erythroid differentiation by retinoic acid and retinol. Eur. J. Haematol. 61, 295-301.

Cragg GM and Newman DJ (1999), Discovery and development of antineoplastic agents from natural sources. Cancer Invest 17: 153-163

Dalle-Donne I., Rossi R., Colombo R., Giustarini D., Milazani A (2006). Biomarkers of oxidative stress in human disease. Clin. Chem. 52, 601-23.

Datta BK, Rahman I and Das TK: Antifungal activity of Indian plant extracts (1998). Mycoses 41: 535-536

De Logu, A., Loy, G., Pellerano, M.L., Bonsignore, L., Schivo, M.L (2000). Inactivation of HSV-1 and HSV-2 and prevention of cell-to-cell virus spread by Santolina insularis essential oil. Antiviral Res. 48, 177–185.

Digrak, M., Ilcim, A., Hakki Alma, M (1999). Antimicrobial activities of several parts of Pinus brutia, Juniperus oxycedrus, Abies cilicia, Cedrus libani and Pinus nigra. Phytother Res. 13, 584–587.

Ducrey M, Huc R, Ladjal M, Guehl JM (2008). Variability in growth, carbon isotope composition, leaf gas exchange and hydraulic traits in the eastern Mediterranean cedars Cedrus libani and Cedrus brevifolia. Tree Physiol 28:689-701.

Elson C (1995). Suppression of mevalonate pathway activities by dietary isoprenoids: protective roles in cancer and cardiovascular disease. J. Nutr. 125, 1666S-1672S.

FAO (2004). Trade in medicinal plants. In: Economic and Social Department, Food and Agriculture Organization of the United Nations. Rome, 2-3.

Ferrea, G., Canessa, A., Sampietro, F., Cruciani, M., Romussi, G., Bassetti, D (1993). In vitro activity of a Combretum micranthum extract against herpes simplex virus types 1 and 2. Antiviral Res. 21, 317–325.

Fiorini C., Fouraste David I.B. and Bessiere J.M (1997). Composition of the flower, leaf and stem essential oils from *Laurus nobilis* L. Flavour Fragr. J. 12, 91-93.

Gambari R (2003a). *In vitro* antiproliferative effects on human tumor cell lines of extracts from the Bangladeshi medicinal plant *Aegle marmelos* Correa. Phytomedicine **10**, 300-308.

Gambari R(2003). The human erythroleukemia K562 cell culture system for identification of inducers of fetal haemoglobin. Minerva Biotecnologica 15, 123-128.

Gambari R and Fibach E: Induction of fetal hemoglobin by DNA-binding drugs. Minerva Biotecnologica 15: 145-151,

Gibson and Williams (2000). In: GR. Gibson and CM. Williams, Editors, Functional foods. Concept to product, CRC Press, Boca Raton.

Graham JG., Quinn ML, Fabricant DS, Farnsworth NR (2000). Plants used against cancer: An extension of the work of Jonathan Hartwell. J Ethnopharmacol 73, 347-377.

Gulluce M, Sokmen M, Daferera D, Agar G, Ozkan H, Kartal N, Polissiou M, Sokmen A and Sahin F (2003). *In vitro* antibacterial, antifungal, and antioxidant activities of the essential oil and methanol extracts of herbal parts and callus cultures of *Satureja hortensis* L. J Agric Food Chem 51: 3958-3965

Hafizoğlu B, Reunanen M (1993). Studies on the components of *Laurus nobilis* from Turkey with special reference to laurel berry fat. Fat Sci. Technol. 95, 304-308.

Halliwell B., Gutteridge JMC (1999). Free radicals in biology and medicine. 3rd Edition. Oxford University Press

Hammer KA, Carson CF, Riley TV (1999). Antimicrobial activity of essential oil and other plant extracts. J. Appl. Microbiol. 86, 985–990.

Harzemasah H (1987). Studies on the chemistry of Cedrus Libani-rich I.J.Holzforshung,41,27-38

Harzemasah H (1987). Studies on the chemistry of Cedrus Libani-rich II.J.Holzforshung,41,73-75

Hedberg I: Botanical methods in ethnopharmacology and the need for conservation of medicinal plants (1993). J Ethnopharmacol 38: 121-128

Heinrich M and Gibbons S: Ethnopharmacology in drug discovery: an analysis of its role and potential contribution (2001). J Pharm Pharmacol 53: 425-432

Hoffman R, Murnane MJ, Benz EJ., Prohaska R, Floyd V, Dainiak N, Forget B.G, Furthmayr H (1979). Induction of erythropoietic colonies in a human chronic myelogenous leukemia cell line. Blood 54, 1182-1187.

Ikeda, T., Ando, J., Miyazono, A., Zhu, X.H., Tsumagari, H., Nohara, T., Yokomizo, K., Uyeda, M (2000). Anti-herpes virus activity of Solanum steroidal glycosides. Biol. Pharm. Bull. 23, 363–364.

Irshad M, Chaudhuri PS (2002). Oxidant-antioxidant system: Role and significance in human body. Ind J Exp Biol 40, 1233-1239.

Iyamu E.W., Adunyah S.E., Fasold H., Horiuchi K., Baliga S., Ohene-Frempong K., Turner E.A., Asakura T (2003). Combined use of non myelosuppressive nitrosourea analogues with hydroxyurea in the induction of F-cell production in a human erythroleukemic cell line. Exp. Hematol. 31, 592-600.

Jose JK, Kuttan G and Kuttan R (2001). Antitumour activity of *Emblica officinalis*. J Ethnopharmacol 75: 65-69

Juliani Jr., H.R., Biurrun, F., Koroch, A.R., Oliva, M.M., Demo, M.S., Trippi, V.S., Zygadlo, J.A (2002). Chemical constituents and antimicrobial activity of the essential oil of Lantana xenica. Planta Med. 68, 762–764.

Jung B, Matzke M, Stoltefuss J (1996). Chemistry and structure activity relationship of glucosidase inhibitors. In: Kulhmann J, Puls W, editors. Handbook of experimental pharmacology: oral antidiabetics, vol. 119. Berlin: Springer.

Khan, M.T.H., Ather, A., Thompson, K.D., Gambari, A (2005). Extracts and molecules from medicinal plants against herpes simplex viruses. Antiviral Res. 67, 107–119.

Khan MT, Lampronti I, Martello D, Bianchi N, Jabbar S, Choudhuri MS, Datta BK and Gambari R (2002). Identification of pyrogallol as an antiproliferative compound present in extracts from the medicinal plant *Emblica officinalis*: effects on *in vitro* cell growth of human tumor cell lines. Int J Oncol 21: 187-192, 2002.

Kar K, Puri VN, Patnaik GK, Sur RN, Dhawan BN, Kulshrestha DK, et al. J(1975). Pharm Sci 64:258.

Katsube N, Iwashita K, Tsushida T, Yamaki K and Kobori M (2003). Induction of apoptosis in cancer cells by Bilberry (*Vaccinium myrtillus*) and the anthocyanins. J Agric Food Chem 51: 68-75,

Kizil M, Kizil G, Yavuz M. Priki (2002). Antimicrobial activity of Resins obtained from roots and stems of Cedrus libani and Abies cilicica. Biochemistry and microbiology, 38:166-8.

Lampronti I., Martello D., Bianchi N., Borgatti M., Lambertini E., Piva R., Jabbar S., Choudhuri M.S., Khan M.T., Gambari R (2003a). *In vitro* antiproliferative effects on human tumor cell lines of extracts from the Bangladeshi medicinal plant *Aegle marmelos* Correa. Phytomedicine 10, 300-308.

Lampronti I., Bianchi N., Borgatti M., Fibach E., Prus E., Gambari R(2003b). Accumulation of gamma-globin mRNA in human erythroid cells treated with angelicin. Eur. J. Haematol. 71, 189-195.

Lampronti I., Saab A.M., Gambari R (2006). Antiproliferative activity of essential oils derived from plants belonging to the Magnoliophyta division. Int. J. Oncol. 29, 989-995.

Lee, K. G., Shibamoto, T (2001). Inhibition of malonaldehyde formation from blood plasma oxidation by aroma extracts and aroma components isolated from clove and eucalyptus. Food Chem. Toxicol. 39, 1199–1204.

Lev E (2002). Some evidence for the use of doctrine of signatures in the land of Israel and its environs during the Middle Ages. Harefuah 141: 651-655

Lin, Z.K., Hua, Y.F., Gong, G.P., Gu, Y.H (1990). Chemical constituents of the essential oils from the leaves of *Laurus nobilis* and tendency in changes of the constituents month by month. Acta Botanica Sinica 32, 878-882.

Lebovit H (1998). Diabets Rev;6:132

Loizzo M, Saab A, Lampronti I,Gambari R,Menichini F,Cinatl J,Dorr W (2008).Phytochemical analysis and in Vitro evaluation of the biological activity against herpes simplex virus type-1(HSV-1)Cedrus Libani essential oil. Phytomedecine 15, 79-83

Loizzo M, Saab A, Sttati G, Menichini F (2007). Phytochemical composition and in vitro α-Amylase inhibitory effect of essential oils from Cedrus libani. A.Rich fitoterapia 78 ,323-326

Loizzo, M.R., Tundis, R., Menichini, F., Saab, A.M., Statti, G.A., Menichini, F., (2007). Cytotoxic activity of essential oils from Labiatae and Lauraceae families against tumor *in vitro* models. Anticancer Res. 27, 3293-3300.

Loizzo, MR., Said, A., Tundis, R., Hawas, UW., Rashed, K., Menichini, F., Frega, NG., Menichini, F (2009). Antioxidant and antiproliferative activity of *Diospyros lotus* L. extract and isolated compounds. Plant Foods Human Nutr. 64, 264-270.

Loizzo, M.R., Menichini, F., Tundis, R., Bonesi, M., Nadjafi, F, Saab, AM, Frega, NG, Menichini, F(2010). Comparative chemical composition and antiproliferative activity of aerial parts of *Salvia leriifolia* Benth. and *Salvia acetabulosa* L. essential oils against human tumor cell in vitro models. J. Med. Food. 13, 62-69.

Loizzo M. R. , Tundis R. , Statti G. A. , Menichini F (2005) " In vitro antiproliferative effects on human tumor cell lines of extracts and jacaranone from Senecio leucanthemifolius Poiret". *Journal of Pharmacy and Pharmacology* Vol. 57, n. 7, pp. 897-902.

Loizzo M.R., Said A., Tundis R., Rashed K., Statti G.A., Hufner A. and Menichini F (2006). Inhibition of angiotensin converting enzyme (ACE) by flavonoids isolated from *Ailanthus excelsa* (Roxb) (Simaroubaceae.P*hytoterapy Research* 21 (1), 32-36

Lu R, Paul C, Basar S, König WA, Hashimoto T, Asakawa Y (2003) Sesquiterpene constituents from the liverwort Bazzania japonica. Phytochemistry 63:581-58

Manzoor-i-Kuda M, Slaque MA (1964).Constituent of Cedrus Deodara wood. Part I. Isolation of dewarene and dewarol. J.Sciences Indian research,6, 201-203.

Mau J, Ko PT, Chyau, C (2003). Aroma characterization and antioxidant activity of supercritical carbon dioxide extracts from *Terminalia catappa* leaves. Food Res. Int. 36, 97-104.

Meepagala, K.M., Sturtz, G., Wedge, D.E (2002). Antifungal constituents of the essential oil fraction of *Artemisia dracunculus* L. var. *dracunculus*. J. Agric. Food Chem. 50, 6989–6992.

Meskin, M.S., Bidlack, W.R., Davies A.J., Omaye, S.T (2002). Phytochemicals in Nutrition and Health, CRC Press, Boca Raton.

Metin D, Ahmet I, Hakki A (1999). Antimicrobial activities of several parts of Pinus brutia juniperus oxycedrus,Abies Cilicia,Cedrus libani and pinus nigra..Phytoterapy.Reseaech, 13: 584-87.

Morse, M., Stoner, G (1993). Cancer chemoprevention: principle and prospects. Carcinogenesis 14, 1737-1746.

Mouhamad I, Moussa JS (1992). Antibacterial diterpenes from the leaves and seeds of Juniperus Excelsa. J. Phytotherapy research, 6,261-264.

Mukherjee AK, Basu S, Sarkar N and Ghosh AC (2001). Advances in cancer therapy with plant based natural products. Curr Med Chem 8: 1467-1486, 2001

Navarro, J., Obrador, E., Carretero, J., Petschen, I., Avino, J., Perez, P., Estrela, J.M (1999). Change in glutathione status and the.antioxidant system in blood and in cancer cells associate with tumor growth in vivo. Free Radic. Biol. Med. 26, 410-18.

Newman DJ, Cragg GM (2007). Natural products as sources of new drugs over the last 25 years. J. Nat. Prod. 70, 461-477.

Pinn G (2001). Herbs used in obstetrics and gynaecology . Aust Fam Physician 30: 351-35

Plattier M, Teisserie P (1972).Contribution a la connaissance de l'huile essentielle de cedre de L'Atlas.131-134

Popov AM, Atopkina LN, Uvarova NI and Elyakov GB (2001). The antimetastatic and immunomodulating activities of ginseng minor glycosides. Dokl Biochem Biophys 380: 309-312

Putievsky E., Ravid U., Snir N., Sanderovich, D (1994). The essential oils from cultivated bay Laurel (*Laurus nobilis*). Isr. J. Botany. 33, 47-52.

Rajesh D, Howard P (2003). Perillyl alcohol mediated radiosensitization via augmentation of the Fas pathway in prostate cancer cells. Prostate **57**, 14-23.

Rathore, R., Elfenbein, G.J (2003). The chronic leukemias: current and novel therapeutic approaches. Med Health R I. 86, 240-242.

Rates S.M.K., 2001. Plants as source of drugs. Toxicon 39, 603-613.

Reichling, J., 2001. Australian and New Zealand tea tree oil as the source of noval antiinfective agents. In: Wildi, E., Wink, M. (Eds.), Trends in Medicinal Plant Research. Romneya- Verlag, Dossenheim, pp. 69–80.

Riaz, M., Ashraf, C.M., Chaudhary, F.M (1989). Studies of the essential oil of the Pakistani *Laurus nobilis* Linn in different seasons. Pak. J. Sci. Indust. Res. 32, 33-35.

Richardson MA (2001). Biopharmacologic and herbal therapies for cancer: research update from NCCAM. J Nutr 131: 3037-3040

Ruffa MJ, Ferraro G, Wagner ML, Calcagno ML, Campos RH and Cavallaro L: Cytotoxic effect of Argentine medicinal plant extracts on human hepatocellular carcinoma cell line. J Ethnopharmacol 79: 335-339, 2001.

Sarkar Ruffa MJ, Ferraro G, Wagner ML, Calcagno ML, Campos RH and Cavallaro L (2001). Cytotoxic effect of Argentine medicinal plant extracts on human hepatocellular carcinoma cell line. J Ethnopharmacol 79: 335-339

Sarkar, A., Bishayee, A., Chatterjee, M (1995). Beta-carotene prevents lipid peroxidation and red blood cell membrane protein damage in experimental hepato carcinogenesis. Cancer Biochem. Biophys. 15, 111-125.

Saab A, Harb Y, Koenig W (2005). essential oil components in heart wood of Cedrus libani and Cedrus Atlantica from Lebanon.Minerva Biotec ,17,159-61.

Saab A, Harb Y, Koenig W (2009). essential oil components in the leaves of Cedrus Libani and cedrus deodara from Lebanon.Minerva Biotec ,21(4),201-6.

Saller, R., Reichling, J., Hellenbrecht, D., 1995. Phytotherapie– Klinische, Pharmakologische und Pharmazeutische Grundlagen. Haug-Verlag, Heidelberg. Schuhmacher, A., Reichling, J., Schnitzler, P (2003). Virucidal effect of peppermint oil on the enveloped viruses herpes simplex virus type 1 and type 2 in vitro. Phytomedicine 10, 504–510.

Sharma N, Trikha P, Athar M and Raisuddin S (2000). *In vitro* inhibition of carcinogen-induced mutagenicity by *Cassia occidentalis* and *Emblica officinalis*. Drug Chem Toxicol 23: 477-484

Sharma PR, Shanmugavel M, Saxena AK, Qazi GN (2008). Induction of apoptosis by a synergistic lignan composition from Cedrus deodara in human cancer cells. Phytother Res. 22,1587-94.

Shashi B, Jaswant S, Madhusudana RJ, Kumar SA, Nabi QG (2006). A novel lignan composition from Cedrus deodara induces apoptosis and early nitric oxide generation in human leukemia Molt-4 and HL-60 cells. Nitric Oxide ,14:72-88.

Sayyah, M., Saroukhani, G., Perovi, A., Kamalinejad, M (2003). Analgesic and anti-inflammatory activity of the leaf essential oil of *Laurus nobilis* Linn. Phytother. Res. 17, 733-736.

Shinde UA, Phadke AS, Nair AM, Mungantiwar A, Dikshit VJ and Saraf MN. Membrane stabilizing activity (1999). A possible mechanism of action for the anti-inflammatory activity of Cedrus deodara wood oil. Fitoterapia 1999; 70:333-339.

Shinde UA, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ, Saraf MN (1999). Studies on the anti-inflammatory and analgesic activity of Cedrus deodara (Roxb.) Loud. wood oil. J Ethnopharmacol; 65:21-27.

Singh SK, Shanmugavel M, Kampasi H, Singh R, Mondhe DM, Rao JM, Adwankar MK, Saxena AK, Qazi GN (2007). Chemically standardized isolates from Cedrus deodara stem wood having anti cancer activity. Planta Medica 73:519-26.

Skocibusic M and Bezic N: Phytochemical analysis and *in vitro* antimicrobial activity of two Satureja species essential oils. Phytother Res 18: 967-970, 2004.

Sokmen A., Gulluce M., Akpulat H.A., Daferera D., Tepe B., Polissious M., Sokmen M., Sahin F (2004). The *in vitro* antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic *Thymus spathulius*. Food Control 15, 627–634.

Suhaj M (2006). Spice antioxidants isolation and their antiradical activity: A review. J. Food Comp. Anal. 19, 531–537.

Tatman D and Mo H (2002).Volatile isoprenoid constituents of fruits, vegetables and herbs cumulatively suppress the proliferation of murine B16 melanoma and human HL-60 leukemia cells. Cancer Lett 175: 129-139, 2002.

Tranchant J, (1995). *Manuel Pratique de Chromatographie en phase Gazeuse*. Masson, Paris.

Tiwari AK, Srinivas PV, Kumar SP, Rao JM. Free radical scavenging active

components from Cedrus deodara (2001). J Agric Food Chem ;49:4642-4645.

Verdian-rizi M (2009). Variation in the essential oil composition of *Laurus nobilis* L. of different growth stages cultivated in Iran. J. Basic Appl. Sci. 5, 33-36.

Wattenberg L (1992). Inhibition of carcinogenesis by minor dietary constituents. Cancer Res. 52, 2085s-2091s.

Whitley RJ, Kimberlin DW, Roizman B (1998). Herpes simplex viruses. Clin. Infect. Dis. 26, 97–109

Wargovich MJ, Woods C, Hollis DM and Zander ME (2001). Herbals, cancer prevention and health. J Nutr 131: 3034-3036

Williamson EM (2001). Synergy and other interactions in phytomedicines. Phytomedicine 8, 401-409.

Yamasaki K, Nakano M, Kawahata T, Mori H, Otake T, Ueba N,Oishi I, Inami R, Yamane M, Nakamura M, Murata H and Nakanishi T (1998) Anti-HIV-1 activity of herbs in Labiatae. Biol Pharm Bull 21: 829-833,

Yesilada E, Gurbuz I, Shibata H (1999). Screening of Turkish anti- ulcerogenic folk remedies for anti-Helicobacter pylori activity. J Ethnopharmacology, 66,289-293

Yesilada E, Fugita T(1979). Screening of some Turkish medicinal plants of their anti-ulcerogenic activities.J. Phytoterapy,7,236-240.

Zhang J, Meltzer P, Jenkins R, Guan XY, Trent J (1993). Application of chromosome microdissection probes for elucidation of BCR-ABL fusion and variant Philadelphia chromosome translocations in chronic myelogenous leukemia.J.Blood,;81(12):3365-71.