

## UPLC analysis of phenolic compounds in *Matricaria chamomilla*

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**Background and Aims:** The effects of honeybee venom therapy have been known since prehistoric times. It has been found to have a marked effect on variety of diseases, including arthritis, angiocardopathy, back pain, musculoskeletal pain, cancerous tumors, anticancer, and multiple sclerosis the immune system, cardiovascular system. It is a typical representative of biologically active peptide drugs with high therapeutic potential. Bee venom contains a complex mixture of biologically active agents, such as peptides, enzymes, biogenic amines, etc. The main components of Bee venom are enzymes: phospholipase A2 (12-15%), hyaluronidase (1-3%), peptides: melittin (approx. 50%), mast cell degranulating (MCD) peptide (2%), apamin, and promelittin. The aim of the study was to survey the amount of melittin as major compound in several honeybee venom dried samples by reverse-phase high-performance liquid chromatography (RP-HPLC).

**Methods:** A HPLC method with photodiode array (PDA) detector was used for determination of melittin and in crude honeybee venom dried powder as the traditional medicine possessing specific biological activity. Melittin of samples was extracted with deionised water followed by HPLC–PDA analysis on a C18 column of Europa Protein with water and acetonitrile containing 0.1% TFA as mobile phase under gradient elution at 220 nm.

**Results:** In this study, by applying this methodology the difference in melittin content of honeybee venom samples was evaluated. The melittin amount ranged from 8.2 to 51.3%. The reason of difference in melittin contents between samples can be probably due to different origin of samples, the use of different sampling methods and drying and maintain conditions.

**Conclusions:** HPLC analysis revealed a considerable difference in melittin of honeybee venom powdered samples. The findings showed that one of the samples is standard and the rest samples have to be subjected to further purification process.

**Keywords:** *Matricaria chamomilla*; Flavonoids; UPLC

## Herb remedies prescriptions in Alang, a village in Golestan province: Medical ethnography's fragments

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**Background and Aims:** Each climate and each land has its own herbal drugs. So there aren't so many herbs in one place and this is the philosophy of creation that each place has its own herbal drugs appropriate to the special diseases that exist in that place. Alang is one of Kordkuy's suburbs of Golestan in Iran. This village is full of various herbal drugs, because it is near Derazno mountain, Radkan and Pateno. This research is going to study the herbal drugs prescriptions of Alang's local doctors.

**Methods:** This article is covering a great study about people's local medicine in Alang village. To collect information interviews with local doctors were held and there were observations of documents that exist. Notes were taken and interviews were recorded and classified and also specified by numbers.

**Results:** People in Alang are using many herbal drugs that exist in their lands to cure diseases. Local doctors are using "Marbo" to cure stomachache, borage to cure pneumonia, plantago to cure cold, violet for fever, cinnamon for stomachache, "Kondos" for microbicide, and it is also anti urea and anti fat. "Valik" as micribicide and gall bladders diseases and haematogenesis. "More" is used to cure new born's stomachache and crab-apple is used to cure fat. In the original and extended part of the text each of these prescriptions is explained in detail.

**Conclusions:** Herbal drugs are common in caring diseases and they are popular in common medicine. If we study them in detail and more seriously then we can improve health cure and remedy in our country. It is essential to record the information in this case which is like a candle and if it isn't recorded, it would be put off in the destroying storm of changes, otherwise it would be too late.

**Keywords:** Herbal drugs; Common medicine; Local doctors; Herb prescriptions; North of Iran

## Herbal drugs in house curing among people of Rahmat Abad in Fazelabad village in Golestan

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**Background and Aims:** providing and finding easy ways to cure different diseases and to quiet pains was important and considerable. According to the tendency of people to use current herb remedies that are natural and available. Researchers decided to study current and popular and traditional herbal drugs among people in Rahmat Abad in Fazel Abad in Golestan.

**MethodS:** In this study there are interviews with people who had experienced curing disease with herbal house curing the interviews were written and recorded, classified and specified by numbers. The instruction of using of these cures were questioned.

**Results:** To cure headache Borage or mixture of Moret Pea and Oat is used. Runny nose is cured with boiled Eucalyptus. Infused Pteridease and Violet for clogged nose. Curing toothache with dried piece of apricot. Curing sputum with boiled hedge mustard's seeds and Plantago. Purgative manna and Hedy Sarum are used to cure new born's icteric. Stone boiling of Mint and Gandejaru for baby stomachache. Infused Mint and Damascus rose for constipation. Using Marambo and Golpure as an antiacid. The kidney stone is cured with Beet. Infused leaves of with fig tree for broken bones. Vaginal infections would be cured with infused Cummin, Sweet root, Coriander or using boiled Noonekalagh and Gondejaru. using Malakarzagh and Alones as suppository. All of this mentioned methods were popular and are currently used among people of Rahmat Abad in Golestan.

**Conclusions:** Common medicine in Iran has an old curing diseases and solving health problems. It's vital to record and keep them for future uses.

**Keywords:** Herbal drugs; House curings; Rural people; Golestan province

## An antidiabetic compound from *Vaccinium arctostaphylos* berries

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**Background and Aims:** *Vaccinium arctostaphylos* L. (Ericaceae) is a compact shrub up to 3 m in height which grows wildly in the northern forests of Iran. Its berries are edible cherry and are used as an antidiabetic agent in Traditional Iranian Medicine. The present study deals with the bioassay guided fractionation of *V. arctostaphylos* berry extract and in vitro  $\alpha$ -amylase enzyme inhibition assay of the extract and fractions for their antidiabetic activities.

**Methods:** Bioassay-guided fractionation of the fruit extract of *Vaccinium arctostaphylos* using preparative PC, and the in vitro  $\alpha$ -amylase inhibition assay, as biological monitor model, were used for the isolation. The isolated compound was characterized by spectroscopic methods.

**Results:** The crude extract showed a suitable dose-dependent inhibitory effect against  $\alpha$ -amylase activity [ $IC_{50}$  = 1.91 (1.89 – 1.94) mg/mL]. The activity guided fractionation of the extract led to the isolation and purification of an anthocyanin from it. The compound was identified as malvidin-3-O- $\beta$ -glucoside by determination of its hydrolytic and spectral data. The compound demonstrated a suitable dose-dependent enzyme inhibitory activity [ $IC_{50}$  = 0.16 (0.16 – 0.17) mg/mL].

**Conclusions:** *Vaccinium arctostaphylos* berries and malvidin-3-O- $\beta$ -glucoside (the active constituent from the berries) showed potent  $\alpha$ -amylase inhibitory activity.

**Keywords:** *Vaccinium arctostaphylos*;  $\alpha$ -Amylase inhibitory activity; Malvidin-3-O- $\beta$ -glucoside

## Analysis of essential oil of *Zingiber officinale* ROSCOE. rhizome cultivated in Isfahan

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**Background and Aims:** *Zingiber officinale* ROSCOE. (Zingiberaceae) grows in hot and humid climate. This plant is used for the treatment of digestion, fever, vertigo and vomit. Ginger is imported to Iran mostly is used as a spices. In this study, ginger rhizome were cultivated in Isfahan and its main chemicals were analyzed.

**Methods:** Rhizomes of ginger were obtained from Thailand, UE, China and Singapor and cultivated in University of Isfahan glasshouse. The growth media was 10 cm diameter pots consists of perlite and cocoperlite. The cultivated rhizomes were extracted and then quality and quantity of chemicals were evaluated with TLC, GC, GC/MS methods.

**Results:** In spite of the different ginger sources, all of them are able to produce the main compounds of originated plant almost similar to parent's plant when cultivated in Isfahan climate. However the parent's plants were richer than cultivated one in constituents.

**Conclusions:** It seems the similarity of the main constituents of the cultivated and the parent's plant rhizomes guide to the conclusion that ginger can be cultivated in Isfahan.

**Keywords:** Ginger; *Zingiber officinale*; Cultivations; Volatile oil

## Analysis of the essential oils from 3 species of cymbocarpum (Apiaceae) from Iran

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**Background and Aims:** Apiaceae is one of the biggest plant families of Iran, holding 111 genera and 350 species. This family contains several aromatic and medicinal species like carrot, coriander, caraway, cumin, fennel, etc. In different organs of these species have been recognized compounds like essential oils and coumarins. Three Species has been reported for Cymbocarpum in flora of Iran including *C. stenocarpa*, *C. erythraeum* and *C. anethoides*. In this article, compositions of essential oil of them have been analyzed.

**Methods:** The first species was collected from Ghazvin Province (July 2006) and others were collected from East Azerbaijan Province (June 2010), (at the flowering period), the samples were stored in the Central Herbarium of ACECR. The essential oils were obtained by Hydrodistillation in a Clevenger type apparatus and were dried over anhydrous Sodium Sulphat. The yields of essential oils were 0.3, 0.4 and 0.5% w/w, respectively. Then, the oils stored at +4°C in the dark place until analysis. The chemical compositions of essential oils were analysis by GC and GC/MS method. The identification of the essential components was based on calculation of retention Indices and comparison of mass spectra with standards.

**Results:** 42, 33 and 80 components were identified in the essential oil of aerial parts, representing of the 96, 95 and 98% of the total oils, respectively. The major constituents of *C. stenocarpa* were 3-dodecen-1-al (37.1%), decanal (18.0%), 2-methylene cyclopentanol (7.7%) and 2-decenal (6.7%). In *C. erythraeum* 2-dodecenal (29.4%), 2-tridecanal (13.7%), nonane (9.1%) and  $\alpha$ -pinene (8.6%) and in *C. anethoides* 2-dodecenal (45.5%), E-2-decenal (17.9%),  $\alpha$ -pinene (8.4%) and E-2-tridecanal (8.35%) were main components.

**Conclusions:** The main class of the compounds was found in the Genus to be aliphatic aldehydes. These components are the major in the essential oil of some other Apiaceous herbs, such as *Ducrosia anethifolia* and *Heracleum* sp.

**Keywords:** Cymbocarpum; *C. stenocarpa*; *C. erythraeum*; *C. anethoides*; Essential oil; Aliphatic aldehydes

## Antibacterial evaluation and preliminary phytochemical analysis of *Microcystis* sp.

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**Background and Aims:** The present study was designed To promote the proper use of cyanobacteria and to determine their potential as sources for new drugs. cyanobacteria , a morphologically diversed class of prokaryotic photosynthetic organisms flourish in static eutrophicated water bodies , dominate microbial assemblage through formation of blooms. the medicinal value of cyanobacteria lies in some chemical substances that produce a definite physiological action on human body.the most important of these bioactive constituents of cyanobacteria are alkaloids , tannins , flavonoids , and phenolic compounds.further investigations into secondary metabolite products of cyanobacteria identified biologically active compounds with antimicrobial properties.

**Methods:** *Microcystis* sp. Collected from a lake of Daneshju park in Rasht in september 2011 .then culitivated to optimise growth and produce yield of species. Enriched BG11 media was used for cultivation of *microcystis* sp.to determine the efficacy of the methanol /sonication extracts of *Microcystis* sp. In response to temperature and extract concentration, the cup plate method were repeated using bacteria *E coli*,*B. subtilis* , *S . aureus* and *p. aeroginosa*. The preliminary phytochemical screening was carried out on 99% methanol extract of sample.Dragendroff's test and Mayer's test were done to assay alkaloids ,foam test for saponins ,shinoda test for flavonoids ,ferric chloride solution test for tannins and phenolic compounds , Libermann – burchard reaction for stroid and borntrager's test for anthraquinone glycosides were done.

**Results:** alkalids,stroids and saponins were present in all samples.flavonoids,tannins and anthraquinone glycosides were absent in *Microcystis* sp. no inhibition was recorded with aqueous and methanolic extract in antibacterial test.

**Conclusions:** The present study carried out on *Microcystis* genus revealed the presence of medicinally active constituents.for example alkaloids.in general alkaloids are a broad group of heterocyclic nitrogenous compounds .they could have neurotoxic ,cytotoxic or dermatotoxic effect.to date no alkaloids have been found in *Microcystis* genus.

**Keywords:** Blue green algae *microcystis*; Antibacterial effect; Phytochemistry

## Composition and biological activities of the essential oil and extracts of *Anisosciadium orientale*

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**Background and Aims:** The plants of the Apiaceae family have about 3000 species in 400 genera. In Iranian ancient medicine, the fruits of *Anisosciadium orientale* have been used for treatment of toothache, diarrhea and epilepsy. This paper describes the chemical analysis of the essential oil from *A. orientale*. Antioxidant activity and total phenol contents were also determined in the essential oil and extracts (methanolic, 80% methanolic and DCM) of the plant.

**Methods:** The essential oil of *A. orientale* was analyzed by gas chromatography (GC) and gas chromatography-mass spectroscopy (GC-MS). The essential oil and extracts were screened for antioxidant activities using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and Folin-Ciocalteu reagent total phenol. For antibacterial activity, the volatile oil and extracts of this plant have been tested with micro dilution method using a gram positive bacterium (*Bacillus subtilis*) and a gram negative bacterium (*Escherichia coli*). The cytotoxic activity was measured on 3 human cancer cell lines including K562 (human chronic myelogenous leukemia), LS180 (human colon adenocarcinoma) and MCF-7 (human breast adenocarcinoma) cells.

**Results:** Sixteen compounds were identified representing 92.86% of the total components of the oil from which myristicine (30.07%), terpinolene (24.54%) and limonene (20.99%) were major constituents. Antioxidant activity was observed for the oil, while no antibacterial activity could be detected. The methanolic extract of the plant showed higher antibacterial activity than the others, and 80% methanolic extract was observed to have highest antioxidant activity. IC<sub>50</sub> values of cytotoxic activity on K562, LS180 and MCF-7 cells were  $396.8 \pm 64.9$ ,  $183.2 \pm 42.8$  and  $159.5 \pm 29.0$   $\mu\text{g/ml}$ , respectively (mean  $\pm$  S.E.M.).

**Conclusions:** Monoterpene hydrocarbons were identified as one of the major fraction of the oil (45.53%). The plant antioxidant activity was due to phenolic constituents.

**Keywords:** Apiaceae (Umbelliferae); *Anisosciadium orientale*; Essential oil; Antioxidant activity; Antibacterial activity



## ***Crataegus pontica* C. Koch; microscopical, macroscopical and chemical investigations and their uses in chemotaxonomy**

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**Background and Aims:** The *Crataegus* genus is widely distributed in Iran. It belongs to Rosaceae family and there are 17 species of that genus in Iran which one of them is *Crataegus pontica* C. Koch.

**Methods:** In this paper some microscopical and macroscopical characteristics of this plant were analyzed, then were compared with other features that were expressed previously in recent reports. All components in *C. pontica*, were analyzed by thin layer chromatography method and then were specified the type of flavonoids and hydroxycinnamic acids in *C. pontica*. Its flavonoids was analyzed quantitatively based on Deutsch Pharmacopoeia method according to hyposide content. Flavonoids are using for determining the chemosystematic relevancies in some species, so in this paper *C. pontica* was compared with three other species of *Crataegus* genus such as *C. monogyna*, *C. melanocarpa* and *C. curvisepala* that are found in Iran, and also with the medicinal standard species of *Crataegus* genus which is called *C. oxyacantha*.

**Results:** Lots of microscopically and macroscopically features of *C. pontica* are indetical to other types of *Crataegus*. Hyposide, rutin and chlorogenic acid are the main flavonoids and hydroxycinnamic acid of the plant. Also these are the main and common structural components in all species of that genus are mentioned above.

**Conclusions:** It seems that researchers can use hyposide, rutin and chlorogenic acid in future chemosystematic studies about *Crataegus* genus and other genera of Rosaceae family.

**Keywords:** Chemotaxonomy; *Crataegus pontica*; Hyposide

## Determination of $\gamma$ -terpinenes in *Bunium persicum* essential oil by voltammetric method using gold nanoparticles modified glassy carbon electrodes

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**Background and Aims:** Several analytical techniques for the determination of  $\gamma$ -terpinene in *Bunium Persicum* essential oil have been reported. In this study, a new method using a glassy carbon electrode modified with gold nanoparticles (GNPS/GCE) was developed and validated for determination of  $\gamma$ -terpinene, most abundant constituent of *Bunium Persicum* fruit essential oil.

**Methods:** The electrochemical behavior of  $\gamma$ -terpinene at the modified sensor was characterized by cyclic voltammetry, chronocoulometry, linear sweep voltammetry, differential pulse voltammetry and rotating disk electrode voltammetry.

**Results:** Electrochemical parameters such as the diffusion coefficient (D), electron transfer coefficient ( $\alpha$ ), electron transfer rate constant (k) and ionic exchange current density ( $i_0$ ) were determined for the oxidation of  $\gamma$ -terpinene on the surface of electrodes. The oxidation of  $\gamma$ -terpinene on the GNPs/GCE in acetonitrile (0.1 M LiClO<sub>4</sub>) at a scan rate of 25 mV s<sup>-1</sup> were successfully conducted by differential pulse voltammetry. The peak current increased linearly with the concentration of  $\gamma$ -terpinene. The results show that the plot of peak current versus  $\gamma$ -terpinene concentration is linear in the range of 1.2×10<sup>-2</sup> M - 1×10<sup>-4</sup> M. From the analysis of these data, we estimate that the detection limit of  $\gamma$ -terpinene is 5×10<sup>-5</sup> M.

**Conclusions:** A new and selective method was developed for the measurement of  $\gamma$ -terpinene. This method was successfully applied to the determination of  $\gamma$ -terpinene in *Bunium Persicum* fruit essential oil.

**Keywords:**  $\gamma$ -terpinene; Gold nanoparticles; *Bunium Persicum*; Voltammetry methods

## Differentiation of *Azadirachta indica* A. juss and *Melia azedarach* L. by using pharmacognostical and preliminary phytochemical methods

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**Background and Aims:** *Azadirachta indica* A. Juss and *Melia azedarach* L. are two species from Meliaceae which are distributed in the south coast and the north forests of Iran respectively. They are both important due to their numerous pharmacological activities and their usage in traditional medicine but they have very often been confused with each other. The present study was carried out to define referential pharmacognostical and phytochemical parameters which can aid rapid and easy identification and differentiation of these two valuable species.

**Methods:** Various evaluations like macroscopic and microscopic characteristics, preliminary phytochemical screening and fluorescence analysis were carried out on pulp and husk, leaves and seeds of both plants in powdered form.

**Results:** The results showed that the two species can be differentiated macroscopically and microscopically but tests of phytochemical analysis were not absolutely felicitous for this aim. In addition, the obtained information about different colors of the powders on reaction with various reagents and fluorescence analyses of them provided supporting referential parameters for identification and differentiation of these two species.

**Conclusions:** Based on the present study, it can be deduced that pharmacognostical study is a practical and affordable method for differentiating of the species, although to catch this benefit, the fluorescence analysis, can be considered as its great complement.

**Keywords:** Pharmacognostical study; Phytochemical; Meliaceae

## Documentation of traditional therapeutic methods in Kermanshah province, ir Iran

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**Background and Aims:** Kermanshah is a city in west of Iran with a specific customs and cultures between the people who are living here. According to historical documents these cultures are very ancient and belong to more than one thousand years. The climate condition in this place force people to find the solution of their problems using the plants and natural facilities. Therefore traditional healers were so active in Kermanshah. From 8000 of plant species in Iran more than 1200 species has grown in Kermanshah. The ancient customs, cultures, traditional medicine and formulations generally used by rural populations was transfer from ancient to modern people.

**Methods:** Documentation of these traditional methods was studied in this research in order to compare and certified the traditional medicine with modern methods and find new dosage forms of drug with botanical source.

**Results:** It was established that about 50 plant species and 8 types of diseases were distinguished and cured by these people.

**Conclusions:** It is also concluding that utilization of these plants approximately the same as application of plants in recent publications.

**Keywords:** Traditional; Medicine; Complementally therapy; Natural plants

## Essential oil analysis of Iranian tropical flea-bane

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**Background and Aims:** Pulicaria species are used in traditional and folk medicines of different parts of the world for treatment of hemorrhoids, haematomas and diabetes and have cancer chemopreventive, cytotoxic, insecticide and mitotic properties. Iranian tropical flea-bane (ladies' false fleabane) or *Pulicaria arabica* (L.) Cass. from Asteraceae family is one of the medicinal plants of Iran that its aerial parts have been used in folk medicine. The aim of this study was determination of the aerial parts essential oil of *P. arabica* gathered from Dezful (Khuzestan province, Iran) for the first time.

**Methods:** The aerial parts of *P. arabica* were powdered and the volatile fraction was isolated by hydrodistillation for 3h according to the method recommended in British Pharmacopoeia. The collected oil was dried using anhydrous sodium sulfate and stored at a fridge. The oil was analyzed by GC-MS analysis.

**Results:** The aerial part of the plant yielded pale yellowish oil with a pleasant herbaceous-spicy odour. The major constituents of its essential oil were alpha-cadinol (17.7 %), germacrenol (17.0%), epi-alpha-cadinol (13.9%), delta-cadiene (11.5 %) and beta-caryophyllene (7.4 %).

**Conclusions:** Although some of our essential oil components were identified in other *Pulicaria* species reported before but most of them and their percentages are different from the samples that are gathered from Middle East and Persian Gulf countries and may be subjects for other chemical, pharmacological and biological surveys.

**Keywords:** *Pulicaria arabica*; Asteraceae; Essential oil; GC-MS; Alpha-cadinol

## Essential oil of *Tanacetum dumosum* as a new source for fragranol

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**Background and Aims:** *Tanacetum* (tansy) belongs to a tribe Anthemideae that is one of the largest tribes of Asteraceae family. The essential oils of several Iranian tansies were analyzed by different authors and found to have monoterpenoids as major constituents. Among them pinenes, 1,8-cineol, camphor, chrysanthenol derivatives and thujones are detected more frequently. To the best of our knowledge this is the first report on characterization of chemical composition of the essential oil of *T. dumosum*.

**Methods:** The aerial parts of the *Tanacetum dumosum* Boiss., was collected from Dena mountain at altitude of 3200 m in July 2011. The essential oil of the leaves and flowers of plant (70 g) was extracted by hydrodistillation method to yield 0.2 ml of pale-yellow oil, dried over anhydrous sodium sulfate and analyzed by gas chromatography-mass spectrometry (GC-MS) and GC- flame ionization detector (GC-FID).

**Results:** Twenty seven mono and sesquiterpenoids were identified in oil using GC retention indices (RRI) and mass spectral (MS) data, obtained from GC and GC-MS experiments. Among them the mono terpene hydrocarbons, santolin triene (2.2%) and  $\alpha$ -pinene (2.2%) and the oxygenated monoterpenes, fragranol (26.7%), 1,8-cineol (5.7%), fragranyl acetate (23.6%) and trans-carvyl acetate (10.1%) were detected as the major constituents. Helifolenol A, Beta-sesquiphellandrene, caryophyllene oxide and germacrene D are the major sesquiterpenes detected in this oil but still in lower quantities compared to the monoterpenoids fraction.

**Conclusion:** monoterpene hydrocarbons were identified as one of the major components of the oil. The good smell of the plant may be due to the presence of fragranol and its acetate ester and may suggest this plant for future use in perfume industries.

**Keywords:** *Asteraceae*; *Tanacetum dumosum*; Essential oil; Fragranol

## Evaluation of 4 *Salvia* species compounds with thin layer chromatography (TLC) technique

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**Background and Aims:** *Salvia*, the largest genus of the Lamiaceae family, has been credited with a long list of medical uses, including: spasmolytic, antiseptic, astringent, antibacterial and antioxidant activities. These activities are due to the presence of phenols and terpenoids. Objective: To compare 3 Iranian native species compounds with *S. officinalis*.

**Methods:** The methanolic extract of the species was enriched with ethylacetate, after the preparation of the TLC plates with the size 3.5×4.5 cm and selection of the *S. officinalis* leaves extract as the standard, a spot of each extract was placed with a 0.5 cm distance from each other, on the TLC plates, and 8 solvent system was used to study the separation pattern of the compounds and the best solvent system has been selected.

**Results:** Among the different solvent systems which has been selected as mobile phase, the solvent system “chloroform-methanol (98:2)” was the best, and among the different species “*S. mirzayanii*” had the most separable compounds and “*S. hydrangea*” had the most similarity to “*S. officinalis*”, so it may be use as an alternation for “*S. officinalis*”.

**Conclusions:** TLC technique can be use as a simple, cheap and available method for recognition and screening of the compounds in different species of the same genus extracts. This method can be use as a rapid one for qualitative evaluation of a plant compounds, and primary screening of different species of a genus can be done by this method.

**Keywords:** *Salvia officinalis*; *S. mirzayanii*; *S. hydrangea*; *S. macrosiphon*

## Evaluation of residual diazinon and chlorpyrifos in children herbal medicine by SPME and GC-FID

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**Background and Aims:** Pesticides are used extensively in agriculture to enhance the food production by controlling the unwanted insects and disease vectors, however it has some hazard on biological system of human especially children. The present study was designed to examine the residual of organophosphorus pesticides; Diazinon, Chlorpyrifos; in children herbal medicines available in the market.

**Methods:** Five children herbal medicines dosage form were extracted with SPME (Solid Phase MicroExtraction) using PDMS-DVB fiber. Then the extracts were injected into a GC. The gas chromatograph was Younglin model YL 6100 equipped with a flame ionization detector. The column was Technokroma 60 m length, 0.53 mm internal diameter and 1.25  $\mu$ m film coated. The presence and quantity of Diazinon and Chlorpyrifos was evaluated using their standard curves.

**Results:** Trace amount of chlorpyrifos and diazinon was detected in the samples. Based on European pharmacopeia, threshold limits of chlorpyrifos and diazinon residues for medicinal plant materials are 0.2 and 0.5 mg/kg. Our analysis results showed that residue limits of these two pesticides in five children herbal medicines are ignorable.

**Conclusions:** The use of herbal products as the first choice in self-treatment of minor conditions continues to expand rapidly across Iran. This makes the safety of herbal products an important public health issue. With this study we recommend to do a study with more herbal product samples using GC-MS.

**Keywords:** Diazinon; Chlorpyrifos; SPME-GC-FID; Children herbal medicine



## Evaluation of the chemical composition and antimicrobial activity of different fennel (*Foeniculum vulgare* Mill.) ecotypes essential oils from Iran

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**Background and Aims:** The present study has evaluated the chemical composition and antimicrobial activity of the essential oils of five Iranian ecotypes of fennel.

**Methods:** The Essential oils of five Iranian ecotypes of fennel have obtained from dried seeds using hydro distillation method. Variations in the essential oil compositions were determined using Gas chromatography coupled to mass spectrometry (GC-MS). Antimicrobial activities were tested against the strains *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans* using disc paper and broth microdilution methods.

**Results:** Our results show that all essential oils have a broad spectrum of antimicrobial activity against all the tested bacterial strains. The essential oils have more sensitivity to gram-positive than gram-negative bacteria, however, some difference in antimicrobial activities of essential oils was observed for several microorganisms, which was attributed to the variation in percentage of the components. The most important identified compounds in all samples of fennel volatile oils were trans-anethole, estragole, fenchone, limonene, alpha-pinene and gamma-terpinene.

**Conclusions:** This plant showed desire antibacterial effects and therefore is a good candidate for future investigation of development of new bactericidal and bacteriostatic agent investigation was developed to purify the essential component and estimate their effects.

**Keywords:** MIC; *Foeniculum vulgare*; MBC; Chemical composition

## Evaluation of UV blockage and SPF determination of seven medical plants extracts

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**Background and Aims:** Skin is one of the most important organs of the body and the main barrier that protects the other organs against external invasion. Damaging factors such as sunlight can cause skin dehydration, skin pigmentation, photo aging, etc. So it seems necessary to protect skin against sun rays. Chemical compounds as sun protecting agents have some adverse effects but some medical plants that contain active ingredients like as flavonoids having blocking effect with low adverse effects.

**Methods:** In this study seven medical plants: *Achillea wilhelmsi* L., *Calendula officinalis* L., *Ruta graveolens* L., *Fumaria parviflora* Lam, *Lawsonia inermis* L., *Nymphaea alba* L., *Punica granatum* which contains high amount of flavonoids were evaluated. Methanolic extracts were prepared and flavonoids were isolated and fractioned by ethyl acetate. Then SPF of these extracts was determined by diluted solution method as an in-vitro method.

**Results:** Among all of the plants, *Ruta graveolens* with SPF 14.62, *Calendula officinalis* with SPF 9.8 and *Achillea wilhelmsi* with SPF 9.3 had the maximum protection against UV rays.

**Conclusions:** Despite the protecting effect of these plants against harmful UV rays and anti-inflammatory effects of these plants, we can recommend use of these extracts in antisolar products.

**Keywords:** SPF; Flavonoids; Skin

## ***Ferulago macrocarpa* (Fenzl) Boiss. fruits as a source of bornyl acetate and 1,10-di-epi-cubenol**

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**Background and Aims:** The genus *Ferulago*, which belongs to the Umbelliferae family consists of about seven species in Iran. *Ferulago macrocarpa* (Fenzl) Boiss. is a perennial herb which grows in western Iran. Previous phytochemical studies of *Ferulago* have led to the isolation of various coumarins and volatile oils. Some of the isolated coumarins have shown antimicrobial, antioxidant, cytotoxic and acetylcholinesterase inhibitor activities. In addition, the essential oils of many other *Ferulago* species have exhibited antimicrobial activities.

**Methods:** The acetone extract of the fruits of this plant was prepared using maceration method. Repeated open column chromatography in normal phase and HPLC using petroleum ether, toluene, ethyl acetate and n-heptane as mobile phase resulted in isolation of these compounds. The structures of compounds were elucidated using NMR and MS spectra.

**Results and Conclusions:** According to spectroscopic data, the isolated compounds were assigned as a bornyl acetate and 1,10-di-epi-cubenol. These compounds are mainly used as antioxidant, antifungal, antibacterial and anti-inflammatory agents.

**Keywords:** *Ferulago macrocarpa*; Bornyl acetate; 1,10-di-epi-cubenol

## Isolation of new disulphides from *Ferula foetida* oleo-gum-resin

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**Background and Aims:** The genus *Ferula* (Apiaceae) comprises about 170 species occurring from central Asia westward to northern Africa. The Iran flora comprises 30 species. *Ferula* spp presents interesting phytochemical features such as the occurrence of sulphides and sesquiterpene coumarins. Many biological features of this genus such as cytotoxicity, antibacterial, antiviral, P-glycoprotein (P-gp) inhibitory and anti-inflammatory activity have been attributed to sesquiterpene coumarins and sulphides. *F. foetida* is one of the species that produce *asafetida* oleo-gum-resin with several traditional uses.

**Methods:** Oleo-gum-resin was collected from Jandagh near Isfahan, central Iran. Air dried material was extracted with hexane using maceration method for 24 h and the solvent was evaporated in vacuo. Repeated open normal phase column chromatographies and HPLCs using n-hexane and ethyl acetate as mobile phases resulted in isolation of several sulphides. The structure of isolated coumarins was elucidated using 2DNMR and MS spectra.

**Results:** Several new sulphides along with known disulphides like foetisulphide A were purified and structures elucidated. **Conclusions:** Since sulphides have biological active functional groups, a plant rich in sulphides can be used as a source of pharmacologically active phytochemicals.

**Keywords:** Disulphide; foetisulphide A; *Ferula*; *Assafetida*; 2D NMR

## Isolation of new disulphides from *Ferula foetida* roots

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**Background and Aims:** *Ferula foetida* is a perennial plant of Apiaceae which is one of the Asafetida oleo-gum-resin bearing genera. This species is native to East, Iran and is scattered in the warm, dry and mountainous areas. Asafetida has been used as spice and herbal remedy for centuries. *Ferula* species contain various compounds like sesquiterpene coumarins and sulfur-containing compounds which the latter play an important role in the odor and taste of asafetida plants. The compounds have shown antioxidant, antigenotoxic and hypoglycemic effects and bear beneficial effects on cholesterol level and platelet aggregation.

**Methods:** Roots of *Ferula foetida* were extracted with acetone and the concentrated extract was fractionated by vacuum liquid chromatography on silica gel. The fractions were purified with HPLC to render pure sulphides. The structures were elucidated with 2NMR and mass spectra.

**Results & Conclusions:** Several new sulphides along with known disulphides like methoxylatifolone were purified and structures elucidated. Since sulphides have biological active functional groups, a plant rich in sulphides can be used as a source of pharmacologically active phytochemicals.

**Keywords:** Disulphide; Methoxylatifolone; *Ferula asafetida*; 2D NMR

## Pharmacognostic evaluation of herbal medicines used for obesity on Isfahan traditional medicine market

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**Background and Aims:** Overweight and obesity have become a major public health issue due to a steep rise in the prevalence of overweight. Overweight and obesity are major risk factors for many diseases, including cardiovascular problem, diabetes, and hypertension. Because of inappropriate diet, lack of adequate physical activity, and sedentary life style; people use medical treatments to lose weight. Herbal medicines have been gaining considerable popularity in recent years. Access to such products is largely unrestricted, without the need for prescriptions. Given the increasing popularity of herbal medicines for treatment of obesity in Iran, there is a need to evaluate these products, which are traditionally provided.

**Methods:** Ten herbal products used for obesity on Isfahan traditional medicine market were collected, their medical information was recorded, and then the drugs were microscopically and macroscopically evaluated. Finally, their therapeutic effects were compared with the indications mentioned in traditional medicine texts.

**Results:** More than 50% of the products contained at least one medicinal plant, which has been used traditionally for treatment of obesity.

**Conclusions:** The providers of traditional medicine should receive required trainings according to texts of traditional medicine. Moreover, they should be continuously monitored and traditional medicine products continuously undergo pharmacognostic testing.

**Keywords:** Obesity; Herbal medicine; Pharmacognostic evaluation

## Pharmacognostical evaluation, phytochemical analysis and antioxidant activity of the roots of *Achillea tenuifolia* Lam.

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**Background and Aims:** *Achillea* L. (Asteraceae) contains about 100 species throughout the world with many therapeutic aspects. *Achillea tenuifolia* is one of the mentioned species that grows wildly in Iran.

**Methods:** In this research the antioxidant activity of methanol and ethyl acetate extracts obtained from the roots of *A. tenuifolia* against free DPPH (2,2-diphenyl-1-picrylhydrazyl) radical together with the total phenol contents of extracts were assayed. Furthermore, preliminary phytochemical analysis of the above mentioned extracts and microscopic characterization of various plant tissues were determined.

**Results:** The results showed that total phenol contents of methanol and ethyl acetate extracts were  $59.4 \pm 1$  and  $70.6 \pm 3.8$  ( $\mu\text{g}/\text{mg}$  EXT), respectively. IC<sub>50</sub> value for BHA, vitamin E, methanol and ethyl acetate in radical inhibition were calculated in the following order: 7.8, 14.2, 145.5 and 320  $\mu\text{g}/\text{mL}$ . The scavenging capacity of methanol extract was higher than ethyl acetate extract. Preliminary phytochemical analysis indicated that both extracts contained sterols and terpenoids, nevertheless, tannins were detected in the methanol extract. Microscopic observations exhibited the presence of undeveloped cypselae, papillae stigma and elongated epidermal cells in the flower tissue, lanceolate leaflet with anomocytic stomata, cubic calcium oxalate prism and oil containing cells in the leaf parts, anomocytic stomata and cicatrix in the stem segments and finally sclereids, pitted and spiral vessels in the root tissue.

**Conclusions:**

Root extracts of *A. tenuifolia* mainly contain tannins, terpenes and sterols, and shows antioxidant activity not necessarily related to their total phenol content. Different plant tissues exhibited characteristic microscopic properties which make it distinguishable from other *Achillea* species.

**Keywords:** *Achillea tenuifolia*; Antioxidant activity; Microscopy; Preliminary phytochemical analysis

## Preparation and characterization of a herbal emulgel for treatment of varicose veins

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**Background and Aims:** Varicose veins are part of continuum of circulatory disease which have been indicated with weakened, enlarged and twisted veins, that have symptoms like tiredness, heaviness, tension and edema (specially in legs). Emulgel (Emulsion in gel) has emerged as one of the useful semisolid drug systems as has been improved the stability of emulsion by incorporating in a gel matrix. The aim of this study was to achieve a suitable formulation of emulgel containing Horse chestnut and *Calendula officinalis* extracts for treatment of varicose veins.

**Methods:** Horse chestnut extract, which was purchased from Indian LEPRO Company, incorporated and standardized based on 10th German pharmacopeia (D.A.B.). *Calendula* extract was prepared based on BP method. Suitable oil in water emulsion base, Horse chestnut extract and gel phase were added to *calendula* extract to forming emulgel. Physicochemical tests such as creaming, coalescence, pH, centrifugal test, viscosity, spreadability, freezing and thawing and content uniformity tests were performed.

**Results:** Results showed that appropriate amounts of sorbitan monostearate and gelling agent are the critical factors in stability of formulations. Most of formulations showed acceptable physical stability. Among physically stable formulations, one of them was selected as choice formulation based on results of all tests.

**Conclusions:** The results revealed that emulgel systems are a perfect choice for preparing topical cream especially containing herbal extract which can interfere with simple emulsion systems.

**Keywords:** Varicose veins; Horse chestnut; *Calendula officinalis*



## Production of lignans in callus culture of *Linum glaucum* Boiss. and Noë

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**Background and Aims:** Medicinal plants are sources of important therapeutic aid for alleviating human ailments. Callus culture can also be used as an alternative to whole plant for the production of secondary metabolites. In this study, the influence of plant growth regulators, explant type and light condition on the callus culture of *Linum glaucum* Boiss. & Noë was investigated.

**Methods:** The callus culture of *L. glaucum* Boiss. & Noë was initiated and established from different explants of seeds (root, hypocotyl and cotyledon) in MS media with four different ratios of plant growth regulators: kinetin (1mg/L) alone and in combination of three type of auxins, 2,4-D, IAA and NAA (0.5mg/L) under light and dark conditions. A factorial randomized design with 3 replicates was used for data analysis.

**Results:** In this research projects, the best callus induction (100%) was recorded on media supplemented with kinetin and NAA in light condition. The callus growth (Callus fresh weight) also was affected by media and light. The best growth rates of callus (189mg) were found in media supplemented with kinetin alone at light condition. Methanol extracts were obtained from in vitro cultured *L. glaucum* and the lignins was detected by TLC.

**Keywords:** *Linum glaucum* Boiss. & Noë; Callus; Lignans

## Production of some volatile components in *Daucus carota* L. callus cultures

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**Background and Aims:** *Daucus carota* L. or carrot from Apiaceae family is a herbaceous and biennial plant that produces an edible root. The aromatic substances and beta-carotene, as an orange and abundant pigment of the fruits and roots are the main components of the plant. Callus cultures of *D. carota* fruits may produce the similar compounds. The aim of this work was to initiate and establishment of callus culture of *D. carota* fruits for volatiles and beta-carotene production as well as determination of the essential oil of *D. carota* fruits for comparing.

**Methods:** A sterile in vitro *D. carota* fruit was prepared by sterilization with ethanol 70% and hydrogen peroxide 30% and subculture of buds, excised aseptically and inoculated into sterile culture jars containing Murashige and Skoog's medium and were incubated at 25 °C. Production of calluses main compounds was evaluated by TLC and GC-MS techniques. The same processes were done on *D. carota* fruits as well. For this purpose the fruits of *D. carota* were powdered and the volatile fraction was isolated by both of BP hydrodistillation method and dichloromethane solvent. The collected oils were analyzed by GC-MS analysis.

**Results:** No beta-carotene was detected on cultures and six compounds were found in the essential oil of *D. carota* fruits including apiol (44.6%). Apiol and myristicin were detected in DCM extract of cultures.

**Conclusions:** According to our results the cell cultures of *D. carota* fruits like as intact plant are able to produce secondary metabolites but it looks different in number of compounds.

**Keywords:** *Daucus carota*; Apiaceae; Callus culture; Essential oil; GC-MS; Apiol

## Reducing toxicity using *Transgenic tobacco (Nicotiana tabacum L.)* plants

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**Background and Aims:** *Nicotiana tabacum* has known toxic compound nicotine. Tobacco plants are used as cigarette smoking and its nicotine cause many health problems. Reducing nicotine content of this plant is necessary. The present study was designed to examine the effect of plant genetic engineering for blocking the pathway of nicotine biosynthesis in order to produce plant with low level of nicotine.

**Methods:** In this research transgenic plants carrying T DNA, and non-transgenic plants (mother plants) were grown on MS medium. Leaves were harvested and dried. The powdered plant materials were extracted with alkali solution. Then nicotine content of extract were analysed using GC.

**Results:** The analysis of extracts showed that nicotine content decreased in transgenic plant (contain T-DNA) in comparison with non-transgenic plants.

**Conclusions:** Variation of nicotine content in transgenic plants might be related to inhibition of key enzymes in nicotine biosynthesis pathway.

**Keywords:** Tobacco; Transgenic; Nicotine; Toxicity; *Nicotina tabacum*

## Screening of some medicinal plants for antidiabetic activity

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**Background and Aims:** Diabetes mellitus is one of the most serious chronic diseases characterized by chronic hyperglycemia. Traditional herbal remedies are still in use by diabetic patients especially in 3rd World Countries. The present work was carried out to investigate the potential antidiabetic effects of some Iranian medicinal plants using an in vitro  $\alpha$ -amylase enzyme inhibition assay.

**Methods:** The ethanol extracts obtained from ten plants (including *Trigonella foenu-graecum*, *Camellia sinensis*, *Urtica dioica*, *Vaccinium arctostaphylos*, *Urtica pilulifera*, *Calendula officinalis*, *Juglans regia*, *Olea europaea*, *Salvia officinalis*, *Arctium lappa*) were tested against  $\alpha$ -amylase and inhibitory activity of each extract, expressed as IC<sub>50</sub> values, calculated from Log concentration-response curve. As positive control acarbose was used.

**Results:** Among the tested samples, *Camellia sinensis* (Theaceae) leaf (IC<sub>50</sub> = 1.54 mg/ml), *Trigonella foenum-graecum* (Leguminosae) seed (IC<sub>50</sub> = 1.87 mg/ml) and leaf (IC<sub>50</sub> = 1.92 mg/ml), and *Urtica dioica* (Urticaceae) leaf (IC<sub>50</sub> = 1.89 mg/ml) revealed  $\alpha$ -amylase inhibitory activities in a concentration-dependent manner.

**Conclusions:** The ethanolic extracts of *Camellia sinensis*, *Trigonella foenum-graecum* and *Urtica dioica* showed the potent  $\alpha$ -amylase inhibition effects.

**Keywords:**  $\alpha$ -Amylase inhibitors; Antidiabetic medicinal plants; Diabetes mellitus

## Structure elucidation of water-soluble polysaccharides of *Trehala Manna*

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**Background and Aims:** Trehala Manna is a cocoon-like, oval and white bulk with an uneven surface that has a smooth hole in the middle and the hole usually occupied by its productive insect. The sweet Manna has laxative and anti-cough properties and is widely used in Iranian Traditional Medicine. This Study has been designed to identify the structure of water-soluble polysaccharides in Trehala Manna.

**Methods:** Water-soluble polysaccharides in Trehala Manna has extracted by Sedimentation in 96% ethanol and then centrifuged. After Lyophilization, polysaccharides were purified by ion exchange and gel filtration chromatography. The carbohydrate content was determined by phenol-sulfuric acid method. Uronic acids and protein content was determined spectrophotometrically. After acid hydrolysis, the derivatized monosaccharides were identified by Gas chromatography - mass spectrometry (GC / MS) analysis. The molecular weight was calculated by the calibration curve obtained by using various standard dextrans.

**Results:** From 9 polysaccharides which were purified, the structures of 3 major polysaccharides were elucidated. These 3 polysaccharides were different in molecular weight (40-670 Kilodalton) and structure. They consisted of variety of monosaccharides such as glucose, mannose, arabinose, galactose and xylose. The uronic acid content of these polysaccharides was 35.33%, 22% and 26.44%, respectively. One of the polysaccharides had glycoprotein structure (2.59% w/w, protein content).

**Conclusions:** The Trehala Manna contains different water soluble polysaccharides which differ in structure and Molecular weight.

**Keywords:** Polysaccharides; *Trehala Manna*; GC / MS

## Study on chemical constituents of the roots of *Ferula szowitsiana* DC and evaluation of their antioxidant activity

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**Background and Aims:** *Ferula szowitsiana* DC widely grows in Turkey, Iran and Afghanistan. The plant has been used as an antihelminic and antiseptic in traditional medicine of Azarbaijan. This study is determination of chemical constituents of the roots of *F.szowitsiana* that growth in Marni lands of East Azarbaijan and assessment of their antioxidant activity.

**Methods:** The powdered roots of *F.szowitsiana* (300g) were soxhlet extracted successively with n-hexane, dichloromethane and methanol. The n-hexane extract (2g) was subject to vacuum liquid chromatography on silica gel using a step gradient of n-hexane:ethyl acetate (100:0, 99:1, 98:2, 97:3 96:4, 95:5, 94:6, 93:7, 92:8, 91:9, 90:10, 80:20, 60:40, 40:60, 20:80, 0:100). Yielded fractions were checked on Thin Layer Chromatography, n-hexane/ethyl acetate as developing solvent. The methanolic extract (2g×3) was fractioned with Solid Phase Extraction method by using Sep-Pak (Octa Decyl Silica) and a step gradient of methanol in water (20:80, 40:60, 60:40, 80:20, 100:0). The resulting fractions were analysed by preparative reversed-phase High Performance Liquid Chromatography. The structures of the purified compounds were determined by <sup>1</sup>H-NMR (Nuclear Magnetic Resonance) and <sup>13</sup>C-NMR spectra. Antioxidant activity of n-hexan, dichloromethane and methanolic extracts were evaluated by 2,2-Diphenyl para-1-Picryl Hydrazyl (DPPH) method.

**Results:** The preparative reversed-phase HPLC analysis of the 20%, 40% and 80% methanolic fractions resulted in numerous pure compounds, which structure of 5 of them determined by <sup>1</sup>H-NMR, <sup>13</sup>C-NMR and Ultra Violet spectroscopy as: Umbelliferon-7-apiosyl β (16) glucoside, P-hydroxy phenylethanoid glucoside, Galbanic acid, Umbelliferon β-D [6' (ferolye)-glucoside] and Umbelliferon. The methanolic extract has shown significant antioxidant activity (8.85 ×10<sup>-2</sup> mg/ml) in the DPPH model, which was comparable with rutin (5.12 × 10<sup>-2</sup> mg/ml).

**Conclusions:** This study shows that *F.szowitsiana* is a reach source of coumarin and furanocoumarin derivatives. The methanolic extract showed remarkable antioxidant effects and it seems that the isolated compounds may pose potent antioxidant property.

**Keywords:** *Ferula szowitsiana* DC; Coumarin; Antioxidant; Galbanic acid

## Treatment of bedsores by Asvad salve in Traditional Iranian Medicine

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**Background and Aims:** Bed sore is a kind of sore that is created in areas of body that they are in constant contact with bed more than 24 hours. The disease mostly occurs in aging due to inactivity. In traditional Iranian medicine (TIM) bed sore kind of ulcers that is very corrupt and is caused due to lack of blood and the improvement is long. Ethnomedicine is cheaper and more successful in healing than modern medicine.

**Methods:** Treatment of bedsores in TIM is based on improving nutrition, eating blood-producing foods, massage around the wound and usage of Asvad salve. This salve has been experienced for a long time in folk medicine and traditional medicine of Iran. Its components are litharge, olive oil, mastic gum, turpentine gum, honey, pine resin and bees wax.

**Results and Conclusions:** litharge is the main part and it has these effects: detergent, resolvent, desiccant, absorbent, corrosive decayed flesh, effective in creating of outgrowing flesh and the healing of deep wounds. On the other hand, each component of this formula is also effective in wound healing, lonely. Since one of the components of litharge is lead oxide, it can increase blood flow to the area around the wound and it is healing faster. Honey, mastic and turpentine gum have anti-bacterial effect. Litharge preparation in TIM has very long process with use of modified drugs for prevention of lead poisoning. Clinical research has been done on this salve very little but its indigenous and traditional usage for a long time has shown that is very effective and without side effects. So we suggest further research on this formula.

**Keywords:** Bed sore; Traditional Iranian Medicine; Asvad salve

## Volatile constituents of the seed Kernel and leaf of *Moringa peregrina* (Forssk.) Fiori, Agricol. cultivated in Chabahr (Iran)

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**Background and Aims:** Volatile constituents of the seed kernel and leaf of cultivated *Moringa peregrina* (Forssk.) Fiori, Agricol. collected after hydrolysis were analyzed by GC and GC/MS. Five glucosinolate degradation products which constituted almost the whole isolated oil of the seed kernel were identified to be: isobutyl isothiocyanate (94.0%), isopropyl isothiocyanate (4.9%), sec-butyl isothiocyanate (0.5%), n-butyl isothiocyanate (0.5%) and benzyl isothiocyanate (<0.1%); while the volatile isothiocyanates which constituted also almost the whole isolated oil of the leaf were found to be: isobutyl isothiocyanate (88.5%), isopropyl isothiocyanate (10.2%), n-butyl isothiocyanate (0.4%) and sec-butyl isothiocyanate (<0.1%).

**Keywords:** Isobutyl isothiocyanate; Isopropyl isothiocyanate; *Moringa peregrina*; *Moringaceae*; Volatile isothiocyanates.



## Volatile oil analysis of white celery fruits as an endemic and rare species of Iran

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**Background and Aims:** The Apiaceae family is one of the most important families within plant kingdom which is rich in volatile oils and secondary metabolites and has high pharmaceutical and economic value. *Hausknechtia elymaitica* Boiss. or white celery, an endemic and rare Iranian plant from this family has been chosen in our study. It is the unique species of the genus and a monotypic genus just found in southwestern provinces of Iran. The aim of this study was to determine the volatile oils of the ripened fruits of *H. elymaitica* from West of Iran for the first time.

**Methods:** The dried and ripened fruits of the plant were chopped in distilled water and its hydro-distilled fraction was isolated by hydrodistillation for 3 h. Volatile oil sample was homogenized and dried over anhydrous sodium sulfate and stored in a refrigerator. The oil was analyzed by the GC-MS analysis.

**Results:** The volatile oil was a pale yellow, clear liquid bearing the characteristic pungent and cool aromatic odor of Apiaceae family plants. The major constituents of the oil were beta-bisabolene (51.1%), trans-asarone (25.0%), lavandulyl acetate (10.2%) and alpha-phellandrene (5.1%).

**Conclusions:** Volatile oil of white celery fruits is a valuable source of beta-bisabolene and trans-asarone. These active natural constituents can be used in pharmaceutical industries. beta-bisabolene demonstrated bactericidal activities and asarone is a potential candidate for managing of cognitive impairment such as Alzheimer's disease.

**Keywords:** *Hausknechtia elymaitica*; White celery; Volatile oil; GC-MS analysis; Apiaceae; Beta-bisabolene

## Scientific evaluation of medicinal plants with protective and healing activity on peptic ulcer in Iranian Traditional Medicine

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**Background and Aims:** peptic ulcer, including gastric and duodenal ulcers is a complex and multi-causal disease. The main reason of ulceration is imbalance between the aggressive factors and the mucosal defensive factors. In Traditional Iranian Medicine (TIM), several medicinal plants are thought to have protective and healing effect on peptic ulcer. The present study was conducted to verify anti gastric ulcer activity of selected medicinal plant used in TIM.

**Methods:** Information from important TIM books were collected and added to scientific reports derived from modern medical databases like Pubmed, Scirus, Scisearch and Scopus. The modern pharmacological mechanisms of action of these plants were obtained.

**Results:** Data showed that many of plants used as anti gastric ulcer in TIM have pharmacological mechanisms in modern investigation. *Tragopogon collinus* known as “lahiat-o-tis” has been claimed the most effective anti gastric ulcer drug in the traditional sources. Its protective activity against ethanol-induced gastric ulcer in rat has been proven in modern investigation. *Berberis vulgaris* known as “ambarbaris” and *Rhus coriaria* “samac”, have cytoprotective and anti-oxidant activity in the modern sources. *Plantago ovata* known as “esfarzah” has wound healing and anti-oxidant activity. *Quercus ilex* known as “ balut” has antimicrobial and anti-oxidant activity. *Morus alba* known as “ toot” can protect gastric cell by increasing nitric oxide and prostaglandin, two mediator of mucosal defense process.

**Conclusions:** It can be conclude that modern studies confirm protective and healing effect of these medicinal plants used as anti gastric ulcer in TIM. Further research to find perfect anti-ulcer mechanisms of these plants is recommended.

**Keywords:** Medicinal plants; Gastric ulcer; Iranian Traditional Medicine

## Herb remedies among Turkish people from Ardebil that live in Gonbadekavoos

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**Background and Aims:** Providing and finding ways to cure different diseases and to quiet pains was important and considerable. According to the tendency of people to use current herb remedies that are natural and available, researchers decided to study current and popular herbal drugs among Turkish people in Gonbad. **Methods:** In this study there are interviews with Turkish people in Gonbadekavoos that experienced herb remedies. The interviews were recorded and classified and also specified by numbers. The instruction of using of these cures were questioned.

**Results:** Curing nose-bleed with leek's water, thore throat by sucking orange-peel, curing pectoralgia with yellow rose, curing inappetence with mushroom, sour grape juice for fat. Sour orange juice for blood pressure, almond for diarrhea, ginger and pepper for cold. Fumitory for arms and legs' itching. Curing knee pain with date palm, curing fever by rubbing kholfé seed on body. Black myrobalan for stomachache, curing fatness with caraway seed. For asthma chervil's root, hazelnut for heart disease. Using barberry water to cure Kobra's poison. All the above mentioned methods are current and popular among Turkish people in Gonbadekavoos.

**Conclusions:** Common medicine in Iran has an old history for curing diseases and solving health problems. It is vital to record and keep them for future uses.

**Keywords:** Herbal drugs; House curing; Turkish; Gonbadekavoos

## Extraction of fatty acids from sunflower seed (*Helianthus annuus*) using supercritical CO<sub>2</sub> in green and high-tech separation process with response surface methodology

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**Background and Aims:** In this present study, Supercritical fluid extraction (SFE) was developed for extraction of seeds oils from Sunflower. This method was used on a large scale for the production of essential oils and pharmaceutical products from plants.

**Methods:** The experimental parameters of SFE such as pressure, temperature, modifier volume, static and dynamic extraction time were optimized by using a central composite design after a 2<sup>n</sup>-1 fractional factorial design. The chemical compositions of the SFE extract were identified by GC-MS. A SFE model suprexmts/225 and a GC-MASS 5890 hp was used in this study.

**Results:** The major components of SFE extracted oils in optimum conditions were: Hexadecanoic acid, 9, 12-Octadecadienoic acid, 9, 12, 15-Octadecatrienoic acid, 11-Eicosenoic acid, Eicosenoic acid, Docosanoic acid, Heneicosanoic acid. Extraction recovery based on the SFE varied in the range of 0.02-9.25% (w/w) under different conditions.

**Conclusions:** These results confirm supercritical fluid extraction compared with other extraction method is relatively rapid because of the low viscosities and high diffusivities associated with supercritical fluids. In this work, under the optimum conditions several fatty acids from sun flower seeds with high recovery were extracted and identified by using SFE-GC-MASS.

**Keywords:** Supercritical fluid extraction (SFE); GC-MS; *Helianthus annuus*

## Supercritical CO<sub>2</sub> extraction of fatty acids from cotton seeds (*Linum usitatissimum*): Experimental design and gas chromatography-mass

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**Background and Aims:** In this present study, Supercritical fluid extraction (SFE) was developed for extraction of seeds oils from Cotton seeds. This method was used on a large scale for the production of essential oils and pharmaceutical products from plants.

**Methods:** The experimental parameters of SFE such as pressure, temperature, modifier volume, static and dynamic extraction time were optimized using a central composite design after a 2n-1 fractional factorial design. The chemical compositions of the SFE extract were identified by GC-MS. A SFE model suprexmts/225 and a GC-MASS 5890 hp Was used in this study.

**Results:** The major components of SFE extracted oils in optimum conditions were: Hexadecanoic acid, 8, 11-Octadecadienoic acid, Octadecanoic acid, Oleic acid, Eicosenoic acid, Extraction recovery based on the SFE varied in the range of 0.31-9.47% (w/w) under different conditions.

**Conclusions:** These results confirm supercritical fluid extraction compared with other extraction method is relatively rapid because of the low viscosities and high diffusivities associated with supercritical fluids. In this work, under the optimum conditions several fatty acids from cotton seeds with high recovery were extracted and identified by using SFE-GC-MASS.

**Keywords:** Supercritical fluid extraction (SFE); GC-MASS; Cotton seeds

## Supercritical CO<sub>2</sub> extraction of fatty acids from sesame seeds: experimental design and gas chromatography-mass

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**Background and Aims:** In this present study, Supercritical fluid extraction (SFE) was developed for extraction of seeds oils from Sesame seeds. This method was used on a large scale for the production of essential oils and pharmaceutical products from plants.

**Methods:** The experimental parameters of SFE such as pressure, temperature, modifier volume, static and dynamic extraction time were optimized by using a central composite design after a 2<sup>n</sup>-1 fractional factorial design. The chemical compositions of the SFE extract were identified by GC-MS. A SFE model suprexmts/225 and a GC-MASS 5890 hp was used in this study.

**Results:** The major components of SFE extracted oils in optimum conditions were: Hexadecanoic acid, 9, 12-Octadecadienoic acid, Octadecanoic acid, Oleic acid, 11-Eicosenoic acid, Eicosenoic acid, Docosanoic acid, 1, 2- Benzenedicarboxylic acid, Gamma-Sitosterol. Extraction recovery based on the SFE varied in the range of 0.20–10.18% (w/w) under different conditions.

**Conclusions:** These results confirm supercritical fluid extraction compared with other extraction method is relatively rapid because of the low viscosities and high diffusivities associated with supercritical fluids. In this work, under the optimum conditions several fatty acids from Sesame seeds with high recovery were extracted and identified by using SFE-GC-MASS.

**Keywords:** Supercritical fluid extraction (SFE); GC-MASS; Sesame seeds

## Study on the phytochemical constituents of *Thymus kotschyanus* Boiss. Et Hohen and its efficacy on the symptoms' improvement of irritant bowel syndrome

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**Background and Aims:** In some regions of Iran, *Thymus kotschyanus*, with local names of “KahliOti” or “KahUti”, is well-known as a natural remedy for eliminating cold temperament of yogurt or its common traditional beverage (named in Persian as Dough). It is also used for treatment of disorders such as mucus losing, flatulence, abdominal pain with diarrhea and/or constipation.

**Methods:** Based on the existing folkloric knowledge on application of this plant for treatment of irritant bowel syndrome, a pre- and post-interventional clinical trial has been performed. The main phytochemical constituents of the plant and chemical analysis of the aerial parts volatile oils were also determined. The clinical survey involved 29 patients suffering from typical signs of IBS. The patients were treated with the infusion made from flowering aerial parts of *T. kotschyanus*, sweetened with crystallized sugar for 4 weeks. The Friedman test and Nonparametric-Wilcoxon signed ranks test were applied.

**Results:** Phytochemical studies revealed presence of considerable amount of flavonoids and volatile oils in the aerial parts. GC-MS analysis of the volatile oils showed presence of large amounts of monoterpene hydrocarbons, sesquiterpene hydrocarbons and their oxygenated derivatives as 26.38%, 6.57%, 62.99% and 2.17% of the total content of oil, respectively. Consequently, the major IBS symptoms including diarrhea or constipation and also the patients main complains of abdominal pain and flatulence were statistically relieved ( $P < 0.001$ ).

**Conclusions:** In conclusion, chemical analysis of the plant's volatile oils reveals the considerable constituents comparing with other species and as a more important achievement, the administration of *T. kotschyanus* seems to be effective for abdominal symptoms in patients suffering from irritant bowel syndrome.

**Keywords:** *Thymus kotschyanus*; Irritant bowel syndrome; GC-MS analysis

## Antioxidant and cytotoxic activity of several plants from Iran

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**Background and Aims:** Iran is one of the most geographically diverse countries in the world and therefore one of the major producers of herbal medicines. Dena mountain region has many medicinal herbs. We collected several plants including *Salix excelsa*, *Cirsium bracteosum*, *Rhamnus cornifolia*, *Dionysia bryoides*, *Dionysia revoluta*, *Solenanthes circinnatus* and *Pterocephalus canus* collected from this region. In this study, we investigated the antioxidant and cytotoxic activity of methanolic extract of these plants.

**Methods:** For evaluation of the cytotoxic activity of these plants, different tumoral cell lines including K562 (human chronic myelogenous leukemia), MCF-7 (human breast adenocarcinoma) and MOLT-4 (human lymphocytic leukemia) were exposed to the 90% methanolic extracts and cytotoxic analysis was performed using MTT colorimetric assay. Furthermore, the antioxidant efficacies of the extracts were studied on the 80% methanolic and methanolic plant extracts employing 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays for testing.

**Results:** Obtained results indicated that a number of the extracts had the capacity to decrease the proliferation of tumoral cells. Among the plants, *Dionysia bryoides* was effective on all 3 cell lines. On the other hand, *S. excelsa* had the highest antioxidant capacity.

**Conclusions:** These results indicated that the extracts used in this study have a high potential for discovery of novel biologically active compounds.

**Keywords:** Antioxidant activity; Cytotoxic activity; Plants



## Caspase-dependent pathway in apoptosis induced by Safranal in alveolar human lung cancer cell line

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**Background and Aims:** Most lung cancer patients resist against conventional chemotherapy and suffer from its side effects. Thus, identification of a novel anticancer drug is urgently needed. Saffron has been shown to exhibit good antioxidant and anticancer activity. However, relatively little is known about the potential benefits of its active constituent, safranal.

**Methods:** A549 Cells were incubated with different concentrations of safranal, then, cell morphological changes, cell growth and apoptosis were determined by the normal invert- microscope, MTT assay, Annexin V and propidium iodide, and flow cytometric analysis, respectively. Activated caspases were detected by treatment of safranal in the lung cancer cells using fluorescein –labelled inhibitors of poly-caspases.

**Results:** The proliferation of A549 cells were decreased after treatment with safranal in a dose-and time-dependent manner. The results indicated that safranal induced morphological changes, decreased percentage of growth and induced apoptosis dose-and time-dependently. Safranal could induce apoptosis in A549 cells and activate caspases system. The levels of caspases involved in safranal-induced apoptosis in the A549 cell line indicating caspase-dependent pathway was induced by safranal.

**Conclusions:** Safranal exerts anti-proliferative effects on the human lung adenocarcinoma A549 cells. The anticancer activity of safranal could be attributed partly to its inhibition of the cell proliferation and induction of apoptosis in cancer cells through caspase-dependent pathways activation.

**Keywords:** Caspases; Apoptosis; Safranal; Lung; Cancer

## Chemical composition and biological activity of the essential oils of *Tripleurospermum caucasicum* (willd.) Hayek growing in Iran

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**Background and Aims:** In this study the chemical composition and antimicrobial and antioxidant activity of the essential oils obtained from the aerial parts of *Tripleurospermum caucasicum* (Willd.) Hayek were carried out.

**Methods:** The oil was obtained by water-distillation and was analysed by GC and GC/MS. Antibacterial activity by disc diffusion method and minimum inhibitory concentration (MIC) at different oil dilutions were screened against Gram positive and Gram negative bacteria, and fungi. The antioxidant activities of the oil was evaluated using ferric reducing antioxidant power (FRAP) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assays.

**Results:** Fifty-six constituents, representing 98.60 % of the total components in the oil, have been identified in the essential oil extracted from the aerial parts of this plant. The essential oil with major compositions of 1,8-cineole (16/70),  $\beta$ -Thujone (15/20),  $\alpha$ -Thujone (11/55) showed an inhibiting activity on disease causing Gram-negative and Gram positive bacteria, the most inhibited being *Escherichia coli* (MIC values of 16  $\mu$ g). This is particularly interesting from a medical point of view because this microbial agent is responsible for severe opportunistic infections. The essential oil showed good antioxidant activities, ( $IC_{50} = 4.21 \pm 0.50$   $\mu$ g/ml) which correlated well with the total phenolic content ( $1.92 \pm 0.50$  mg catechin /g essential oil) of the oil.

**Conclusions:** The results indicating that *T. caucasicum* has potential use in phytotherapy. This is particularly important because in many developing countries about 80% of available drugs come from medicinal plants and in industrialized countries plants make up the raw material for processes, which synthesize pure chemical derivatives.

**Keywords:** Essential oil; *Tripleurospermum caucasicum*; Antibacterial; Antioxidant

## Chemical constituents and cytotoxic activity of the essential oil of *Libanotis transcaucasica* Schischk from Iran

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**Background and Aims:** The genus *Libanotis* L. belongs to the family Apiaceae. The plants of this family possess peculiar botanical characteristics such as the typical umbellate inflorescences and are distributed widely from tropical to temperate regions where they often are used as spices or drugs. *L. transcaucasica* is a perennial plant distributed in Europe, northwest of Iran and also Caucasia. *Libanotis montana* var. *lasiopetala*, *seseli libanotis* var. *armeniacum* and *seseli transcausicum* are the other names for this plant. In the present study, chemical constituents of the essential oil from *L. transcaucasica* aerial parts and the cytotoxic activity of the oil are investigated.

**Methods:** The essential oil was isolated by hydrodistillation and analyzed by combination of gas chromatography (GC) and gas chromatography/mass spectroscopy (GC/MS). The cytotoxic activity was evaluated by MTT assay on four human cancerous cell lines (HeLa, LS180, MCF-7 and Raji).

**Results:** The GC/MS analysis of the oil resulted in 54 compounds, making up 84% of the total composition. Germacrene B (20.2%) was the most abundant constituent in this oil, followed by isospathulenol (11.0%), germacrene D (9.2%) and kessane (5.5%). The essential oil showed weak to moderate cytotoxic activity in the studied cancer cell lines.

**Conclusions:** Sesquiterpene hydrocarbons were identified as the main components of the essential oil (48.3%) and the cytotoxic activity observed in the oil may be contributed to the existence of this group of hydrocarbons in the plant.

**Keywords:** *Libanotis transcaucasica*; Essential oil; Cytotoxic activity

## Cycloartanes from *Euphorbia connata* Boiss

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**Background and Aims:** Cycloartanes are the major intermediates in the phytosterols biosynthesis. In addition, these compounds are used as specific chemotaxonomic markers in *Euphorbia* genus, which comprises well over 2000 species in tropical and temperate zones of Asia and other parts of the world. In this study the authors decided to isolate a number of cycloartanes from this species in order to introduce them as possible lead compounds for new drug discovery.

**Methods:** The compounds were purified using column chromatography run by Merck, Silica gel, and HPLC column YMC Pack-Sil (25\*300 mm). The structures of the isolated compounds were elucidated by <sup>13</sup>C- and <sup>1</sup>H-NMR as well as 2D-NMR, IR and by the aid of mass fragmentation pattern and comparing with the literature.

**Results:** In this research, dried acetone: chloroform extract (1:2) of aerial parts of *Euphorbia connata* collected from the Kerman in the east part of Iran, afforded a number of triterpenes included: cycloart-23-ene-3 (1), 25-diol, cycloart-23-en-25-methoxy-3-ol (2), cycloartan-3-ol (3), cycloeucalenol (4) for the first time from this plant.

**Conclusions:** Isolation and identification of a Cycloartanes for the first time from this plant as a new source for this type of compounds, allowing them to further study in the future, through the assessment of biological and toxic effects.

**Keywords:** *Euphorbia connata*; Terpenoids; Cycloartanes

## Determination of melittin in several honeybee venom powder samples by HPLC

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**Background and Aims:** The effects of honeybee venom therapy have been known since prehistoric times. It has been found to have a marked effect on variety of diseases, including arthritis, angiocardopathy, back pain, musculoskeletal pain, cancerous tumors, anticancer, and multiple sclerosis the immune system, cardiovascular system. It is a typical representative of biologically active peptide drugs with high therapeutic potential. Bee venom contains a complex mixture of biologically active agents, such as peptides, enzymes, biogenic amines, etc. The main components of Bee venom are enzymes: phospholipase A2 (12-15%), hyaluronidase (1-3%), peptides: melittin (approx. 50%), mast cell degranulating (MCD) peptide (2%), apamin, and promelittin. The aim of the study was to survey the amount of melittin as major compound in several honeybee venom dried samples by reverse-phase high-performance liquid chromatography (RP-HPLC).

**Methods:** A HPLC method with photodiode array (PDA) detector was used for determination of melittin and in crude honeybee venom dried powder as the traditional medicine possessing specific biological activity. Melittin of samples was extracted with deionised water followed by HPLC-PDA analysis on a C18 column of Europa Protein with water and acetonitrile containing 0.1% TFA as mobile phase under gradient elution at 220 nm.

**Results:** In this study, by applying this methodology the difference in melittin content of honeybee venom samples was evaluated. The melittin amount ranged from 8.2 to 51.3%. The reason of difference in melittin contents between samples can be probably due to different origin of samples, the use of different sampling methods and drying and maintain conditions.

**Conclusions:** HPLC analysis revealed a considerable difference in melittin of honeybee venom powdered samples. The findings showed that one of the samples is standard and the rest samples have to be subjected to further purification process.

**Keywords:** Honeybee venom; Melittin; HPLC

## Determination of triterpene contents of *Euphorbia osyridea*

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**Background and Aims:** Recent studies in natural product sources have resulted in many compounds that are being developed to treat inflammatory diseases. *Euphorbia* species are traditionally used in folk medicine to treat inflammations and tumors. Previous investigation by the authors on triterpenes also led to the isolation of some pentacyclic triterpenes with marked immunoinhibitory effects. Therefore, in this study, phytochemical research on *E. osyridea* was carried out by our group in order to find a suitable lead compounds with low toxicity for researches on their anti-inflammatory activities.

**Methods:** Plant material was collected from North East part of Iran (Khorasan province) and identified by Department of botany, herbaceous sciences research center at Ferdowsi University of Mashhad. The dried plant was extracted with dichloromethane:acetone (2:1) and after defatting, transferred repeatedly on silica columns using hexane:ethylacetate as mobile phase. Finally the fractions were purified by HPLC column YMC Pack-Sil, (20\*250 mm). The structures of the isolated compounds were elucidated by mass and different NMR spectroscopic methods.

**Results:** In this research, dried acetone:chloroform extract of aerial parts of *Euphorbia osyridea*, afforded a number of penta cyclic triterpenes for the first time from this plant.

**Keywords:** *Euphorbia osyridea*; Pentacyclic triterpenes; NMR spectra; Chemosystematic

## Essential oil component in leaf and flower of Lemon balm (*Melissa officinalis* L.)

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**Background and Aims:** In this research, *Melissa officinalis* L. which is valuable compound plants in Iran was studied for essential oil component and focuses on the analysis of the chemical composition *M. officinalis* leaf and flower essential oil.

**Methods:** Leaf and flower of *Melissa officinalis* L. were collected from the botanic garden in the Noshahr province in Iran; These parts of plant were carefully cleaned, air-dried and distilled separately for 4 h with a Clevenger apparatus. Essential Oil samples were over anhydrous sodium sulfate and stored at low temperature before analysis. The chemical components of the leaf and flower oil of *M. officinalis* were analyzed by GC/MS.

**Results:** Our results showed that 16 and 24 substances were identified in leaf and flower of *M. officinalis*, respectively. The main of *M. officinalis* flower oil contained  $\alpha$ -pinene (25.61%),  $\alpha$ -bisabolene (13.06%), caryophyllene oxide (10.74%) and  $\Delta$ -cadinene (7.89%) and flower oil of this plant contained, caryophyllene oxide (24.01%),  $\alpha$ -pinene (14.98%),  $\Delta$ -cadinene (8.64%),  $\gamma$ -muurolene (5%) and germacrene D (4.70%). However, (z)-  $\beta$  - Ocimene, E-  $\beta$  - Ocimene,  $\alpha$ - methyl Benzene methanol,  $\alpha$  - Campholenal, Borneol, Pulegone, Piperitenone, Piperitenone Oxide, Phenol acetate <2, 6-dimethoxy->, 8- Amorphene and  $\beta$ -Eudesmol were identified in flower oil and were not detected in leaf oil. But,  $\alpha$ -Cedrene,  $\alpha$ - Bisabolene and Elemol only were identified in leaf oil of *M. officinalis*.

**Conclusions:** The citronellal, citronellol, linalool and geraniol as major chemical compositions of the essential oil of the lemon balm have been previously reported. However reported the oil from dried plant material exhibited relatively higher concentrations of citronellal and caryophyllene oxide, compared with the oil from fresh herb. In this study, citronellal was 2.20% and 3.23% in leaf and flower oil, respectively.

**Keywords:** *Melissa officinalis*; Extraction; GC/MS

## Evaluation of antimicrobial effect of ethanol and chloroform extracts of *Stachys byzantina*

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**Background and Aims:**In this study antimicrobial effects of ethanol and chloroform extracts of *Stachys byzantina* which were used as antimicrobial agent in Iranian folk medicine were evaluated against 11 strain of gram positive and negative bacteria by Determination of MIC and MBC value.

**Methods:**Preliminary antibacterial activity study of the ethanol and chloroform macerated extracts of *S. byzantina* were investigated against eleven bacterial strain by cup plate method. After primary evaluation of the antibacterial activity of ethanol and chloroform extracts of *S. byzantina* MIC and MBC of extracts were determined by broth macro dilution method against the test organisms as recommended by the national committee for clinical laboratory standards NCCL 2006.

**Results:**The ethanol extract of *S. byzantina* showed more antimicrobial effects against all tested Gram positive and Gram negative bacterial strains with the largest diameter of inhibition zones 28 mm against *Staphylococcus aureus* (ATCC 29923) with lowest MIC and MBC value equal to (1.5, 6.25 mg/ml). The other MIC and MBC values against *Micrococcus luteus* (ATCC9341), *Klebsiella pneumoniae* (ATCC10031), *Escherichia coli* (ATCC8739), Methicillin Resistance *Staphylococcus Aureus* (ATCC33521), *Pseudomonas aeruginosa* (ATCC27853), *Streptococcus sanguis* (ATCC1447), *Streptococcus sorbinas* (ATCC1601), *Streptococcus mutans* (ATCC35608), *Streptococcus salivarius* (ATCC9222), *Streptococcus faecalis* (ATCC1373), with were assessed to be (0.15,3.1), (3.1,50), (3.1,100), (12.5,25), (12.5,50), (6.25,100), (12.5,50), (12.5,100), (12.5,100), (12.5, up to 200) mg/ml, respectively. The chloroform extract hasn't show good track of antimicrobial effect in comparison to ethanol extract.

**Conclusions:** The results concluded that the ethanol extracts of *S. byzantina* has a potential antimicrobial effect due to its flavenoids part of extarct which reduced in chloroform extract. Ethanol extract of *S. byzantina* had the best antimicrobial effect on the tested micro organisms.

**Keywords:** MIC; MBC; Antimicrobial



## Evaluation of phenolic and flavonoid compounds in inflorescence organs of three Iranian Achillea species

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**Background and Aims:** Achillea is a genus belongs to Asteraceae with 19 reported species in Iran. Achillea plants have long been used in traditional medicine as an anti-inflammatory agent for treatment of rheumatic pain. Phenolics and flavonoid compounds are the most important medicinal metabolites of Achillea species. Flavonoids have been reported to exert a wide range of biological activities including: anti-inflammatory, antibacterial, antiviral, anti-tumor effects. Because of wide spread of Achillea species in Iran and pharmaceutical importance of their metabolites, the aim of this study was determination of total phenolic and flavonoid contents and characterization of some flavonoids in inflorescence of three Iranian Achillea species.

**Methods:** Flowering samples of *A. kellalensis*, *A. wilhelmsii* and *A. vermicularis* were collected from different natural regions. Samples were extracted in ethanol and total phenolic and flavonoid contents of extracts were determined spectrophotometrically. The existence of some flavonoids in samples was demonstrated by thin layer chromatography (TLC) method and their contents were determined by high performance liquid chromatography (HPLC) technique.

**Results:** The highest total content ( $75.08 \pm 2.40$  mg/g DW) of phenolic compounds was obtained for inflorescence sample of *A. kellalensis* (Boein Zahra population) and the highest amount ( $4.55 \pm 0.33$  mg/g DW) of total flavonoids was determined for inflorescence sample of *A. kellalensis* (Qazvin population). On the basis of HPLC data the amounts of quercetin, apigenin and rutin flavonoids in the samples ranged 0-0.32, 0-0.3 and 0-0.8 mg/g DW, respectively. The highest content of quercetin and apigenin was calculated in inflorescence of *A. kellalensis* (Boein Zahra population) and the highest amount for rutin was obtained in inflorescence of *A. wilhelmsii* (Shiraz population).

**Conclusions:** It is suggested that inflorescence organs of some populations of Achillea species growing wild in Iran are potent natural sources for phenolic and flavonoid compounds.

**Keywords:** Achillea; Flavonoids; Phenolic compounds; Spectrophotometry; TLC; HPLC

## Flavonoid constituents in some of *Endemic salvia L. (lamiaceae)* species in Iran

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**Background and Aims:** *Salvia* Linnaeus (L.) genus is considered one of the most important medicinal genera of Lamiaceae. This study is concerned the flavonoid compounds in five endemic *Salvia* species from Iran namely as *S. macrosiphon* Boissier (Boiss.), *S. spinosa* L., *S. reuterana* Boiss., *S. sharifii* Rechinger and Esfandiari (Rech. & Esfand.) and *S. mirzayanii* Rech. & Esfand. which were collected from natural habitats of Iran and analyzed for their flavonoid constituents.

**Methods:** The flavonoid solution was extracted from air-dried leaves using crude 85% MeOH at 60°C. The extract was analyzed by two-dimensional maps on silica gel thin layer chromatography. Spots' detection with natural product identifiers (5% H<sub>2</sub>SO<sub>4</sub> in MeOH) was performed under UV-366 nm. The purification of flavonoid compounds of each species was carried out using column chromatography with sephadex LH20. The fractions were subjected to one dimensional map on silica gel plates. Identification of purified compounds was performed on the basis of their UV spectra (366 nm), MeOH solution and shift reagents.

**Results:** Based on the findings, total numbers of spots obtained for each species were 22-34. The yellow and blue spots were common in *Salvia* species. The patterns of flavonoid variations in *Salvia* species displayed more diversity. The highest flavonoid variations are related to hydroxylation (70%) and methoxylation (50%). 60 flavonoid compounds were identified namely as flavones, flavonols, isoflavones, flavanones and chalcones. Moreover, the highest flavonoid classes among five *Salvia* species were flavones (88%) and the lowest were chalcones (8.1%). Some of the flavonoid compounds in *Salvia* species studied were first reported for Iran such as sakuranin, pseudobaptigenin, tectorigenin and tectorigenin-7-o-glucoside.

**Conclusions:** It can be concluded that hydroxylation, methoxylation and glucosylation patterns may be considered to be specific to the *Salvia* species. Their presence could be significant in chemotaxonomy and pharmaceutical researches.

**Keywords:** Endemic; Flavonoid; *Salvia*; Iran

## Four-week ingestion of fresh and commercial orange juice on pro-inflammatory cytokines levels: A randomized cross-over study in healthy volunteers

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**Background and Aims:** Orange juice (OJ) consumption has become a worldwide dietary habit. OJ product has a great flavonoid content; such as polymethoxylated flavones, hesperitin and naringin. This juice is a rich source of dietary flavonoids. Flavonoids have antioxidant and anti-inflammatory properties that preventing the development of atherosclerosis. The aim of this study was to examine the effects of 4-wk intake of fresh OJ (*Citrus sinensis*) and commercial OJ on pro-inflammatory cytokines levels in healthy volunteers.

**Methods:** Twenty-two healthy subjects (age 18–59Y) were included in a randomized crossover study; they were randomly divided into two groups. Throughout the two 4-wk periods, volunteers daily consumed with 500 mL/day, twice a day with breakfast and dinner, of either fresh OJ or commercial OJ. There was a 2-wk washout period between each treatment. Volunteers made 4 visits to the clinical research unit on the before (visits 1, 3) and after (visits 2, 4) day of each of experimental period. Fasting blood was obtained at baseline (visits 1, 3) and after 4-wk (visits 2, 4) treatment periods.

**Results:** This study indicated fresh and commercial orange juice were significantly decreased ( $p \leq 0.05$ ) Intercellular cell adhesion molecule1 (-20.28% and -18.95%;  $p \leq 0.000$ ), vascular cell adhesion molecule1 (-22.53% and -20.50%;  $p \leq 0.000$ ), High-sensitivity C-reactive protein (-37.07% and -35.00%;  $p \leq 0.000$ ) and Eselectin (-28.62% and -24.61%;  $p \leq 0.000$ ) after 4 wk consumption relative to baseline. While interleukin-6 (-1.41% & -2.38%;  $p \geq 0.05$ ) has not significantly changed.

**Conclusions:** Consumption of 500 ml/day of OJ for 4 weeks, effectively decreased biomarkers of inflammation. While reactivity compared differences mean for fresh OJ and commercial OJ respectively were no significantly. Our study suggests both OJ ingestion significantly prevent the development of atherosclerosis reactivity.

**Keywords:** Orange juice; Pro-inflammatory cytokines levels; ICAM; VCAM

## Identification of anticholinesterase compound from *Berbers integrima*, *Rheum ribes* and *Levisticum officinale*

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**Background and Aims:** The present study was designed to investigate the acetylcholinesterase inhibitory compound from *Berbers integrima*, *Rheum ribes* and *Levisticum officinale*.

**Methods:** Initially dried powder of 100 plants were extracted successively in Methanol and tested for the presence of acetylcholinesterase inhibitory activity. Among them *Levisticum officinale*, *Berbers integrima* and *Rheum ribes* have inhibitory activity on mentioned enzyme. We identified the active compound of these three plants by thin layer chromatography (TLC) bioautography. TLC chromatograms revealed the presence of secondary metabolites in these plants. The phytochemical tests carried out on the active methanolic extracts made it possible to emphasize the various phytochemical groups presents in the plants.

**Results:** Phytochemical analysis of 3 active extracts demonstrated the presence of common phytoconstituents like terpenoides, alkaloids and phenols. Our findings indicated that the inhibitory activity of *Levisticum officinale* maybe due to the presence of some terpenoide compounds in this plant. The inhibitory effect of *Rheum ribes* maybe due to both terpenoides and alkaloids and the inhibitory effect of *Berberis integrima* maybe totally due to presence of compounds belong to alkaloids in these plant.

**Conclusions:** Results of this study showed that the alkaloid and terpenoid compounds are probably main responsible for the acetylcholinesterase inhibitory activity of these plants. Therefore these plants offer a unique candidate to find out potential new inhibitor for acetylcholinesterase enzyme.

**Keywords:** Acetylcholinesterase; Alkaloids; Terpenoids; *Levisticum officinale*; *Berbers integrima*; *Rheum ribes*

## Isolation and structural elucidation of natural products in *Euphorbia erythradenia*

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**Background and Aims:** The genus *Euphorbia* is the largest in the plant family Euphorbiaceae, comprising more than 2000 species. Over 70 species of *Euphorbia* are so far reported from Iran, out of which 17 species are native. Studies on Iranian *Euphorbia* indicates that these plants contain secondary metabolites such as diterpenoids with myrsinane skeletons, flavonoids, tannins, sterols, mono-, sesqui- and triterpenoids etc, which have interesting biological activities. Diterpenoids, from Iranian *Euphorbia* species have some biological activity such as skin-irritating, tumor-promoting, co-carcinogenic, analgesic, DNA-damaging activity, enzyme inhibition, etc. In this study *Euphorbia erythradenia*, endemic specie growing in Iran, is investigated for its chemical constituents.

**Methods:** The aerial part of the plant after grinding was extracted with dichloromethane and methanol. The two extracts were combined and divided into three different phases (n-hexane, ethyl acetate and water) using liquid-liquid extraction. The hexane layer of the plant extract was subjected to different chromatography systems including column-, flash column- and thin layer-chromatography on various stationary and mobile phases. The molecular structures of the purified compounds were determined by spectroscopic methods including <sup>1</sup>H NMR, <sup>13</sup>C NMR, MS, IR.

**Results:** Two cycloartane triterpenoids as the major constituents of the plant in addition to  $\beta$ -sitosterol,  $\beta$ -sitosterol glycoside, and a lipid-glycoside were among the identified compounds.

**Conclusions:** The plant can be used as a new source for isolation of the cycloartane triterpenoids and steroids.

**Keywords:** Keywords: Euphorbiaceae; *Euphorbia erythradenia*; Cycloartane triterpenoids;  $\beta$ -sitosterol

## Isolation of triterpenes from *Euphorbia macrostegia*

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**Background and Aims:** Euphorbiaceae is one of the largest families of the phylum Anthophyta. In this family the largest genera is Euphorbia which comprises well over 2000 species in tropical and temperate zones of Asia and other parts of the world. In Iran 70 species are reported that 17 of them are endemic. In traditional medicine Euphorbia was used as treatment of intestinal parasites and skin diseases. However, multidisciplinary biological screening tests carried out in recent years have shown that some of them were useful as anti-tumors, pesticides and antiviruses.

**Methods:** In this research, *Euphorbia macrostegia* Boiss. was collected from Yasuj, and the compound were purified using column chromatography run by Silica gel, and HPLC column YMC Pack-Sil, (25\*300 mm). The structures of the isolated compounds were elucidated by <sup>13</sup>C- and <sup>1</sup>H-NMR as well as 2D-NMR, IR and by the aid of mass fragmentation pattern and comparing with the literature.

**Results:** Triterpenes were isolated from dried acetone:chloroform extract (1:2) of aerial parts of *Euphorbia macrostegia* Boiss for the first time.

**Conclusions:** These findings confirm that this plant is a new and non expensive source for this group of compounds, and suggest further study in the future for drug discovery.

**Keywords:** *Euphorbia macrostegia* Boiss; HPLC; NMR; Triterpenes

## New insight in ezetimibe /garlic combination in hypercholesterolemic mice

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**Background and Aims:** Using herbs for lowering blood glucose and lipids to normal levels is clinically important. In this respect, garlic is one of the medicinal plants, which has shown hypocholesterolemic effects. Ezetimibe is also a novel and effective lipid lowering medicine that is well tolerated by the patients and has a safety profile similar to that of placebo. This study was aimed to evaluate the combination effect of aquatic extract of garlic and ezetimibe on lipid profile and glucose in hypercholesterolemic mice.

**Methods:** A total of forty N-mary male mice were randomly divided into five groups. Group 1 received: chow+2% cholesterol+0.5% cholic acid, group 2: chow+4% garlic extract+2% cholesterol+0.5% cholic acid, group 3: chow+0.005% Ezetimibe+2%cholesterol+0.5% cholic acid, group 4: chow + 4%garlic+0.005% Ezetimibe+2% cholesterol+0.5% cholic acid, and group 5: chow only. After four weeks mice were sacrificed, blood was collected, liver weight was measured and lipid profile and glucose levels were determined enzymatically.

**Results:** Compared with hypercholesterolemic mice, ezetimibe plus garlic significantly decreased cholesterol level ( $P<0.000$ ), low-density lipoprotein cholesterol levels ( $P<0.000$ ), liver weight ( $P<0.001$ ), %liver/body weight ( $P<0.02$ ) and atherogenic index ( $P<0.005$ ).

**Conclusions:** The findings showed that the combination of garlic and ezetimibe was more effective than garlic and ezetimibe alone in improving the lipid profile.

**Keywords:** Garlic; Ezetimibe; Hypercholesterolemic mice

## Novel aromatic sesquiterpene from the sponge *Halichondria*

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**Background and Aims:** Curcuphenol (1) is a biologically active sesquiterpene phenol that has been isolated from the marine sponges. It has shown antimicrobial, antifungal, antimalarial and also cytotoxic activities. In this work, isolation and structure elucidation of new Curcuphenol derivatives from the sponge *Halichondria* was done.

**Methods:** The sponge (700g) was collected, identified and extracted with MeOH: EtOAc (1:1). The extract was subjected to a Kupchan solvent-partition scheme yielding hexane, CH<sub>2</sub>Cl<sub>2</sub>, BuOH and H<sub>2</sub>O soluble material. The hexane and CH<sub>2</sub>Cl<sub>2</sub> extracts were further fractionated over several Si gel flash columns and RPHPLC. The pure compounds were elucidated by spectroscopic data.

**Results:** Curcuphenol (1) was obtained from hexane extract along with a new curcuphenol derivative sesquiterpene (2).

Compound 1: Yellow oil, <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 1.25 (d, 3H, J = 6.9 Hz), 1.49–1.75 (m, 2H), 1.56 (s, 3H), 1.70 (s, 3H), 1.93–1.99 (m, 2H), 2.29 (s, 3H), 2.94–3.06 (m, 1 H), 4.74 (s, 1H), 5.15 (t, 1H, J = 6.9 Hz), 6.60 (s, 1H), 6.74 (d, 1H, J = 7.8 Hz), 7.05 (d, 1H, J = 7.8 Hz). MS (EI): m/z 218 Anal. Calcd for C<sub>15</sub>H<sub>22</sub>O. Compound 2: <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 1.25 (d, 3H, J = 6.9 Hz), 1.75, 1.85 (m, 2H), 1.66 (s, 3H), 1.9–2.04 (m, 2H), 2.29 (s, 3H), 2.94–3.06 (m, 1 H), 6.45 (dt, 1H, J = 1,6), 6.60 (s, 1H), 6.74 (d, 1H, J = 7.8 Hz), 7.05 (d, 1H, J = 7.8 Hz), 9.35 (s, 1H). MS (EI): m/z 232 Anal. Calcd for C<sub>15</sub>H<sub>20</sub>O<sub>2</sub>.

**Conclusions:** Compound 2 differs from 1 in the terminal aldehyde group. This was confirmed by the observation of singlet at 9.3 ppm in <sup>1</sup>H NMR along with a signal at 195.45 ppm from CNMR. So compound 2 was proposed as a new member of curcuphenol derived sesquiterpenes.

**Keywords:** *Halichondria*; Curcuphenol; Sesquiterpene



## Pharmacologic effects of *Origanum vulgare* related to GABA<sub>A</sub> receptors

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**Background and Aims:** Today role of herbal plants in treatment of disease is one of important controversies in medicinal associations. Oregano oil has a wide range of traditional uses including the treatment of various digestive upsets, asthma, colds, flu, bronchitis, headaches, rheumatism, muscular pain, insect bites, and warts (naghbi2005). The objective of this study is to evaluate possible involvement of the GABA<sub>A</sub> system in analgesic effect of aqueous extract of *Origanum Vulgare* in male rats.

**Methods:** The rats were anaesthetized by ketamine (80mg /kg) and xylazine (10mg /kg) and the cannula was inserted into left ventricle using stereotaxic apparatus (Paxinos, 2005). after 5-7 days dose of extract determined 3µg/rat that was administered intraventricularly. Control group given saline 0.5ml.i.p/ saline 5µl, i.c.v or ORG 3µg/rat,i.c.v. GABA<sub>A</sub> receptor agonist (Muscimol, 1mg/kg,i.p), GABA<sub>A</sub> receptor antagonist (Bicuculline 5mg/kg,i.p). The aqueous extract of ORG was administered 20 min after drug /vehicle injection. The latency response of rats recorded by Tail-Flick test.

**Results:** Intracerebroventricular administration of the ORG extract resulted in significant and dose-dependent increase in the latency response in the Tail-Flick test (p < 0.001). Gaba<sub>A</sub> receptors can contribute in this analgesic effects. Analgesic effect of this plant has been probably related to oxygenated compounds such as Borneol, one of compounds of that. Borneol is a two circulated monoterpene that have analgesic effect and increasing effect on gabaergic receptors.

**Conclusion:** The results of this study shown that analgesic effect of ORG maybe mediated by Gaba receptors.

**Keywords:** *Origanum Vulgare*; GABA<sub>A</sub>; Pain

## Phenylethanoid glycosides from *Eremostachys azerbaijanica* Rech. F.

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**Background and Aims:** The genus *Eremostachys* is one of the largest genera of the Lamiaceae family that about 16 species of this genus are growing in Iran. In traditional medicine of Azerbaijan region of Iran, roots of some species belonging to the genus *Eremostachys* are topically used in treatment of bruises, superficial and local inflammations and pains. In this research, roots of *E. azerbaijanica* were phytochemically studied until perhaps by identification of chemical content of this plant, a step can be taken towards correct use from this natural product in treatment of diseases.

**Methods:** In this research, the roots of *E. azerbaijanica* were sequentially extracted with petroleum ether, chloroform, ethyl acetate and methanol using maceration method. The dried methanolic extract was preliminary fractionated by SPE method on ODS sorbent, and the compounds existing in resulted fractions were purified by preparative HPLC (C18 column and acetonitrile-water solvent system). Extremely, the chemical structures of all compounds separated from MeOH extract were determined by using methods of UV/Visible and one dimensional NMR spectroscopy.

**Results:** Three known phenylethanoid glycosides named Forsythoside B, acetoside and alyssonoside were identified from MeOH extract of *E. azerbaijanica* roots.

**Conclusions:** Since all compounds identified from *E. azerbaijanica* structurally belong to the group of phenylethanoid glycosides with anti-inflammatory and analgesic effects, therefore it is expected that traditional and local application of this plant's roots in treatment of local inflammations and pains is reasonable and beneficial. On the other hand, in laboratory studies conducted on the anti-inflammatory and analgesic effects of several species of the genus *Eremostachys*, such effects in plants belonging to this genus have been proved.

**Keywords:** *Eremostachys azerbaijanica*; Phenylethanoid glycosides; HPLC; NMR

## Phytochemical analysis and antimicrobial effect of essential oil of *Artemisia kermanensis* of Faryab area by agar dilution method

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**Background and Aims:** Essential oil has many applications in food industry. Their antimicrobial activity affects different classes of microorganisms. This study aims to compare of the chemical composition and antimicrobial activity of the essential oils obtained from the aerial parts of *Artemisia kermanensis* of Faryab area.

**Methods:** The oil was extracted by hydro distillation method with efficiency 1.82 % (W/W) and was analyzed by GC-MS technique. Antimicrobial effects of this essential oil were carried out by Agar dilution method on *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Candida albicans*, *Aspergillus niger* microorganism.

**Results:** The main constituents of this oil were follows: 1, 8- Cineole (26.93%), Camphor (16.97%), alpha-Thujone (7.52%), Borneol (7.47%) and alpha-Terpineol (5.77%). The presented results of Minimal Inhibition Concentration (MIC), in the following: *Staphylococcus aureus* (4µg/ml), *Salmonella typhi* (32µg/ml), *Escherichia coli* (32µg/ml), *Candida albicans* (8 µg/ml), *Aspergillus niger* (4 µg/ml). Microorganisms of *Salmonella typhi* and *Escherichia coli* in the highest dilution and microorganisms of *Staphylococcus aureus* and *Aspergillus niger* in the least dilution were bound by essential oil.

**Conclusions:** The results of this examination are completely adapted with results of GC-MS analysis of essential oil that confirms the existence of 70.60 oxygenated compounds. This proper antimicrobial feature of essential oil is in full adaptation with the chemical composition and percent of oxygenated terpenoids.

**Keywords:** Chemical composition; Antimicrobial; Essential oil; *Artemisia kermanensis*; Agar dilution method

## Phytochemical analysis and antioxidant activity of *Fumaria parviflora*

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**Background and Aims:** *Fumaria parviflora* one of the endemic Iranian perennial herb with a long history of medicinal use, was studied to detect some biologically active chemical constituents of the aerial parts of plant.

**Methods:** The air dried powder (150g) of the plant was Soxhlet-extracted successfully with solvents of increasing polarity; n-hexane, dichloromethane and methanol. The resultant methanol extract was subjected to Sep-Pack fractionation (using a step gradient of methanol–water mixture). The preparative reversed phase high-performance liquid chromatography analysis of the 40% and 60% methanolic Sep-Pak fraction afforded two purified compounds. Next, the structures of the isolated compounds were elucidated on the basis of UV and NMR spectroscopic techniques. Besides, the alkaloid content of the methanol extract were taken up in hydrochloric acid that were filtered and the aqueous acid solution brought to pH 8 with concentrated ammonium hydroxide and extracted with dichloromethane. Subsequently, a gas chromatography–mass spectrometry technique was applied for determination and identification of tertiary bases in alkaloid extracts of *F. parviflora*. Furthermore, the in vitro antioxidant activity of the extracts were assessed by free radical scavenging capacity of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals.

**Results:** According to the patterns of <sup>1</sup>H NMR and UV spectrums for the compounds isolated from HPLC their structures were suggested as 1) 7-O-Glucosyl coumarin, 2) 4-Methoxy-3-rutinosyl-kampferol. GC-MS analysis of the prepared extract determined four known alkaloids 3) Fumariline 4) Dihydro fumariline 5) Fumaritine 6) Oxyberberine within *F. parviflora*. Moreover, the antioxidant activity for different solvent extracts varied according to the presence of compounds in each of them, where the methanol extract with RC<sub>50</sub> of 72×10<sup>-3</sup> mg/ml exhibited the potent activity.

**Conclusions:** *F. parviflora* with assorted valuable compounds possessed favorable antioxidant properties that could be assigned to the variety of its medicinal usages.

**Keywords:** *Fumariaceae*; *Fumaria parviflora*; Alkaloids; gas chromatography–mass spectrometry; DPPH

## Phytochemical constituents of root and aerial parts of *Scrophularia striata*

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**Background and Aims:** *Scrophularia striata* Boiss is used for the treatment of eczema, wounds and has shown various biological activities such as antimicrobial, antitumoral and anti-inflammatory properties. In this study the amounts of total phenolic, flavonoid, soluble sugar and anthocyanin contents of root and aerial part of *Scrophularia striata* are compared.

**Methods:** Samples were extracted using ethanol %80 as a solvent. The amount of total phenolic compounds in all sample extracts was determined with the Folin-Ciocalteu's reagent according to Meda et al. (2005). Results of total phenolic contents were expressed as milligrams of gallic acid equivalents (GAE) per gram dry weight (DW). Total flavonoids compound was measured by the aluminum chloride colorimetric assay based on Chang et al. (2002). Flavonoid compound of extracts were expressed as mg quercetin equivalent/g dry weight (DW). The soluble sugars were determined spectrophotometrically by the phenol-sulfuric acid method based on Kuchert method (1985). For aerial part, total anthocyanin content was measured based on Mita et al. method (1997).

**Results:** Aerial part exhibited higher total phenolics (TP) and total flavonoids (TF) compared to root, 4.60 mg galic acid equivalent (GAE)/g DW and 1.34 mg quercetin equivalent (E)/g dry weight (DW) respectively. Anthocyanins contents (ANT) in aerial part was 0.043. Total soluble sugars for aerial part and root were 36.08 and 37.24 mg glucose equivalent (GAE)/g DW respectively.

**Conclusions:** Results showed the potential of this plant as a source of and flavonoid phenolic compounds especially from the aerial part. So, it is proposed that this plant possess antioxidant activity.

**Keywords:** Anthocyanins; Flavonoids; Phenolic compounds; *Scrophularia striata*; Soluble sugars

## Phytochemical investigation on *Euphorbia macrostegia* (persian wood spurge)

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**Background and Aims:** Euphorbia species have been used in folk medicine for treatment of diarrhea, gonorrhea, migraines, cure skin diseases, wart remover, inflammation and swellings. The chemical constituent of some species of genus Euphorbia have been found to include macrocyclic and polycyclic diterpenoids and triterpenoids that shows different biologically activities such as; enzyme inhibition ( $\alpha$ -glycosidase, urease, HIV-1 reverse transcriptase), tumor promoting, DNA damage activity etc. These features promoted us to investigate the chemical constituents of Euphorbia macrostegia.

**Methods:** The aerial part of the plant was extracted by maceration in methanol and dichloromethane (DCM) respectively. The crude extracts were mixed together and subjected to Liquid-liquid extraction. The extraction has afforded four different phases, from non polar to polar: n-Hexane, DCM, 1-butanol and water. The hexane layer was subjected to isolation and purification of its chemical components using column chromatography over silica gel, flash column and thin layer chromatography. The structure of all compounds were elucidate using spectroscopy methods including 1 and 2D-NMR, <sup>1</sup>H NMR, <sup>13</sup>C NMR, MS, IR and by comparison with the literature survey.

**Results:** Four cycloartane triterpenoids,  $\beta$ -sitosterol, and four oxidized unsaturated fatty acids were suggested for the structure of the purified compounds

**Conclusions:** The triterpenoids as the chemical markers of the genus Euphorbia have been identified, but the presence of oxidized unsaturated fatty acids is reported rarely in this genus.

**Keywords:** Keywords: Euphorbiacea; *Euphorbia macrostegia*; Cycloartane Triterpenoids; Oxidize unsaturated fatty acids

## Volatile composition of the essential oil *Salix excelsa* from Iran

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**Background and Aims:** *Salix excelsa* is a genus belongs to Salicaceae, which distributed in Dena Mountain and Golestan province. The willow family of plants, *Salix*, contains notable amounts of phenolic compounds, so they have antioxidant activity. Antibacterial activity was also reported for other species of this family. The chemical constituents of the essential oil from *S. excelsa* is investigated at the present work.

**Methods:** The essential oil was isolated by hydrodistillation and analyzed by combination of gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS).

**Results:** Forty-nine components were identified, constituting approximately 86.3% of the oil. The major constituents of essential oil were salicylaldehyde (36.0%), pentadecanal (6.1%), (Z)-3, 7, 11, 15-tetramethyl-2-hexadecene-1-ol (5.5%),  $\beta$ -eudesmol (3.8) and (2E)-nonene-1-al (3.6%).

**Conclusions:** The aldehydes were identified as the major constituents of the essential oil of the plant (50.6%). Due to the highest amount of salicylaldehyde in the volatile oil of this genus, the plant might have good antibacterial activity.

**Keywords:** *Salix excelsa*; Essential oil; Salicylaldehyde

## Study of antioxidant activity and total phenolic content of 22 plants from *Compositae* family

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**Background and Aims:** Several biochemical and physiological processes are involved in the production of free radicals. Increase in free radical production could cause many diseases such as atherosclerosis, cancer, diabetes. Medicinal plants are good sources for natural antioxidants. For this reason some plants in *Compositae* family were chosen for studying antioxidant activity.

**Methods:** Methanolic extracts of the plants were prepared by maceration method. Antioxidant activity of the extracts were studied by FRAP and DPPH assays. Total phenolic content of the extracts were measured by Folin-Ciocalteu method.

**Results:** The results showed high antioxidant activities for most plants. *Anthemis trumfettii* showed the highest and *Achillea eriophora* showed the lowest antioxidant activities. There was a good correlation between antioxidant activity and total phenolic content.

**Conclusions:** These findings show that different plants from the *Compositae* family possess interesting biological activities and may be used for discovery and development of novel pharmacologically active compounds.

**Keywords:** Antioxidant; DPPH; FRAP; *Compositae*; Phenolics



## Study of seasonal changes of antioxidant enzymes of Lichen, *Physcia biziana* in Zirab region, Mazandaran, Iran

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**Background and Aims:** A Lichen is a symbiotic life form between a wide range of fungi, algae, or cyanobacteria, or possibly of all three. Antioxidants are the main factors for neutralizing the free radicals which are active and harmful for lichen.

**Methods:** The antioxidant enzyme of species was studied during period of summer, autumn and winter 1390 in Zirab region, Mazandran, Iran. The biochemical analysis involved measuring some antioxidant enzymes such as DPPH radical scavenging, superoxide dismutase, catalase and ascorbat peroxidase activities in the lichen using spectrophotometric methods.

**Results:** The tested extracts had strong antioxidant activity. The DPPH radical scavenging activity was as average of  $10.46 \pm 0.4$  mg/ml. The superoxide anion scavenging was showed activity between  $8.2 \pm 0.2$  to  $10.26 \pm 0.6$   $\mu$ g/mg of protein.

**Conclusions:** The results obtained in the present study indicate that *P. biziana* is a potential source of natural antioxidant.

**Keywords:** Antioxidant activity; Superoxide dismutase; Lichen; *Physcia biziana*

## The HPLC analysis of Iranian pomegranate (*Punica granatum*) seed oil phytosteroids

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**Background and Aims:** *Punica granatum* is small tree, belonging to the puniceae family. Pomegranate is grown mainly in Iran, India and the USA, but also in most Near and Far East countries. The oil that extracted from seeds (PSO) was mentioned that had many effects in Iranian traditional medicine.

**Methods:** the oil that is extracted from seeds that collected of different part of Iran. In HPLC analysis, an isocratic elution method using 35% aqueous acetonitrile solution at 1.0 ml/min with photodiode-array (PDA) detection at 225 nm and 254 nm was found to optimally separate and identify the steroid hormones from the pomegranate samples with a run time of less than 30 min. the type of column of HPLC was reverses and exactly C18 column was used.

**Results:** Estrone was detected but testestorn and estradiol as same as standard retention time was not detected.

**Conclusions:** This paper described the identification and re-evaluation of several steroid hormones analysis in PSO using HPLC–PDA and showed that could be good replacement for chemical drugs. The estrone is one of important phyto-estrogen that pomegranate seed oil is rich source of these hormones.

**Keywords:** Pomegranate seed oil; PSO; *Punica granatum*; Esteroids

## Triterpenes from *Euphorbia spinidens* with their immunomodulatory activity

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**Background and Aims:** Recent studies in natural product sources resulted in many compounds that are being developed to treat immunosuppressive disorders. *Euphorbia* species are traditionally used in folk medicine to treat inflammations and tumors. In recent studies by the authors, cycloartanes isolated from *euphorbia* species has showed immunostimulatory properties. In this study the authors decided to isolate a number of triterpenes from this genus and evaluate their immunomodulatory effects for further pharmaco-immunological research.

**Methods:** The compounds were purified using column chromatography run by Merck, Silica gel, and HPLC column YMC Pack-Sil, (25\*300 mm). The structures of the isolated compounds were elucidated by <sup>13</sup>C- and <sup>1</sup>H-NMR as well as 2D-NMR, IR and by the aid of mass fragmentation pattern and comparing with the literature. In phagocyte chemiluminescence assay, oxidative burst by phagocyte cells, different concentrations of compound were incubated with human whole blood in triplicate and the chemiluminescence activity of phagocytic cells were measured by using serum opsonized zymosan and luminol. For lymphocyte proliferation assay, peripheral human blood lymphocytes were incubated with different concentrations of the test compound in supplemented RPMI-1640 along with 5.0 µg/mL phytohemagglutinin (PHA) at 37° C in CO<sub>2</sub> environment for 72 hours and proliferation level was determined by Beta-scintillation counter.

**Results:** In this research, dried acetone:chloroform extract of aerial parts of *Euphorbia spinidens* collected from the khorasan province in North East part of Iran, afforded two triterpenoids, lup-20(29)-ene-3, 28-diol, commonly known as betulin and (3β,23E)-Cycloarta-23-ene-3,25-diol for the first time isolated from this plant. In phagocyte chemiluminescence assay, betulin showed moderate inhibitory effect on oxidative burst in neutrophils while addition of betulin triterpene to phytohemagglutinin (PHA) stimulated peripheral human blood lymphocytes (PBLs).

**Conclusions:** *Euphorbia spinidens* could be considered as a new source for (3β,23E)-Cycloarta-23-ene-3,25-diol (0.03% of plant dry weight).

**Keywords:** *Euphorbia spinidens*; Lupane type pentacyclic triterpenes; Cycloartane