



**7<sup>th</sup> National Congress on Medicinal Plants**  
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# **Oral Presentations**

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**Nature as Source for New Hit and Lead Compounds**

**Hermann Stuppner**

*Institute of Pharmacy/Pharmacognosy, CMBI, University of Innsbruck, Innsbruck, Austria*  
*E-mail: hermann.stuppner@uibk.ac.at*

The treatment of inflammatory disorders in Western medicine relies heavily on the use of non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids [1]. However, currently available treatment options are often unsatisfactory. Natural products (NPs) have always been an important source of new drug leads. Almost half of the drugs currently in clinical use are of natural product origin and even today, in the post genomic era, plants, fungi, marine organisms, and microorganisms are still an important source for the development of new drugs [2].

In the course of a national research network project, we aimed to identify and characterize anti-inflammatory NPs capable to combat inflammatory processes specifically in the cardiovascular system. The combined use of computational techniques with traditional knowledge, high-tech chemical analysis and synthesis, and a broad range of in vitro, cell-based, and in vivo pharmacological models led to the identification of a series of promising anti-inflammatory drug lead candidates. Mechanistic studies contributed to a better understanding of their mechanism of action and delivered new knowledge on the molecular level of inflammatory processes. Highlights of this interdisciplinary project will be presented.

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**Keywords:** Natural products, Hit compounds, Inflammation

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## New Perspectives in the Analysis of Medicinal and Aromatic Plants, applying Spectroscopic Techniques - Opportunities and Limitations

**Hartwig Schulz\***, Andrea Krähmer, Christopf Böttcher

*Julius Kühn Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Königin-Luise-Strasse 19, 14195 Berlin-Dahlem, Germany*

*E-mail: hartwig.schulz@julius-kuehn.de*

Quality control of medicinal and aromatic plants as well as products derived from them usually comprises correct botanical identification of the plant material as well as quantification of the individual active principles. For this, testing of plant material such as phytopharmaceutical products is usually performed in accordance with validated standard methods described in the Food Chemical Codex, the European Pharmacopeia, the United States Pharmacopoeia and others. Contrary to this approach, there is some need to apply also various rapid high-throughput methods aiming to characterise simultaneously several quality parameters and to reduce efforts for sample preparation to a minimum. In this context new vibrational spectroscopy methods (ATR-IR, NIR and Raman spectroscopy) in combination with various chemometric algorithms are presented which allow efficient monitoring of numerous plant samples within a short time [1]. Especially Raman spectroscopy has been found to be a reliable and non-destructive method for rapid discrimination of different plant species or chemotypes if characteristic key bands can be observed in the spectrum. But also NIR and ATR-IR spectroscopy has made the handling of powdered as well as liquid samples very quick and simple. Today, portable IR and Raman spectrometer systems are available which need only small sample amounts of a few microliters or milligrams for analysis. In most cases, vibrational measurements can be performed directly on plant tissues as well as on fractions isolated from the plant material by hydro-distillation or solvent extraction. Based on individual marker bands, spectroscopic analyses in principle allow the discrimination of different species, and even to classify chemotypes among the same species. Combination of vibrational spectroscopy and hierarchical cluster analysis provides a fast, easy and reliable method for chemotaxonomic characterization. The ability to rapidly monitor various plant components provides the possibility to efficiently select high-quality single plants from wild populations as well as progenies of crossing experiments. Today, vibrational spectroscopy is already introduced in industry in order to perform fast quality checks of incoming raw materials and continuous controlling of production processes. Furthermore, a study is presented which demonstrates the variation of metabolite profiles of onion bulbs (*Allium cepa* L.) depending on genetic and environmental factors. Using a recently established metabolite profiling approach based on liquid chromatography-coupled electrospray ionization quadrupole time-of-flight mass spectrometry [2], polar and semipolar metabolites which belong to different compound classes determining nutritional, sensory, and technological quality of onion bulbs such as saccharides, flavonoids, S-substituted cysteine conjugates, amino acids, and derived  $\gamma$ -glutamyl peptides were relatively quantitated in parallel. Statistical analyses of the obtained data indicated that depending on the compound class genetic and environmental factors differently contributed to variation of metabolite levels. For saccharides and flavonoids the genetic factor was the major source of variation, whereas for cysteine sulfoxides, amino acids, and peptides both genetic and environmental factors had a significant impact on corresponding metabolite levels. The described comprehensive metabolite profiling approach targeting all relevant compound classes has been performed in an exemplary manner and can be applied in a similar way to examine other plant species such as medicinal and aromatic plants with respect to individual aspects of their biological activity.

**Keywords:** Quality control, breeding, Raman, infrared, mass spectrometry

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## New Avenues for Good Old Natural Products (NPs)

### Pascal Richomme

*University of Angers, Dpt of Pharmacy, SONAS/SFR QUASAV, Campus du végétal, 42 rue Georges Morel 49070 Beaucouzé, France*

*Email: [pascal.richomme@univ-angers.fr](mailto:pascal.richomme@univ-angers.fr)*

NPs remain a great source of inspiration when developing analytical methods as well as bioactive compounds. We will illustrate this statement by presenting three concrete examples. The rapid and targeted chemical characterization of complex mixtures of secondary plant metabolites has become essential to many areas of NPs research such as dereplication studies, metabolomics profiling, and quality control. In this respect high-performance liquid chromatography coupled with mass spectrometry (HPLC-MSn) is commonly considered as method of first choice, while others such as matrix assisted laser desorption ionization (MALDI) as well as matrix free laser desorption ionization (LDI) are hardly discussed. As an introduction, the present lecture will highlight some recent advances in MALDI and LDI such as the development of highly selective MALDI matrices for the detection of alkaloids as well as the matrix free LDI detection of UV absorbing NPs. Indeed MALDI as well as LDI-MS may provide useful complements or alternatives to classic analytical approaches in NPs' research.

Conventional chemical fungicides commonly used to protect crops against fungal infections present the drawback to be highly pollutant for the environment, especially for soil and water supply, whereas these products may be highly toxic for humans. The so-called "Alternaria Leaf Spot" is a common disease of crucifers caused by the fungal pathogen *A. brassicicola* which affects different crops including cabbage, kale, Brussels sprout, cauliflower and broccoli. Indole phytoalexins camalexin and brassinin play in plants a key role in crop protection against this necrotrophic agent. However it has been shown that mutants become phytoalexin-resistant by activating at least three signaling pathways named as Cell Wall Integrity (CWI), High Osmolarity Glycerol (HOG) and Unfolded Protein Response (UPR). The latter is particularly involved in the fungus protection against phytoalexins since UPR deficient avirulent mutants of *A. brassicicola* appear as hypersensitive to camalexin and brassinin. Since very few UPR inhibitors such as the synthetic STF-083010 are known we decided to develop an original screening assay, detecting the production of a HAC1 fluorescence-induced protein, i. e. a transcriptional activator involved in the UPR pathway, in *Saccharomyces cerevisiae* cultures. The preliminary screening of an in-house NPs library [c.a. 70 compounds] revealed four compounds as potential UPR inhibitors. Finally, RT-PCR validated the true inhibitory effect of 2-deprenyl-rheediaxanthone B and griffipavixanthone which clearly appeared as good candidates for inhibiting UPR and, consequently, alternative crop protections.

The last example deals with functional lipidomics, i. e. a combination of comprehensive lipid mediator profiling together with mechanistic and cell-biological studies aiming at unravelling the molecular mechanism of bioactive agents. This approach was developed during a fruitful collaborative project, involving different European universities, focusing on the role of vitamin E ( $\alpha/\beta/\gamma/\delta$ -tocopherol/tocotrienol), which inhibits pro-inflammatory leukotriene formation and protects from leukotriene-related diseases (e.g., asthma, cardiovascular disease and cancer) but with controversial clinical evidence. As far as 5-lipoxygenase (5-LO) activity was concerned, it was shown that  $\omega$ -oxidation of vitamin E derivatives leads to a variety of long-chain hydroxy- and -carboxy metabolites which potently inhibited the enzyme. Further structural optimization revealed garcinoic acid ( $\delta$ -tocotrienolic acid, GA) -isolated from *G. kola* seeds- as one of the most potent inhibitors of 5-LO within this series. GA was then used as a scaffold to semisynthesize potent specific 5-LO inhibitors.



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## Liquorice Triterpenoids are Good or Bad?

**H Malekinejad**<sup>1</sup>, **P Akbari**<sup>2</sup>, **J Fink-Gremmels**<sup>3</sup>

<sup>1</sup>Department of Pharmacology & Toxicology, School of Pharmacy, Urmia University of Medical Sciences, Urmia, Iran

<sup>2</sup>Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science

<sup>3</sup>Division of Veterinary Pharmacy, Pharmacology and Toxicology, Utrecht University, Utrecht, The Netherlands

Liquorice extract from the roots of *Glycyrrhiza glabra* L. (Fabaceae) is one of the herbal medicines that are used widely in various countries. Extracted liquorice, containing glycyrrhizin (GA) and its main component 18 $\beta$ -glycyrrhetic acid (18 $\beta$ -GA) have been used as an additive for flavoring and sweetening of tobacco, candies, and beverages. Licorice has been traditionally prescribed for treating conditions like asthma, dry cough, and other “pectoral diseases”. We in our previous studies demonstrated the hepatoprotective and renoprotective effects of liquorice extract. The most reliable route of administration for liquorice extract often is oral method. There are however, little is known about the effects of licuorice compounds on integrity of intestinal tract. Therefore, the current study aimed to highlight any impact of aforementioned compounds in Caco-2 cells. Two methods of MTT and LDH were used to estimate the cytotoxicity of used compounds. To evaluate the effect of licuorice compounds on paracellular flux, Lucifer yellow (LY) as a membrane-impermeable molecule was used and the permeability of LY was measured after 24 exposure to various concentrations of glycyrrhizin and two metabolites. The fluorescence intensity of LY in the basolateral compartment was measured. Moreover, the integrity of cellular monolayer was assessed by measuring the TEER at 0, 3, 6, 12 and 24 h after exposure to increasing concentrations. The expression of junctional adhesion molecules following exposure to GA and its metabolites for 24 h also was analyzed both at mRNA and protein levels. Only 18 $\beta$ -GA and at the highest given concentration could significantly reduce the cell viability, which was measured with two LDH leakage and MTT metabolic activity determination assays. We found that 18 $\beta$ -GA at 100  $\mu$ M concentration remarkably reduced the TEER values following 3 h exposure and the mentioned values were further declined in a time-dependent manner. The lowest antioxidant capacity and the highest ROS production potency were found in the group of CaCo-2 cells which were exposed to the highest concentration of 18 $\beta$ -GA. Only 100  $\mu$ M of 18 $\beta$ -GA was able to significantly ( $P < 0.05$ ) increase the Lucifer yellow permeability via CaCo-2 cell's tight junctions.

Our data suggest that despite of plenty of medically beneficial effects of liquorice extract, much caution must be taken in account in the oral and frequently usage of GA and in particular its 18 $\beta$ -GA metabolites.

**Keywords:** Liquorice extract; CaCo-2 cells; Transepithelial Electrical Resistance



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***Kelussia Odoratissima* Mozaff. (Apiaceae): Insights from Ethnobotany to Phytochemistry and Biological Activity**

**Mahdi Ayyari<sup>1,2,\*</sup>, Mohammad Rezaei<sup>2</sup>, Safiollah Raeissi<sup>3</sup>, Maxime Le Bot<sup>4</sup>, Shahram Eisa-Beygi<sup>5</sup>, Sara Pahlavan<sup>2</sup>, Gilda D'urso<sup>6</sup>, Mahya Hosseini<sup>2</sup>, Aniseh Alimohammadpour<sup>1</sup>, Mehrnaz Namiri<sup>2</sup>, Alexander D. Crawford<sup>7</sup>, Dimitri Bréard<sup>4</sup>, Seyed Hamzeh Hossieni Kahnouj<sup>8</sup>, Ingrid Freuze<sup>4</sup>, Sonia Piacente<sup>6</sup>, Pascal Richomme<sup>4</sup>, Hossein Baharvand<sup>2,9</sup>**

<sup>1</sup>Department of Horticultural Science, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran

<sup>2</sup>Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

<sup>3</sup>FTSS Co., Incubator Center of Chaharmahal & Bakhtiari Science and Technology Park, Sharekord, Iran

<sup>4</sup>SONAS, SFR4207 QUASAV, University of Angers, Beaucouzé, France

<sup>5</sup>Developmental Vascular Biology Program, Division of Neonatology, Department of Pediatrics, and Department of Obstetrics and Gynecology, Children's Research Institute, Medical College of Wisconsin, Milwaukee, Wisconsin, USA

<sup>6</sup>Dipartimento di Scienze Farmaceutiche, Università degli Studi di Salerno, Via Ponte Don Melillo, 84084 Fisciano, Salerno, Italy

<sup>7</sup>Department of Basic Sciences and Aquatic Medicine, Faculty of Veterinary Medicine, Norwegian University of Life Sciences (NMBU), Oslo, Norway

<sup>8</sup>Department of Biology, Faculty of Science, Jiroft University, Jiroft, Iran

<sup>9</sup>Department of Developmental Biology, University of Science and Culture, Tehran, Iran

E-mail: [m.ayyari@modares.ac.ir](mailto:m.ayyari@modares.ac.ir); [mahdiayyari@gmail.com](mailto:mahdiayyari@gmail.com)

*Kelussia odoratissima* Mozaff. Known as “Kelos” or “Karafse Kouhi” in Iran from the Apiaceae family is an endemic and endangered plant growing in the central Zagros Mountains of Iran at altitudes of 2500 m and above. Kelos is one of the most popular wild culinary herb used in salads, pickles, soups and yogurt by local people, especially in the *Chaharmahal & Bakhtiari* province. It is also well known in Isfahan, Shiraz and Ahvaz as part of the traditional remedies and spice [1]. There are a plethora of biological activities traditionally associated with the pharmacologically of this plant, including treatment for rheumatism, indigestion, hypertension, inflammation, ulcers and cardiovascular diseases [2, 3]. In this study, the ethnobotanical background, extinction risks and regeneration and domestication of the plant and popularity of the *Kelussia* will be explained. The metabolite profiling of *kelussia* different parts has been carried out using GCMS and LCMS (LC-ESI/LTQOrbitrap/MS and LC-PDA-ESI-MS<sup>n</sup>). The major components of this plant are phthalide as mono and dimers. The properties, stability and isolation and identification of some bioactive compounds have some challenges and will be described. Several biological activities, including anti-angiogenesis (in zebrafish and HUVECs), antiepileptic (in zebrafish) and antioxidant properties are studied and will be presented.

**Keywords:** *Kelussia odoratissima*, Metabolite profiling, Zebrafish, Antiangiogenesis, Phthalides

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**Enhanced Production of Tropane Alkaloids in Hairy Root Cultures of *Hyoscyamus kurdicus* Bornm. Treated with Methyl- $\beta$ -Cyclodextrin and Coronatine**

**Farnoosh Fattahi<sup>1\*</sup>, Abdolali Shojaeiyan<sup>2</sup>, Javier Palazon<sup>3</sup>, Elisabeth Moyano<sup>3</sup>, Laura Torras Claveria<sup>3</sup>**

<sup>1</sup>*Department of Range management, Faculty of Natural Resources and Marine Sciences, Tarbiat Modares University, Noor, Iran*

<sup>2</sup>*Department of Horticultural Sciences, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran*

<sup>3</sup>*Department of Plant Physiology, Faculty of Pharmacy, Barcelona University, Barcelona, Spain*

*E-mail: f.fattahi@modares.ac.ir*

One of the best biotechnological methods for plant bioactive compounds production is hairy root cultures. On the other hand, application of biotic or abiotic elicitors in hairy root cultures can enhance the secondary metabolites production [1] so in this research two biotic elicitors, methyl- $\beta$ -cyclodextrin ( $\beta$ -CD) and coronatine (Cor), were used in hairy root cultures of *Hyoscyamus kurdicus* in order to increase the capacity of hyoscyamine (HYO) and scopolamine (SCO) production. In this regard the *Agrobacterium rhizogenes* A4 was used for infection of 2 month pot plant leaves of *H. kurdicus*. one gr fresh weight of selected hairy root line were inoculated in 30 ml of liquid half-strength B5 (B5/2) medium supplemented with 3% sucrose and placed in a shaker incubator (110 rpm) at 25 C° in dark situation. The old medium was replaced with 30 ml fresh medium on day 14th containing the elicitor treatments including: 50 mM  $\beta$ -CD, 0.5  $\mu$ M Cor and joint treatment of 50 mM  $\beta$ -CD and 0.5  $\mu$ M Cor with 3 replications. The sampling was performed on day 21 (T1) and 28 (T2) of culture. According to HPLC analysis the maximum content of HYO in hairy root cultures was observed in control at T1 (1.06 mg/g dw), Cor alone at T1 (0.94 mg/g dw) and combination of  $\beta$ -CD and Cor at T2 (0.92 mg/g DW) treatments respectively with no significant difference between them and also  $\beta$ -CD alone showed negative impact on HYO production. On the other hand, the highest amount of SCO was detected in treated hairy root cultures with Cor alone at T1 (4.57 mg/g dw), combination of  $\beta$ -CD and Cor at T2 (4.06 mg/g dw) and Cor alone at T2 (3.73 mg/g dw) with no significant difference between them. The results indicated  $\beta$ -CD alone didn't have significant effect on SCO production.

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**A Comparative Study on the Effect of Medicinal Drops of Damperly and Placenta in the Treatment of Sinusitis-Double Blind Randomized Clinical Trial.**

**Mahmud Khorushy<sup>1,\*</sup>, Mahrokh Sepahvand<sup>1</sup>, Asghar Pourhasan<sup>1</sup>, Roghayyeh Khorushy<sup>2</sup>, Ramez NasiriSaleh<sup>1</sup>, Mohsen Arzanlou<sup>3</sup>, Soureh AllafAkbari<sup>2</sup>, Raouf MollaJavadi<sup>2</sup>, Bahman Azizi<sup>4</sup>**

<sup>1</sup>*Incubator of Iranian Academic Center for Education Culture and Research, Ardabil Branch, Ardabil, Iran.*

<sup>2</sup>*Laboratory of Sabalan Hosbital, Ardabil, Iran.*

<sup>3</sup>*Administrator, Deputy Food and Drug, Ardabil, Iran.*

<sup>4</sup>*Pharmacy Department, Ardabil University of Medical Science, Ardabil, Iran.*

*E-mail: azhyaneh.co@gmail.com*

Herbal medicinal plants such as black currant, olive and rosemary have been identified as antibacterial agents in different herbal remedies. In this study, we tried to investigate the comparative effect of diphtheria drops and placebo in the treatment of sinusitis in a randomized, double blinded clinical trial. This double-blind clinical trial was conducted in which 50 patients with sinusitis were selected based on the presence of two major symptoms or major and two minor, according to the definition of sinusitis and the table published in this regard and randomly divided into two groups of 25. The control group received placebo (drops of distilled water) and the group received dipped herbal drops. For both control and treatment groups, routine sinusitis, which included Co-Amoxiclav antibiotics (in the case of sepsis sensitivity), washed serum and Fluticasone inhaler. Signs of the patients were evaluated before entering the study and two weeks after the treatment. All statistical analyses were done in SPSS software. There were differences between the groups for the underlying variables. Dairy herbal drops were more effective than placebo in reducing and even eliminating some of the symptoms of sinusitis. In this regard, the droplet of dairy and placebo did not have the same effect and there was a significant difference between them ( $P < 1.000$ ). Dairy herb receptor group was more effective in reducing the symptoms of sinusitis than in the placebo group. Hence, it has a certain superiority.

**Keywords:** Sinusitis, Bronchitis, Treatment, Drip drops, Placebo

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**Analysis of Genes Expression Pattern Involved in Biosynthesis of Secondary Metabolites of *Hypericum perforatum* L. in Response to Ultraviolet Radiation Stress**

Saeedeh Mafakheri,<sup>1</sup> Akbar Karami <sup>1,\*</sup>

<sup>1</sup> Department of Horticultural Science, College of Agriculture, Shiraz University, Shiraz, Iran.  
Email: akbarkarami@shirazu.ac.ir; akarami2004@gmail.com

Saint John's wort (*Hypericum perforatum* L.) is an important medicinal plant which have been used to cure diseases for long times. It has active biological components such as hypericin, hyperforin and pseudohypericin; these compounds are reported to have antiviral, antidepressant and anticancer activities. However, the content of these compounds in plants is very low. Treating plants with UV lights and especially UV-B is a technique to increase the content of secondary metabolites in medicinal plants. So, to study the possibility of using this technique in Saint John's wort, this experiment was conducted in the greenhouse of Horticulture Department, Shiraz University College of Agriculture, Shiraz, Iran, in 2016 and 2017. The experiment was conducted in factorial in the form of a completely randomized design (CRD) with three replications. In this experiment, plants were treated with UV-B light (20 w/m<sup>2</sup>) for 10, 20, 40 and 80 minutes and then hypericin content was measured in different times after treatment (12, 24, 48 and 96 h after treatment). Results indicated that hypericin content was significantly increased when plants were treated with UV-B light for 10 min, and the best time to harvest plant tissue was 12 h after the treatment. On the other hand, gene networks were drawn for genes affecting the biosynthesis pathway of secondary metabolites in Saint John's wort. Among these genes, *PAL* and *4CL* genes from shikimic acid pathway and *PKS* and *HYP-1* genes from the biosynthesis pathway of hypericin were selected to study the gene expression under UV treatment. Studying the genes with real-time qPCR indicated that gene expression was significantly reduced; showing the effect of UV treatment on DNA molecules destruction.

**Keywords:** Gene network, Hypericin, Ultraviolet radiation, *HYP-1*, *PKS*, *PAL*, *4CL*

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**Antioxidant Potential and Secondary Metabolites of *Ferulago Angulata* (Schlecht) Boiss at Various Habitats**

**Soleyman Bagherifar<sup>1</sup>, Mohammad Mahmoodi Sourestani<sup>1\*</sup> and Maryam Zolfaghari<sup>1</sup>**

<sup>1</sup>Department of Horticultural Science, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Iran

Email: m.mahmoodi@scu.ac.ir

Chavil (*Ferulago angulata*) is a perennial medicinal plant that belongs to the Apiaceae family. The plant spreads in Turkey, Syria, Lebanon, Iraq, as well as Iran [1]. The long-term use of plant prevents cancer and cardiovascular disease. In addition, the plant oil has high antimicrobial activity at high concentrations and is used as a food preservative, especially for dairy products [2]. Different habitats affect the plant growth as well as secondary metabolites. So this research was conducted to evaluate the essential oil content and composition as well as phenolic compounds and antioxidant activity of Chavil in different habitats from five locations in east of Khuzestan province; North Mongar altitude 2000 (M 2000 N), M 2500 N, South Mongar altitude 2500 (M 2500 S), M 3000, North Milhe Amir altitude 2300 (MA 2300 N), MA 2600 N, North Kohsyah altitude 1800 (KS 1800 N), South Tagaka altitude 2600 (T 2600 S) and North Gandomkar altitude 2600 (G 2600 N). Sampling was carried out on June and plant was dried on shade and ambient temperature. Essential oil was extracted by Clevenger apparatus and then analyzed by GC and GC-MS. Extraction was done by ultrasonic and the antioxidant activity was evaluated through DPPH and ORAC methods by plate reader. The phenolic compounds were measured by spectrometer. The results showed that secondary metabolites of plant change significantly at various habitats. The highest (1.34%) and the lowest (0.18%) essential oil contents were obtained in M 2500 S and MA 2600 N habitats, respectively. There were noticeable differences in the amounts of several compounds amid nine habitats. The main oil components were alpha pinene (20.84-49.06%), beta-E-ocimene (5.95-25.70%), transverbenol (3.01-6.97%) and Bornyl acetate (5.15-20.63%). Interestingly, G 2600 N had different oil profile and compounds such as linalool, transverbenol, bicyclogermacrene and gamma cadinene were higher than the rest of populations. The highest and the lowest flavonoid content were registered at M 2000 N and MA 2600 N region, respectively. In contrast, M 2500 S and M 2000 N had the highest and lowest total phenol, respectively. In both DPPH and ORAC methods, the maximum and minimum antioxidant activity was obtained in M 2500 N and T 2600 S habitats, respectively. In summary, results suggest that the quality and quantity of chemical compounds and their antioxidant activity vary depending on the locations which refer to both genetic and environmental situations.

**Keywords:** Chavil; Habitat;  $\alpha$ -pinene;  $\beta$ -E-ocimene; Bornyl acetate; Antioxidant activity; Flavonoid

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## Study of Variability in Saffron with more than Three Stigmas Using Molecular Markers

**Mohammad Ali Behdani\***, Ali Izanloo

*Saffron Research Group, University of Birjand, Birjand, Iran.*

*E-mail: mabehdani@birjand.ac.ir*

Saffron is the most valuable spice in the world. It is genetically a monomorphic clone. However, differences in phenotype and quality have been reported. The most important agro-economically phenotypic variation is the appearance of flowers with more than three stigmas. The main objective of this study was to study the genetic variability of saffron clones with more than 3 stigmas using SSR and ISSR molecular markers. In this research, saffron clones with more than three stigmas were collected along with the corm and the root from Saffron fields of Qaen and Sarayan, South Khorassan province, then transferred as a whole to the Biotechnology Laboratory of the Faculty of Agriculture, University of Birjand. The number of stigmas in each flower was counted. Genomic DNA was extracted according to CTAB method with minor modifications from leaves of the flower with more than three stigmas. Flower with more than three stigmas was larger and had more petals than ordinary ones. The most frequent number of flowers with more than three stigmas was related to four and five stigmas with 38%. Six-spice samples with a frequency of 14% were observed. Among the collected samples, only one specimen with seven stigmas was observed. Of the 48 tested ISSR primers on the bulk of DNA, only 16 primers amplified bands and selected. The results of agarose gel electrophoresis for ISSR primers amplified the bands ranged from 100 to 1000 bp. By examining the bands formed for ISSR primers, no significant polymorphism was observed between different clones of saffron. Therefore, based on this marker system, no sign of genetic diversity was observed between clones with different number of stigmas. Among the tested SSR markers, 10 primer pairs showed amplified band among the clones. The results of correlation analysis based on Spearman correlation coefficient showed that there was no statistically significant correlation between microsatellite marker alleles and number of stigmas.

**Keywords:** Epigenetics, Genetic diversity, SSRs

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**The molecular det of Dendrosomal Curcumin (DNC) as a Therapeutic Agent for Modulating the Expression of p53 in both Wild Type and Mutant Type Cancer Cells**

**M. Montazeri<sup>1</sup>, N. Zarghami<sup>2</sup>, M. Sadeghizadeh<sup>3</sup>**

<sup>1</sup>*Department of Medical Biotechnology, Tehran University of Science, Tehran, Iran.*

<sup>2</sup>*Department of Medical Biotechnology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran.*

<sup>3</sup>*Department of Genetics, School of Biological Sciences, Tarbiat Modares University, Tehran, Iran.*

The aim of this paper is to investigate the effect of dendrosomal curcumin (DNC) on the expression of p53 in both p53 mutant cell lines SKBR3/SW480 and p53 wild-type MCF7/HCT116 in both RNA and protein levels. Curcumin, derived from *Curcumin longa*, is recently considered in cancer related researches for its cell growth inhibition properties. p53 is a common tumor-suppressor gene involved in cancers and its mutation not only inhibits tumor suppressor activity also promotes oncogenic activity. Here, p53 mutant/Wild-type cells were employed to study the toxicity of DNC using MTT assay, flow cytometry and Annexin-V. Real-time PCR and Western blot were used to analyze p53, BAX, Bcl-2, p21 and Noxa changes after treatment. DNC during the time increased the SubG1 cells and decreased G1, S and G2/M cells, early apoptosis also indicated the inhibition of cell growth in early phase. Real-Time PCR technique showed an increased mRNA of BAX, Noxa and p21 during the time with decreased Bcl-2. The expression of p53 mutant decreased in SKBR3/SW480, and the expression of p53 wild-type increased in MCF7/HCT116. Consequently, p53 plays an important role in mediating the survival by DNC, which can prevent tumor cell growth by modulating the expression of genes involved in apoptosis and proliferation.



**6(17)-Epoxylythyrane Diterpenes from *Euphorbia sogdiana* Popov with Cytotoxic Activity**

**Zeinab Yazdiniapour<sup>1,\*</sup>, Behzad Zolfaghari<sup>1</sup>, Mustafa Ghanadian<sup>1</sup>, Virginia Lanzotti<sup>2</sup>**

<sup>1</sup>Department of Pharmacognosy, Isfahan University of Medical Sciences, Isfahan, Iran.

<sup>2</sup>Dipartimento di Agraria, Università di Napoli Federico II, Portici, Napoli, Italy.

E-mail: yazdiniapour@pharm.mui.ac.ir

*Euphorbiasogdiana* is a rare species known since Roman times and more recently as component of the Iranian flora in the Kopetdagh region in Northeast of Iran. Diterpenoids with wide range of their potentially valuable biological activities and their broad structural diversity are interest for new drug discovery. Lathyranes are a class of diterpenoid metabolites and their structure is based on a five-membered ring condensed with an 11 membered ring. In this study we performed isolation and structure elucidation of diterpenoids from *Euphorbia sogdiana* and it was resulted isolation and identification of three new and a known 6(17)-Epoxylythyrane diterpenes. Air-dried plant materials were powdered and exhaustively extracted with acetone-dichloromethane (2:1). The extracts were subjected to solvent-solvent partitioning between H<sub>2</sub>O and EtOAc. Non-polar fractions were adsorbed onto RP-18 chromatographed by MPLC. Diterpene-containing extract which eluted with MeOH-H<sub>2</sub>O (7:3) chromatographed by MPLC on a silica gel using a linear gradient solvent system from n-hexane 100% to EtOAc 100%. TLC and <sup>1</sup>H NMR analysis indicated that some fractions contained a series of diterpenoids, which were purified by further chromatographic procedures (preparative and analytical HPLC and PTLC). <sup>1</sup>H NMR, <sup>13</sup>C NMR, 2D NMR, ESIMS, HRMS and IR techniques were used to elucidate of isolated compounds. Cytotoxicity assay of *E. sogdiana* Lathyrene diterpenes were evaluated on Jurkat T-leukemia and EJ-138 bladder cancer cells. Phytochemical analysis of *Euphorbia sogdiana* Popov, Euphorbiaceae, afforded the isolation of three new diterpenes based on the rare 6(17)-epoxylythyrane skeleton, along with a lathyrene previously isolated from *Euphorbia aellenii*. Their chemical structures were established through a combination of nuclear magnetic resonance spectroscopy and mass spectrometric methods. The epoxylythyrans were tested to evaluate their cytotoxic activity against Jurkat T-leukemia and EJ-138 bladder cancer cells and their chemical analogy allowed to propose some structure activity relationships.

**Keywords:** *Euphorbia sogdiana* Popov, Lathyrene diterpenes, 6(17)-Epoxylythyrane

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**Herbosome; Potential Active for Herbal Constituents**

**Nastaran Khojastehpour<sup>1\*</sup>, Mohammad Naseri<sup>1</sup>, Bhama Sundaram<sup>2</sup>, Sambathkumar Ramanathan<sup>2</sup>**

<sup>1</sup>J.K.K.Nattraja College of Pharmacy, The Tamilnadu Dr M.G.R Medical University, India.

<sup>2</sup>Department of Pharmaceutics, J.K.K.Nattraja College of Pharmacy, The Tamilnadu Dr M.G.R Medical University, India.

E-Mail: n.khojastehpour@gmail.com

The term “Herbo” means plant while “some” means cell like. Herbosomes technology is one of such systems that incorporate phospholipids into standardized active ingredients of herbal extracts. Most of the biologically active constituents of plants are polar or water soluble molecules. However, water soluble phytoconstituents are poorly absorbed either due to their large molecular size; which cannot absorb by passive diffusion or due to their poor lipid solubility, severely limiting their ability to pass across the lipid rich biological membrane resulting poor bioavailability when taken by mouth. The effectiveness of any herbal medication is dependent on the delivery of effective level of the therapeutically active compounds. Herbosomes absorption in GIT is greater resulting in increased plasma level than individual component. Herbosomes novel complexes which are prepared by reacting from 3-2 moles but preferably with one mole of natural or synthetic phospholipids like phosphatidyl choline, phosphatidyl ethonolamine or phosphatidylserine with one mole of component like flavolignans, either alone or in the natural mixture in aprotic solvent such as dioxane or acetone. The herbosome complex can be then isolated by precipitation with non-solvent such as aliphatic hydrocarbons or lyophilisation or by spray drying. In the complex formation of herbosomes the ratio between these two moieties is in the range from 0.5-2.0 moles. The most preferable ratio of phospholipids to flavonoids is 1:1. Herbosomes results from the reaction of stoichiometric amount of phospholipid with standardized herbal extract or polyphenolic constituents like (flavonoids, terpenoids, tannins, xanthenes, etc) in nonpolar solvents. Herbosomes improve the *in vivo* bioavailability of herbal drugs. Herbosomes have also improved pharmacokinetic and pharmacological parameters which advantageously be used in treatment of various disease as the more amount of active constituent becomes available at site of action at similar or less dose as compared to the conventional herbal extracts.

**Keywords:** Herbosome, Phospholipids, Flavonoids, Polyphenols, Herbal extract

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**Novel Technologies in Extraction of Active Pharmaceutical Ingredients from Medicinal Plants**

**Reza Ghafarzadegan**

*Department of Pharmacognosy and Phytochemistry, Institute of Medicinal Plants, ACECR, Karaj, Iran*  
*Email: reza.ghafary@yahoo.com*

Extraction is the separation of medicinally active portions of plant using selective solvents through standard procedures. The purpose of all extraction is to separate the soluble plant metabolites, leaving behind the insoluble cellular marc (residue). Several of the commonly used extraction methods are Traditional methods such as maceration and Soxhlet extraction are commonly used at the small research setting or at Small Manufacturing Enterprise (SME) level. Significance advances have been made in the processing of medicinal plants such as the modern extraction methods; microwave-assisted (MAE), ultrasound-assisted extraction (UAE) and supercritical fluid extraction (SFE), in which these advances are aimed to increase yield at lower cost. Other methods such as accelerated solvent extraction (ASE) and supercritical fluid extraction (SFE) are also being used in the extraction of plant materials.

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**Comparison between essential oil of Lavender from Iran and Delberg Germany**

**Elahe Kashani<sup>1</sup>, Mehdi Mehran<sup>2</sup>**

<sup>1</sup>Validation Department, Barij Essence Pharmaceutical, Kashan, I.R. Iran

<sup>2</sup>Phytochemistry Group, Barij Medicinal Plants Research Center, Kashan, I.R. Iran

E-mail: Elahekasahni90@yahoo.com

Lavender (*Lavandula anagustifolia*) is plant that flowering season of lasts from first to end August. Lavender oil has benefits such as reduce anxiety and emotional stress, improve brain function, relieve pain, and improve sleep. This study describes the chemical composition by using gas chromatography of two essential oil of lavender from Iran and Delberg Germany. Compounds such as limonene, 1,8 cineol, linalool,  $\alpha$ -terpineol, borneol and camphor in aerial part of essential oil of Iranian lavender essential oil were 2.64, 21.74, 31.7, 1.36, 10.15 and 6.13 w/w%, respectively. while the amount of limonene, 1,8 cineol, linalool,  $\alpha$ -terpineol, borneol and camphor in essential oil of Delberg Germany were 12.3, 35.8, 9.4, 0.3, 0.2 and 33.7 w/w%, respectively.

**Keywords:** Lavender, Essential oil, Chemical composition, Gas chromatography

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**Effect of Inoculation of *Mycorrhizal arbuscular* Fungi on Survival and Morphological Traits of *Myrtus communis* L. under Drought Stress.**

**Soghra Azizi, Masoud Tabari Kouchaksaraei\***

*Department of Forestry, Faculty of Natural Resources and Marine Sciences, Tarbiat Modares University, Noor, Iran.*

*E-mail: mtabari@modares.ac.ir*

The medicinal plant of *Myrtus communis* L. that distributed in arid and semi-arid regions of the country, due to contain many medicinal active compounds such as essential oil, tannin, phenol and flavonoids, its have many drug uses. The purpose of this study was to investigate the mycorrhizal fungus inoculation on the improvement of growth characters under drought stress in controlled conditions of greenhouse. The experiment was carried out in a factorial of  $4 \times 3$  in a completely randomized design with 3 replicates (each replicate had 4 seedlings). The treatments applied in this experiment consist of water deficit stress in 3 levels of control (100% FC (field capacity)), moderate (60% FC) and severe (30% FC) and inoculation in 7 levels (control (no inoculation), inoculation with mycorrhizals of *Glomus mosseae*, *G. intraradices*, combination of *G. mosseae* + *G. intraradices*, inoculation with rhizobacterias of *Pseudomonas fluorescens*, *P. putida* combination of *P. fluorescens* + *P. putida*) that conducted in a 6 months period. The results of Two Way ANOVA showed that the interaction of drought stress and inoculation of mycorrhizal fungus at 1% and 5% level on growth and morphological traits such as survival, leaf area, root length, root volume, root biomass and total biomass of the seedlings were significant but did not significant effect on leaf and stem biomass. So that with increasing levels of drought stress all of the seedlings characters showed a decreasing trend, but inoculation with mycorrhizal fungi led to improve the performance of plant under drought stress, so that in all traits, treatment of drought stress 100% FC and combination of two types of mycorrhizal fungi was the highest and the treatment of 30% FC and non-inoculated showed the lowest. The results of the study also showed that the effects of drought stress and inoculation of mycorrhizal fungus alone had a significant effect on all morphological traits of seedlings. Overall, the results of this experiment indicated that the symbiosis with Arbuscular mycorrhizal fungi can led to increase the morphological traits under drought stress conditions and also improve resistance to drought stress of seedling. Effect of both species of *G. intraradices* and *G. mosseae* was almost identical.

**Keywords:** *Myrtus communis*, Mycorrhizal symbiosis, Drought stress, Leaf area, Total biomass



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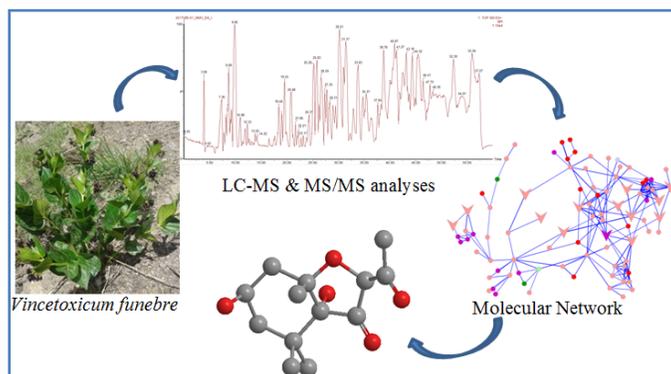
## A Molecular Networking Technique for Dereplication of Ache Inhibitory Compounds from the Medicinal Plant *Vincetoxicum Funebre*

Mahdi Abbas-Mohammadi<sup>1,2</sup>, Mahdi Moridi Farimani<sup>1,\*</sup>, Peyman Salehi<sup>1</sup>, Samad Nejad Ebrahimi<sup>1</sup>, Ali Sonboli<sup>1</sup>, Celine Kelso<sup>2</sup>, Danielle Skropeta<sup>2</sup>

<sup>1</sup>Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran.

<sup>2</sup>School of Chemistry, University of Wollongong, Wollongong, NSW 2500, Australia.

In drug discovery of natural products, reliable and fast tentative identification of known metabolites is mandatory for exploration of new bioactive natural compounds. Actually, computational tools constitute great alternatives in order to find hit compounds saving time and cost. Herein, a molecular networking technique using UHPLC-QTOF-MS and MS/MS was performed to rapidly dereplicate the chemical composition of the ethyl acetate extract of the medicinal plant *Vincetoxicum funebre*, as an active extract against the acetylcholinesterase enzyme. In addition, the inhibitory activity of the identified compounds was evaluated against the acetylcholinesterase enzyme via molecular docking analysis. Molecular networking analysis led to the dereplication of fifty compounds, of which a glycosylated flavonoid indicated the highest affinity to the enzyme with a docking score value of -13.43 kJ/mol. Semi-preparative HPLC analysis based on profiling of *V. funebre* also allowed the isolation of one new compound as well as four known compounds.



**Keywords:** Molecular networking, LC-MS, Molecular docking, Acetylcholinesterase inhibition

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**Market study of Migraine Cut<sup>®</sup> (Nasal Spray).**

**Reza Ramazani Khorshidoost<sup>1\*</sup>, Farid Semsarha<sup>2</sup>, Saleh Ramazani Khorshidoost<sup>3</sup>, Pooya Paknejad<sup>3</sup>, Vahid Naghshineh Arjmand<sup>4</sup>**

<sup>1</sup>*Faculty of Industrial Engineering Systems Management, Amirkabir University of Technology, Tehran, Iran.*

<sup>2</sup>*R & D Department, MIM Daroo Pharmaceutical Co, Tehran, Iran.*

<sup>3</sup>*Aramesh Multi-Professional Pain Clinic, Tehran, Iran.*

<sup>4</sup>*Department of Business Management, Faculty of Management, Central Tehran Branch, Islamic Azad University, Tehran, Iran.*

*E-mail: ramazani@aut.ac.ir*

Migraine headache is a prevalent, temporary, and crippling disease. More than 11 percent of the world population which amounts 800 million are affected by migraine. Based on a report of WHO, migraine is one of the 30 disabling diseases in the world, which results in clinical care of the patients. The burden of social and economic costs of migraine rests on both patients and society [1]. The costs include “direct costs” (like cost of doctor, cost of drug, and clinical costs) and indirect costs (such as cost of absenteeism and cost of decrease in productivity). For instance, the annual economic cost of migraine in the USA exceeds 21 billion dollars and in Europe amounts 18.5 billion euros. For the last 21 years the collaboration of the researchers of three medical science universities and two research centers, and one multi-professional pain clinic resulted in a new curative herbal-based medicine for migraine headaches. The final product is MigraineCut<sup>®</sup>, a nasal spray. An extensive pre-clinical R&D and clinical trials based on IHS documents and US FDA directives were completed. The curative and herbal-based nature of MigraineCut<sup>®</sup> is unique in the world. The final results of MigraineCut<sup>®</sup> efficacy on more than 1100 patients indicates 81 percent cure and 88.5 satisfactions of migraine patients. This effort is possessed by MIM Daroo Company, established by the initiators of the project. A preliminary prudent market study indicates that the ROI of MigraineCut<sup>®</sup> sales is very attractive and also the investment in the production of such herbal products causes to remarkable national saving.

**Keywords:** Migraine, MigraineCut<sup>®</sup>, market, ROI.

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## Resveratrol Treatment Improve Renal Function in Pyelonephritic Rats

**Sattar Gorgani-Firuzjaee**

*Department of Medical Laboratory Sciences, School of Allied Health Medicine, AJA University of Medical Sciences, Tehran, Iran*

Urinary tract infections is a common causes of outpatient referrals to medical centers. Acute pyelonephritis is often treated by injectable antibiotics. Side effects, especially resistance to antibiotics, considered use of non-antibiotic compounds. Resveratrol which is found in grapes and its products is a powerful antioxidant, anti-inflammatory and anti-bacterial as well. In this study, the effect of resveratrol on renal function in pyelonephritis rats was evaluated. Pyelonephritis induced by direct injection of ATCC25922 strain of E. Coli by surgical expose of right kidney in 20 male Wistar rats. Induced urinary tract infection confirmed by urine culture and 72 hours after induction of pyelonephritis, rats treated with resveratrol (10 mg / kg) and ceftriaxone via intraperitoneal injection for a week. After 6 weeks plasma markers of renal function such as urea, creatinine and cystatin C determined by Hitachi biochemistry autoanalyser. Urea, creatinine and cystatin C significantly increased in pyelonephritis which show renal function is impaired ( $p < 0.001$ ). Resveratrol treatment almost neutralize the effects of pyelonephritis and the plasma markers effectively reduced compared with the control group. Resveratrol and Ceftriaxone combination therapy probably via synergistic effects, more effectively improved renal function. ( $P < 0.05$ ). Totally our finding demonstrated that resveratrol improved renal function of pyelonephritis rats. This study provides evidence that resveratrol suggest a protective role almost equal with the ceftriaxone against kidney damage induced by pyelonephritis.

**Keywords:** Pyelonephritis, resveratrol and renal function



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**Domestication and Cultivation Practices of Liquirice (*Glycyrrhizaglabra*L.) in Iran**

**F Najafi<sup>\*1</sup>, L Tabrizi<sup>2</sup>, H Rezadust<sup>1</sup>, E Talebi<sup>1</sup>, M Amirshahi<sup>1</sup> A Raisi<sup>1</sup>**

<sup>1</sup> Medicinal Plants and Drugs Research Institute of Shahid Beheshti University, G.C, Tehran, Iran

<sup>2</sup> Faculty of Agricultural Science and Engineering, College of Agriculture & Natural Resources, University of Tehran, Karaj, Iran

Email: f\_najafi@sbu.ac.ir

Liquirice (*Glycyrrhizaglabra* L.) is a valuable medicinal plant which its roots are widely used in different industries like pharmaceutical, food, cosmetic and cigarette industries. Raw materials of this plant are still collected from wild habitats and the growing market and use by industries cause to threatened the wild populations in different countries so domestication and cultivation of this species is the only way to support industries and decrease the pressure on wild habitats. The aim of this research was to study the ability of domestication this plant in low input agricultural systems. Three different experiments are conducted in two years of 2016 and 2017 to study the effect of irrigation regimes, plant density and organic fertilizers on growth, yield and phytochemical properties of different wild populations of Eqlid, Ramjerd, Baft and Lalehzar. Results showed that yield parameters decreased by increasing of water evaporation. The highest ground yield per plant and per square meter, 512.92 g and 2052 g.m<sup>-2</sup> in the second year of study were observed in 100 mm evaporation but it was not a significant difference with 200 mm evaporation. So it seems that after plant establishment in second year increasing of irrigation times did not increase ground yield significantly. Results showed that with decreasing of irrigations from 100 mm evaporation to no irrigation the ground yield decreased nearly 50 % but still at no irrigation this plant show a valuable amount of 861.5 g.m<sup>-2</sup> in second year. Our observations indicated that the Baft population in first year grew very slowly but in the second year and after establishment grew very fast and the highest ground yield was observed in this population but the amount of glycyrrhizic acid and glabridin was significantly higher than other populations. The highest ground yield was obtained in a plant density of 16.66 pl.m<sup>-2</sup> and the growing and yield parameters did not react to different organic fertilizers which shows that this plant can be used for domestication in low input agricultural systems.

Keywords: medicinal plant, glabridin, cultivation, water deficiency, plant density

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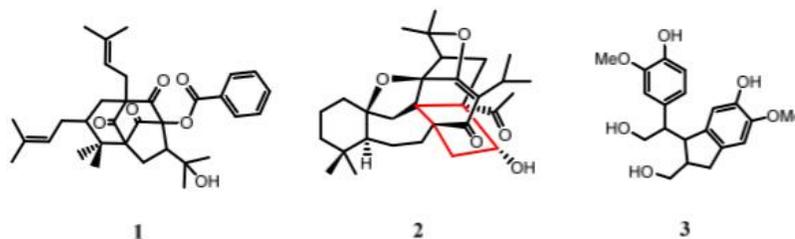
**Iranian Medicinal Plants as Sources of New Bioactive Compounds with Unprecedented Architectures**

**Mahdi Moridi Farimani**

*Department of Phytochemistry, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran.*

*E-mail: m\_moridi@sbu.ac.ir*

Natural products research continues to explore a variety of lead structures, which may be used as templates for the development of new drugs by the pharmaceutical industry. These substances, representative of very wide chemical diversity, continue to demonstrate the importance of compounds from natural sources in modern drug discovery efforts. Nowadays, one of the strategies for drug discovery is extraction and identification of chemical constituents of the plants and assessment of their bioactivities through cellular or animal studies. Our recent studies, aimed at identifying structurally interesting and bioactive metabolites from Iranian endemic species, resulted in the isolation of several new compounds; some of them possess unusual and unique structures (1-3). The structures were elucidated by a combination of 1D and 2D NMR, HRESIMS, and X-ray crystallographic analyses. Plausible biosynthetic pathways toward these new skeletons were proposed. Biological properties of the new compounds were also investigated. Some of these compounds showed good *in vitro* antiplasmodial and anticancer activities at submicromolar concentrations.



**Keywords:** 2D NMR, Structure elucidation, Biosynthetic pathway, Biological activity

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**Medicinal Herbs in Cosmeceuticals: a Review**

**Leila Fotouhi\***

*Business Development Unit, Zardband Pharmaceuticals, Tehran, Iran*

*E-mail: fotouhi.l@zardband.com*

The market research shows an incredible growth in trade of cosmeceuticals and also in popularity among consumers as a loss of faith in chemical and synthetic skin remedies. Medicinal plant extracts are believed to induce better results by delivering nutrients and less side effects. So herbal cosmetic industry plays a major role in fulfilling this worldwide demand for natural products [1]. Many phytochemicals have been evaluated for their cosmetic-therapeutic potential. For example, clinically proven ingredients such as antioxidants, anti-inflammatory agents and depigmentation factors which are widely used in cosmetic formulations, are claimed to address a variety of skin conditions in beauty lines; the application of natural essential oil in aromatherapy has opened a new market in fragrance industry and pharmaceutical-style benefits of herbal extract is expanding into other beauty categories, such as haircare and nail care products and etc. too [2]. In addition new herbal skin care treatments are emerging, presenting dermatologists and their patients the challenge of understanding the science behind these cosmeceuticals. Therefore the need for cosmeceuticals research, regulation and clinical trial is more than ever necessary. This speech is going to highlight the broad applications of botanical extracts and essential oils in the current marketplace, and to expose some of brand new standardized extracts produced in Zardband Pharmaceuticals for beauty and personal care industry. Hopefully, more questions will be raised than answered and some ideas for intellectual discourse will be provided to fulfil this gap in near future.

**Keywords:** Medicinal plants; Cosmeceuticals; Aromatherapy; Herbal extract; Phytochemicals.

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**Evaluation of Peppermint (*Mentha piperita* L.) and Rosemary (*Rosmarinus Officinalis*) as Antibiotic Growth Promoter Substitutions on Immunological Responses of Japanese Quails (*Coturnix japonica*)**

**Seyed Hadi Goldani,<sup>1,\*</sup> Mohsen Daneshyar,<sup>1</sup> Keyvan Azizi,<sup>1</sup> Mehrdad jalalifard,<sup>1</sup> Seyed Meysam Abtahi<sup>2</sup>**

<sup>1</sup>Department of Animal Science, Faculty of Agriculture, Urmia Univesrity, Urmia, Iran

<sup>2</sup>Department of Microbiology, Veterinary Faculty, Urmia University, Urmia, Iran

E-mail: S.hadigoldani@gmail.com

Botanical products, also known as phytogetic feed additives (PFA's), are a relatively new class of feed additives that use as antibiotic growth promoter substitutions in poultry nutrition and our knowledge is still rather limited regarding their modes of action and aspects of their application. This research was conducted in two separate experiments to evaluate the effects of different levels of Peppermint (*Mentha piperita* L.) powder and Rosemary (*Rosmarinus Officinalis*) powder on immune system responses of Japanese quails (*Coturnix japonica*). For each experiment, 200 one-day-old quail chicks, were used in 4 treatments and 5 replicates each (10 birds per replication). The experimental treatments consisted of four levels of Peppermint and Rosemary 0.0, 0.25, 0.5 and 0.75 percent. Results showed that, the consumption of 0.5 and 0.75% peppermint powder significantly reduced the severity of respiratory burst ( $P < 0.01$ ). No effects of experimental treatments were observed for heterophil, lymphocyte, heterophil to lymphocyte ratio and monocyte counts ( $P > 0.05$ ). Different levels of peppermint powder caused to decreased antibody production against sheep red blood cells (SRBC) and humoral immune suppression ( $P < 0.01$ ). In addition, different levels of peppermint powder enhanced the cellular immune system through increased lymphocyte proliferation and significant increase in the thickness of the membrane between the toes in response to SRBC injection ( $P < 0.01$ ). All the rosemary levels increased the cellular immune response whereas 0.75% rosemary decreased the humoral immune response ( $P < 0.05$ ). The respiratory burst decreased by increasing the rosemary consumption level whereas the lymphocyte proliferation increased by 0.75% rosemary level ( $P > 0.05$ ). In this study, the consumption of the highest rosemary level (0.75%) to 35 days causes the decreased humoral response and increased cellular response in Japanese quail.

**Keywords:** Japanese quail; Rosemary; Immune system; Peppermint; Phytogetic



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***Molecular characterization of diversity and sex determination in Cannabis accessions from Iran***

**Maryam Rahimi, Majid Shokrpour\*, Seyed Alireza Salami, Leila Riahi, Mohamad Reza Taheri, Azizollah Khandan Mirkouhi, Faezeh Aghaei.**

*Department of Horticultural Science, College of Agriculture and Natural resources, University of Tehran, Karaj, Iran.*

**E-mail: shokrpour@ut.ac.ir**

*Cannabis* (*Cannabis sativa* L.) is an important plant with various uses in pharmaceutical and paper production industries which grows in different areas of Iran and some other countries. However, there is just little information about of Iranian cannabis accessions and also early determination of sex and evaluation of diversity in cannabis are one of the major concern of researchers and producers due to the higher priority of female and male cannabis plants for pharmaceutical uses and fibre industry, respectively, and quality reduction of the pharmaceutical products by pollination and fertilization. Seeds from 26 accessions from different regions of Iran along to one accession from Afghanistan were planted in the field based on the Randomized Complete Block Design. For a detailed evaluation of diversity among accessions, 10 SSR primers were used. The analysis of molecular variances showed 30 and 70% variation between and within accessions, respectively. The low amount of Fst mean (0.178), confirmed the low variation among the accessions. The maximum number of alleles belonged to C08-CANN2 (six alleles). Also the maximum Shannon information index (0.99) was observed in this primer. The highest observed heterozygosity belonged to B01-CANN1 and D02-CANN1 (0.98). The maximum Shannon information index was observed in Esfahan that showed the highest variation within this accession. Generally, the results showed a great genetic variation in the studied accessions. To test sex determination in this plant, five female and five male plants were sampled from each accession for molecular analyses. Thirteen ISSR and two SCAR primers were used. The highest mean of heterozygosity were found in ISSR3 and the lowest in UBC825 primers. Overall, 143 polymorphic loci and one polymorphic locus were obtained in ISSR and SCAR primers, respectively. Out of 143 polymorphic ISSR loci, only 10 markers had significant relations with gender. The SCAR primer of MADC5 showed polymorphism for gender while the MADC6 couldn't determine male and female individuals.

**Keyword:** Sex determination, Female, Male, Cannabis, Molecular marker, Shannon index

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