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In the name of Allah

On behalf of the National Network of Research and Technology in Medicinal Plants it is a great pleasure to welcome you to the 4th National Congress of Medicinal Plants (NCMP, 2015).

The first congress was held successfully on May 2012, in Kish Island, the second on May 2013 in Tehran and the third was held on May 2014 in Mashhad. In the fourth congress, more than 1800 abstracts were received to the secretariat office that about 1200 of them will be presented as posters and oral lectures.

The topics of the congress covers all of the main areas of medicinal plants including pharmacy, medicine, agriculture and natural resources, biotechnology, basic sciences, business and industry.

Aside from the excellent scientific program, we have also prepared an exhibition that will provide the participants an opportunity to exchange their experiences and negotiate with the producers and business sectors of medicinal plants and herbal medicines.

I would like to thank our many colleagues in scientific and organizing committees who have helped us to put the congress together. Finally, the generous financial support of the sponsors in gratefully acknowledged.

Peyman Salehi

Congress Scientific Chair



4th National Congress on Medicinal Plants
12, 13 May 2015
Tehran- Iran



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4th National Congress on Medicinal Plants
12, 13 May 2015
Tehran- Iran



ORAL PRESENTATIONS



4th National Congress on Medicinal Plants
12, 13 May 2015
Tehran- Iran



EVIDENCE BASED HERBAL MEDICINE AND MENTAL HEALTH

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Herbal medicines include a range of pharmacologically active compounds: in some cases it is not well understood which ingredients are important for a therapeutic effect. The supporters of herbal medicine believe that isolated ingredients in the majority of cases have weaker clinical effects than whole plant extract, a claim that would obviously require proof in each case. Generalizations about the efficacy of herbal medicines are clearly not possible. Each one needs systematic research including a variety of animal studies and also randomized clinical trials. Indeed, clinical trials of herbal medicines are feasible much in the same way as for other drugs. Physicians need to understand the biochemical and evidential bases for the use of herbs and nutrients to diagnose and treat patients safely and effectively, to avoid interactions with standard medications, and to provide patients with the benefits of alternative treatments. Saffron is a perfect example for evidence based herbal medicine. Indeed, it is a Persian herb with a history as long as the Persian Empire itself. In the past decade, several clinical trials showed the efficacy of saffron in the treatment of depression, Alzheimer's Disease, premenstrual syndrome (PMS) and sexual dysfunction. To date, six published randomized controlled trials have been published about effects of saffron on depression. Traditionally, saffron was thought to improve sexual functioning. From an evidence-based point of view, *Crocus sativus* L. and its constituents improved all components of sexual function in male rats. In addition, several constituents of saffron including safranal, crocin, crocetin, and carotenoids have shown neuroprotective properties in animal models of ischemic, oxidative, traumatic, and inflammatory brain injury.



**INVESTIGATION OF THE EFFECTS OF CURCUMINOID
SUPPLEMENTATION IN PATIENTS WITH CHRONIC PULMONARY AND
CUTANEOUS COMPLICATIONS DUE TO SULFUR MUSTARD
INTOXICATION**

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Curcuminoids comprising curcumin demethoxycurcumin and bisdemethoxycurcumin, are phytochemicals with documented safety and antioxidant activity and also remarkable anti-inflammatory properties that are derived from dried rhizomes of the plant *Curcuma longa* L. (turmeric). Pulmonary problems are among the most frequent chronic complications of sulfur mustard (SM) intoxication and are often accompanied by deregulated production of pro-inflammatory cytokines. Oxidative stress plays a key role in the development of chronic pulmonary complications of sulfur mustard (SM) that suppress systemic inflammation in patients suffering from SM-induced chronic pulmonary complications. In an randomized double-blind placebo-controlled trial investigation in Baqiyatallah University of Medical Sciences, subjects were recruited with curcuminoids(1500 mg/day) + piperine (15 mg/day) combination for a period of 4 weeks. Regarding the promising effects of curcuminoids on the measures of systemic oxidative stress, clinical symptoms, these phytochemicals may be used as safe adjuvants in patients suffering from chronic SM-induced pulmonary complications who are receiving standard treatments. Therefore, short-term adjunctive therapy with curcuminoids can suppress systemic inflammation in patients suffering from SM-induced chronic pulmonary complications [1, 2]. Chronic cutaneous complications such as pruritus are among the very frequent complaints of sulfur mustard (SM)-exposed patients. In a randomized, double-blind trial research on Iranian veterans who were suffering from chronic SM-induced pruritic skin lesions were assigned to curcumin 1 g per day for four weeks. Curcumin supplementation effectively mitigates inflammation in patients suffering from chronic SM-induced cutaneous complications. This anti-inflammatory effect might account for the observed pruritus alleviation and quality of life improvement by this phytochemical.

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540

**CLINICAL EVALUATION OF EFFICACY OF *ALCEA DIGITATA* AND
MALVA SYLVESTRIS COMPOUND IN THE IMPROVEMENT OF
RADIATION-INDUCED XEROSTOMIA IN PATIENTS WITH HEAD-AND-
NECK CANCERS (HNC)**

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xerostomia (dry mouth) is a common side effect of radiotherapy for head and neck cancers (HNC) [1, 2]. Xerostomia affects important aspects of life, such as speaking, chewing, swallowing, eating and the wearing of dental prostheses [3]. Despite significant advances in radiation delivery therapy methods; there is not any completely effective treatment for radiation induced xerostomia [1, 3]. The aim of this randomized clinical trial was evaluating efficacy of *Alcea digitata* and *Malva sylvestris* compound in compare with artificial saliva (HypoZalix) in improvement of the xerostomia symptoms and quality of life (QOL) in patients with HNC. Total of 75 patients based on the arrival of patients were randomly allocated into two groups, the experimental compound (experimental group) or HypoZalix (control group) were administered. The duration of the study was four weeks. Efficacy was appraised by visual analogue scale (VAS) and the grading of xerostomia; for appraising QOL, the EORTC QLQ-H&N 35 questionnaire was used. In both groups, there was a significant difference between the mean of VAS and QOL score before and after intervention ($p < 0.001$). Additionally, there was a significant difference in VAS and QOL scores between two groups four weeks after intervention. In the experimental group there was a statistically significant difference between the grade of xerostomia before and after intervention ($p < 0.018$), while there was no change in the grade of xerostomia in the control group. The results of the this study demonstrate that *Alcea digitata* and *Malva sylvestris* compound significantly reduced the symptoms of xerostomia and improved QOL in patients with HNC and can use as alternative medicine.

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**EVALUATION OF ANTICONVULSANT EFFECT OF THREE DIFFERENT
MEDICINAL PLANTS USING EXPERIMENTAL MODELS**

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In Iranian traditional medicine, many herbal drugs have been used for treatment of epilepsy. *Foeniculum vulgare*, *Heracleum Persicum*, and *Lippia citriodora* are three important medicinal plants used as anticonvulsants in Iran. In this study, anticonvulsant effect of extract of *F.vulgare* seeds, extract of *L. citriodora* leaves, and extract of *H.persicum* seeds were evaluated using maximal electroshock and pentylentetrazole models in NMRI mice. The results showed that the essential oil of *F. vulgare* seeds, methanolic extract of *L. citriodora* leaves, and extract and fractions of *H. persicum* seeds have anticonvulsant effects in experimental models.



857

**ANTICANCER EFFECTS ON HUMAN BREAST CANCER T47D CELLS
TREATED WITH EXTRACTS OF *TUSSILAGO FARFARA* L. IN
COMPARISON TO DOXORUBICIN**

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The flowers and leaves of *Tussilago farfara* L. (Colt's-foot) belonging to the Compositae family which is locally named Fanjiun or Pay-e khar, were collected from the mazandran province in the north of Iran. TIM (Traditional Iranian Medicine) literatures says that it is used for chronic cough, asthma, lung cancer and wounds. The infusion of aerial parts of the plant has been used in common cold and coughs [1,2]. The dried plant materials were extracted via maceration method using hydro alcoholic solvent for achieving a total extract. The total extract fractionated by Methanol, Ethyl acetate, Dichloromethane and Petroleum Ether. Different dilutions of total extract of leaf and flower and each fractions were investigated for in-vitro cytotoxicity activity against T47D (breast ductal carcinoma) using MTT assay compared to doxorubicin. A stock extract solution in DMSO has provided and diluted with PBS and sterilized by a 0.22 micron filter. Different dilutions provided freshly by the help of RPMI 1640 implant environment at experiments time. T47D cancer cells implanted in 96 well plates and effected by different dilutions of hydro-alcoholic extract of leaf and flower of Colt,s foot were compared to doxorubicin and no-drug control on cell-reproduction with MTT method at different times evaluated. Comparing of leaf and flower total hydro-alcoholic extracts, showed that the leaf extract has more noticeable effect on growth of T47D cells. Also comparing of methanol, ethyl acetate, dichloromethane and petroleum ether fractions of the leaf, showed that petroleum ether fraction effected as like as doxorubicin on T47D cells. Phytochemical tests indicated that the leaves total extract and also the petroleum ether fraction, were contained noticeable amounts of flavonoid and sesquiterpene. In conclusion, it can be said that significant content of flavonoid and sesquiterpene in total hydro-alcoholic extract and also in petroleum ether fraction of the leaves, gives this probability that flavonoid and sesquiterpene are the main effective chemical compounds of this species.

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1752

**GENETIC, PHYTOCHEMICAL AND MORPHOLOGICAL EVALUATION
OF HAWTHORN (*CRATAEGUS* SPP.) GROWN IN IRAN**

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The genus *Crataegus* L. (Hawthorn) belongs to the Rosaceae family and has recently become quite a popular herbal drug in phytotherapy due to its positive effects on the cardiovascular system. Iran is one of the important biodiversity centres of *Crataegus* with more than 27 species. The fruits, leaves and flowers of several *Crataegus* species containing *C. pentagyna*, *C. pseudomelanocarpa*, *C. monogyna*, *C. meyeri*, *C. songarica*, *C. azarolus* var. *aronia*, *C. azarolus* var. *pontica*, *C. curvisepala*, *C. pseudoheterophylla*, *C. szovitisii*, *C. persica*, *C. arosanguinea*, *C. orientalis*, *C. sakranensis*, *C. Turkestanica* from different parts of Iran, with 56 accessions, have been collected. This study represented the genetic (based on internal transcribed spacer (ITS) regions), phytochemical (such as soluble carbohydrates, total carotenoids, chlorophyll a and b, total flavonoids, total phenols, antioxidant activity, total carotenoids, chlorophyll a and b etc.) and morphological (such as fruit weight, dimensions fruit and seed, fruit skin color, flesh/seed ratio etc.) features of the collected hawthorn organs. In addition to above phytochemical properties, the individual phenolic compounds such as, chlorogenic acid, vitexin-2"-*O*-rhamnoside, vitexin, rutin, hyperoside, isoquercitrin and quercetin of various organs of all accessions were quantified by HPLC. Some of the species are a rich source of these flavonoids and phenolic acid. A phylogenetic tree based on ITS sequence data has been constructed and compared with the tree of morphological data. Result showed that DNA sequencing is a good tool for species identification and estimation of genetic distance. The study revealed that there were differences in terms of phytochemical and morphological characteristics among hawthorn species and organs and thus better quality hawthorn genotypes can be selected within the species and organs. Different organs and species of the genus *Crataegus* L. showed a high level of phenolic compounds as well as antioxidant capacity. These results showed that different species are promising sources of natural antioxidants and other bioactive compounds beneficial to be used in the food or the pharmaceutical industries [1, 2].

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370

**MINOR IRIDOID GLUCOSIDES FROM *SCROPHULARIA OXYSEPALA* AND
ANTIOXIDANT ACTIVITIES**

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The Scrophulariaceae family consists of 220 genera this genus is known for the historical therapeutic uses and phytochemical compounds. The Scrophularia genus consist of 60 species in the flora of Iran that the roots and stems of many of them used as antipyretic, febrifuge and antibacterial also used as a remedy in traditional central and west Asian medicine for treatment of erythema, inflammations, ulcerous, and cancer according to these uses many pharmacological researches conducted on this genus , that experimental observations confirmed many of traditional uses. Previous investigations have been shown that this genus is a rich source of Iridoid glycosides and phenyl propanoid glycosides. In this research, we report isolation and structure determination of chemical compounds of the aerial part of *S. oxysepala* and antioxidant activities of them. We isolate scrolopioside E (1), gmelinoside M (2), scrophuloside A7 (3), scrophuloside A3 (4) Scropolioside D(5) 7-O-actyl loganin (6), 8-O-actyl muralioside (7) and loganic acid (8). The aerial parts of *S. oxysepala* were collected from East Azerbaijan province 30 kilometer to Kalibar town, Garehdagh mountain in the during flowering period. A voucher specimen (2821) has been deposited at the Herbarium of the Researches center for agriculture and natural resources, East Azerbaijan, Iran. The air-dried and powdered aerial parts of *S. oxysepala* (1800g) were Soxhlet-extracted with n-hexane, dichloromethane (DCM), and methanol (2 L each). All these extracts were separately concentrated using a rotary evaporator at a maximum temperature of 45°C. A portion of the methanol extract (2g) was subjected to solid phase extraction (SPE) on a Sep-Pak (10g) C18 cartridge using a step gradient of methanol: water mixture (10:90, 20:80, 40:60,60:40, 80:20,100:0). The preparative HPLC (Dr. Mainsch GmbH ODS column 20 µm,250mm×20mm); liner gradient 0-45 min 20-90% methanol in water; isocratic gradient 90% methanol in water during 45-50 min;liner gradient 50-52 min 90-100% methanol in water; isocratic gradient 52-55 min 100 methanol; liner gradient 55-58 min100-20% methanol in water; isocratic gradient 20% methanol in water during 58-65min; flow rate = 8ml/min detection at 190-400 nm to yielded: 7-O-actyl loganin (6), 8-O-actyl muralioside (7) and loganic acid (8) from10% methanol SPE fraction, liner gradient 0-24min 60-90% methanol in water; isocratic gradient 90% methanol in water during 20-40min; liner gradient 40-43 min 90-100% methanol in water; isocratic gradient 43-45 min 100% methanol; liner gradient 45-47min 100-60% methanol; isocratic gradient 60% methanol in water during 47-50min; flow rate =8ml/min detection at 190-400 nm)to yielded: scrolopioside E (1), gmelinoside M (2), scrophuloside A7 (3), scrophuloside A3 (4) scropolioside D (5) from 60% methanolic fraction.



545

**NEW SAPOGENINS FROM *ALLIUM UMBILICATUM* BOISS. WITH
CYTOTOXIC ACTIVITY**

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Since ancient times medicinal plants have been used as a source of remedies and healthcare preparations. Recently a renewed interest in medicinal plants re-emerged for restoring and maintaining health [1]. Plants of *Allium* genus have been a part of the folk medicine being used since Egyptian times as medicinal herbs. Epidemiological studies have suggested that *Allium* consumption may protect against several diseases (e.g. carcinogenesis, hypercholesterolemia, type 2 diabetes, hypertension, cataract, and microbial infections) [1-5]. Many of the biological effects of these plants are related to the thiosulfonates- responsible of their characteristic pungent aroma and taste- saponins, and flavonoids. *Allium umblicatum* Boiss. is a species subendemic of Iran, Afghanistan and Pakistan and found at considerable altitude. It is a wild species that could be also easily cultivated and is used in some places as a vegetable and condiment. As a part of our research project on the isolation of bioactive compounds from *Allium* species, we decide to plan the phytochemical study of this plant. The air-dried flowers of the plant were extracted in a four step extraction method with hexane, chloroform, chloroform-methanol (9:1) and methanol. The methanolic extract was finally partitioned between butanol and water. Butanolic extract was fractionated by MPLC, selected fractions were subjected to HPLC and interesting compounds were purified. Chemical structure of the compounds was elucidated by comprehensive spectroscopic analyses including 1D- and 2D-NMR and MS spectroscopy. Analysis of the extract has led to the isolation of two new saponins, named secoumbilicagenin A and B, possessing a unique chemical structure based on a 3,4 secospirostane skeleton. Interestingly, the isolated compounds exhibited cytotoxic activity on J-774, murine monocyte/macrophage, and WEHI-164, murine fibrosarcoma cell lines.

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4th National Congress on Medicinal Plants
12, 13 May 2015
Tehran- Iran



1260

**OPTIMIZATION OF THE SUBCRITICAL WATER EXTRACTION OF
BIOACTIVE COMPOUNDS FROM SAFFRON PETAL USING RESPONSE
SURFACE METHODOLOGY**

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Reactive free radicals, such as superoxide anion, hydroxyl radical and peroxy radical may lead to the disruption of membrane fluidity, lipid peroxidation, protein denaturation, oxidation of DNA and variation of platelet activities in the human body [1]. Crude extracts of fruits, herbs, vegetables, cereals, and other plant materials rich in phenolics are increasingly of interest in the food industry and pharmacy because of their multiple biological effects, including antioxidant activity. In recent years, considerable attention has been directed towards identification of natural antioxidants that may be utilized for human consumption [2]. *Crocus sativus* L. prevalently known as saffron is a perennial plant widely cultivated in different regions of the world, especially in Iran [3]. Saffron is traditionally exploited to attain the dried stigmas of the flower, which is considered the most valuable spice of the world [4]. Petal is the huge amount of saffron by-product which is not practical for the farmers. Conventional extraction techniques based on organic solvents may have undesirable effects on the environment and on food components; hence, “green” technologies would be more desirable [5]. Subcritical water extraction (SWE) is an extraction technique using water as the solvent, but with modified physical properties; it is considered a recent alternative for the isolation of antioxidant constituents from plant materials [6]. Box-Behnken design was employed to optimize the water-to-solids ratio, temperature and time obtaining extract from saffron petal with high total phenolic contents and high antioxidant activities. Analysis of variance showed that the contribution of a quadratic model was significant for the responses. An optimization study using response surface methodology was performed and 3D response surfaces were plotted from the mathematical models. The optimal conditions based on combination responses were: $x_1= 1:33$ w/v, $x_2= 160^\circ\text{C}$ and $x_3=20$ min. These optimum conditions yielded total phenolic contents of 550 mg gallic acid equivalents/100 g, %DPPHsc of 89.2% and FRAP values of 3.58 mM. Close agreement between experimental and predicted values was found. This methodology could be applied in the extraction of bioactive compounds in the natural product industry.

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THE EFFECT OF LAVENDER SYRUP ON FATIGUE AND WORK-RELATED STRESS IN NURSES

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Nurses due to the conditions of employment, are more susceptible to fatigue and job stress. Considering the impact of fatigue and job stress on nurses' family lives, it is essential to handle stress and fatigue and conflicts resulting from them in life. Nurses require physical, psychological and social health in their respective roles not only in their personal life but also in their careers. This study is a clinical trial to determine the effectiveness of lavender syrup on nurses' fatigue and job stress. In this study, 94 nurses were selected randomly and divided into three groups: control, placebo and case. Data sources were obtained from demographic questionnaire, multidimensional questionnaire assessing fatigue and Taft Anderson job stress questionnaire. In this study, the average fatigue in lavender syrup consumer groups, before and after consumption was not at the same level, given the average value before (62.11) and after taking the syrup (49.14), average fatigue after taking the syrup dropped ($P=0.001$). The average fatigue after taking the syrup in the control group (57.89), placebo (54.23) and case (49.14) was not at a same level and syrup consumption in fatigue level was significantly different for the three groups ($P=0.0001$). In the case group, the job stress level after taking the syrup significantly decreased than before consumption ($P=0.007$). Level of stress after taking the syrup in control (58.51), placebo (52.66) and case group (48.60) was statistically different and this rate was clearly lower in the case group ($P=0.01$). Fatigue and job stress levels before and after the intervention in placebo and control groups statistically did not differ. This study shows that the use of lavender syrup reduces fatigue and stress levels among nurses in the case group.



4th National Congress on Medicinal Plants
12, 13 May 2015
Tehran- Iran



1765

**EVALUATION THE YIELD, ESSENTIAL OIL AND ADAPTABILITY OF
CULTIVATED SATUREJA SPECIES**

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Satureja.spp growing in all over the world. In Iran some species often growing in stony mountains. More than 15 species of the genus in 9 species are endemic to Iran. The essential oil of *Satureja* species, consist of abundant in thymol and carvacrol as important ingredients. In this experiment, 8 species with 29 accessions of *Satureja* was conducted in Damavand during 2010-2014. The results showed the 4.24% of the essential oil of *S.rechingeri* as maximum, then *S. mutica* 3.02 percent, *S. sahendica* to 2.94 percent, *S. spicigera* by 2.9 percent, *S. bachtiarica* with 2.47 percent, *S. khuzistanica* to 2.46 percent, *S. macratha* with 1.89 percent and the lowest essential oil yields of the species was belong to *S. isophylla*. Dry matter yield in *S. mutica* was 500gr/plant which was significant different with other species. Also the least dry matter yield recorded in *S. isophylla*. Cumulative dry matter increased in almost all *Satureja* species during experiment unless *S. khuzistanica* and *S. reschigeri*. Of course these two mentioned, were rich of carvacrol by 90% which is considerable in medicinal plants components, although could not growth well in cold and semi cold climates. This experiment showed the adaptability and productivity of *Satureja spp* due to domestication and mass production. Further studies on some cultivation treatments in different condition of Iran, could determine the valuable *Satureja* species productivities potentials.



455

**EVALUATION OF MELATONIN ON TALL FESCUE THROUGH MARKER
BASED PARENTAL SELECTION**

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Cultivars of outbreeding medicinal grasses such as tall fescue (*Lolium arundinacea* Schreb.) are usually synthetic populations derived from intercrossing several selected parents using the polycross method. The aim of this study was to investigate whether the amount of melatonin in tall fescue could be improved by using genetically diverse parents selected from a large breeding pool. Phenotypic and molecular markers (EST-SSR) were evaluated for their potential to optimise genetic diversity in a polycross breeding program. For both types of markers, two polycrosses of six parental plants with contrasting levels of genetic diversity were composed. A fifth polycross population was composed using six genotypes with highest general combining ability. The amount of melatonin was investigated by HPLC. The Syn1 progeny from parents with high general combining ability generally showed lower values of melatonin. Progeny from parents selected for high molecular genetic diversity on the other hand showed higher values (6 ng/g) than the other progenies for this trait. The results of this study demonstrate that EST-SSR markers provide a reliable means to predict genetic diversity in Syn1 progenies which may allow efficiently selecting parental genotypes and enhancing tall fescue breeding programs.

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4th National Congress on Medicinal Plants
12, 13 May 2015
Tehran- Iran



1374

PLANT GROWTH STRATEGIES OF TWO ECOTYPE OF THYME (*THYMUS DAENENSIS SUB SPDAENENSIS*) IN RESPONSE TO VARIOUS ENVIRONMENTAL CONDITIONS

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Thymus daenensis is an important endemic medicinal plant of Iran. In this study, to evaluate the impact of changes in ecological factors on *thymus daenensis* three experiments were performed as follows. The test for determining the upper limit and lower limit temperature, the required number of both ecotypes thyme at five different temperatures of 15 °C to 35 °C were placed in light germinator. Result showed that increasing the temperature to 30 °C in *Esfahan* ecotype and 20 °C in *Elam* ecotype of the increased vegetative and reproductive dry weight and after these temperatures were decreased them. *Esfahan* ecotype in 25 °C-30 °C Chasa maximum length and width of leaf, internode length, stem length, plant height, plant diameter, number of lateral branches, number of gland on the leaf, number and length of inflorescence, flower weight relative to the total shoot, root weight was produced but in *Elam* ecotype most of the traits measured between 15 °C-20 °C has reached its maximum. The maximum percentage and yield of essential oil and important ingredient, thymol and carvacrol, obtained by 30 °C and 20 °C in *Esfahan* ecotype and in *Elam* ecotype respectively. All of antioxidant enzymes such as Catalase, SOD, GPX and APX showed similar trends. In the next experiment, behavior of the two ecotypes were examined at three levels of water stress (30, 60 and 90% of field capacity). *Esfahan* ecotype in 90% had most of the vegetative and reproductive dry weight, seed and essential oil yield and root weight. In *Elam* ecotype the vegetative and reproductive dry weight decreased but increased oil yield, because of increased water stress due to the increase of essential oil percentage. Increased tension in both the ecotypes reduced leaf length and width, internode length, inflorescence length, plant height, stem diameter and plant, the number of glandular trichomes on the leaf and number of lateral stem. Tensions increased leaf thickness and percentage of dried leaves in *Esfahan* ecotype but *Elam* ecotype was the opposite. Increase stress in *Esfahan* ecotype led to the ratios of dry weight reproductive/ vegetative reduced but in *Elam* ecotype increased. The tension raised essential oil, but the oil yield in *Esfahan* ecotype at 60% and in *Elam* ecotype at 30% were maximum. The water stress caused thymol and carvacrol decreased, but increased p-simen. In *esfahan* ecotype of antioxidant enzymes in humidity of 60%, were at their lowest levels. Increase in antioxidant enzymes in 30 and 90% moisture level can be due to water stress and flooding. *Elam* ecotype at 30% field capacity the maximum amount of essential oil yield was observed. All of antioxidant enzymes were investigated in this study showed a similar pattern in response to changes in moisture so that almost the lowest enzyme activity was observed. In the next experiment to determine the optimal light intensity for the assembly and function of the photochemical effects of light levels (100%, 75%, 50% and 25% of natural light) on the above two ecotypes were studied during two years in field conditions. The results showed that decreasing light intensity in *Esfahan* ecotype, decreased biomass, seeds and essential oil yields and root dry weight. In *Elam* ecotype at 75% light intensity these features were at its highest level but at levels higher or lower than 75%, these features decreased. Decreasing light intensity to 75% first increased leaf length and width, length of internode, length of stem and inflorescence, plant height and its diameter and then decreased them. In *Elam* ecotype these features increased even in 25% light intensity. In *Esfahan* ecotype, decreasing light intensity decreased flowering, but *Elam* ecotype, flowered even in the lowest light intensity. The highest percentage of reproductive/ vegetative in *Esfahan* ecotype obtained in natural light and *Elam* ecotype 75% light intensity. While highest percentage of thymol, in *Esfahan* ecotype obtained in 75% light intensity and *Elam* ecotype in 50% light intensity. Carvacrol and Caryophyllene increased by decreasing intensity in *Esfahan* ecotype, but in *Elam* ecotype Carvacrol increased at 50% light intensity and Caryophyllene increased at 25% light intensity. Increasing light intensity, increased "Glutathione peroxidase" enzyme activity in *Esfahan* ecotype more than *Elam* ecotype that indicate to tolerating light stress. Chlorophyll content in *Esfahan* ecotype declined with increasing light intensity but in *Elam* ecotype at 75% light intensity increased and then declined it. It can be concluded that *Elam* ecotype adapted itself to lower light intensities and is Quantitative light plant but *Esfahan* ecotype is an obligate light plant. Seem that *Elam* ecotype better adapted to low temperature is probably due to the escape of heat and dryness of summer habitat and the habitat it happened. While *Esfahan* ecotype of seasonal rainfall for most of the season and in the spring and summer in their original habitat has been adapted to higher temperatures.

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1227

**IDENTIFICATION AND ISOLATION OF A GENE ENCODING MYB
TRANSCRIPTION FACTOR IN *PAPAVER SOMNIFERUM***

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Plants produce thousands of secondary metabolites of diverse chemical nature. Many secondary metabolites are valued for their pharmaceutical properties [1]. Many transcription factors have been characterized for their roles in regulating biosynthetic pathways of secondary metabolites at the transcriptional level [2]. The MYB superfamily constitutes one of the most abundant groups of transcription factors and plays central roles in many plant developmental processes, growth and defense responses and widely present in all eukaryotes. The MYB gene family encodes proteins characterized by a highly conserved DNA-binding domain that typically contains one to four imperfect repeats the majority of which are of the type R2R3 (with two MYB-motifs) [3]. Opium poppy (*Papaver somniferum* L.) is an economically important medicinal plant that produces a large number of pharmaceutical alkaloids such as morphine, codeine, papaverine, noscapine, and sanguinarine [4]. Despite the vital roles of MYB proteins in biosynthesis of secondary metabolites, no MYB genes have described for *P. Somniferum*, yet. In the present study, we used EST library of *P. somniferum* L. and assembly tools in order to reach a consensus sequence containing R2R3 MYB transcription factor gene. Then, Specific primers were designed based on the consensus sequence. The designed primers were used to run 3'RACE-PCR. A full length CDS of *P. somniferum* L. encoding a R2R3 MYB transcription factor was obtained and sequenced. The isolated R2R3 MYB transcription factor gene, consisted of 1029 bp submitted to NCBI database (GeneBank Accession Number: KP411870).

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**THE COMPARISON OF MICRO ELEMENTS AND HEAVY METALS
IN THE SOIL OF PERENNIAL FARMS OF SAFFRON (*CROCUS SATIVUS,L*)
IN SOUTHERN KHORASAN**

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In order to study concentrations of soil micro and heavy metal elements in some saffron planting regions of Birjand Province, an experiment was performed as factorial layout based on a completely randomized block design with three replications at year 2013. Treatments were three field ages (annual, triennial and quinquennial) and five saffron regions including Aryan shahr, Hosseinabad, Khosef, Golferiz and Mahmoei. Soil microelement and heavy metal concentrations such as Fe, Zn, Mn, Co, Cr and Cd of soil were measured. The results showed that the concentrations of Fe, Zn, Mn and Cr in soil were significantly affected by field age ($p \leq 0.05$). Effect of planting region was significant on soil Fe, Zn, Mn, Co and Cr concentrations ($p \leq 0.01$). Interaction effects between field age and planting region were significant on Fe, Zn and Co concentrations ($p \leq 0.01$). By increasing in field age soil Zn concentration was declined and heavy metal concentrations such as Co, Cr and Cd of soil were enhanced. Mn concentration in the quinquennial fields was 29 and 34% higher than annual and triennial first fields, respectively. Co content in the quinquennial fields was 53 and 46% higher than annual and triennial first fields, respectively. The maximum and minimum Fe concentrations were observed with 1.65 and 0.77 ppm for the fields of Khosef and Hosseinabad, respectively. The highest and lowest Fe concentrations were obtained with 2.436 and 0.77 ppm for the annual fields of Khosef and Hosseinabad, respectively. The highest Co concentrations were recorded in Hosseinabad and Khosef fields with 8.7 and 4.31 ppm, respectively. Thus, it is recommended to use ecological managements such as reducing the application of chemical fertilizers and improving the organic fertilizers to decline the concentrations of these elements in saffron fields.

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4th National Congress on Medicinal Plants
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**THE ROLE OF ESSENTIAL OILS IN TREATMENT OF
INFECTIOUS DISEASES**

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The rapid development and spread of multi-drug resistant pathogens around the world is the most serious threats to public health [1]. Furthermore, chemical antimicrobial agents are sometimes associated with adverse effects [2]. Therefore, there is an interesting to develop new alternative antimicrobial agents. One approach is to screen the plant's essential oils as sources of novel antimicrobial compounds [3-4]. Essential oils or plant's secondary metabolites serve as plant defense mechanisms against invasion of microorganisms, insects and herbivores [3]; therefore the essential oils may have antimicrobial activities. On this hypothesis, BarijEssence Pharmaceutical Company (Kashan, Iran) has been produced some natural products for treatments of different kind of infectious diseases. These products are effective in treatment of related infections. They include: Acneherb topical gel for treatment of *Acne vulgaris* with main ingredients of *Lavandula stoechas* essential oil and *Pelargonium graveolens* essential oil [5]. Mycoderm spray for treatment of cutaneous fungal infections with main ingredients of *Artemisia sieberi* essential oil [6-8]. Lamigex Otic Drop for treatment of acute otitis externa with main ingredients of *Eugenia caryophyllata*, *Lavandula stoechas* and *Pelargonium roseum* essential oils. Denta Barij for treatment of dental pain and adjuvant therapy of teeth and gum infections with main ingredients of *Eugenia caryophyllata* essential oil, *Salvadora persica* and propolis extracts. Matrica mouth washes for inflammatory lesions of gum & buccal mucosa and as antiseptic with main ingredient of *Matricaria chamomilla* essential oil Leucorex for vaginal bacterial and fungal infections with main ingredient of *Zataria multiflora* essential oil. The antimicrobial potencies of these products and many others have been confirmed against infectious diseases in many experimental and clinical studies. The potencies of these natural products are comparable with chemical antimicrobial agents. Furthermore, the essential oils have other biological activities such as anti-inflammatory, analgesic, antinociceptive and antioxidant activities that enhance the efficacy of these products. Also, In spite of chemical antimicrobial agents, the natural products have been shown no adverse effects in clinical trials.

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4th National Congress on Medicinal Plants
12, 13 May 2015
Tehran- Iran



**HYDROSOLUBLE ESSENTIAL OILS FROM MEDICINAL PLANTS: AN
ALTERNATIVE TO TRADITIONAL FLOWER WATERS**

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Traditional flower waters known as hydrosols, hydroflorates or distillates are widely extracted either as a flavor or medicinal uses. In considering the problem of using traditional flower waters such as lack of appropriate standard methods, lack of active substance in medical dosage and inappropriate packaging methods, therefore it is necessary to produce a product with similar function as traditional flower waters. Zarband Pharmaceuticals Company formulated hydrosoluble essential oils as an oral drop which depends on high or low medical dosage is consumed in medical uses, flavorful or as an alternative product for traditional flower waters. Some advantages of this product are as follow: soluble in water, standardized active substance and proper packaging.



1757

**OPTIMIZATION OF EFFECTIVE PARAMETERS IN SOLID-LIQUID
HERBAL EXTRACTION**

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Medicinal plants have been increasingly used for the production of herbal drugs as a reliable alternative or supplement to the conventional medicines. One of the most critical steps for the development of herbal products is the extraction of active ingredients from the medicinal plants. The extraction process enormously affects the quality of these products, which is a key issue in the development of herbal medicines [1]. It also influences the production cost and commercial use of these products. Various extraction methods have been used to obtain natural compounds from herbs. Among those is the percolation which has been frequently used in pharmaceutical industry. There are several key parameters, such as time and temperature which affect the performance of percolation. In this study, we have investigated the effect of some major parameters on the extract quality of thyme, which is among aromatic plants with therapeutic features. Thymol and its isomer carvacrol are the main phenolic component found in thyme essential oil [2]. In addition to the influence of percolation time and temperature, the effects of relative motion of the solvent and herbal powder through solvent circulation or mixture stirring have been studied. In this article, we provide a discussion on these parameters and how to obtain an optimized extraction condition. Results show that heating the powder-solvent mixture during percolation improves the extraction performance. By increasing the temperature from 20 °C to 40 °C, the extraction yield is significantly enhanced during 24 hours of percolation. Also, continuous stirring of the mixture increases the extraction efficiency by at least 25%.

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4th National Congress on Medicinal Plants
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**THE DEVELOPMENT OF HERBAL MEDICINE PRODUCING
PROCEEDING IN IRAN**

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The industry of herbal medicine producing is a newly fledged industry and on the way of development which, during the 30 past years has been facing with various difficulties and obstacles. The way of herbal producing companies' formation, the obstacles and challenges which stood and have been standing on the way of this newly fledged industry and its lacking factors will be discussed and considered in this lecture.



1144

METHYL JASMONATE EFFECTS ON TRIGONELLIN CONTENT OF FENUGREEK

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Fenugreek (*Trigonella foenum-graecum* L.), grows in parts of Europe and western Asia. The leaves are edible, but it has the small brown seeds that are famous for their use in medicine. It is known to have hypoglycemic and hypocholesterolaemic effects. The seeds are used as tonic and blood sugar lowering in Iranian traditional medicine. Seeds of fenugreek contain Flavonoids, amino acids like as Isoleucine, 4-Hydroxyisoleucine, Alkaloid- trigonellin and diosgenin [1]. Different techniques have been used for increasing the yield potential of plants. The yield potential of medicinal plants can be defined as the total produced secondary metabolite. Jasmonates (JAs), fatty acid derivatives synthesized via the octadecanoid pathway, play critical regulatory roles in plant defense responses, especially against herbivores and necrotrophic pathogens. JA signaling induces the production of a wide range of defensive secondary products and has been widely used to improve the production of useful phytochemicals [3]. The objective of the present study was to evaluate the effects of JA in elicitation of trigonellin production in Fenugreek seeds. The experiment conducted in completely randomized design with three treatments and five replications in greenhouse. Treated plants were sprayed with MJ solution (30, 60, 90 and 120 μM) three times every seven days in flowering stage and control plants were sprayed with dH_2O . Shoot and root length, shoot and root dry weight was determined after 30 days. Data regarding yield and yield contributing parameters including pod per plant, seeds per pod and seed dry weight were recorded at maturity. Trigonelline was extracted with 80% methanol and analyzed with HPLC method. The results showed that, No significant differences were found between treated and non treated plants for shoot and root length, shoot and root dry weight. The lowest seed dry weight and pod number were observed in fenugreek treated by 90 μM JA. The most striking result to emerge from the data is that, trigonellin content was significantly increased from 0.018 mg g^{-1} DW in control plants to 0.032, 0.035 and 0.036 mg g^{-1} DW in JA treated plants (30, 60 and 120 μM , respectively). A reduction in trigonellin content (0.026 mg g^{-1} DW) was observed in 90 μM JA treated plants. Based on these results, a concentration of 60 μM was chosen for future molecular analysis. Biochemical and molecular approaches is necessary for understanding the elicitation effects of JAs in secondary metabolites production.

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**DISCOVERY OF NEW BIOACTIVE COMPOUNDS FROM PLANTS BY
HPLC BASED ACTIVITY PROFILING**

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Terrestrial plants represent a rich source of new molecules with interesting pharmacological properties that could be used as lead compounds for the development of new drugs. During past decades, the interest in the investigation of the secondary metabolites from plants has been increased and led to the introduction of several important new drugs, such as anti-malarial drug artemisinin, anticancer substances vinblastine and vincristine and taxol. One of most critical steps in drug discovery is identification and isolation of active compounds from complex matrix of plant extracts. Bioassay-guided microfractionation was undertaken using HPLC to localize the active compounds [1, 2]. Time based microfractionation of the HPLC-UV chromatogram were linked to bioactivities. In parallel to this HPLC-based activity profiling, HPLC-PDA-ESI-MS and HPLC-TOF-HRMS were used for the early identification of some of the compounds present. The targeted isolation of the active compounds was performed by medium pressure liquid chromatography (MPLC-UV) and further semi-preparative HPLC steps. Structures of the isolated compounds were elucidated by spectroscopic methods including UV, NMR, MS and HRMS. Their absolute configuration was elucidated by electronic circular dichroism (ECD) spectroscopy. Successful application of this methodology for discovery new lead compounds on different targets will be discussed.

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4th National Congress on Medicinal Plants
12, 13 May 2015
Tehran- Iran



1394

**BIOLOGICALLY ACTIVE NATURAL PRODUCTS FROM MARINE RED
ALGA FROM THE PERSIAN GULF, DICHOTOMARIA OBTUSATA**

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Marine red alga, *Dichotomaria obtusata* (J.Ellis & Solander) Lamareck is found in Kenyan [1] and Cuban coastal area [2, 3]. Different solvent extracts of the Cuban specie showed analgesic and anti-inflammatory activity and were found to contain phenolics and steroidal natural products [2, 3]. However, to the best of our knowledge its cytotoxic and chemical constituents were not characterized precisely. The red alga, *D. obtusata* collected from Bushehr coasts at the depth of 2.5 m in the Persian Gulf is now searched for its potential anticancer natural products against three human cancer cell lines namely; LS180 (human colon adenocarcinoma), MOLT-4 (human lymphoblast leukemia), and MCF-7 (human breast adenocarcinoma) [4]. The bioassay guided fractionation and purification of the active extract of the marine alga resulted in purifying three compounds with moderate cytotoxic activity (IC₅₀ 15.9-53.4 µg/ml) which was then subjected to MS, and ¹H and ¹³C NMR, HSQC and HMBC spectral analyses to elucidate their structures. In this paper, I will describe the purification methods and structural elucidation of the isolated metabolites using classical chromatography and semi-preparative HPLC and 2D NMR spectral data elucidation of the above bioactive natural products.

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4th National Congress on Medicinal Plants
12, 13 May 2015
Tehran- Iran



1741

**HISTORY OF TRADITIONAL MEDICINE AND MEDICINAL PLANTS IN
IRAN**

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Iran is considered as one of the pioneers of humans' civilization with a brilliant historical reputation and vast area, which with respect to its diversity of languages, variety of tribes, and various geographical and regional conditions, have used plants based on their necessities. The emergence of Islam and its expansion in Iran, parts of Africa and Europe, establishing legislative court by 'Mamon' and 'Motevakeh Abbasi' during the lunar years period of 232 to 247, and the necessity of accessing the pharmaceutical plants data enforced the Greek and Arab's scientists to write, translate and adapt the existing knowledge related to pharmaceutical plants in Arabic language. They translated the only accessible source: 'Simple Medicinal Substances' by Dioscorides which was a difficult task since most of the plants stated in that book were not found in Arabic countries' region. Thus the mentioned plants names were unfamiliar and not translatable. Therefore, individuals namely Stephan Benbesil and Haninebne Eshagh (194-260 lunar Calendar) Arabicized extended words and occasionally rewrote the aforementioned names written in 'Simple Medicinal Substances' by Dioscorides in Arabic calligraphy which caused a great confusion and disarray regarding pharmaceutical plants and their synonymous names. During that era, within the period of 250 to 313 (lunar calendar) (864-925), Mohammadebne Zakaria Razi, one of the Iranian scientists, made an effort to write and collect data related to pharmaceutical plants and his book: 'Alhavi fi Teb' and his other books are the evidence for this issue. Subsequently, 'Abureyhan Beiruni (362-440 lunar calendar / 937-1048), living in the same era as 'Abu Ali Sina', wrote the book called: 'Siedne Fi Teb' who collected the past data up to his period; moreover, its translation is presently accessible. In the field of pharmaceutical plants knowledge, Ziaedin Abu Mohammad ebne Bitarolmaleghi known as Ebne Bitar can be mentioned among Islamic scientists whose books highly influenced the traditional medicine practiced by the people of Europe and Iran. He lived in the second half of sixth century (lunar calendar) and collected and adapted his own data from Dioscorides's book and other scientists prior to his life and has added several topics to them. Furthermore, another Islamic scientist called: Ali ebne Hossein Ansari (known as Haj Attar 729-806 lunar calendar) can be mentioned who wrote a book called: 'Ekhtiarat Badi' related to pharmaceutical plants. As a matter of fact, Mohammad Momen Tonkaboni (1077-1105 lunar calendar / 1677) should be mentioned, who was one of the physicians during 'Safavieh Dynasty'; moreover, 'Tohfato Momenin' or 'Tohfe Hakim Momen' is among his published works. Mohammad Hossein Aghili Khorasani (1183 lunar calendar / 1769) holds a brilliant status in Iranian traditional medicine. He intended to analyze and collect the data prior to his era by publishing and collecting a book called: 'Makhzan ol Advieh'. One of the features in his book is Latin names used (European languages) and Arabic terminology.



4th National Congress on Medicinal Plants
12, 13 May 2015
Tehran- Iran



1066

PREPARATION OF LABELED TAXOL FROM *TAXUS BACCATA* FOR
TUMOR TARGETED IMAGING

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Paclitaxel was discovered as a result of a screening program, being isolated from the bark of the Pacific yew, *Taxus brevifolia*, thus its name "taxol". Paclitaxel is approved in the UK for ovarian, breast and lung, bladder, prostate, melanoma, esophageal, and other types of solid tumor cancers. Paclitaxel's mechanism of action involves interfering with the normal breakdown of microtubules during cell division. Unlike other tubulin-targeting drugs such as colchicine that inhibit microtubule assembly, paclitaxel stabilizes the microtubule polymer and protects it from disassembly. Chromosomes are thus unable to achieve a metaphase spindle configuration. This blocks progression of mitosis, and prolonged activation of the mitotic checkpoint triggers apoptosis or reversion to the G-phase of the cell cycle without cell division [1,2]. ^{99m}Tc-based conjugates of taxol is attractive due to the availability of low cost ⁹⁹Mo/^{99m}Tc generator, favorable physical characteristics of ^{99m}Tc ($t_{1/2}$ of 6 h, γ 140 keV 89% abundance) and high specific activity of radionuclide. taxol labeled with ^{99m}Tc was investigated further. Complex was prepared by labeling with ^{99m}Tc in the presence of SnCl₂·2H₂O. The in vitro stability and lipophilicity of the complex were investigated. The biodistribution and in vivo image were obtained in tumor bearing mice. After different time post-injection, the weight and radioactivity of each organ were measured and gamma camera image was obtained. High labeling yield was obtained by thin layer chromatography evaluation. Labeled complex showed good stability in the presence of human serum. Biodistribution studies in mice showed that labeled taxol was accumulated in tumor and was cleaned fast from normal organs and showed clearance through urinary and hepatobiliary systems. The gamma camera image was obtained post injection showed selectively localized in tumor. Labeled with a gamma emitter radionuclide such as ^{99m}Tc, this natural compound extracted from medicinal plant may be useful for tumor imaging.

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858

**ROLE OF HYDROALCHOLIC AND CHLOROFORMIC EXTRACTS OF
SALVIA CANDIDISSIMA ON HYPERALGESIA, EDEMA AND SERUM TNF-
A LEVEL DURING ADJUVANT-INDUCED ARTHRITIS**

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Inflammatory symptoms such as edema and hyperalgesia were induced by some mediators like cytokines in response to irritant stimulus. The aim of this study was investigation the role of hydroalcoholic and *chloroformic* extracts of *salvia candidissima* on hyperalgesia, edema and serum TNF- α level during adjuvant-induced arthritis. Inflammation was induced by single subcutaneous injection of CFA into the rats' hindpaw. Hydroalcoholic and chloroformic extracts of *salvia candidissima* prepared from aerial part of plant and different dose of extracts (25, 50 and 100/kg) were administrated during the 21 days study via intraperitoneally. CFA-treated rats which received 5mg/kg doses of indomethacin considered as positive control group. Hyperalgesia, edema and serum TNF- α were assessed on 0, 3, 7, 14 and 21 days of study by radiant heat, plethysmometer and ELISA methods respectively. The results showed administration of 50 mg/kg dose of hydroalcoholic and chloroformic extracts of *salvia candidissima* (as effective dose) caused significant reduction in paw edema, hyperalgesia and serum TNF- α level on days 0, 3, 7, 14 and 21 day in comparison to control group ($p < 0.001$). Moreover, our results demonstrate that, hydroalcoholic extract of *S. candidissima* was significantly more effective than chloroformic extract in those variations. It can be concluded that hydroalcoholic and chloroformic extracts of *Salvia candidissima* dose dependently reduce paw edema, hyperalgesia during CFA-induced arthritis which those efficacy can be mediated via decreasing serum TNF- α level.



4th National Congress on Medicinal Plants
12, 13 May 2015
Tehran- Iran



1248

OPTIMIZATION AND SCALE-UP ISOLATION OF SAFFRON MAJOR
ACTIVE COMPONENTS USING PREPARATIVE HPLC

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There are many reports on biological activity of saffron, the dried stigmata of *Crocus sativus* L., especially in both neuro-protection and anti-depressive therapy [1]. Saffron main metabolites could be divided in three categories including colors due to crocins, flaover comes from safranal and bitter comes from picrocrocin [2]. According to previous studies all cis and trans-crocins, especially all-trans-crocin-1, are lead compounds for potential NMDA-antagonistic effects in the central nervous system [3]. Here and according to difference in UV-Vis pattern, at first an analytical RP-HPLC-DAD-MS using water and acetonitrile as mobile phase and a 250 × 4.6 mm column were designed for simultaneous separation of Picrocrocin, Safranal and all crocins. Secondly factors including loading factor and flow rate in analytical separation were scaled up to a preparative reversed-phase column in 50×2 cm column. Eventually all isolated peaks were applied to mass spectrometry and nuclear magnetic resonance to be confirmed.

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**IMPACT OF NEXT GENERATION SEQUENCING TECHNOLOGIES ON
MEDICINAL PLANTS RESEARCHES:
A CASE REPORT OF CANNABIS**

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Medicinal plants are rich resource of secondary metabolites which are commercially important both in traditional and modern medicine systems and there is increasingly a high demand for metabolites such as terpenoids, alkaloids, flavonoids and cannabinoids as essential ingredient of various drugs. Towards better understanding the very intricate and complex secondary metabolic pathways and therefore producing higher and more qualified secondary molecules, scientists are always looking for the best strategies to associate the genomic and transcriptomic data to metabolome, thereby allowing genome-enabled identification of candidate genes in a specific pathway through correlation of gene expression with the production of specific pharmaceutically relevant metabolites. Unfortunately, scattered and generally unlinked information associated with medicinal plants limiting the potential to identify new sources for drugs and other products. However, a sudden upsurge in broadening the knowledge base on omic approaches in parallel with high throughput sequencing of genomes and transcriptomes (RNA-Seq) using next generation sequencers in initially 1000 plants genome project has accelerated the progress of research across the life sciences and provided new insight into plant metabolic processes with the aim to design drugs that destroy cancer cells and cure other diseases as well. One of these important medicinal plants and a premier in this list is *Cannabis* which has acquired considerable attention all over the world due to its medical properties to treat pain, depression, nausea, insomnia, asthma, AIDS, cancer and multiple sclerosis. As a valuable medicinal model plant, cannabis genome and transcriptome has been sequenced and extensively reviewed which provided tremendous insight into metabolic processes and specific pathways. In conclusion, to better understanding of the gene-product relationship and practical use of metabolite molecules, unifying the "OMICS" approaches into the system biology concepts would be necessary and "next" and "next-next" generation of high throughput sequencing technologies and their related established datasets have provided a powerful tool to address questions regarding secondary metabolites production and their related pathways.



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12, 13 May 2015
Tehran- Iran



1763

NEW *IN VIVO* AND *IN VITRO* APPROACHES FOR THE PRODUCTION OF GALANTAMINE AS AN IMPORTANT ANTI ALZHEIMER 'S DISEASE COMPOUND

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Alzheimer's disease (AD) is an irreversible, progressive brain disease that slowly destroys memory and thinking skills, and eventually even the ability to carry out the simplest tasks. In 2010, there were between 21 and 35 million people worldwide with AD and this number is predicted to increase three-fold in 50 years [1]. Acetylcholine (ACh) is one of the major compounds by which electrical impulses carried by nerve cells are transmitted to another nerve cell or to voluntary and involuntary muscles. Acetylcholinesterase (AChE), a key enzyme in the nervous system of animals, hydrolyses the neurotransmitter ACh at the cholinergic synapses, thus leading to loss of stimulatory activity and causing AD. Inhibition of AChE serves as a strategy for the treatment of AD [2]. Galantamine (GAL), a morphine like alkaloid, is an important AChE inhibitor that is obtained naturally from the bulbs and flowers of Amaryllidaceae members such as *Galanthus* sp., *Narcissus* sp., *Leucojum aestivum*, and *Lycoris* sp [3]. In the present work, new *in vivo* and *in vitro* approaches for the production of this medicinally important compound were presented. As *in vivo* attempt, *Galanthus nivalis* populations which are growing wild in Mazandaran, Guilan and Golestan provinces and *Narcissus tazetta* populations, native to southern regions of Iran (Fars, Khozestan and Bosuhehr provinces) were subjected to the HPLC analysis for their GAL content. Maximum content of GAL was measured in the Zirab population of *G. nivalis* (0.36 mg/g DW) and Ghaemshahr population of *N. tazetta* (0.33 mg/g DW), respectively. *In vitro* mass bulb production of the plants in order to the production of GAL as well as isolation and identification of GAL producing endophytic fungi were also conducted. Our results revealed that maximum number of bulbs (31±0.58) was achieved on the MS medium supplemented with 2 mg/l NAA and 2 mg/l BAP in light condition. The highest amount of GAL was measured in the *in vitro* bulb regenerates of *G. nivalis* (0.25 mg/g DW). For the first time, *Penicillium verruculosum* Peyronel and *Penicillium pinophilum* Hedgcock were isolated and identified as GAL producing endophytic fungi according to their morphological characteristics, ITS nuclear rDNA gene sequence as well as HPLC analysis.

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**EVALUATION OF ENVIRONMENTAL IMPACTS FOR SAFFRON
AGROECOSYSTEMS OF KHORASAN BY USING LIFE CYCLE
ASSESSMENT (LCA)**

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Life cycle assessment (LCA) is a methodology to assess all environmental impacts associated within a product by accounting and evaluating its resource consumption and emission to environment. This study evaluated the environmental impacts of saffron agroecosystems of Khorasan based on nitrogen levels by using life cycle assessment (LCA) methodology, mean corm weight and consumed inputs in saffron agroecosystems of Khorasan per one ha were defined during years of 1999 to 2013. Four steps including goal definition and scoping (D & S), inventory analysis (IA), life cycle impact assessment (LCIA) and integration & interpretation (I & I) were considered based on ISO (International Organization for Standardization) 14040 methods. Impact categories were acidification, aquatic and terrestrial eutrophication and global warming. Functional unit was considered as one tone corm of saffron. The results showed that by increasing in nitrogen fertilizer level from 200 to more than 350 kg.ha⁻¹ acidification and aquatic and terrestrial eutrophication impact categories enhanced up to 37, 36 and 37%, respectively. The highest global warming potential was computed with 1128.17 CO₂ equiv./t corm for 350 kg N ha⁻¹. The maximum eco-Index for saffron agroecosystems of Khorasan were calculated in 350 kg N ha⁻¹ with 0.62 PO₄ equiv./t corm and 0.12 CO₂ equiv./t corm, respectively.

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**MEDICINAL PLANT ESSENTIAL OILS REDUCE RUMINAL AND FECAL
ESCHERICHIA COLI O157:H7 POPULATION IN BEEF CATTLE**

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Cattle are reservoirs of the pathogenic bacteria *E. coli* strain *O157:H7*, and approximately 30% of feedlot cattle shed *Escherichia coli O157:H7*. Feedlot cattle are fed high grain rations in order to increase feed efficiency. It has been reported that populations of *E. coli O157:H7* were higher in grain-fed than in forage-fed cattle, and when cattle were abruptly switched from a high grain diet to an all hay diet, total *E. coli* populations declined 1000-fold [2]. Therefore, strategies that reduce *E. coli O157:H7* prior to slaughter will reduce human exposures to this virulent pathogen. However, a dietary switch to forage in feedlots is not advocated due to feasibility, weight loss and other logistical issues, other feedstuffs rich in phenolics or essential oils (EO) may be a more feasible alternative strategy to decrease *E. coli O157:H7* populations [2]. Essential oils are secondary metabolites present in many plants, and data available show a strong bactericidal activity of a number of EO against pathogenic bacteria such as *E. coli O157:H7* [1]. Among the EO, thyme (THY) and cinnamon (CIN) oil have attracted considerable attention in several research studies because of their potential antimicrobial activity against ruminal microorganisms [1]. The objective of the present study was to evaluate the effects of THY and CIN on *E. coli O157:H7* population in the rumen and feces of feedlot calves fed high-concentrate diets. Sixteen growing Holstein calves (213±17 kg initial BW) were used in a completely randomized design and received their respective dietary treatments for 45 d. Treatments were: 1-control (no additive), 2-THY (5 g/d/calf), 3-CIN (5 g/d/calf) and 4- forage (sudden switch from grain diet to forage diet in the last week of experiment; as positive control). Calves were fed *ad libitum* diets consisting of 15% forage and 85% concentrate. Ruminal and fecal samples were collected from animals at the end of the experiment. Quantitative detection of *E. coli O157:H7* in samples were done using specific primers and real-time PCR method. The relative abundances of *E. coli O157:H7* were determined using total bacteria as reference according to the $2^{-\Delta\Delta Ct}$ method and reported as fold change compared with control. The relative abundances of *E. coli O157:H7* decreased ($P<0.05$) in the rumen of calves when supplemented with additives. Treatments also decreased *E. coli O157:H7* population in the feces of feedlot calves. Results suggest that the medicinal plant EO could potentially be used to control enterohemorrhagic *E.coli* colonization in cattle.

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4th National Congress on Medicinal Plants
12, 13 May 2015
Tehran- Iran

