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BIOASSAY GUIDED PURIFICATION OF CLERODERMIC ACID AS AN APOPTOSIS INDUCING ANTICANCER COMPOUND FROM *SALVIA NEMOROSA*

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The genus *Salvia* is the largest member of the Lamiaceae family with broad biological activities [1]. This genus is represented in Iran with 64 species [2]. In the present work, 12 crude extracts from 4 Iranian *Salvia* plants were screened for their cytotoxicity against A549 cancerous cell line using a bioassay guided isolation approach based on MTT assay. Purification proses led to the isolation of clerodermic acid, a diterpenoid, as the active principle compound. Chemical structure of clerodermic acid was confirmed using 1D and 2D NMR spectra and also by comparing with the literature. Several bioassays were performed for approving its apoptotic activity such as flow cytometry, cell cycle arrest, DAPI staining, and DNA fragmentation analysis. Moreover, western blotting and RT-PCR studies were carried out for investigation of its mechanism of action. Results showed that clerodermic acid has antiproliferative activity with IC_{50} value of 36 µg/mL against A549 cells. Findings suggest that this diterpenoid compound could be considered as a promising anticancer product.

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TRADITIONAL HERBAL MEDICINE IN COMBINATION WITH A CALORIE-RESTRICTED DIET CAN IMPROVE BIOMARKERS OF SYSTEMIC INFLAMMATION IN OBESE WOMEN: A RANDOMIZED DOUBLE-BLIND, PLACEBO-CONTROLLED CLINICAL TRIAL

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Inflammation is one of the primary mechanisms involved in the development of metabolic complications. The aim of the present study was to determine the effects of "Mohazell", a traditional herbal formula consisting of Origanum vulgare, Carumcarvi, Trachyspermum copticum and Ruta Graveolen in combination with a calorie-restricted diet on biomarkers of systemic inflammation in adult obese women. In this double-blind placebo-controlled randomized clinical trial, 68 volunteer obese (Body mass index: ≥ 30kg/m2) women aged 20–50 years were recruited. Participants were randomly divided into two groups, an intervention group (n=34) and a placebo group (n=34). Each group received either: (1) a low-calorie diet with 3 g/day of 'Mohazell' or (2) a low-calorie diet with 3 g/day placebo for 8 weeks. Patient's weight was measured, their BMI was calculated and biochemical parameters were measured at baseline and after the intervention. Subjects in the intervention group did not report any side effects with the 'Mohazell' supplementation. 'Mohazell' decreased serum levels of tumor necrosis factor-alpha ($p=0.001$) in both groups and high Sensitivity C-reactive protein ($p=0.04$) in the treatment group. Additionally, significant reductions were observed for weight, BMI, Energy and macronutrients ($p<0.05$). The 'Mohazell' supplementation combined with a calorie-restricted diet may modulate systemic inflammatory biomarkers in obese women. However, more studies are needed to clarify the efficacy of 'Mohazell' as an adjunct therapy to improve inflammatory parameters in obese subjects.

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COMPARISON OF FLOWERING AND GROWTH OF SAFFRON IN NATURAL AND CONTROLLED CULTURE SYSTEMS

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Soilless plant culture has permitted the grwoth of several plants under conditions in which normal cultivation is difficult. In this cultivation method, the growth environment and nutrition of the plants can be curefully controlled, resulting in higher yield and generally better quality product. In addition, in controlled culture the lower water is needed for plant production, which is an advantage in areas affected by drought stress. Accordingly, soilless culture system could be perhaps offer an alternative to the current field culture of saffron production [1, 2]. In this study saffron corms went to flowering phase at the natural environment (NE) and flowering chamber (=FC: soilless culture with 8 hours light and 16 hours of darkness, and temperature of 17 ˚C). Then, the corms of FC were transferred to NE or greenhouse. Therefore, the treatmens were included: 1- Saffron growing in the natural environment (NS), 2- Transplanted saffron from FC to greenhouse (GS) 3- Transplanted saffron from FC to natural environment (TS). The growing substrates in NE and greenhouse were garden soil and sand, respectively. Flowers were harvested during the second half of November, while chlorophyll fluorescence and growth parameters were determined on 14th February. Results showed that percentage of flowering corms in NE was 6%, while in FC was 40%. There was no-significant difference between NS and GS in terms of chlorophyll fluorescence parameters (Fo, Fm and Fv/Fm). Leaf growth improved significantly in plants grown under greenhouse condition. This observation was due to the higher transport rate of nutrient reservoirs from mother corm to vegetative parts, where mother corm weight was 2.59, 2.17 and 1.25 g in TS, NS and GS, respectively. Number of initiated replacement corms for NS, GS and TS were 3.66, 4 and 4.33 corms per plant, respectively. Root number and length were higher in NS (45 root per plant with 5.38 cm), followed by TS (45 root per plant, 2.9 cm) and GS (14 root per plant, 2.1 cm). It should be noted that both root number and length were higher in GS one week after flowering phase. In addition, more contractile roots were produced in TS treatment. There is little information on economic feasibility as well as qualitative and quantitative indices of saffron produced in controlled environment. However, it has been shown that the implementation of this production method is technically possible [1]. In a study on saffron it was concluded that production of stigmas and the concentration of the main stigma constituents was similar in soil, aeroponics and hydroponics culture systems. Overall, our results revealed that GS had more flowering, considerable leaf growth but more root vanishing rate. Therefore, it seems that soilless culture can be recommended for saffron production, especially in areas that are faced with water shortage.

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THIN-LAYER CHROMATOGRAPHY–DIRECT BIOAUTOGRAPHY AND GC-MS ANALYSIS FOR SCREENING OF ANTIOXIDANT CONSTITUENTS IN ESSENTIAL OILS OF TWO *THYMUS* SPECIES FROM IRAN

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Thymus species are well known to have significant amount of phenolic compounds and exhibit strong antioxidant activities. This study is designed to analyze the essential oils of two Iranian *Thymus* species, (*T. kotschyanus* Boiss. et Hohen and *T. pubescense* Boiss. et Kotschy ex Celak) obtained by hydrodistillation of aerial part of these plants, using GC-FID and GC-MS and evaluate the *in-vitro* antioxidant activities in quantitative and qualitative methods (namely $DPPH^{\cdot}$ and $ABTS^{\cdot+}$, β-carotene/linoleic acid bleaching assay and TLC-DB) to determine the total phenolic content of the species (assayed by colorimetric techniques) and to study the possible composition-antioxidant activity relationship [1,2]. According to the results, It was found that essential oil of *T.pubescens* had more activity in $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ tests. Both of essential oils showed high antioxidant activity in β-carotene/linoleic acid bleaching assay. Also, between the studied essential oils, *T. pubescens* contained more phenolic content. The major aroma constitutes in the essential oil of *T. pubescense* were found to be thymol (38.7 %), γ-terpinene (7.5 %), *p*-cymene (5.5 %), α-terpenyl acetate (3.8 %) and β-bisabolene (3.7 %) while in the essential oil of *T. kotschyanus*, α-terpineol (16.9 %), 1,8-cineol (14.4 %), linalool (9.6 %), thymol (7.2 %) and geranyl acetate (5.4 %) were the main compounds. The isolation and characterization of antioxidant essential oil compounds were performed by Direct Bioautography on TLC, using $DPPH^{\cdot}$ As a detection reagent and identified by GC-MS. In both of essential oils, Thymol and Carvacrol were the active constituents.

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EVALUATION OF THE EFFECT OF *NEPETA BRACTEATA* BENTH. ON ALLERGIC RHINITIS PATIENTS, A RANDOMIZED DOUBLE BLIND CLINICAL TRIAL

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Allergic rhinitis is one of the health problems in the world. It's necessary to develop new treatment procedure for control of this disease. The aim of this study was to assess the effect of *Zofa (Nepeta bracteata* Benth*)* on allergic rhinitis patients. In this double blind clinical trial study, 71 patients (37 patients in treatment and 34 in placebo group) participated. In treatment group, N. bracteata syrup was used for four weeks as three times a day. The efficacy of the drug regarding allergic rhinitis symptoms (rhinorrhea, sneezing, nasal obstruction, itchy nose and ocular symptoms) were evaluated through a visual analog scale (VAS) by 0–10 before administration and at the end of the whole treatment period. There were significant differences in symptoms such as rhinorrhea, sneezing, nasal congestion, itchy nose and ocular symptoms before and after the treatment with. N. bracteata syrup ($P \leq 0.05$); also, VAS before (mean = 7.1±1.92) and after (mean = 2.37±1.76) the treatment was significantly different. The results of this study indicate that. N. bracteata Benth has significant effects on improving the symptoms of allergic rhinitis. So, it can be a good alternative to current temporary and partial treatments.

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NANO PARTICLE ASSISTED-EXTRACTION OF EUGENOL FROM CLOVE OIL: MATRIX SOLID-PHASE DISPERSION

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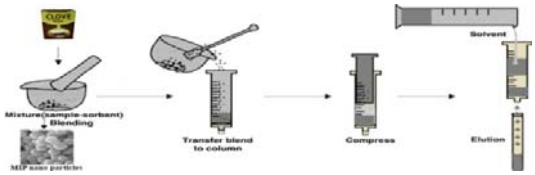
Eugenol is a component of clove oil. The name is derived from the scientific name for clove, *Eugenia aromaticum* or *Eugenia caryophyllata*. In medicine, eugenol is used as an antiseptic and an anesthetic. The results showed that eugenol derivatives inhibit the activity of the enzyme 15-lipogenase which is involved in many diseases such as asthma and lung cancer [1]. In this study, a simple, fast, and selective method for extraction of Eugenol from clove oil has been developed by molecularly imprinted polymer (MIP) nano particles. The MIP nano particles were synthesized and applied as special solid sorbent for matrix solid-phase dispersion (MSPD) to improve the selectivity. The MIP nano particles were prepared by using Eugenol as template, methacrylic acid as functional monomer, trimethylolpropane trimethacrylate as cross linker and methanol as porogen. The produced polymer nano particles were characterized by SEM and FT-IR techniques. The properties involving adsorption dynamics, static adsorption and selective recognition capacity were evaluated. Furthermore, the performance of the MIP nano particles as MSPD was investigated in detail. As the result, highly uniform imprinted nanospheres were obtained with average mean diameter of 82 nm. Good binding for Eugenol was observed in MIP adsorption experiments with 12.8 selectivity factor. The best Eugenol extraction conditions were as follows: the ratio of MIP to sample was 1:1, the dispersion time was 11 min, washing solvent was 2% aqueous methanol and elution solvent was acetic acid–methanol (2:98, v/v). Mean recovery of Eugenol from clove oil samples at different spiked levels was between 94.5 and 98.2%, with RSD values within 1.3–2%. According to USP (United States Pharmacopeia) standard, eugenol product must have purity higher than 98 %. These results are in accordance with European Community guidelines, which recommend recovery in the range 80– 110% of the target concentration and RSD below 15% (European Commission, Regulation 2002/657/EC). Moreover, the developed MIP nano particles–MSPD method was successfully applied to direct extraction and determination of Eugenol in clove oil. This method has some advantages, such as higher selectivity, lower cost, easier preparation and higher extraction yield compared with other method.



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AUTHENTICATION OF MEDICINAL PLANTS FROM MARKETS USING INNOVATIVE METHOD OF DNA BARCODING

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Medicinal plants in markets and traditional herbal shops are normally traded as dried plant parts, or in processed forms such as powdered parts, mixtures, or extracts. Such plant material lacks many of the morphological characteristics necessary for accurate identification by retailers and customers. Reliable identification and authentication of these products using macro- and micro-morphological and organoleptic methods can be time-consuming, error-prone and requires expertise and reliable references [1]. Moreover, substitution and adulteration of plant ingredients in herbal products can cause challenges for safety and quality controlling authorities. DNA barcoding can provide an accurate and reliable identification for checking the authenticity of herbal products. It can be used to identify and discriminate species in any developmental or processed stage from which DNA can be extracted. in a study on the authenticity of herbal products sold in North America using DNA barcoding it has been found that more than half (52%) of the products did not contain the species reported on the label. In this study, DNA barcoding-based molecular identification of market samples collected from *Attaris* in Northern Khorasan was performed to check the authenticity of claimed plants. Plant samples were collected from *Attari* shops and identified using morphology and vernacular names with relevant floras and pharmacopoeias. Then the 68 samples were identified using DNA barcoding. Two DNA markers, the nuclear ribosomal internal transcribed spacer, nrITS, and the plastid marker, *trnL* were used for barcoding. The BLAST similarity-based method and an integrative identification approach were used for final identification. With the BLAST search for *trnL* marker, 16.7% (10 samples) were identified to species level, 55.0% (33) to genus level, and 28.3% (17) to family level. For nrITS marker 35.4% (17 samples) to species level, 58.3% (28) to genus level, and 6.3% (3) to family level. Combining data from both markers, the sequence matching method identified 36.7% to species level, 47.1% to genus, and 16.2% to family level. In integrative identification approach, combining both markers resulted in 80.9% (55 samples) species level identification and 19.1% (13) genus level identification. The integrative identification approach resulted in a 2.9, 0.9 and 1.2 fold increase for *trn*L, ITS and both combined respectively, over objective sequence matching. DNA barcoding of traded plant material requires objective strategies to include data from multiple markers, morphology, and traditional knowledge to optimize species level identification success.

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COMPREHENSIVE HPLC-DAD BASED FINGERPRINTING ANALYSIS OF IRANIAN SAFFRON AND ITS GEOGRAPHICAL CLASSIFICATION

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The main concern behind Saffron, the dried stigmas of *Crocus sativus* L., is quality control [1]. Crocins, Crocetin, Safranal and Picrocrocins are the main constitutes of Saffron in which they have resposilbity for color, odor and bitter taste respectively [1,2]. In this study a comprehensive Geographical dependent quality of saffron based on HPLC-DAD analysis was investigated. Also a similarity chromatographic fingerprinting was made. Our results show a wide metabolite distribution of 0.83±0.09 to 38.82±1.50 ,0.003±0.000 to 0.103±0.004 , 4.93±0.11 to 30.23±0.21 And 0.021±0.002 to 0.579±0.014 for Crocins, Crocetin, Picrocrcin and Safranal respectively. Also the statistical fingerprinting analysisdemonstratingCrocins are the main constitituteof Safforn which could be used for saffron geographical classification.

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A COMPREHENSIVE METABOLOMICS OF IRANIAN WILD *PUNICA GRANATUM* L. (POMEGRANATE) POLYPHENOLS BY MEANS OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH DIODE ARRAY AND ELECTROSPRAY IONIZATION-MASS SPECTROMETRY DETECTION

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The present study was aimed at the development of ananalytical method for analysis of polyphenols inIranian wild *Punica granatum L.* (pomegranate) peel.23 wild pomegranates collected from north of Iran were used toestablish chromatographic fingerprints. ultrasound-assisted extraction (UAE)with a mixture of water and ethanol 50:50 (v/v) with 10% of glacial acetic acid as the extraction solvent was used [1]. The qualitative analysis of pomegranate polyphenols was performed by high-performance liquid chromatography with diode array and electrospray ionization-mass spectrometry detection. For the fingerprint analysis, 8 characteristic peaks [Digalloyl-hexoside, Gallagyl -hexoside (punicalin), Punicalagin, Digalloyl-gallagyl-hexoside, Galloyl-HHDP-DHHD Phexoside (granatin B), Caffeic acid der, Ellagic acid-hexoside, Ellagic acid] were selected to evaluate the similarities of pomegranate peels. The acquisition of the metabolite fingerprinting of pomegranate fruit with an efficient and reliable method also represents a powerful tool for the assessment of the authenticity of pomegranate-based products and for use in pharmaceutical and nutritional investigations, since synergistic effects are involved in the activity of these compounds.

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EFFECTS OF CURCUMINON IRON OVERLOAD IN B-THALASSEMIA MAJOR PATIENTS: A DOUBLE-BLIND RANDOMIZED CONTROLLED CLINICAL TRIAL

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B-Thalassemia major is the most common inherited anemia in the world and Iran. Iron overload, the main complication of β-thalassemia major is caused by frequent blood transfusion and an increased absorption of iron in the gastrointestinal tract due to suppression of hepcidin production. Given thatdeferoxamineusage is associated with difficulties, finding an oral iron chelator of plant origin could be useful in management of β-thalassemia. Curcumin (diferuloylmethane), the active polyphenol in turmeric has therapeutic properties such as cancer preventive, antioxidant and iron binding activities. The objective of this study was to evaluate the efficacy of curcumin on iron overload in these patients. This double-blind randomized controlled clinical trial was performed on 68 male and female β-thalassemia major patients between 18 and 40 y old, who were on medication with deferoxaminein Amirkola thalassemia center in Mazandaran. Ethical Committee of National Nutrition and Food Technology Research Institute of ShahidBeheshti University of Medical Sciences approved the study protocol. The registration ID of this study in Iranian Registry of Clinical Trials was: IRCT2016053028165N1. Subjects in curcumin group (n=34) received daily two 500 mg curcumin capsules and patients in placebo group (n=34) took daily 2 placebo capsules for 12 weeks. Dietary intakes, anthropometric measures and biochemical parameters were assessed at the beginning and the end of intervention.Statistical analysis was performed using SPSS software. Mean dietary intake of zinc was significantly lower in curcumin group than placebo group ($p=0.038$). Results of analysis of covariance showed, non-transferrin bound iron (NTBI) significantly decreased ($p<0.01$) in curcumin group compared to placebo group, adjusted for dietary intakes of iron, zinc and baseline values.The paired sample t test revealed thatcurcumin also significantly decreased serum levels of NTBIat the end of trial in comparison with pretreatment values ($p<0.01$). A considerable fall in ferritin and a slight elevation in hepcidinlevel was observed in curcumin group during the study period; however these changes did not attain statistical significance ($p>0.05$).Differences in Hb and serum iron levels also were not significant in any of the 2 groups. Coadministration of curcumin and deferoxamine resulted in an additive effect on reducing iron burden in patients with β-thalassemia major. Curcumin may be useful as a complementary treatment to ameliorate iron loading in these patients.

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EFFECTS OF POLYPLOIDY ON SOME MORPHOLOGICAL AND CYTOLOGICAL CHARACTERISTICS OF PERSIAN POPPY (*PAPAVER BRACTEATUM* LINDL)

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Persian poppy (*Papaver bracteatum* Lindl.) is a wild perennial medicinal plant that grows natively in the Alborz Mountains in Northern of Iran. It is mainly known for the high amounts of valuable thebaine alkaloid. Today, *In vivo* induction of polyploidy techniques by using chemical mutagen such as colchicine, widely used in medicinal plant breeding. In this study the effect of different concentrations of colchicine (0, 0.5, 0.1, 0.2, and 0.5 %) and three exposure times (24, 48, 72 hours) in the greenhouse medium were investigated. Flow cytometry is the pre-eminent method for evaluation of the induced polyploidization. However, alternative confirmation methods, such as *Chlorophyll* contents and some morphological, physiological and cytogenetic characteristics are also used. The *Chlorophyll* contents, the length and width of the stomata of *P. bracteatum* treated plants were analyzed. ANOVA Results showed significant difference between polyploidy candidates and diploid plants ($P<0.01$). The highest and lowest average of *Chlorophyll a* (0.9 and 0.52 mg/g fw) and *Chlorophyll b* (0.65 and 0.58 mg/g fw) was obtained in 0.2 % of colchicine in 24h and control plants Also, there is significant difference between both control and treated plants in length and width of the stomata. The maximum length of stomata (37.5 µm) and width of stomata (11.2 µm) was observed on 0.2 % of colchicine in 24h. The minimum length of stomata (21.4 µm) and width of stomata (6.3 µm) was observed on 0 % of colchicine in 24h. Thus, we can conclude that morphological and physiological traits of induced tetraploid plants were changed.

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PART, AN INDUSTRIAL CHICORY GENOTYPE FOR INULIN PRODUCTION IN IRAN

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Industial chicory (*Cichorium intybus* L. var. *sativum*) is an important root crop for inulin production which is widely used as food ingredient due to its health promoting properities for human and animal husbandry (1). Root yield of Iranian chicory genotypes for inulin extraction was disappointing (2) and so 14 selective plants among 5 root chicory cultivars (Orchies, Tilda, Hera, Melci and Schepenes) were cloned based on root yield and inulin percent to improve a synthetic cultivar for moderate climate zones of Iran. These clones were planted in an isolate plot in Latin Square Design (LSD) at the beginning of March, 2013. Seed of S_0 was obtained in June, 2014. This seed was again planted in an isolate plot with large area about 2000 m^2 to reach to S_1 seed in 2016 after omiting unwanted plants. The S_2 seed will be produced in large quantity to produce suitable root chicory by selected farmers for industrial inulin production in RIFST (Research Institute of Food Science and Technology) pilot. Assessing inulin percent and inulin quantity was detected by HPLC-RI during enhancing Part varitie. Average root yield of this synthetic cultivar, Part, was 4.56 kg/m^2 of fresh root weight which was significantly higher than all used cultivars in Mashhad, Iran. Finally, Farasoodmand-e Part company hopes to be the first producer of inulin and its byproducts by using his own cultivar in the middle east.

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QUANTITATIVE AND QUALITATIVE CONTROL OF SOME OF HERBAL TEA-BAGS AVAILABLE IN THE PHARMACEUTICAL MARKET IN TEHRAN

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Herbal tea can be used for therapeutic or nutritional purposes, depending on the chemical constituents present. In this research, the quantitative and qualitative control of herbal tea bags including Chamomile, Fennel, Lavender, Wild Mint and Thyme were conducted. In terms of the kind of the plant, the ash, the essential oil and their active substance were determined. The essential oil was prepared by hydro-distillation and identification of the chemical compounds was performed by gas chromatography. By micrographic study, compatibility of the herbal contents were confirmed in tea bags withIranian Herbal Pharmacopoeia (IHP). According to the TLC tests results, R_f values of anethol in Fennel essential oil, linalyl acetate in Lavender essential oil, thymol and carvacrol in Thyme essential oil and carvone in Mint were 0.88, 0.75, 0.55 and 0.5 respectively. Chamomile sample had not essential oil. So, the TLC and GC analysis were not performed for this plant. The total ash and acid-insoluble ash were 1% and 8%, respectively for fennel, 5% and 13%for lavender, Were2.03 % and 7.5%, respectively for chamomile, 2.5% and 8%.for Wild Mint and 1% and 11%for Thyme. Based on GC analysis, amount of anethol in the essential oil of fennel, carvone in Wild Mint essential oil, thymol in Thyme essential oil and linalool in Lavender essential oil were 0.098 gr / mL, 0.621 gr/ mL, 0.019 gr/ mL, 0.164 gr/ mL respectively. According to the results, R_f values were corresponded with standard indicators listed in references. The total ash and acid-insoluble ash of Fennel, Wild Mint and Chamomile were also correspond with the values available in Iranian Herbal Pharmacopoeia (IHP) but the mentioned values for Lavender and Thyme were not in compliance with reference. Chamomile sample has not essential oil which could be due to inappropriate storage condition or using the old plant sample for preparing of herbal tea.The outcome of this study can serves as a contribution to knowledge in establishing quality parameters for the standardization of herbal teas in the market.

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SILYMARIN APPEARS TO BE A REAL MIRACLE

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Silymarin (SMN) is a complex mixture of flavonolignans extracted from seeds of the milk thistle (*Silybum marianum*). Traditionally, SMN has been used as a natural remedy for digestive problems and in particular for diseases of the liver and the biliary tract, for menstrual disorders and varicose veins [1]. There are increasing data indicating beneficial effects of SMN on various disorders and diseases in different tissues. We in our investigations during the last ten years found that SMN exerts remarkable protective and regulatory effects on drug and xenobiotic biotransforming enzymes in experimentally-induced diabetic animals. At the same series of investigation we explored that although both SMN and melatonin treatment was able to normalize the antioxidant status, while only SMN administration could restore the β cells of Langerhans islets in diabetic rats. The anti-inflammatory property of SMN on mono-iodoacetate-induced osteoarthritis and antinociceptive effects on acetic acid-induced reaction were also clarified. In another study the SMN protective and preventive effects on doxorubicin-induced carbonyl stress, DNA damage, and its capability in the alteration of c-myc gene expression were demonstrated. SMN beneficial effects on mycophenolate mofetil–induced duodenal disorders was found attribute to its capability in the reduction of NO and MDA levels and myeloperoxidase activity, suggesting it's not only antioxidant but also anti-nitrosative capacities.

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OPTIMIZATION OF SUBSTRATE FOR A MEDICINAL MUSHROOM CULTIVATION (*PLEUROTUS ERYNGII)* USING LIGNOCELLULOSIC AFFORDABLE WASTES

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King Oyster Mushroom (*Pleurotus eryngii*) belongs to Basidiomycota division, Agaricomycetes class and Pleurotaceae family. This mushroom generally is Known as a edible medicinal mushroom and grows on wood wastes of Apiaceae family. Type of substrates for mushroom growing depends on available plant or agricultural wastes. In this research, *P. eryngii* strain (ID code of KS004) was donated by Edible Mushrooms Research Laboratory of Gyeongsang University of South Korea. Sawdust as the main material was composed of beech (50%) and populous (50%) tree. Calcium sulfate (3%) and Calcium carbonate (3%) added to each treatment. Substrates sterilized at 121 C and 1.5 bar for 2 hours. After cooling, substrates, inoculated with *P. eryngii* commercial spawn (3%). experiment was done in completely randomized design with five Treatments and 4 Replications. The growth indices including the amount of mycelium growth (AMG) in substrates; the number of fruiting body (NFB) of mushroom; the weight of mushroom and biological efficiency (BE) were measured. The amount of mycelium growth in substrate in test tube (50ml) and also in plate with substrates extract was measured in different pH levels (5.5, 7 and 8.5). Data were analyzed by statistical software of JAMP 4.0 and graphs were drawn by Microsoft Excel 2007 and SigmaPlot 12.0 software. Mycelium growth in substrate extracts measured in three different pH levels(5.5, 7 and 8.5). Measuring the average of mycelium growth after 7 days in experimental treatments extract showed that the best pH for mycelium growth for all treatments was pH=7. Measuring AMG of *P. eryngii* in different treatments showed that among 5 treatment substrates, the most amount of Mycelium growth was obtained in substrate No. 1 and the less amount of mycelium growth was obtained in substrate No. 5. The higher number of mushrooms was related to substrate No. 1 and the less one was related to substrate No. 5. In term of the number of fruiting body, there was no significant difference ($p \leq 0.05$) between substrates No. 1 and 3 and substrates No. 2, 4 and 5. the biological efficiency percentage in experimental treatments No. 1, 2, 3, 4 and 5 were 64.81, 49.74, 59.22, 28.72 and 19.8, respectively. The results showed that treatment No. 1 had the most biological efficiency (64.81%) and treatment No. 5 had the less biological efficiency (19.8%). The mean comparison of experimental treatments showed that treatment No. 1 and 3 and also No. 4 and 5 had no significant difference ($p \leq 0.05$).

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LABELING OF A VINCA ALKALOID FROM CATHARANTHUS ROSEUS WITH TECHNETIUM-99m AND EVALUATION AS A TUMOR IMAGING AGENT

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A phytochemical investigation of the plant Vinca rosea has demonstrated that a number of alkaloidal substances can be obtained with antitumor activity. Over 30 alkaloids have been obtained, of which four including vinblastine, vinleurosine, vincristine, and vinrosidine are known definitely to be active. They represent a new class of large complex dimeric alkaloids containing both indole and dihydroindole moieties. Among these compounds vinblastine has proved effective in a number of types of cancer including Hodgkin's disease and other lymphomas, non-small cell lung cancer, bladder cancer, brain cancer, melanoma, and testicular cancer by. It is given by injection into a vein and after that by binding to tubulin, the assembly of microtubules will be inhibited [1,2]. In this study labeling of vinblastin with ^{99m}Tc was caried out in order to achive a tumor imaging agent with potential that could be used in clinics. Due to ideal properties such as short half-life (6 h) and 140 keV gamma-rays emission ^{99m}Tc has become the most major radioisotope for medical imaging. Vinblastine was labeled with 99mTc via direct labeling after reduction of ^{99m}Tc with $SnCl_2 \cdot 2H_2O$ as a reducing agent. Radiochemical purity and labeling yield were determined by Thin layer chromatography and HPLC. The stability in human serum was determined. Biodistribution study in mice with tumor was performed and whole body gamma scan was obtained. ^{99m}Tc-Vinblastin was prepared with high radiochemical purity. Radiocomplex showed long stability in the in vitro medium. Tumor acumulation with hepatobiliary excrition were observed. Tumor was visulized by gamma imaging at 1 h post injection. This natural radiocomlex could be a potential imaging agent in tumor diagnosis in nuclear medicine.

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IDENTIFYING OF MALE STERILE GENOTYPES AND ITS IMPORTANCE IN BREEDING OF DIFFERENT ECOTYPES OF THYME SPECIES (*THYMUS* SPP.)

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One of the main methods for production of new varieties in breeding programs of Lamiaceae is inter-intraspecific hybridization. Because of the small size of flowers, dense inflorescence and non-synchronicity opening of flowers in Lamiaceae, the manual emasculation and pollination is very difficult. Therefore, an effective solution to overcome this limitation is identifying of male sterile genotypes and their application in hybridization which is very important in breeding programs of Lamiaceae such as thyme. So, in order to identify the male-sterile genotypes in different populations of Thymus spp., an experiment was conducted based on randomized complete block design with three replications in research station of Department of Horticultural Science, University of Tehran, Iran, during 2015–2016. The seeds of 25 populations of 4 thyme species (*T. daenensis, T. vulgaris, T. kotschianus and T. loncifolius*) were planted in greenhouse in February 2015 and the transplants were planted in farm in May 2015. In the spring of the second year (2016) in the flowering stage, the male sterile genotypes were identified from the appearance of flowers. The results showed that except ecotypes of Lorestan (*T. daenensis*), male sterile genotypes were observed in 24 other ecotypes. The highest (84%) and lowest (8%) ratio of male sterile plants were observed in ecotypes of Ilam (*T. daenensis*) and ecotype of Markazi (*T. loncifolius*), respectively. The average ratio of male sterile plants in species of *T. daenensis*, *T. vulgaris, T. kotschianus* and *T. loncifolius* was 25.7, 61.2, 27.3 and 59.2 %, respectively. So, according to this results it could be concluded that the male sterile genotypes can facilitate inter- intraspecific hybridization in Thymus spp. and can be usefull in breeding program of thyme to produce new hybrid varieties.

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EXTRACTION OF *OCIMUM BASILICUM* PEROXIDASE AND COUPLE WITH GLUCOSE OXIDASE IN DETERMINATION OF BLOOD GLUCOSE LEVELS

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Ocimum basilicum (Basil) callus obtained from the leaf cell culture of Basil ,was subcultured regularly on Murashige and Skoog (MS) medium containing sucrose (15 gr/L), 2, 4-Dichlorophenoxyacetic acid (10^{-6} M) and kinetin (10^{-5}M) under darkness conditions at 25°C [1]. After 31 days of subculturing, it was 0.2 mg Pox per 1gr callus, thus *O. Bacilicum* could be as an appropriate source of plant peroxidase. Extraction of *O.Basilicum* callus peroxidases (OBPoxs) were performed using Tris-buffered saline (0.01 M, pH 6) and PMSF as serine proteases inhibitor. Proteins were precipitated by using Ammonium sulfate (80%) salt [2]. Following this, purification was carried out using G-50 Sephadexgel-filtration and Sepharose-S (Cationex change) chromatography column and Tris-buffered saline (0.01 M, pH 6) as liquid phase [3]. Fractions that had peroxidase based on maximum absorbance in 280 nm and enzyme activity assay was chosen [4]. SDS-Polyacrylamide gel electrophoresis (1% SDS, 7.5%T) of these purified proteins showed a band of about 40 kD. OBPox was purified 117.78 foldand its specific activity was 12962.83 U/mg. Optimum temperature and pH for OBPox was 35°C and 5.5, respectively. Studies showed that OBPox is a cationic heme-containing enzyme that is classified in type III plant peroxidases. Peroxidases have different commercial uses, for example as a component of clinical diagnostic kits and for immunoassay. For this purpose, Purified peroxidases coupled with glucose oxidase in aspectrophotometric method [5], using Guanidin-SO_3H [1], a non-carcinogenic, high sensitive substrate for peroxidase, was used to quantitation of blood glucose in patient and healthy samples. This method is able to determine a minimum concentration of glucose about 16.6uL, (R^2=0.92). Comparing biochemical properties of OBPox with Horseradish peroxidase [6], show that OBPox has the competition ability in immunoassays with commercial Pox for determine the amount of blood glucose levels.

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AN OUTLOOK TO IRAN POTENTIAL IN FLAVONOIDS PRODUCTION: GERMPLASM, CLIMATE, HOW TO START- AND SCALE UP

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Changing consumer lifestyles, focus on preventive medicine and rising demand for beauty and health supplements are reasons for highly global demand of flavonoid-containing products. The backbone of present article and lecture is our recent studies on flavonoid containing-plants. The plants we work on their flavonoids are *Dracocephalum kotschyi*, *Capparis spinosa, Salvia reuterana, Satureja hortensis* and *Rheum ribes* [1-3]. Since the primal sources of plants has been collected form natural habitat therefore it can provide a good opportunity for us to discuss about their germplasm. Novel *in-vitro* techniques for flavonoid production and problems we face with them, high efficient methods for extraction, new simple and advanced analytical methods are the other subjects of present study. With available data, exist potential for plant origin drugs, and growing rate of cardiovascular disease, diabetes, and cancer, Iran has the potential to become one of the world's largest flavonoid producer and consumer in the new future. Therefore to reach this goal, getting of fundamental managements and strategies are essential instruments to shortening the time taken or even increasing the success rate.

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SELECTION OF SUPERIOR GENOTYPES OF *ECHINACEA PURPUREA* L. BY ANALYSIS OF GENERAL COMBINING ABILITY IN HALF SIB FAMILIES UNDER DROUGHT STRESS CONDITION

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Echinacea purpurea L. is widely used in traditional medicine around the wold. All parts of the plant especially its roots contains valuable metabolites to combat cold, flu, cough, sore throats and many other ailments. Due to frequently occurring water deficit condition in the most agricultural fields of Iran, selection of tolerant and high yielding genotypes may be considered as a good choice to overcome the limitations. To achive the aims, this study was conducted to select the best genotypes based on their progenies tests in a diverse population. Around 100 plants were selected for yield components such as flower number, plant height and shoot dry weight. Then the seeds of each selected plant were separately harvested. Since open-pollination behaviour of *Echinacea*, the seeds of each plant can be considered as a half-sib family. To evaluate genotypic value of the selected plants under drought stress, two separate experiments were run during two years of 2015-2016. First experiment was designed on the basis of a simple lattice design with two replications using the 100 half-sib families as treatments under normal irrigation (100% FC). Second experiment was run like the first one, but the irrigation was made by 50% FC. Phenotyping assay were done by measuring of 14 morphophysiological traits. Combined analysis of variance of the collected data revealed that the half-sib families and their interaction with water stress were significantly different ($p>0.01$) based on the studied characters such as leaf area, flowering commencement, bud number, flower number, plant height, flower diameter, plant fresh and shoot dry weight. The water stress led to decrease all the studied traits. According to the results, based on the means and GCA of the main economical traits and yield components such as number of buds and flowers, plant height, and dried weight, some families were the most drought tolerant, for which by combining their parent seeds to form the next improved population might be optimistic. This experiment was the first cycle of selection by ear to row method that can be recurrently continued to reach an improved variety..

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IRAN HERBAL MEDICINE AND NATURAL HEALTH PRODUCTS: CONDUCTING CLINICAL RESEARCH BASED ON ICH GUIDELINES FOR MARKET REGISTRATION AND EXPORT TO CANADA AND EUROPEAN UNION

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Iran Herbal Medicines (HM) and Natural Health Products (NHP) have a long history of usage in Iran and in neighbor countries. The aim of this presentation is to introduce the international guidelines in order to test clinically the safety, and efficacy of NHP and HM produced by Iranian companies and clinical scientists for conducting clinical trials based on the International Council for Harmonization (ICH) guidelines for market registration and expanding export activities to Canada and European Union countries. Based on Canada and EU requirements, NHP and HM must meet the regional regulatory requirements for quality, efficacy and safety. In Canada, regulations mandate that a manufacturer, packer, labeler or importer need to have a prior registration with Health Canada before commencing any such activity. The process involves registration of the manufacturing site along with the products. Complete data on clinical studies, product composition, standardization, stability, microbial and chemical contaminant testing methods and tolerance limits, safety and efficacy along with ingredient characterization, quantification by assay needs to be submitted. The authority mandate that NHPs must comply with the contaminant limits and must be manufactured as per the ICH-GMP norms. In Europe, The European Medicine Agency have laid down two ways of registration of herbal medicinal products: (1) A full marketing authorization by submission of a dossier providing information on quality, safety and efficacy of the medicinal products, physicochemical, biological or microbial tests and pharmacological, toxicological and clinical trials data and (2) For herbal medicinal products, which do not require medical supervision, a simplified procedure exists. Iran herbal medicine has excellent potential to obtain product registration from Canada and EU for reaching these important markets. In order to start or expand Iranian exportation of herbal medicine to these economic regions, the knowledge and application of ICH guidelines for conducting clinical trials and reporting of adverse reactions is mandatory.

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VARIETY SELECTION AS A KEY TO HERBAL MEDICINAL PRODUCT QUALITY

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In times of ever increasing regulation, the selection of plant material for herbal medicinal products has a strong impact on issues of quality, toxicology and clinical uses. Increasing knowledge in pharmacognosy and pharmacology, and the strict documentation of adverse effects, frequently causes restrictions by European regulators for the protection of consumers from theoretical risks derived from the properties of certain isolated constituents (e.g., carvachrol in thyme or menthofuran and pulegone in peppermint), or from factual risks derived from poor quality (e.g., as observed in the case of the South Pacific plant "kava", *Piper methysticum*). Both cases call for action with the plant on the field as a starting point, preferably with local varieties adapted to the local climatic conditions. Non-compliance to this point led to a disaster with St. John's wort (*Hypericum perforatum*) and kava in the past, by planting under non-suitable climatic conditions causing fungal and/or viral diseases. Frequently forgotten is the traditional knowledge, which may lead to unpleasant surprises when a plant material traditionally known as safe is exchanged for mere monetary reasons against other plant parts and other varieties for which there is no such experience. The selection of a potentially toxic variety by one (!) manufacturer almost killed the entire kava production in the South Pacific, with tremendous repercussions to the local economies. Finally, in cases where there is a rather uniform plant quality on the market, defined by pharmacopoeial specifications (e.g., *Harpagophytum procumbens*), the use of high performance varieties can also be a unique selling point in the marketing of the plant materials and the products manufactured from them. Examples of plants grown in Iran with superior quality are *Matricaria chamomilla*, *Thymus vulgaris*, *Echinacea purpurea*, *Galega officinalis*, *Tanacetum parthenium* or *Cucurbita pepo var. styriaca*. The regulatory burdens related to GACP and GMP can ultimately be a great opportunity for farmers: Although to date the payment system is rather by mass instead of by class, the demand for distinctly superior quality is growing, and thus the chance for a better income in the regions where the material originates. Consequently, the use of specific high performance varieties adapted to local conditions and traditional knowledge is not only positive for the pharmaceutical companies and their products, but may also be considered a stabilising factor for the societies in the regions of origin.

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MEDICINAL AND AROMATIC PLANTS IN UMBELLIFERAE FAMILY IN IRAN

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Umbelliferae family in Iran including 122 Genus and about 400 species, widespread in temperate and cold temperate region of Iran, some species of them are Medicinal and aromatic and some of them used as vegetable, spice and Medicinal plants. Regarding to variation of usage, plantsin this family can be divided to:

Vegetables
Anethum graveolens (Shevid), *Apium graveolens* (Karafs), *Coriandrum sativum* (Geshniz), *Daucus Carota* var. *sativa* (Havij), *Petroselinum crispum* (Jaafari).

Spices
Bunium persicum (Zire siahe, Zire Kermani, Zire Irani), *Carum Carvi* (Zire siahe Europaei), *Cuminum Cyminum* (Zire sabz), *Echinophora platyloba*(Khusharize), *Foeniculum vulgare* (Raziane), *Heracleum persicum*(Golpar), *Kelussia odoratissimum*(Kelus),*Pimpinella Anisum*(Anisum, Badiane Roumi),
Trachyspermum Copticum (Zenyan, Nankhah.

Lactiferous Medicinal, poisonous and industrial species
Dorema Ammoniacum (Koma kandal, Vasha), *Ferula Assa-foetida* (Anghuze kazeb, Gande koma), *Ferula foetida* (Anghuze), *Ferula galbaniflua*(Ghasni, Barije), *Ferula persica* (Sakbinej).

Poisonus plants
Chaerophyllum aureum (Jaafari Farangie khaldar), *Conium maculatum* (Shukaran), *Diplotaenia damavandica* (Kozal), *Oenanthe aquatic* (Abchekan), *Prangos Uloptera* (Vaye, Kharkul)
Plant species which traditionally used by domestic people, soon in the spring as wild vegetable
Dorema Aucheri (Bilhar), *Eryngium caucasicum* (Chuchakh, Chuchagh, Anarije), *Falcaria vulgaris* (Ghazyaghi), *Froriepia subpinnata* (Zeleng), *Muretia amplifolia* (Baraza), *Pimpinella affinis* (Tartizake Baghi)
Some most well Known medicinal plants of the Umbelliferae family are
Ammi majus (Alafe Osghofi, Ami), *Anethum graveolens* (Shevid), *Anthriscus Cerefolium*(Jaafarie vahshie Yeksale), *Apium graveolens* (Karafs), *Bunium persicum* (Zire Kermani, Zire Irani), *Carum Carvi* (Zire siahe Europaei), *Centella asiatica* (Ab Boshghabi), *Conium maculatum* (Shukaran), *Coriandrum sativum* (Geshniz), *Cuminum Cyminum* (Zire sabz), *Daucus Carota* var. *sativa* (Havij Farangi), *Foeniculum vulgare* (Raziane), *Levisticum officinale* (Anjadan), *Petroselinum crispum* (Jaafari), *Pimpinella Anisum* (Anisun, Badiane Roumi), *Trachyspermum Copticum* (Zenyan, Nankhah)

Fodder plants
Bilacunaria microcarpa(Raziane Asbi),*Diplotaenia cachrydifolia*(Kozale Jashiri, Gorz),*Ferula ovina*(Koma),*Ferulago angulata*(Chavil),*Prangos ferulacea* (Jashir)

Some Aromatic plants which are not well known for domestic people

Aziliaeryngioides (Azil), *Chaerophyllum* spp (Jaafarie Farangi), *Cymbocarpum anethoides* (Shevidak),
Kalakia marginata (Kalaki), *Malabaila Secacul* (Shaghaghole Sahraei), *Peucedanum angustifolium* (Raziane Kuhi), *Pycnocycla* spp. (Sag dandan).