

**THE 13TH INTERNATIONAL CONGRESS
PHYTOPHARM 2009**

**29-31 July 2009,
Bonn, Germany**



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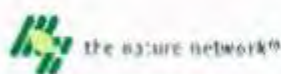
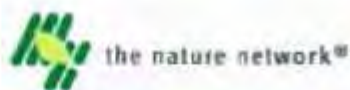
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Interregional Center "Adaptogen"
Saint-Petersburg, Russia
Tel./fax +7 812 3225605
E-mail: adaptogen@sp.ru, phyto2009@mail.ru

ISBN 978-5-9651-0365-2



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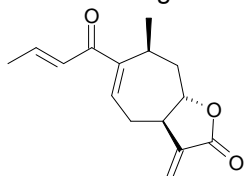
STRONG ZOOSPORICIDAL ACTIVITY OF A NEW FLAVONE ISOLATED FROM SUDANESE *XANTHIUM BRASILICUM* VELL (ASTERACEAE)

Muna Ali Abdalla¹, Tofazzal Islam², Hartmut Laatsch¹

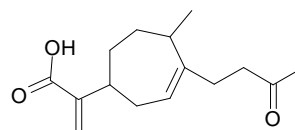
¹Institut of Organic and Biomolecular Chemistry, Georg-August, Universität Göttingen, Tammannstrasse 2 D-37077 Göttingen, Germany

²Department of Crop Sciences- Plant Pathology and Plant Protection, Georg-August-Universität Göttingen, Grisebachstraße 6, 3707 Göttingen, Germany

The widespread naturally occurring and agriculturally used weed [1] *Xanthium brasiliicum* is in use in Sudanese folk medicine as a decoction for the treatment of malaria. Recently an antimalarial activity of *X. brasiliicum* *in vitro* and *in vivo* has been reported [2]. Various essential oils, terpenes, and flavonoids were previously isolated thereof, and related species are rich in flavonoids and sesquiterpenes as well. To isolate the active constituents, the aerial parts of *X. brasiliicum* were dried, crushed and extracted with CH₂Cl₂ and EtOAc. Subsequently, the crude extract was subjected to flash silica gel column chromatography using a cyclohexane/EtOAc gradient. Further separation afforded three compounds: a new flavone and two sesquiterpenes 1 and 2 were isolated from *X. brasiliicum* for the first time, in addition to four known sterols. 2-(3,4-Dihydroxy-phenyl)-3,7-dihydroxy-5,6-dimethoxy-chromen- 4-one gave the molecular weight 346 and the molecular formula C₁₇H₁₄O₈ by HRESI MS. The structure was elucidated by detailed analysis of spectroscopic data such as ¹H NMR, ¹³C NMR and 2D spectra. Compounds 1 and 2 were identified as anhydrodehydroivalbin and 4-oxobedfordia acid. ESIMS displayed the molecular weights 246 and 250, respectively, while HRESI MS afforded the molecular formulas C₁₅H₁₈O₃ and C₁₅H₂₂O₃. In previous investigations, compounds 1 and 2 showed antifungal activity against *Candida albicans*, *Candida glabrata*, *Aspergillus fumigatus*, and antileishmanial activity against *Leishmania infantum* and *Leishmania mexicana* [3]. Additionally, we have found an interesting high zoosporicidal activity at a very low concentration of isolated flavone 1 against Oomycetes.



1



2

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PRODUCTIVITY AND PHYTOCHEMICAL STUDY OF *ELEAGNUS ORIENTALIS* L. FRUITS INTRODUCED IN MOSCOW REGION

E.A. Abizov¹, S.P. Zavadsky¹, O.N. Tolkachev², S.D. Maltsev³

¹Moscow Sechenov Medical Academy, Moscow, Russia

²All-Russian Research Institute of Medical and Aromatical Plants, Moscow, Russia

³Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Moscow, Russia

The purpose of the study was to evaluate the productivity and chemical composition of *Eleagnus orientalis* L. fruits introduced in Central part of Russia.

Materials and methods: Productivity study was estimated on 10 samples of *E. orientalis* L. introduced in 1995 in Moscow region from seeds obtained from the Institute of Botany National Academy of Sciences (Armenia). Fruit were collected and drying for raw material. Actual fruit productivity was determined by calculation and weighing of ripe fruits collected from every plant. For phytochemical study fractional extraction method and common qualitative reactions were used. Individual components were identified using TLC, IR and NMR-spectroscopy.

Fruit formation for *E. orientalis* L. in Moscow region were observed for the 7-8 years plants. The 7-th year plants produced 2.5 kg per plant and 11-th year plants yield 7.2 kg each. Reducing sugars content in fruits 50.67-55.75%, total sugar (sucrose) – 60.0±5.0%, total content of soluble and non-soluble pectines – 3.6±0.3%. Fruits contains flavonoids rutin, quercetin, isorhamnetin, eleagnoside, kempferol and caffeic acid. Fruit flavonoids and phenolcarboxylic acids contents were 1.35±0.15%. Two hydroxycoumarins and p-cumaric acid also were found. Steroid saponins content (1.96±0.52%) were determined in fruits according to Fontan-Candela method. Aminoacids: Asp, Thr, Ser, Glu, Pro, Gly, Ala, Val, Met, Ileu, Tyr, Phe, His, Lys, Arg, Try, CysH, cysteinic acid were identified in fruits pericarpium. Raw material contains vitamins ascorbic acid (5.6 mg%), *b* -carotene (17.5 mg%), vitamins E and K. Tannin content (catechines and tannins) in fruits were 5.03±0.05%. *b* -Carboline alkaloids and fatty oils also were identified.

After obtained results we can conclude the prospects to use this plant material for preparation of medicinal drugs and food stuffs.

ESSENTIAL OILS YIELD AND HEAVY METALS CONTENT OF SOME AROMATIC MEDICINAL PLANT GROWN IN ASH-SHOUBAK REGION-SOUTH OF JORDAN

Mohammad Sanad Abu-darwish

Ash-Shoubak University College, Al-Balqa Applied University, Al Salt 19117, Jordan

The use of aromatic medicinal herbs to relieve and treat many human diseases is increasing in Jordan and worldwide due to their mild features and low side effects [1]. It is important to have a good quality control for aromatic medicinal herbs in order to protect consumers from contamination [2]. The aim of the present study was to carry out a comparative evaluation of essential oils yield and heavy metals content in some medicinal herbs such as *Thymus vulgaris* L., *Thymus serpyllum* L. and *Salvia officinalis* L. grown in Ash-Shoubak region-south of Jordan. The essential oils were hydro-distilled from studied dried aerial herbs using Clevenger- type system. The heavy metals content in collected herbal samples were analyzed by using atomic absorption flame emission spectrometry by method described by Al-Alawi & mandiwana. Analytical results have evaluated by statistical analysis system (SAS, 2006). The mean values of essential oils yields of *T.vulgaris*, *T. serpyllum*, and *S. officinalis* were 4.0, 2.5 and 0.9%, respectively. The contents of heavy metals in the samples were determined in the ranges of 1.26-32.03, 0.47-23.85, 7.66-13.23, 14.7-44.0, 15.8-114.91, 141.3-756.17 ppm for Pb, Ni, Cu, Mn, Zn, Fe, respectively. Cobalt has been detected only in *T. serpyllum*, while Cd and Cr were not detectable in all studied samples. The highest Pb, Ni and Cu content has been detected in *T. vulgaris* (32.03 ppm, 23.85 ppm, and 13.23 ppm, respectively). *S. officinalis* had. The highest Mn, Zn, and Fe content 44.0 ppm, 114.91 ppm and 756.17 ppm, respectively. The essential oils and heavy metals contents in studied plants are affected by environmental conditions. Moreover, the obtained results showed that the studied plants grown in Ash-shoubak region (with respect to the lead content in *T. vulgaris*) can safely be used for pharmaceutical and ethno-pharmacological purposes without any hazardous effect.

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SURGICAL TREATMENT OF METASTATIC SPINAL TUMORS AND OPTIMISATION OF EARLY POSTOPERATIVE PERIOD WITH THE HELP OF FASTACTING ADAPTOGENS "VITAVIS"

A. Antonov, U. Antonov, A. Osipov, A. Grechko, V. Hailenko, M. Tcimbal

State clinical hospital №81, Moscow, Russia

Army Medical College, St. Petersburg, Russia

Oncology advanced training department of Russia State Medical University, Moscow, Russia

The aim of our study was to evaluate effectiveness of decompressive stabilizing operations of metastatic affect of vertebral column.

The patients were treated with standard schemes of wave- or chemotherapy (cytostatic-, hormone-therapy), monotherapy or wave-therapy combined with citone therapy (Metasatron or Stroncium chloride Sr-89), that reduce pain and side effects of wave-therapy. Complex of fast-acting adaptogens-immune modulators "VITAVIS" was administered 3-5 days before and 15-45 days after the surgery and during further rehabilitation period in 47 patients, who underwent pediculo-corporal osteosynthesis of vertebrae with spondylectomy as a second step, corporectomy with autoplasmic with huckle-bone. To stabilize anterior surface of spinal column auto transplants and endoprotheses of vertebral body "Arete" made of titan were used. In all patients after radical or palliative surgery "VITAVIS" showed good protective rehabilitation characteristics.

47 patients with verified diagnosis of lung, kidney, large intestine and mammary cancer with metastases to cervical, thoracic, lumbar and sacral spine affecting 1-2-3 adjacent vertebrae underwent combined therapeutic and surgical treatment. After the surgery regress or neurological symptoms and pain reduction were observed. The use of the complex of fast-acting adaptogens-immune modulators "VITAVIS" allowed to optimize postoperative dynamic of biochemical and immune rehabilitation, reduce postoperative complications, quicken wound healing, engraftment of auto transplants and endoprotheses, reduce postoperative period. A median of postoperative life span corresponded with clinical prognosis of 3-5 years.

In the study effectiveness of combined treatment of metastatic bone tumors with pediculo-corporal osteosynthesis in combination with wave-, chemotherapy and adaptive pharmacological immunocorrection of acute period of surgical trauma was confirmed.

MODERN OPPORTUNITIES OF SURGICAL TREATMENT OF TUMOROUS OBTURATIVE INTESTINAL OBSTRUCTION IN COMBINATION WITH FASTACTING ADAPTOGENS "VITAVIS"

A. Antonov, V. Hailenko, E. Malishev

State clinical hospital №81, Moscow, Russia
Oncology advanced training department of Russia State Medical University, Moscow,
Russia

Aim of the study: comparison and analysis of various surgical techniques of treatment of obturative intestinal obstruction, possible approaches of single-stage surgery.

69 patients with tumorous obturative intestinal obstruction with tumor localization in the left part of colon underwent surgery in 2005-2007. Among them 18 had primary anastomosis (1st group), 51 underwent Hartman left-side hemicolectomy (2nd group). Average patients' age in the 1st group was 61±5, 62±5 in the second. M: F ratio in the 1st group was 1:3.9, 1:3.5 in the 2nd. Disease duration in the 1st group was 26±6 hours, 25±6 hours in the 2nd, time spent at hospital before surgery 12±4 hours in both groups. Duration of postoperative observation - 30 days. During surgery microcirculation level in the intestine wall before and after decompression and basal blood flow of intestine wall of two ends of resected intestine before and after its treatment with chlorhexidine 0.02% solution were measured, using LDF (Laser Doppler Flowmetry) technique carried out using the device LAKK 02. Sterile conductor of LAKK 02 was applied to intestine wall above the obstruction area for 3 min before and after its elimination. During operation prophylaxis of secondary infectious complications and of thromboembolic disorders was carried out.

After intraoperative gut lavage an improvement of microcirculation was observed, as shown by increased blood flow due to activation of endothelial secretion. It proves that disturbance of blood supply of colon wall by obstruction is reversible and is restored rather quickly after decompression. Before and after surgery all patients were treated with fast-acting adaptogens-immune modulator "VITAVIS". Complications: postoperative wound infection – 2 patients in the 1st group, 3 in the 2nd.

Left hemicolectomy is preferable if tumor is located in the left part of colon. Intraoperative colon decompression provides fast regional blood flow restoration. That allows fact to form primary anastomosis. Adaptogens-immune modulators "VITAVIS" optimize early postoperative period and improve life quality.

CLINICAL EXPERIENCE OF USAGE OF RUSSIAN BONE CEMENT "POLACRIS" IN SURGICAL TREATMENT OF BONE TUMORS WITH ADAPTOGEN ADMINISTRATION

A. Antonov, U. Antonov, A. Osipov, V. Hailenko, V. Parkhalin

State clinical hospital №81, Moscow, Russia
Oncology advanced training department of Russia State Medical University, Moscow, Russia

The aim of our study was to evaluate effectiveness of Russian bone cement "POLACRIS" in patients, who underwent conservative surgery of bone, joint and vertebral tumors.

Our study involved 127 patients who underwent surgery. Among them: 23 with primary tumors, 104 with bone metastases. Preoperatively we carried out laboratory tests, MRT, CT, US and X-ray investigations of the tumor. Intraoperatively we made express biopsy, excision of regional lymph nodes, preparation of spinal canal with iodine essence within 3-4 min before cement insertion. The final diagnosis was confirmed histologically. Patients underwent unadjuvant, adjuvant, wave and systemic wave therapy with Stroncium-89 (Sr-89). We administered complex of fast-acting adaptogens-immune modulators "VITAVIS" 2-3 days before surgery and in early postoperative period. We evaluated postoperative dynamic: 1) During first 2 weeks – blood analyses, x-ray evidences, general condition; 2) During 3rd - 5th weeks – clinical and laboratory investigations, x-ray control; 3) During first year – patient's complaints, clinical and laboratory investigations, dynamic of x-ray data; 4) Life quality was evaluated with Karpovsky scale.

We revealed no symptoms of allergy, rejection, fistulas, slowed wound healing, no deterioration of blood test data. X-ray showed absence of bone destruction in the region of interfacial contact of bone tissue with cement, absence of unrolling of prosthesis legs of intermedullar rods.

We confirmed high effectiveness of plastic material "POLACRIS", its combination with metal polymer endoprosthesis in primary and metastatic bone tumors, its combination with wave-, chemo-, hormone-therapy and adaptive pharmacological immunocorrection in acute period of surgical trauma. The simplicity of "POLACRIS" usage, storage and its cheapness allow us to recommend it in orthopedical surgery and oncology in particular.

COMPARATIVE ESTIMATION OF CYTOTOXIC ACTIONS OF MYCELIUM WATER EXTRACTS OF HYPHOSYGIUM ULMARIS BASIDIOMYCETE OBTAINED BY LABORATORY AND INDUSTRIAL METHODS

*E.S. Bekchanova*¹, *I.A. Rodina*¹, *T.V. Shevchenko*¹, *L.M. Krasnopolskaya*²,
*V.P. Tikhonov*¹

¹DIOD Company, JSC Plant of Ecological Equipment and Ecological Nutrition, Moscow, Russia

²F.G. Gauze Institute of New Antibiotics Research (State Institution) of Russian Medical Science Academy, Moscow, Russia

Basidiomycetes contain some fractions of water-soluble polysaccharide and other metabolites that have cytostatic actions on tumour cells and stimulate the immune system. These effects were described for a number of Basidiomycete species that have already been used for creation of such preparations as Lentinan, LEM [Meloxicam], Chrestin, Ganopoly, Immuno-Assist, etc.

The purpose of this study is a comparative estimation of the cytotoxic action of mycelium water extracts of *Hypsosygius Ulmaris* basidiomycete obtained by laboratory and industrial methods.

The culture of human uterine cervix carcinoma cells HeLa was used. The cells were incubating with basidiomycete mycelium extracts in concentrations: 9; 4.5; 2.25; 1.12; 0.56 and 0.28 mg/ml during 1, 2, 3 and 7 days. After the cell viability was assessed on the base of the cell respiratory system activity, using tetrazolium/formazan (MTT-assay). 5-fluorouracil (5-FU) in the 15 mkg/ml doze was used as the positive control. 5-FU is an antitumor drug used for uterine cervix carcinoma treatment. HeLa cells cultivated in a preparation-free medium were the control group. The water extracts were prepared from dry ground mycelium of *Hypsosygius Ulmaris* basidiomycete, produced by the submerged culture cultivation method in laboratory and test industrial conditions.

The cytotoxic effect depended on the substance concentration and the time of cell incubation with preparations, was observed in HeLa cell culture. The cell incubation at the maximal concentration of basidiomycete mycelium extracts (9 mg/ml) within 1-3 days exerted the cytotoxic effect comparable with 5-FU: 50% on the 3rd day of incubation with basidiomycete mycelium extracts, 40% – with 5-FU. The fluorouracil cytotoxicity was higher on long incubation times (7 days): 74% and 84% respectively. The cytotoxic action did not exceed 50% over the observation time at lower than 9 mg/ml *Hypsosygius Ulmaris* water extract concentrations while 5-FU caused the loss of 84% of the cells on the 7th incubation day. The biological activity of *Hypsosygius Ulmaris* basidiomycete mycelium extract produced in industrial conditions was similar to laboratory.

STUDY OF ANTIPROLIFERATIVE ACTIVITY OF BASIDIOMYCETE MYCELIUM EXTRACTS BY THE INTRAVITAL COMPUTER MORPHOMETRY METHOD ON PLANARIANS

E.Ju. Belyankina, I.A. Rodina, T.V. Shevchenko, V.P. Tikhonov

DIOD Company, JSC Plant of Ecological Equipment and Ecological Nutrition, Moscow, Russia

The purpose is the study the antiproliferative activity of mycelium extracts of basidiomycetes: *Ganoderma lucidum*, *Hypsisygyus ulmoris*, *Trametes versicolor* (1:1:1) by the intravital computer morphometry method using invertebrate animals - planarian worms.

Regeneration of the head end of a body of asexual planarians *Dugesia* (*Girardia*) *tigrina* (line worms) was used as a biological model. After decapitation the worms were placed in the experimental solutions with basidiomycete mycelium extracts (BME) of the concentrations 0.5; 1.0; 2.0; and 4.0 mg/ml. The regeneration of planarians was registered by a videocamera 72 hours after the decapitation. The morphological parameters of the regenerating planarians were measured by the specialized software (Plana 4.1) and the regeneration criterion was calculated (the ratio of the growing head end (blastema) to the total body square). Cyclophosphamide ($0.28 \cdot 10^{-6}$ mg/ml) was used as the positive control because of its cytostatic activity. The passive control group was regenerating in water planarians. It has been shown that cyclophosphamide ($0.28 \cdot 10^{-6}$ mg/ml) inhibited the regeneration of planarians in $35.7 \pm 2.3\%$ in comparison with the passive control group. The similar concentration dependent effect was observed for the basidiomycete mixture extract: inhibition of the planarians' head part regeneration was 16.7 ± 1.8 - $47.2 \pm 3.6\%$ at the concentration of 0.5-4.0 mg/ml.

Basidiomycete mycelium extracts showed the antiproliferative effect on the invertebrate animal regeneration model.

STUDY OF ANTI-ARRHYTHMIC ACTION OF NATURAL SUBSTANCES IN MODEL OF ARRHYTHMIA ON CHICKEN EMBRYOS

E.Ju. Belyankina, I.A. Rodina, T.V. Shevchenko, V.P. Tikhonov

DIOD Company, JSC Plant of Ecological Equipment and Ecological Nutrition, Moscow, Russia

The study of new preparations of anti-arrhythmic action on laboratory animals are labour-consuming and expensive. Therefore a new model for heart rhythm disorder have been developed for screening of active substances.

The purpose of present work was development of the arrhythmia model on chicken embryos that arises at cardiac glycoside poisoning, and screening natural substances which have potential anti-arrhythmic actions.

A 10-day chicken embryos were used as biological model.

Ultrasonic dopplerography, based on the Doppler Effect ("Minimax-Doppler-K" device made by MINIMAX Company) was applied. The cardiac rate (on chorioallantoic membrane vessels) and death of embryos were observed.

Arrhythmia was modeled by application of "Strophantinum K" preparation on chorioallantoic membrane vessels. After Strophantinum K administration both the stable arrhythmia formation with its subsequent fading, and death of embryos, starting with the 10th minute of the experiment were observed. Arrhythmia was observed in 82% of embryos on the 10th minute of the experiment and 10% of embryos were dead. In the end of experiment arrhythmia was observed in 45% of embryos and 46% were dead. The preventive administration of Unithiol (a universal antidote) 10 minutes before the experiment resulted in the decrease of arrhythmia to 6% on the 10th minute of the experiment and to 34% at the end; and prevented death of embryos during the whole experiment. A hawthorn extract (6.2 mg/ml) and extract of leaves of red grape (4.3 mg/ml) – reduced the Strophantinum K effect in 20-30% approximately.

The method developed allows the screening of anti-arrhythmic properties of natural substances. The advantages of the proposed test are: express results, effectiveness, and compliance with modern ethical requirements.

RESVERATROL: RED WINE'S MAGIC ANTI-AGEING COMPOUND?

Wolfgang Blaschek

Pharmaceutical Institute, Department of Pharmaceutical Biology Christian-Albrechts-University of Kiel, Gutenbergstr. 76, D-24118 Kiel, Germany

The "French Paradox" was the observation that mortality from cardiovascular disease is relatively low in France despite high levels of dietary saturated fat and cigarette smoking. Regular consumption of polyphenol containing red wine soon was thought to provide health protection. One of these polyphenols, namely resveratrol, became an important candidate for various beneficial health effects.

Resveratrol (3,5,4'-trihydroxystilbene) is synthesized by some plants in response to stress like injury, drought, heat, fungal infection, and UV-radiation. It is found in grapes (the skin only) and some other plants. The amount of resveratrol in grape skins varies with the grape cultivar and its cultivation area; in wine it depends on the contact time of grape skins during fermentation (more resveratrol in red wine).

Resveratrol at least in vitro inhibits the oxidation of low density lipoprotein (LDL). Cardiovascular disease prevention is discussed to be caused in addition by the inhibition of inflammation, promotion of vasodilation by enhanced production of NO and inhibition of platelet aggregation. Caloric restriction is known to extend lifespan in many species including mammals, (but this is an option only for few people). Resveratrol could be shown to increase life time in yeast, a worm, a vertebrate fish and in mice. These effects are thought to be caused via histone deacetylases (Sirtuins) thereby influencing the activity of distinct gene regions resulting e.g. in an improved DNA-repair. Resveratrol also has been found to inhibit in vitro the proliferation of various cancer cell lines. Concerning cancer prevention in vivo various mechanisms of action are proposed, reaching again from anti-inflammatory effects via induction of apoptosis to inhibition of angiogenesis.

Although resveratrol after oral administration is well absorbed by humans, its bioavailability is very low due to its rapid metabolism. Most studies concerning beneficial effects of resveratrol have been performed at concentrations that are substantially higher than peak concentrations detectable in human plasma.

Currently there still is limited convincing evidence that resveratrol has pronounced beneficial health effects in humans.

PHYTOMIX-40 EFFICACY FOR PARKINSON DISEASE

***E. Bocharov*¹, *V. Kucheryanu*¹, *V. Poleshuk*³, *I. Ivanova-Smolenskaya*³,
*G. Kryzhanovsky*¹, *O. Bocharova*²**

¹Institute of General Pathology and Pathophysiology RAMS, Moscow, Russia

²N.N. Blokhin Cancer Research Center RAMS, Moscow, Russia

³Institute of Neurology RAMS, Moscow, Russia

Parkinson's disease (PD) is the result of the damage of dopaminergic neurons thus reducing the dopamine (DA) level in the striatum. Phytomix-40 (Phm-40) contains the components of forty plants (e.g. ginsenosides, eleutherosides, flavonoids). It is known that ginsenosides possess neurotrophic and neuroprotective properties. The aim of this study was to evaluate the effect of Phm-40 on the DA level and its metabolites, on malon dialdehyde (MDA) production, locomotor activity, rigidity, and caspase-3 activity in the striatum of C57BL/6-mice with experimental PD as well as on the immune and antioxidant status and cortisol and cytokine levels in peripheral blood serum together with clinical symptoms using the Unified Parkinson's Disease Rating Scale (UPDRS) in 30 PD patients (based on standard treatment including L-DOPA-preparations) compared to age-matched control subjects. PD in old mice was induced by the neurotoxin MPTP. Muscle rigidity and motor activity was measured in the automated test system Opto-Varimex-3. PhM-40 was administered to mice per os. Levels of DA and its metabolites were analyzed in the striatum ex vivo by HPLC/ED. Caspase-3 activity was assayed in the substantia nigra by the extrication rate of Ac-DEVD-p-nitroanilide. Content of cortisol, superoxide dismutase, catalase, glutathione and glutathione-S-transferase in the blood serum as well as production of cytokines by lymphocytes was examined by immuno-enzyme methods. Phm-40 administration resulted in an increase of animals' motor activity ($p < 0,05$), reduced rigidity ($p < 0,05$) and caspase-3 activity ($p < 0,05$), prevention of MDA formation ($p < 0,05$) and increase of the levels of DA ($p < 0,05$) and its metabolites ($p < 0,01$) in the striatum. Three months after the start of administration of Phm-40 an improvement of clinical symptoms according to UPDRS was observed. Lymphocyte production of IL-6 ($p < 0,05$) and TNF- α ($p < 0,05$) was down-, that of IFN- γ ($p < 0,001$) was up-regulated. The data demonstrate the beneficial effect of including preparations with neuroprotective activity in the treatment of PD patients.

CHEMICAL ANALYSIS OF PHYTOMIX-40 BY NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

V. Sheichenko¹, O. Bocharova², O. Sheichenko¹, E. Uytova¹, E. Bocharov², R. Karpova², I. Kazeev²

¹All-Russian Research Institute of Medicinal and Aromatic Plants, Moscow, Russia

²N.N. Blokhin Cancer Research Centre RAMS, Moscow, Russia

Phytomix-40 (PhM-40) is a biologically standardized original phytoadaptogen-parapharmaceutical demonstrating immunomodulatory, hormonomodulatory, antioxidant, radio-protective, antimutagenic and anti-tumour activities. Physicochemical research is needed for methodological basis creation, production and application of this formulation. The purpose of the work was to develop a nuclear magnetic resonance (NMR) method for the analysis of the multicomponent plant preparation as the basis for chemical standardization. The NMR spectra were measured on Gemini spectrometers (200 and 400 MHz). The type of solvents, conditions for the sample preparation and the parameters for measuring the spectra were determined. ¹H NMR spectra and two-dimensional COSY spectra were obtained for each of the 40 water-alcohol plant extracts as well as the spectra of PhM-40 ethyl acetate and butanol extracts, containing relatively small quantity of carbohydrates. Carbohydrates prevail in Phytomix-40 and in all the plant extracts studied. The major carbohydrate occurred to be glucose possessing two characteristic doublets in the NMR spectra. Terpenoids, flavonoids glycosides, tannins and other aromatic compounds are found in considerably smaller amounts. Intensive signals (two doublets and one singlet) in the low field belong to arbutin. Besides arbutin and glucose, compounds such as hydroquinone, sucrose, ginsenosides (aglycones – panaxadiol and panaxatriol) and aralosides A, B, C – derivatives of oleanolic acid were identified in PhM-40 using NMR spectroscopy. Data obtained demonstrated that NMR spectra are effective for studying the chemical composition of complex plant preparations. The specific and well separated cross-peaks observed in the spectra of the plant extracts which are also observed in the spectra of PhM-40 indicate that two-dimensional spectra can be used for identification and the authenticity proof of PhM-40 and its plant components.

PHYTOADAPTOGENS COMPLEX IS EFFECTIVE FOR FAR-ADVANCED GASTRIC CANCER PATIENTS

A. Klimenkov, O. Bocharova, R. Karpova, S. Chulkova, E. Bocharov, V. Ilyenko

N.N. Blokhin Cancer Research Center RAMS, Moscow, Russia

Phytomix-40 (PhM-40) consists of forty water-ethanol plant extracts including adaptogens (Panax ginseng; Rhodiola r. L. etc.). Animal models have proved its low toxicity, anti-metastatic, immunomodulatory and antioxidant properties. In this study a comparative clinical analysis was performed to evaluate efficacy of PhM-40 administration to patients with far-advanced gastric cancer under different schemes of treatment. The patients with gastric adenocarcinoma were given palliative surgery + etopozid, leucovorin, 5-fluorouracil (ELF) polychemotherapy (1-st group, n=19) or surgery (3-d group, n=16) supplemented with PhM-40 administration (including 7 days before surgery). The control groups patients were given operation + ELF polychemotherapy (2-nd group, n=20) or only operation (4-th group, n=27). PhM-40 was taken orally. Following parameters were measured: immune cell subpopulations; serum carbohydrate antigen (CA 19-9), carcinoembryonic antigen (CEA), cortisol, malondialdehyde (MDA) levels; the status of the serum antioxidants superoxide dismutase (SOD), catalase (CAT), glutation and glutation-S-transferase (G-S-T). With progress of gastric cancer percentage of lymphoid cells expressing markers for T-cells (CD3+), T-helpers (CD4+), B-cells (CD20+), monocytes and natural killers (CD11b+ and CD16+) were decreased. Serum CEA, CA 19-9 and cortisol levels were increased. The results of our study also have shown higher MDA production, as well as increased glutathione level and SOD activity. PhM-40 administration resulted in normalization of immunity and antioxidant profiles. PhM-40 also reduced carbohydrate antigen 19-9 ($p < 0.05$), MDA ($p < 0.05$), cortisol serum content ($p < 0.05$) down to normal levels. The life span was increased in 1 (14.3 months, $p < 0.001$) and 3 (15.1 months, $p < 0.001$) groups. No obvious effect was observed in 2nd and 4th groups (5.4 and 6.3 months respectively). The results suggest that administration of multiadaptogen included into the complex of anticancer treatment to far-advanced gastric cancer patients is effective.

PHYTOADAPTOGENS - GEROPROTECTORS AND ONCOLOGY

*O. Bocharova*¹, *M. Davydov*¹, *A. Baryshnikov*¹, *V. Tutelian*²

¹N.N. Blokhin Cancer Research Center RAMS, Moscow, Russia

²Nutrition Institute RAMS, Moscow, Russia

The incidence of cancer increases with age in humans. Phytoadaptogens (PA) *Panax g.*, *Rhodiola r.*, *Eleutherococcus s. M.* etc. have long been used in the East medicine as general tonics or adaptogens to promote longevity. PA are biological response modifiers due to their ability to increase resistance to physical, chemical and biological stressors. PA show antimutagen, antioxidant, radioprotective, hormonomodulating properties (including sexual function normalization); improve the heart work regulating arterial pressure; show antidepressant and central nervous system activities due to their ability to regulate neurohormones levels (dopamine, endorphins); modulate the immune system activities improving IFN- α and IFN- γ production. PA are considered to be inducers of tissue differentiation because of the normalizing intercellular adhesion interaction that can be resulted in repression of tumour cells proliferation. The anti-tumour activity of PA is also associated with induction of cancer cells apoptosis because of intercellular adhesive molecules (ICAM) expression regulation. PA can enhance in tumours the expression of adhesive ligands (ICAM-1) for immune effectors which can kill cancer cells reducing the immune escape mechanism for tumours. PA can suppress vessels formation in tumours ought to the inhibiting the expression of angiogenesis factors (VEGF, bFGF). "Occupying" the multidrug resistance molecules in cancer cells PA make it possible for cytotoxic drugs not to be transported out of the cells. So, PA can increase the efficacy of chemotherapy reducing its side effects. The combination of the PA and specific anti-tumour therapy improves quality of life and increases survival time of patients. The efficacy of PA is due to the similarity of their bioactive components structure with steroids. However the patients quickly become resistant to monopreparations of PA and thus it is necessary to change standardized preparations for the success in the therapy. These problems call for a new approach. Mixture of forty medical herbs (including some PA) bioactive components certificated as parapharmaceutic Phytomix-40 was researched to be effective for cancer and age-related diseases patients who did not get resistance during its administration.

IMMUNOBIOLOGICAL AND CLINICAL EFFICACY OF ADAPTOGEN PHYTOCOMPLEX FOR HUMAN ORAL LEUKOPLAKIA

*M. Pozharitskaya*², *O. Bocharova*¹, *T. Chekalina*², *M. Lyzhenkova*¹

¹N.N. Blokhin Cancer Research Center RAMS, Moscow, Russia

²Moscow State Medico-Stomatological University, Moscow, Russia

Phytomix-40 (PhM-40) includes the components of forty medical herbs: adaptogenes, anti-inflammatory, sedative, stimulating urination and biliation plants and also some berries (source of vitamins). Oral leukoplakia (OL) is classified as precancer. The conventional treatment of OL is therapy with vitamin A (VA) preparations. In this study comparative clinical and immunobiological effects of PhM-40 (28 OL patients and 20 volunteers without OL) and VA (26 OL patients) were measured. The immune, antioxidant, interferon profiles, cortisol level in peripheral blood serum, expression of differentiation antigens in keratinocytes of oral mucosa in premalignant lesions were evaluated. PhM-40 administration improved IFN status parameters: the elevation of IFN- α ($p=0.01$) and IFN- γ ($p=0.01$) levels to normal values, increase in the sensitivity of patients to IFN- α , IFN- γ preparations. PhM-40 resulted in a significant improvement of the immunity. PhM-40 reduced cortisol blood serum content down to normal ($p=0.002$) in both groups. No alteration was observed after VA treatment. Raised keratin 17 (the marker of the basal keratinocytes) and decreased Fas APO1 antigen expressions were observed in leukoplakia lesions compared to normal mucosa. However, the CD54- and CD29- antigens expression were found to be normal in case of this precancer. Treatment with PhM-40 resulted in enhanced CD54 expression, although no changes were observed after VA treatment. This finding could imply that immune effectors in the leukoplakia lesions are activated by PhM-40. As a result high clinical effect in patients treated with PhM was demonstrated: epithelialization of erosions, reduction of hyperkeratosis size and density. Our study demonstrated that leukoplakia as precancer appeared to have infringements in differentiation program of oral mucosa keratinocytes associated with no specific alterations in immunity. PhM-40 can be used in the treatment of OL. Modulation of keratinocyte differentiation with PhM-40 may be an exiting clinical possibility in the control of malignant transformation of precancers.

PHYTOMIX-40: ADAPTOGEN FOR BENIGN PROSTATIC HYPERPLASIA

V. Matveev, O. Bocharova, R. Karpova, A. Aksenov, A. Goroshanskaya, M. Lyzhenkova, B. Matveev

N.N. Blokhin Cancer Research Center RAMS, Moscow, Russia

Phytomix-40 (PhM-40) was designed as adaptogen or biological response modifier for cancer patients and as anti-aging agent. It is a mixture of components of 40 medical herbs, including phytoadaptogens. We evaluated the clinical efficacy of administration of different doses of PhM-40 in elderly patients (n=79) with benign prostatic hyperplasia (BPH). The immune, hormone, antioxidant, interferon profiles, the prostate-specific antigen (PSA) level, the size of hyperplasia, the International Prostate Symptom Score (IPSS), the frequency of chromosome aberrations in lymphocyte cells were evaluated. Optimum PhM-40 dosage was established in this study (near 45 ml a day). Clinical studies indicated that PhM-40 normalized biological reactivity of patients with BPH. PhM-40 treatment caused a decrease in size of hyperplasia (down to 73%; $p<0.001$) and up-regulated sexual function. The lower urinary tract symptoms improved significantly (IPSS 14→7, $p<0.001$). The immunomodulatory effect of PhM-40 resulted in the normalization of the T-helper/T-suppressor cells ratio ($p=0.001$), in an increase in the number of B-cells ($p=0.001$), macrophages, natural killer cells ($p=0.001$) and lymphoid cells expressing the interleukin-2 receptor ($p=0.001$). The raised percentage of lymphocytes expressing the adhesive molecule ICAM-3 ($p=0.001$) correlated with an up-regulated expression of the apoptosis receptor (CD 95; $p=0.001$). PhM-40 caused a significant increase of testosterone levels ($p<0.001$) accompanied by a decrease of estradiol ($p<0.001$), cortisol, ($p<0.001$) and PSA ($p<0.001$) levels. PhM-40 decreased the frequency of chromosome aberrations in lymphocyte cells ($p<0.05$) with high effectiveness. By the way antiandrogens used in the treatment of BPH and prostate cancer reduce PSA levels, but also suppress the immune and hormone status. PhM-40 causing anti-PSA activity at the same time demonstrated normalization of immunity and the hormone, antioxidant, interferon profiles as well as anti-mutagenic activity, altogether producing antiaging effects. The data are considered to provide a basis for further investigations on PhM-40 to be included in prostate cancer treatment for improvement of efficacy.

MICROPROPAGATION OF *ORIGANUM SIPYLEUM* L., ENDEMIC AND MEDICINAL PLANT OF TURKEY

Esin Akçam Oluk, Ali Çakir

Ege University Science Faculty Biology Department 35100 Bornova-Izmir, Turkey

Origanum sipyleum L. (Lamiaceae) is an endemic to the Western Anatolia. Tissue culture studies were not encountered; however, few reports were available on chemical content of this species [1]. In the present study, rapid micropropagation protocol was aimed for this medicinally important plant. Seasonally field collected seeds were germinated on water-agar (96%). Germinated seedlings were transferred into Murashige-Skoog (MS) [2] or modified MS (MSM) for better growth. MSM was containing increased CaCl₂ (550 mg/L) according to our previous work [3]. Micropropagation through apical tips of the aseptic seedlings grown on MSM was achieved in Murashige-Skoog (MS) medium containing 1 mg/L benzylaminopurin (BAP) within 2 months. This type of cytokinin and amount of was decided from the literature concerning several tissue culture studies of other species of *Origanum* [4]. Rooting was examined in either 0.5 mg/L or 1 mg/L indole-3-butyric acid (IBA) added MS. After 3 weeks, numerous adventitious roots were observed in the presence of 0.5 mg/L IBA, whereas 1 mg/L IBA resulted poor rooting (in 12% of plantlets). It is observed that, 0.5 mg/L IBA increased shoot vigor and number, besides rhizogenesis. All the experiments were performed in 100 ml erlenmeyer flasks containing 20 ml of medium. Rooted plantlets were acclimatized successively (98%) *ex vitro* in the pots covered and containing a peat:sand:perlite (1:1:1; v/v) mixture. They adapted into outdoor conditions with 76% survival rate. These *in vitro* derived microplants are already under the evaluation for their essential oil composition, in our laboratory.

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SAM SO EUM AS THE REMEDY FOR ASTHMA IN MICE

Hyung Woo Kim, Su In Cho

Division of Pharmacology, School of Oriental medicine, Pusan National University, Republic of Korea

In the theory of Oriental medicine, Sam So Eum (SSE), which contains 12 species of herbal plants, expels wind-cold, discharges phlegm and remedies coughs. For these reasons, SSE has been widely used for several symptoms that are induced by wind-cold, such as headache, fever, cough and rhinorrhea. In recent years, it is known that SSE prevents pulmonary damage from ozone, and SSE is also known as an anti-allergic agent. We studied Sam So Eum (SSE) application as the herbal drug to treat asthma in mice and discussed the mechanism of restoring the immuno-modulating cytokines such as IL-10 and IFN- γ . In this experiment, we investigated the effects of SSE on airway hyperresponsiveness to methacholine, cytokine levels in BALF and antigen specific antibody in serum. Prednisolone (PD) was used as positive control. According to obtained results, the mice treated with SSE did not show any significant variation in their body weight and they looked very similar to the controlled ones. The SSE-treated mice showed reduced levels of airway responsiveness to methacholine (fig. 1), and these levels were initially elevated by the induction of asthma compared to the control group. The SSE elevated production level of IFN- γ (fig. 2), which was down-regulated upon induction of asthma.

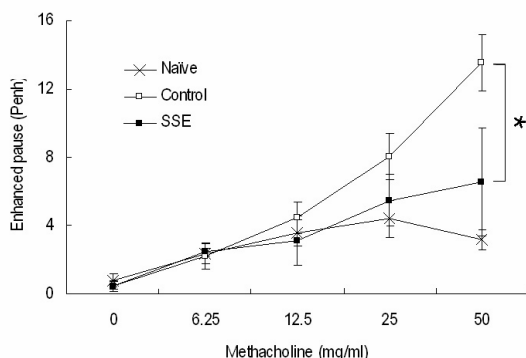


Figure 1. Effects of SSE on airway hyperresponsiveness to methacholine in asthmatic mice

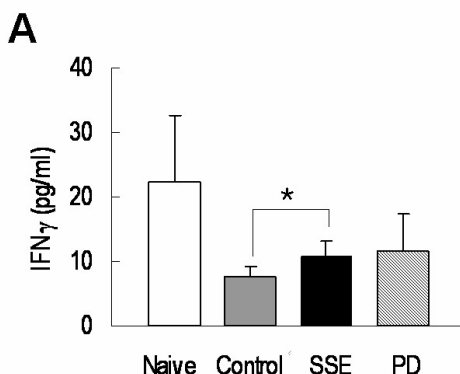


Figure 2. Effects of SSE on production levels of IFN- γ in BALF

This result implies that SSE can change the Th1/Th2 ratio through Th1-skewing reactions, and that SSE can decrease airway hyper responsiveness by changing the Th1/Th2 ratio. The treatment with SSE also restored the IL-10 level to that of the naive condition (fig. 3).

B

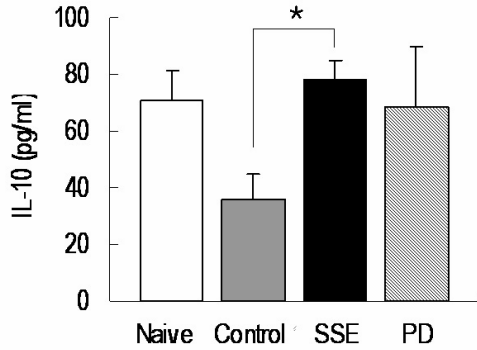


Figure 3. Effects of SSE on production levels of IL-10 in BALF

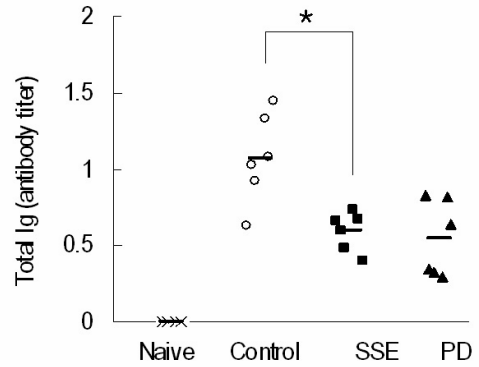


Figure 4. Effects of SSE on levels of OVA-specific total antibody in serum

This means that SSE reduced the airway inflammation through this pathway. The ovalbumin (OVA)-specific antibody (total Ig) production in the serum was also decreased upon SSE treatment (fig. 4). The effectivity of SSE was almost the same as that of PD. As a conclusion, obtained results suggest the possibility of using SSE for the treatment of patients with asthma, and its therapeutic efficacy involves restoring the IFN- γ and IL-10 levels.

THE EFFECT OF DIFFERENT TEMPERATURES AND LIGHT INTENSITIES ON THE GROWTH AND ALTERATIONS IN PROTEIN, TOTAL CARBOHYDRATE, EXOPOLYSACCHARIDE AND PHYCOBILIPROTEIN CONTENT OF *PORPHYRIDIVM CRUENTUM*

Sevde Hatipoglu, Meltem Conk Dalay

Ege University Engineering Faculty, 35100, Bornova, Izmir, Turkey

Porphyridium cruentum (Rhodophyta) - has an increasing interest because of its ability to produce metabolites such as phycobiliproteins, sulfated exopolysaccharides and polyunsaturated fatty acids like arachidonic acid (AA) and eicosapentaenoic acid (EPA). The valuable chemicals that produced by *P. cruentum* are used widely in cosmetics, food industry and pharmaceuticals.

The exopolysaccharide produced by *P. cruentum* cells has antiviral activity against some of the *Herpes simplex* strains (HSV-1 and HSV-2) and *Varicella zoster* virus and also have commercial usage in different industrial areas as gelling and thickening agents, stabilizers and emulsifiers.

The polyunsaturated fatty acid content of these cells allows them to be used as pharmaceuticals; EPA has protective effects against some heart diseases, inflammation and some of the carcinomas while AA is one of the main components of mother's milk and it is the precursor molecule in prostaglandin biosynthesis. Arachidonic acid is also valuable for being one of the important ingredients in artificial baby food formulations.

Phycobiliproteins are used as colorants in food industries in food and cosmetics in Japan, Thailand and China. It was shown that *P. cruentum* has immunomodulating and anticancer activity.

This study has been done to investigate the growth and biochemical composition change of *P. cruentum* under different culture conditions.

The algae *P. cruentum* (Ege Macc-9) provided from Ege Macc (Ege University Microalgae Culture Collection) and cultured in 2 L bottles at different temperature conditions as 16°C, 20°C ve 25°C and different light intensities as 3000 lux ve 5000 lux, in f/2 medium. Analyses were carried out on protein, total carbohydrate, exopolysaccharide, and phycobiliprotein content (R-phycoerythrin, allophycocyanin and B-phycoerythrin) of biomass grown in these conditions.

The maximum specific growth rate was calculated as 0.111 day⁻¹ for the culture grown under 5000 lux irradiance at 25°C. The maximum specific protein and total carbohydrate production rates were found to be 0.003 mg/L day and 0.175 mg/L day for the cultures under 5000 lux irradiance at 16°C and 5000 lux irradiance at 20°C, respectively. The maximum specific exopolysaccharide production rate was determined as 0.144 mg/L day, for the culture under 3000 lux irradiance at 25°C.

The results for the three major phycobiliprotein contents (R-phycoerythrin, allophycocyanin and B-phycoerythrin) of the *P. cruentum* cells were respectively as 0.161 mg/L day (16°C, 5000 lux); 0,1885 mg/L day (20°C, 5000 lux) and 0.111 mg/L day (16°C, 5000 lux).

It was concluded that light intensifies and temperatures significantly effect the growth and biochemical composition.

SPIRULINA; AS A NUTRITIVE SUPPLEMENT FOR HEALTH

Meltem Conk Dalay

Ege University Engineering Faculty, 35100, Bornova, Izmir, Turkey

Spirulina is a microscopic, filamentous prokaryotic organism which has today emerged as a great nutraceutical phenomenon. Worldwide medical research has discovered that *Spirulina*, with good quality proteins, fatty acids, antioxidant vitamins and minerals in its content, has helped to combat many health problems like diabetes, arthritis, allergy, cancer and so forth. *Spirulina* is able to stimulate variety of immune functions. It also shows antibacterial and antiviral activities. Its usage for human consumption was first reported in 1521. Lake Texcoco, in Mexico City and Lake Chad, in Africa are the alkaline lakes where people harvest and eat *Spirulina* for centuries. Outdoor mass production of *Spirulina* for commercial purpose dates back to the 1970's. Today, there are many big or small companies commercially grow *Spirulina* and its usage is rising throughout the world.

Spirulina, the blue-green algae, has some potent probiotic compounds that enhance health with more than 60% good quality proteins, vitamins, β -carotene, and γ -linolenic acid, and has become a favored material of health care. Therefore, spraydried *Spirulina* was incorporated in various recipes to exploit its nutritional properties and to see its efficacy in terms of glycemic and lipemic responses. *Spirulina*, which contains single cell protein of high biological value, was added to the recipes at 2.5 g level. From the results of the present study, it is clear that the multiple components present in *Spirulina* could have played a role in eliciting relatively lower glycemic and lipemic responses as compared to the corresponding recipes without *Spirulina*. It has been well established that amino acids affect the postprandial glucose concentration. It could be speculated that the addition of 2.5 g of *Spirulina*, which is rich in protein, may bring in the insulin peak earlier in the recipes with *Spirulina* compared to recipes without *Spirulina*. This could be one of the possible mechanisms by which a lowered glycemic response was seen in recipes supplemented with *Spirulina* than in recipes without *Spirulina*. Further *Spirulina* contains γ -linolenic acid and antioxidants, which may modulate the lipid metabolism favorably. It has been reported that low-GI diets bring about a 20% reduction in the TG levels in patients with hypertriglyceridemia [1, 2]. The positive shifts in the glycemic and lipemic responses have been substantiated by clinical trials with long-term *Spirulina* supplementation (2 g/day) for a period of 2 months in diabetics [3]. All these observations confirm the efficacy of *Spirulina* as a hypoglycemic and hypolipidemic agent [4].

In animals, *Spirulina* is able to stimulate a variety of immune functions, including macrophage phagocytosis and production of cytokines, chemokines and other inflammatory mediators, NK cell activity, B cell antibody production, and T cell proliferation and possibly cytokine secretion. Despite its ability to induce proinflammatory cytokines, *Spirulina* has also been shown to significantly inhibit inflammatory responses in a variety of animal models, including models of rheumatoid arthritis, colitis, and IgE-mediated local and systemic allergic reactions. This may indicate that *Spirulina* possesses truly immunomodulatory activities, enhancing suboptimal immune responses, while dampening immune system hyperactivity. Acute and chronic toxicity studies in animals indicate that *Spirulina* does

not pose a health risk, and isolated reports of possible adverse effects in humans are not clearly attributable to the consumption of *Spirulina* [5].

Spirulina excretes variable quantities of products from its metabolism, such as organic acids, vitamins, and phytohormones, and extracts of *S. maxima* have shown antimicrobial activity against *Bacillus subtilis*, *Streptococcus aureus*, *Saccharomyces cerevisiae*, and *Candida albicans*. The presence of high quantities of acrylic acid in *Spirulina* was substantiated at the end of the seventies and this substance shows antimicrobial activity at 2 mg/L of biomass concentration. In addition, other bioactive compounds including propionic, benzoic, and mandelic organic acids were also found [7]. To date, relatively few studies have been undertaken examining the antibacterial activity of *Spirulina* and its extracts. One of them, de Mule et al. (1996) tested crude methanolic and aqueous extracts of *S. platensis* on the growth of three microorganisms. In *Candida albicans*, growth was inhibited 17.6% by aqueous extracts and 7.8% by methanolic extracts; in *Staphylococcus aureus* and *Lactococcus lactis* there was growth promotion by both extracts, ranging from 7.5 to 14.7%. Ozdemir et al. (2004) demonstrated that extracts of *S. platensis* were active against four Gram-positive and six Gram-negative bacteria and a yeast, *Candida albicans* ATCC 10239. In this study, the methanol extracts of *S. platensis* (comparable to tobramycin (10 µg/disc), especially against *S. faecalis*, *S. epidermidis*, and *C. albicans*) showed more potent antimicrobial activity than the ethanol, hexane, acetone, and chloroform extracts. Generally, when compared with the standard, tobramycin, all extracts, except the methanol extracts, exhibited low antimicrobial activity. The methanol and ethanol extracts showed antifungal activity against *C. albicans*, but less than that of the standard nystatin [9].

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EVALUATION AND COMPARATION OF BIOMETRIC AND SPECIFIC QUALITY PARAMETERS (TRITERPENIC ACIDS CONTENT) OF THREE MEDICINAL PLANT SPECIES: *SALVIA OFFICINALIS*, *OCIMUM BASILICUM* AND *SATUREJA HORTENSIS*

M. Constantinovici, M. Tilica, V. Plugaru, L. Olariu

S.C. Biotehnos S.A., 3-5 Gorunului Str., Otopeni, RO 075100, Romania

Aim of this study is evaluation of conventional and ecological agricultural technologies, towards these three medicinal plant species' production of standardized vegetal raw material in triterpenic acids content [1]. The plant probes from each phenological phase or were allotted for biometric determinations, evaluation of biomass production and applied drying methods, as well as for chemical analysis of their triterpenic acids content; for *Salvia*, being a perennial culture in the first year of cultivation, it was evaluated just one phenophase. The most important parameters for quality evaluation of these plant species productions were biomass quantity and triterpenic acids content which were in good agreement at later phenophases (III-V) when the cultures are well-grown. The standard deviation of the presented data was <0.1.

| Species | Phenophase/ Vegetation year | Biometric data | | | | | | Triterpenic acid content (% from dried matter) | |
|-----------------|-----------------------------|--------------------------|---|-------------------------|--------------------------|---|-------------------------|--|------------|
| | | Conventional | | | Ecological | | | Conventional | Ecological |
| | | Medium plant weight (cm) | Medium leaves/ inflorescence weight (g) | Medium stalk weight (g) | Medium plant weight (cm) | Medium leaves/ inflorescence weight (g) | Medium stalk weight (g) | | |
| <i>Salvia</i> | I/ I | 86.22 | 59.19 | 27.82 | 84.62 | 63.46 | 21.16 | 3.26 | 2.94 |
| <i>Ocimum</i> | IV | 49.17 | 15.73 | 18.42 | 46.83 | 14.96 | 16.18 | 2.19 | 2.89 |
| | V | 35.42 | 9.18 | 13.35 | 30.47 | 10.47 | 12.72 | 2.97 | 3.16 |
| <i>Satureja</i> | III | 41.82 | 20.31 | 17.63 | 38.47 | 18.55 | 15.47 | 4.06 | 3.76 |
| | IV | 28.74 | 10.39 | 13.58 | 24.51 | 8.93 | 12.15 | 3.61 | 3.99 |

These data will be used to correlate and apply the optimization of triterpenic acid production of these plant species.

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NANOSIZED SUPRAMOLECULAR COMPLEXES OF UBIQUINONE

Yu.V. Dadali², V.A. Dadali¹, V.G. Makarov²

¹St. Petersburg State Medical Academy named after I.I. Mechnikov, Russia

²IC "Adaptogen", St. Petersburg, Russia

Ubiquinone (coenzyme Q₁₀) has gained importance today in the prevention and treatment of cardiovascular diseases. Being a highly hydrophobic compound (Hansch hydrophobicity constant 20.9 ± 1.0), ubiquinone Q₁₀ is not soluble in water. Due to this fact, it exhibits a poor level of digestibility and low accessibility to body tissues. It is possible to increase its accessibility to body tissues and digestibility by its reduction and formation of water soluble complexes of ubiquinone and hydrophilic polymers. The goal of this work is to obtain water soluble forms of ubiquinones and ubiquinolins as their complexes with hydrophilic polymers and to prove the existence of such complexes.

We have been able to obtain molecular complexes of ubiquinones Q₂ and Q₁₀ and their reduced forms H₂Q₂ and H₂Q₁₀ with β -cyclodextrin (β -CD). It has been shown that such compounds are easily soluble in water and make slightly opalescent solutions. We suppose this could be related to the appearance of either a micro- or nanoheterogeneous medium due to the long hydrophobic chain of Q₁₀ and H₂Q₁₀ molecules. To prove the formation of the molecular complex and the Q₂ and Q₁₀ molecules entering the β -CD cavity, good solubility of β -CD with insoluble ubiquinones in water should be mentioned. The obtained complexes H₂Q₁₀- and Q₁₀- β -CD are brought about by noncovalent bonding to form complexes according to the "owner-guest" model. Based on the quantum chemistry calculations, it has been demonstrated that the structure with nonlinear and enclosed configuration in the shape of an entangled cluster with a mobile cavity inside (-150.1 kcal/mol) is the most thermodynamically favorable one, in comparison to the spiral and linear structures of the Q₁₀ and H₂Q₁₀ molecules (-143.7 kcal/mol). We suggest that the nanosized complex H₂Q₁₀- and Q₁₀- β -CD can demonstrate the characteristics of simultaneous penetration of the chemical groups of the Q₁₀ and H₂Q₁₀ ring into the cavity of the β -CD molecule (0.78 nm), on one side, and penetration of the whole β -CD molecule (1.53 nm) into the cavity of the hydrophobic chain of Q₁₀ and H₂Q₁₀ molecules (1.47 nm), on the other side. It decreases the energy of the system and makes this nanosized complex favorable. Formation of aggregates of a larger size, consisting of two or more complexes, may not be excluded either. In such a way, noncovalently bonded compounds of ubiquinones and ubiquinolins with β -cyclodextrin have been experimentally obtained in the form of water soluble powders, which would make ubiquinone more digestible and more easily accessible to body tissues. The presence of molecular complexes of ubiquinones and ubiquinolins with β -cyclodextrin in them has also been confirmed.

ON REDUCTION OF UBIQUINONE BY FLAVONOIDS

Yu.V. Dadali², V.A. Dadali¹, V.G. Makarov²

¹St. Petersburg State Medical Academy named after I. I. Mechnikov, St. Petersburg, Russia

²IC "Adaptogen", St. Petersburg, Russia

The redox pair ubiquinone – ubiquinol is known to be an important component of the mitochondrial respiratory chain and is involved in the shuttle mechanism of electron transfer from flavin dependent enzymes to the cytochrome system. Taking into consideration the importance of the antihypoxic and antioxidant effect of the pair ubiquinone – ubiquinol to the cell, it appears worthwhile to study the possible effects of some exogenous reducing agents on the electron transfer processes in the system including ubiquinone *in vitro*.

The goal of this work is a comparative study of the special features of the reduction of ubiquinones Q₀, Q₂, and Q₁₀ by various flavonoids in homogenous solutions (system I) and in heterogeneous system II, containing polymer ultramicroparticles.

In both systems, the direct effect of ubiquinone reduction by ascorbic acid was observed, while, in homogenous solutions, reduction by flavonoids was not observed. In system II, containing polymer ultramicroparticles, there appeared a new absorption peak at 452 nm (a bathochromic shift of 72 nm) instead of the expected direct ubiquinone reduction by flavon-3-ols, which could be explained by the fact that only flavon-3-ols directly interact with the surface of the polymer particles. This effect is not observed with other types of flavonoids. With quercetin, a new absorption peak is detected in the system even in the absence of ubiquinone, which is related to the appearance of a new form of quercetin stabilized on the surface of the particles by a strong molecular complex with a transfer of charge.

The influence of the tested solvent on the polymer particles in system II has been shown to lead to the renewal and activation of their surface, to formation of ultradispersed particles in the shape of entangled nano- and microclusters containing the sorbed molecules of flavon-3-ols and ubiquinone. It has been established that, in system II, reduction of ubiquinone by quercetin was possible through formation of molecular complexes on the surface on the ultradispersed particles. The main event preceding ubiquinone reduction is the quercetin chemisorption on the surface, which lowers the activation energy for the next stages of electron transfer to ubiquinone.

BETULIN FORMULATIONS WITH RAMIFIED CYCLODEXTRINS AND THEIR INTERVENTION IN A RAT PHOTODAMAGE SKIN RECOVERY

Cristina A. Dehelean¹, Camelia Peev¹, Codruta Soica¹, Zoltan Aigner²

¹University of Medicine and Pharmacy Victor Babes Timisoara, Faculty of Pharmacy, Eftimie Murgu Square no.2, 300041, Timisoara, Romania

²University of Szeged, Faculty of Pharmacy, Hungary

A photodamaged skin means a development in skin carcinoma, cutaneous melanoma, naevi, actinic keratoses and other pathologic signs [1]. UVB radiations lead to abnormal cell proliferation and other important perturbations. In these cases topical therapy with cytotoxic agents is a direct solution or a complementary intervention [1, 2]. Betulin is in higher content in birch tree outer bark in important quantities and birch bark was tested in many tumor interventions, more important as ointment in actinic keratosis treatment [3]. Our goal was the elaboration and application of a semisolid formulation with betulin dissolved with gamma ramified cyclodextrin (2,6-O-dipentyl-gamma cyclodextrin) and the analysis of the final product on a photodamaged rat skin. The cream content in betulin was 5% and the application of the treatment was under observation for 20 days. The 3 groups of rats were Sprague Dawley type, female and UVB exposed at 294 nm for 25 days, 5 min/day, 3 days/week. The cream with betulin was applied also as a protector (group 2) from the day one of UVB exposure. The evaluation was histological and with a Mexameter. The results indicated that betulin intervene in treatment and also in prophylaxis of the photodamage reactions. It helps to a good recovery for external skin layer in 95% of cases and the damages are reduced if the cream is applied for protection. The main conclusion is that betulin alone, in a proper formulation, could improve skin recovery in photo exposure processing.

Acknowledgements to the GRANT CNCSIS PN 2 ID 1257 for financial support.

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A POSITIVE INFLUENCE OF GLUCOSAMINE HYDROCHLORIDE ON REPRODUCTIVE FUNCTIONS OF MALE RATS

la.V. Diakonova, A.V. Zajchenko, L.V. Yakovlyeva, I.G. Butenko, A.V. Andriyanenkov

The National University of Pharmacy, Kharkov, Ukraine

Treatment of sexual dysfunction in men is a challenge of modern pharmacy.

A study of influence of glucosamine hydrochloride (GGh) on gonads and spermatogenesis was carried out on the model of serotonin affection of testicles. Mature male rats were divided into 3 groups. 1st group – intact animals. Animals that were made hypodermic injections of serotonin hydrochloride (SGh) 5 mg/kg (2nd group). Males, who had got GGh at the dosage of 90 mg/kg intragatricum before 2 days, during the injections and two days after having finished the injections of SGh (3rd group). An estimation of effectiveness of GGh had been made according to the changes of level of testosterone in blood serum, content of fructose in a tissue of prostate, morphofunctional state of testicles and indices of spermogram. The study was made on white male rats with decreased sexual activity. GGh has been introduced at the dosage of 90 mg/kg every hour intragatricum for 7 days. In 4 hours after the last introduction of GGh, ovariectomized female rats were put to males for 20 minutes. Those females had got hypodermic injections of 25 mkg of synoestrole and 500 mkg of progesterone 4 hours earlier. A number of inputs, intromissions, ejaculations has been registered, copularitical effectiveness and intercopularitical interval have been counted, the number of animals who had made the whole copularitical cycle was registered.

The indices of spermogram have normalized the level of testosterone and fructose in male rats who have been affected by SGh has increased under the influence of GGh. Gonad protective effect GGh verifies the data of morphological examination of testes. The introduction of GGh to males with little sexual activity has caused the decrease of number of putting animals, increased copularitical effectiveness and number of ejaculations, increased the number of animals who had made the whole copularitical cycle till the level of animals with high sexual activity.

We can make a conclusion that GGh at the dosage of 90 mg/kg with an input of SGh at the background has a gonade protecting action. The input of GGh during 7 days at the dosage of 90 mg/kg to male rats stimulated potentation. GGh can be used as the corrector of male reproductive functions.

THE STUDYING OF ADAPTOGENIC ACTIVITY OF THE BIOLOGICALLY ACTIVE FOOD ADDITIVE "IMUNOZAHIST"

la.V. Diakonova, V.S. Kyslychenko, L.V. Brun, V.Ye. Dobrova

The National University of Pharmacy, Kharkov, Ukraine

Representatives of the Echinacea genus are known for medicinal properties for a long time. Pharmaceutical industries of the USA, Canada and some European countries manufacture a considerable quantity of medical products and biologically active food additives (BAFA) on the basis of raw materials of plants of the Echinacea genus. The rich chemical composition of the plants of this genus conditions a wide spectrum of pharmacological activity of medical products on their basis.

Our phytochemical research is concerned with Echinacea Pallida Nutt. "Krasunya prerii" cultivated in Ukraine. The extract from the roots of Echinacea pallida obtained on the basis of the experimental data enters into the composition of BAFA "Imunozahist". The studying of pharmacological effects of "Imunozahist" was the further purpose of our work. The studying of adaptogenic activity was conducted on nonlinear rats. The animals were divided into two groups. The animals of the first group were given "Imunozahist" in a dose of 100 mg/kg. The animals of the second group were given treated water. In 30 minutes after the oral introduction of the specimen the animals were placed in the prepared pool. The water temperature in the pool was 0°C. The time of the animals' swimming was defined by the collapse of their impellent reflexes. The temperature 0°C in the pool was maintained by means of ice.

For definition of statistical reliability of differences between control and experimental groups the One-Way ANOVA has been carried out. The parameter "swimming time" was considered as fixed factor and groups of animals – as dependent variable. It is established that distinctions between these groups on parametre "swimming time" are statistically significant ($F=312,392$; $p_{level}=0.0001$, i.e. $p < 0.05$). Hence, application of "Imunozahist" leads to statistically authentic change of results of the test on adaptogenic activity. The statistical processing of the received results of the experiment shows that adaptogenic activity of "Imunozahist" makes 27%.

The results of the conducted research indicate that "Imunozahist" has adaptogenic activity and is perspective for depressions, physical and nervous exhaustion and for maintenance of protective forces of a human body.

PHYTOCHEMICAL STUDY OF *ALLIUM CEPA* L. ONION SKIN

Ia.V. Diakonova, I.M. Shevtsov, I.O. Zhuravel, A.A. Brasheveckaya

The National University of Pharmacy, Kharkov, Ukraine

Onion – *Allium cepa* L. is a traditional agricultural plant.

Allilchep and allilglicer, bow onion-based preparations, are present at the pharmaceutical market of Ukraine. They are used as laxative and antibacterial remedies. *Allium cepa* L. bulbs are used to produce Ceba injel (Germany), and fitolizin (Poland) in Europe. Onion-skin is food industry waste. In folk medicine it is widely used for treatment of dermatological, gastroenterology and cordial diseases. Dyeing properties of onionskin found their application in textile and food industries as well as in cosmetology for dyeing hair.

A valuable practical application of *Allium cepa* L. (onion) skin and insufficient knowledge of its chemical composition explain the interest to its phytochemical study.

Qualitative reactions, thin layer and paper chromatography were used to detect flavones, organic acids, glycosides, triterpenoid and steroid saponins, ascorbic acid and tannins.

Quantitative analysis of biologically active substances was conducted by means of spectrophotometry, gravimetry, and titrimetry using universally accepted methods. Polysaccharides made up 9.84%, ascorbic acid – 0.08%, sums of oxidable phenols – 7.24%, organic acids – 5.28%, oxycinnamic acids – 0.13%. Thus, an onionskin phytochemical study revealed the presence of certain biologically active substances in the raw material and determined their quantitative content.

These results suggest good prospects for a further study of *Allium cepa* L, (onion) skin as well as for the development of effective medicines on its basis.

ELECTROPHYSIOLOGICAL SURROGATE PARAMETERS FOR SUBSTANTIATION OF MENTAL HEALTH CLAIMS IN THE PRESENCE OF MILD COGNITIVE IMPAIRMENT IN HUMANS

W. Dimpfel

Justus-Liebig-University Giessen c/o NeuroCode AG. Sportparkstr. 9, D 3578 Wetzlar, Germany

The present work was undertaken to identify surrogate parameters of mental fitness derived from source density processed EEG during concomitant performance of various psychometric tests. Identification of mild cognitive impairment was achieved by selection of volunteers after performance of the interactive questionnaire "DemTect" with a score between 9 and 13. EEG was recorded on 17 channels according to 10-20 system during eyes open and in the presence of mental work (d2-Test, concentration-performance and memory-test). Data were Fast Fourier transformed and absolute power values were used to characterize the electric changes during test performance. Using baseline data (before administration of drugs) from two independent studies with $n=30$ and $n=16$ volunteers. Three major results emerged:

1. The ratio of frontal theta and parietal alpha2 waves during the recording condition "eyes open" could be related to the DemTect score values in a statistically significant manner (Pearson Corr.-Coeff.: $r = -0.75$ at $p=0.0000001$).
2. Successful performance in the d2-test could be related to frontal-temporal beta power ($r=0.53$ at $p=0.0001$).
3. Success in the concentration-performance-test (CPT) could be related to a ratio of fronto-temporal theta waves and temporal alpha waves ($r= -0.73$ at $p=0.0000001$).

These results provide evidence that frequency analysis of the source density EEG allows to define electrophysiological surrogate parameters, which can be used for diagnosis of mild cognitive impairment and also to follow drug effects on cognition. Due to the high sensitivity of the methodology and the use of surrogate parameters clinical studies become possible with small numbers of participants.

THE PECULIARITIES OF CULTIVATION OF MEDICINAL PLANTS IN CONDITIONS OF VOLGO-VYATSKY REGION OF RUSSIA

T.L. Egoshina

Russian Research Institute of Game Management and Fur Farming, Russia, 610000 Kirov, Engels st., 79. e-mail: etl@inbox.ru, +7(8332)353715

Vascular plants flora of Volgo-Vyatsky region includes 2000 species, 130 out of them are in the list of State Pharmacopoeia of Russia. 100 more species are used in folk medicine. More than 50 species used in folk and scientific medicine have significant stocks or are rare and in need of protection or included in Red Books of Russia and regions. Majority of these species (16) are marked in family *Orchidaceae*. Significant press is made on populations of *Adonis sibirica*, *A. vernalis*, *Centaurea sumensis*, *Paeonia anomala* and some more.

One of the ways of preserving natural populations of medicinal plants is cultivation. As a result of primary introduction of more than 40 species it was marked that main limitation factors in conditions of Volgo-Vyatsky region situated in forest zone of Russia are low winter temperatures, short vegetation period, prevalence of podzol soils. About 20 species are noted as successful: *Bidens tripartita*, *Calendula officinalis*, *Echinaceae purpurea*, *Inula helenium*, *Hypericum perforatum*, *Leonurus quinquelobatus*, *Matricaria chamomilla*, *Mentha piperita*, *Origanum vulgare*, *Ononis arvensis*, *Plantago major*, *Polemonium coeruleum*, *Rhodiola rosea*, *Valeriana officinalis*.

Adaptation technology of cultivation which allows to gather stable crops including application of mineral and organic fertilizers, pesticides, recommendations for soils, seeds preparation, quality control; is worked up. Influence of growth stimulators (heteroauxin, humate, aeline) is investigated.

Studies of cultivation peculiarities and reintroduction possibilities of *Anemone sylvestris*, *Lilium martagon*, *Centaurea sumensis*, *Dianthus versicolor*, *Paeonia anomala* are carried now. Basic attention is given to the studies of ontogenetic structure of populations and properties of generative reproduction of plants – markers of species' strategy which define population stability in different conditions, cultivation prospective and economical importance.

ASSAY OF TOTAL ANTHOCYANINS CONTENT IN BIOLOGICALLY ACTIVE FOOD SUPPLEMENT "MALVOVYY" DEVELOPMENT AND VALIDATION

O.V. Evdokimova^{1,2}, ***V.V. Obukhova***¹

¹JSC "Krasnogorskleksredstva", Krasnogorsk, Moscow region, Russia

²I.M. Sechenov's Moscow medical academy, Moscow, Russia

According to the modern requirements for biologically active food supplements (FS), the content of biologically active substances should be regulated. The main component of FS "Malvovyy" is hibiscus flowers (57%), which contain plenty of anthocyanins. Therefore total anthocyanins was chosen as a parameter for the FS quantification.

The purpose of our work was to elaborate and validate the method for total anthocyanins assay for FS "Malvovyy". The optimal conditions of spectrophotometric determination method of total anthocyanins calculated on cyanine were selected on the base of official method recommended for blue cornflower (*Centaurea cyanus* flowers) analysis and our own received data. The method validation was performed according to the recurrence and repeatability of the method. The method recurrence was explored on one sample of raw material in 6 replications ($S_0=3.4\%$). Determination of the method repeatability was performed by 2 engineer-chemists on 3 samples in 3 replications ($S_0=7.6\%$). The acceptability criterion of the method recurrence and repeatability was 10%. The industrial batches analysis has revealed that the total anthocyanins content determined by the proposed method is fluctuating between 11.40 and 17.85 mg per one filter-bag, which allows us to suggest the standard content of active substances not less than 10 mg/filter-bag.

The assay of total anthocyanins content in FS "Malvovyy" was developed. The obtained data were included into "Krasnogorskleksredstva" Technical standards 9197-007-00481146-08. Development and validation of analysis methods for FS is not only the guarantee of product quality, but also a stage directed on requirements harmonization for both FS and medicinal products.

QUALITATIVE TLC-TEST BIOLOGICALLY ACTIVE FOOD SUPPLEMENT "MALVOVYY" DEVELOPMENT AND VALIDATION

O.V. Evdokimova^{1,2}, V.V. Obukhova¹

¹JSC "Krasnogorskleksredstva", Krasnogorsk, Moscow region, Russia

²I.M. Sechenov's Moscow medical academy, Moscow, Russia

The purpose of our study was to develop and validate the method for determination of the main groups of biologically active substances in biologically active food supplement (FS) "Malvovyy".

FS "Malvovyy" was object of the study; TLC was chosen as analytical method for determination of flavonoid compounds in the sample.

The best flavonoid compounds resolution was achieved in the ethyl acetate – anhydrous formic acid – water (14:3:3) system in TLC plates ("Sorbfil" HPTLC-P, Russia). After the 10% oxalic acid solution treatment in the daylight it is detected not less than two areas with crimson color on the test solution chromatogram. These areas are located on the level of crimson color areas of authentic sample hibiscus flowers aqueous alcoholic extract (R_s of about 1.0 and 1.3). The method validation was performed according to the chromatography system specificity and appropriateness. The method specificity was evaluated by coincidence of FS different batches chromatography profiles by the main areas among themselves and their compliance with the method description. The quantity of the studied FS batches stock was not less than 3. Chromatography profiles of different batches of FS "Malvovyy" have coincided among themselves by the main areas and been compliant with the method description. As the chromatographic system suitability index the resolution between the different typical areas with the least index value has been chosen. For the present method the resolution between the areas with R_s of about 1.0 and R_s of about 1.3 was selected. The resolution value between the indicated areas was not less than 1.5.

The method of FS "Malvovyy" identification by TLC has been developed and validated. The received results were included into "Krasnogorskleksredstva" Technical standards 9197-007-00481146-08.

QUALITATIVE TLC-TEST FOR PECTORAL TEA №4 DEVELOPMENT AND VALIDATION

O.V. Evdokimova^{1,2}, V.V. Obukhova¹

¹JSC "Krasnogorskleksredstva", Krasnogorsk, Moscow region, Russia

²I.M. Sechenov's Moscow medical academy, Moscow, Russia

The purpose of our study was to develop and validate the method for qualitative determination of the main groups of biologically active substances in Pectoral tea №4.

Pectoral tea №4 was object of the study; TLC was chosen as analytical method for determination of flavonoid compounds in samples.

Ethanol – toluol – water (50:100:1) appeared to be the best mixture for extraction and the best flavonoid compounds resolution was achieved in the ethyl acetate – toluol – anhydrous formic acid – water – glacial acetic acid (30:10:6:3:1) system in TLC plates ("TLC Silica gel 60 F254", aluminium sheets, MERK, Germany). At viewing test solution chromatogram in UV-light (365 nm) it was detected the area with violet fluorescence (R_s (by quercetin) of about 1.0), which corresponded to *Camomile flowers*. After the 1% diphenylboryloxyethylanmin solution treatment in UV-light (365 nm) it was detected the following areas on the test solution chromatogram: 2 areas with green fluorescence (R_s (by quercetin) of about 0.05 and 0.1), which corresponded to *Calendula flowers* and *Viola herb*, respectively; 2 areas with orange fluorescence (R_s of about 0.3 and 0.6), which corresponded to *Ledum sprigs*; 2 areas with pale blue fluorescence (R_s of about 0.85 and 1.2), which corresponded to *Peppermint leaves* and *Licorice roots*, respectively; and the area with violet fluorescence (R_s of about 0.9), which corresponded to *Viola herb*. The method validation was performed according to the chromatography system specificity and appropriateness. As the chromatographic system suitability index it has been chosen the resolution between the typical areas of rutin (R_s of about 0.15) and quercetin (R_s of about 1.0) standard samples. The resolution value between the indicated areas was not less than 15.

The method of Pectoral tea №4 identification by TLC has been developed and validated. The method allows to detect all 6 components of the tea. The received results were included into the draft version of "Krasnogorskleksredstva" House monography.

QUALITATIVE TLC-TEST FOR VIOLA HERB RAW MATERIAL DEVELOPMENT AND VALIDATION

O.V. Evdokimova^{1,2}, V.V. Obukhova¹

¹JSC "Krasnogorskleksredstva", Krasnogorsk, Moscow region, Russia

²I.M. Sechenov's Moscow medical academy, Moscow, Russia

The purpose of our study was to develop and validate the method for determination of the main groups of biologically active substances in Viola herb raw material.

Viola herb was the object of the study; TLC was chosen as analytical method for determination of flavonoid compounds in the samples.

The best flavonoid compounds resolution was achieved in the ethyl acetate – anhydrous formic acid – water (65:15:20) system in TLC plates ("Sorbfil" TLC-P, Russia). After the 1% diphenylboryloxyethylanmin solution treatment it was detected not less than two areas on the test solution chromatogram in the daylight: one of them had sulphur color (R_s (by rutin) of about 0.8) and the other – honey dew (R_s of about 1.0); and not less than two areas in UV-light (365 nm): one of them was with canary fluorescence (R_s of about 0.8) and the other – with honey dew (R_s of about 1.0).

The method validation was performed according to the chromatography system specificity and appropriateness. The method specificity was studied on 7 raw material batches. The resolution between the different typical areas with the least index value has been chosen as the chromatographic system suitability index. For the present method the resolution between areas with R_s of about 0.8 and R_s of about 1.0 was selected. The resolution value between the indicated areas was not less than 1.5.

The method of Viola raw material identification by TLC was developed and validated. The received results were included into the "Krasnogorskleksredstva" House monographies 42-8253-06 and 42-8254-06.

LIGNINE ACTIVE CHARCOALS USED FOR PURIFICATION OF SOLUTIONS FROM INORGANIC CATIONS

T.I. Fomicheva, E.V. Koluzhnikova, J.S. Fomichev, N.V. Mihajlova

St.-Petersburg State Wood Technology Academy, S.-Petersburg, Russia
"Farmagen Ltd", St.-Petersburg, Russia

Active charcoals are one of most often used sorbents. The active coals produced from wastes of wood processing, possess high sorption properties in relation to gases, steams and impurity sorption from water solutions [1]. The lignin granulated coals can be used as sorbents of inorganic components from solutions. With the help of lignin active charcoals the liquid wastes of hydrolytic processing of vegetative raw materials – the most complicated polydispersional systems - have been subjected to purification. The chemical analysis of such systems shows that it contents about 85% of the organic connections, aromatic structure and double communications and 15-20% of inorganic substances. Method of atom absorption spectrophotometry allows analyzing inorganic components content in biologically cleared sewage after additional flocculation processing with cationic polyelectrolyte and additional absorption purification by the lignin granulated charcoals. Analysis was carried out on spectrophotometer AAS-1N. The results of the deposits analysis after preliminary flocculation processing and additional purification by the activated coal are in the table. The metal cations - in mg per 1g weight of a deposit (deposit mg/g). The flocculation method allows allocating high-molecular organic connections. It is possible that metal cations coagulate as a part of salts of organic and inorganic acids and are deduced from system together with activated charcoals.

| The metal cation content in deposits, mg/g | | | | | | | | | |
|--|------|----------|------|----------|-----|----------|------|----------|------|
| Ca | | Mg | | K | | Na | | Fe | |
| deposits | Δ | deposits | Δ | deposits | Δ | deposits | Δ | deposits | Δ |
| 13,5 | 14,4 | 30,5 | 20,3 | 1,8 | 1,2 | 0,2 | 1,6 | 4,5 | 0,2 |
| Zn | | Mn | | Sr | | Cu | | Mo | |
| deposits | Δ | deposits | Δ | deposits | Δ | deposits | Δ | deposits | Δ |
| 0,2 | 0,1 | 0,1 | 0,1 | 0,1 | 0,1 | 0,01 | 0,01 | 0,02 | 0,02 |

The metal cation content is essentially reduced after physical and chemical clearing of the waste waters. The content of such elements as Ca, Mg, Na, K, Fe decreases, and Zn, Mn, Sr, Cu, Mo, Cd, Co, Pb, Cr are completely removed.

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STRIPPING VOLTAMMETRY ANALYSIS OF LEAD, CADMIUM, ZINC AND COOPER INTO MEDICINAL HERBS

*N. Kondratieva*¹, *T. Fomicheva*¹, *A. Klionkovsky*², *V. Kondratiev*³

¹St.Petersburg State University of Low-Temperature and Food Technologies, Russia

³Scientific & Technical Company "Volta", St. Petersburg, Russia

Chemical analysis of medicinal herbs is usually performed for active components, which have a medical effect for patients. Various chemical/physical methods are used to determine the percentage of active principle components, like alkaloids, flavonoids, enzymes, vitamins, essential oils, fats, carbohydrates or others into herbs. However, for save quality control of herbal raw materials it is required more extended analysis of some contaminants, in particular, heavy metals. It is especially important due to the deterioration of ecological situation on the territories of the gathering of medicinal herbs and the well known effect of accumulation some chemical elements by herbs. It is worth to emphasize here, that the precise chemical analysis of elements may also serve for the proper identification of herbal source materials, providing a chemical "fingerprints" of harvesting of the gathering of medicinal herbs.

Lead, cadmium, zinc and cooper belong to group of heavy metals that have an extremely toxic even at low concentration and they can be accumulated in life organisms. Therefore, the determination of these metals is essential for control of quality of foods and drugs.

Stripping voltammetry was applied for the determination of heavy metals in medicinal herbs. Lead, cadmium, copper and zinc were analyzed in different herbs samples at rotating disk glassy carbon electrode (RDE-GC) in Britton-Robinson buffer solution of pH 2. Sample preparation was performed by boiling of 2-5 g of finely pulverized plant samples for 30 min into HNO₃ (1:5), cooled, filtered. Aliquote of solution (5 ml) was completed to 20 ml measuring flask by buffer solution. The optimal preconcentration times and potentials for proper detection of heavy metals in samples have been studied. The levels of metal contaminations was ranged from 0.005 to 2.000 µg per g of samples. Comparison of data obtained with parallel results of atomic absorption spectroscopy have been made. Good correlation of results obtained by two methods was found. The statistical parameters of stripping voltammetry measurements have been calculated.

ESSENTIAL OILS COMPOSITION OF NATURALLY GROWN AND MICROPROPAGATED *HEDEOMA MULTIFLORUM* BENTH PLANTS

S. Diaz, L. Palacio, M. Goleniowski

Ministerio de Ciencia y tecnología. Subsecretaria CEPROCOR. Arenales 230, B°. Juniors, (CP X5004 AAP) Córdoba, Argentina

Hedeoma multiflorum Benth. (Lamiaceae) is an aromatic herb endemic to Argentina used in popular medicine as a digestive, anti-rheumatic and abortive [2]. Plant parts usually harvested for medicinal purposes include roots, leaves, fruits and seeds; as a result the native population was seriously declined. Plant cell culture represents a useful production alternative to direct extraction of valuable secondary metabolites from the wild plant.

To determine the effect of different growth regulators on the growing rates and essential oil production, nodal segments from in vitro germinated and wild plants were incubated on half-strength MS (Murashige & Skoog) salts medium, at different ratios (0.01, 0.1, 0.5 mg/l) of α -naphthaleneacetic acid (NAA) and N⁶-benzyladenine (BA). The one-way ANOVA at 95% confidence level was used for all statistical analysis.

The maximum principal shoot length and node number were found at 0.01-0.5 mg/l of both growth regulators with the addition of 0.5 mg/l of GA₃ and rooting percentages were achieved up to (87.5-100%) without a rooting media.

Twenty-three volatile compounds were identified for the first time by GC-MS from the foliage of in vitro regenerated plantlets, 14 of them are common at the naturally grown plants. The major compounds were (+)-pulegone and menthone at 51% and 36% respectively in wild plants. The (+)-pulegone yield increased significantly by up to 76% on in vitro plants. Other common components found were α -pinene, β -pinene, mircene, limonene, trans- β -cimene, isomenthone, cis-Isopulegone, α -copaene, (+)aromandendren, germacrene-D, bicycle-germacrene and germacrene-A.

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WINTER OAT IS A MEDICINAL PLANT

G.N. Gudkova

Adige State University, Maikop, Russia

Wide and various using of oat (*Avena sativa* L., Poaceae) such as corn, green mass and straw is the reason of great attention to this plant material. Green or vegetative mass is used as granulated fodder for domestic young animal and poultry. Oat corn contents high quantity of amino acid, lysine and triptophan, mineral substances, vitamins, proteins and especially of oil. Oat grain is richer by P, Fe and Se than wheat, peeled buckwheat and husked millet. Energetic value of 100 g of oat is 420 calories. Recently oat was included to official medicinal plant, phytodrug "Ovesol" was created and investigated. But there is not information about influence of oat varieties, periods of its harvesting and cultivation conditions for obtain raw material with the best quality.

The aim of our work was selection and growing of winter oat for the fodder.

Four winter oat varieties created in Adige Agriculture Institute were materials for the present study. The chemical composition (vitamin C, carotin, Se, Si, protein, fat) of green mass and of corn obtained at the different harvesting time was studied in laboratory of Adige State University. The high-yield varieties from world oat collection and property selection were also tested.

Maximum vitamin C content was obtained for Kabardin oat plants in stem extension phase. Maximum of carotin was determined in variety Taggart (USA). The content of Se in winter oat corn is more high than in winter wheat.

New varieties are remarkable for high yield of corn and green mass as compared with spring oat. Production of winter oat is more ecological purity and economical profitable, than spring oats production. Winter oat is not spring drought and leaf vermin suffered.

As a result more valuable varieties of winter oat were determined. They are useful raw material for food and pharmaceutical industry.

COMPARATIVE ANALYSIS OF THE ELEMENT COMPOSITION OF *ARMENIACA VULGARIS* LEAVES AND EXTRACT

***I.G. Guryeva*¹, *L.V. Upyr*¹, *O.A. Puzak*¹, *N.V. Tolkacheva*²**

¹The National University of Pharmacy, Kharkov, Ukraine

²Nikitsky Botanical Gardens – National Scientific Center

Apricot (*Armeniaca vulgaris*) is recommended in adult's and child's diet because of high content of carotene, vitamin C, potassium, organic acids. It is recommended for people suffered from anemia, atherosclerosis, coronary insufficiency as source of well assimilated potassium and iron. It is reported that consumption of apricot in great amount promotes longevity.

In Ukraine folk medicine fresh leaves of apricot are used as analgesic to treat toothache, stomatitis, and for cleaning teeth.

Dry extract of apricot leaves obtained in our laboratory was studied. Moderate antiinflammatory activity of extract in comparison to control has been determined on animal. About 12.4-13.1% of oxidizable phenolic compounds in it was quantificate after permanganatometric titration. About 35.1-38.1% of water-soluble polysaccharide complex was determined in the extract gravimetrically.

The aim of our further investigation was comparative analysis of element composition in dry extract and leaves. The quantification of 15 elements was done by atomic emission spectrometry based on vaporation of undertesting object ash in electric arc, photographic registration of factored in spectrum emission and measuring of intensity of spectral streaks of elements. Research was carried out on diffraction spectrograph DFS-8, diffraction grating 600 line/mm and three-lens system of illumination of slit. Result represents in the table.

Content of elements in leaves and dry extract of *Armeniaca vulgaris*

| element | leaves | dry extract | element | leaves | dry extract |
|---------|--------|-------------|---------|--------|-------------|
| Fe | 70 | 125 | Ca | 650 | 500 |
| Si | 620 | 880 | Na | 150 | 750 |
| Mg | 280 | 720 | Cu | 0,15 | 0,3 |
| P | 150 | 380 | Zn | 0,5 | 2,5 |
| K | 2310 | 8750 | Mn | 8 | 10 |
| Al | 40 | 25 | Sr | 3 | 2,5 |

Content of Pb, Mo, Ni was insignificant. Content of elements in dry extract was in 1.5-3 time higher than in leaves, except Ca, Al and Sr.

QUALITATIVE COMPOSITION OF FATTY ACIDS AND TOCOPHEROLS IN SOUR AND SWEET CHERRY LEAVES, FRUITSTEMS STEAMS

I.G. Guryeva, L.V. Upyr, V.P. Popovich, V.S. Kyslychenko

The National University of Pharmacy, Kharkov, Ukraine

Fatty acids are essential components of all biological membranes; tocopherols are natural antioxidants, defended alive organisms from oxidative processes and aging, assisted in biosynthesis of protein, tissue respiration and regeneration.

Aim of our work was qualitative analysis of fatty acids and tocopherols composition of leaves, fruitsteams, steams of sour (*Cerasus vulgaris* Mill) and sweet cherry (*Cerasus avium* L), widely cultivated in Ukraine.

Leaves and steams were gathered in May, fruitsteams - in period of gathering rape fruit in Donets (Ukraine). Lipofilic fractions were obtained from all kind of raw material by chloroform exhaustion extraction. Qualitative analysis of acids by gas-liquid chromatography on chromatograph "Chrom-5" on metallic column 2.6 m in length was carried out. Column contains sorbent "Chromaton super" with 10% polyethylene glycol succinate. Preliminarily fatty acids were methylated to obtain low-boiling esters. Identification of fatty acids was carried out by comparison their time retention with time retention of known fatty acid methyl ester.

Division and identification of tocopherols was carried out on such conditions: column 2.6 m in length with solid carrier "Inerton-super"; temperature of analysis – 190°C, temperature heating of Flame Ionization detector – 240°C, speed of gas -carrier, nitrogen of high purity – 40 ml/min.

8 saturated (monodecanoic, lauric, myristic, pentadecanoic, palmitic, heptadecenoic, stearic, arachic) and 5 unsaturated (oleic, linoleic, linolenic, arachidonic and eicosapentaenoic) fatty acid have been determined in all kinds of raw materials. Unsaturated fatty acid prevailed in leaves, fruitsteams, steams of sour and sweet cherry; linoleic and linolenic acid dominated among them. Palmitic and stearic acids are in the greatest quantities among saturated fatty acids. Investigated raw materials contain α_1 , α_2 , β , γ , δ -tocopherols, among them δ -tocopherol dominated. Fruitsteams of sour and sweet cherry contain tocopherol more than other kind of raw materials. Content of tocopherols and unsaturated fatty acid indicate of availability of raw material to create drug.

CURRENCY OF VEGETABLE AND SYNTHETIC ACTIVE SUBSTANCES COMBINING IN MEDICINAL PREPARATIONS

I.G. Guryeva, V.A. Georgiyants, N.B. Burd, O.A. Vasilyeva

The National University of Pharmacy, Kharkov, Ukraine

The tendency of the modern therapy is rational combination of fast-acting and efficiency of the synthetic medications and soft correcting influence of the phytopreparations. Modern technologies make it possible to get the phytopreparations, proper the standards of high quality, possessing the different types of the biological action. Increasing popularity of the usage of phytotherapeutics is in a large measure conditioned by spreading of the side effects and undesirable reactions on the application of chemotherapeutics. Among the most frequent side effects is the lesion of gastrointestinal tract, nervous system, metabolic derangements. Allergic reactions at children meet in 3 times more frequent, than at adults. We analysed the widespread general therapeutic charts at different diseases in which combines the use of chemopreparations and phytotherapeutics. One of the reasons of growing of the frequency of side effects is polypragmasy, increasing negative action on a liver where biotransformation of the medicinal preparation is carried out.

The purpose of our work was rationalization of therapeutic charts. Taking into account the necessity of the reduction of number of the simultaneously applied preparations and economic factors, it is expedient to use the exactly combined remedies, possessing a necessity by a pharmacological action and simultaneously impedimental development of side effects. Practice to combine the chemotherapeutics and phytopreparations is traditional for coughings up preparations (Ambroxol with the plant extracts with mucolytic and emollient action), antihemorrhoidal preparations (phlebotropic action of bioflavonoids and anaesthetic effect of local anesthetics), in dermatology (antifungal and antibacterial properties combine with reparative plants complexes) etc.

It was determined that the considerable risk of origin of side effects, increasing at the application over a long period of time, is due with the use of antibacterial preparations, that especially undesirable in pediatric practice.

The obtained results of the primary pharmacological screening showed the perspective of development of the combined medications, containing antibacterials with the herbal hepatoprotective complex.

EVALUATION OF COMBINED ACTION OF RESVERATROL AND INDOLE-3-CARBINOL

***G.V. Guseva, N.V. Trusov, M.A. Uskova, L.I. Avrenyeva, I.V. Aksenov,
L.V. Kravchenko***

Institute of Nutrition, Russian Academy of Medical Sciences, Moscow, Russia

Polyphenol resveratrol (RES, 3,5,4'-trihydroxystilbene) and indole-3-carbinol (I-3-C) are biologically active components of food and have been reported to possess a variety of properties beneficial for human health. Their biological activity is first of all associated with antioxidant (antiradical) properties and ability to regulate gene expression of key enzymes of xenobiotic metabolism and antioxidant defense.

In the present study we examined combined effect of RES and I-3-C on antioxidant status markers and xenobiotic metabolizing enzymes' activities of male Wistar rats. During one week four groups of animals were given either control semipurified diet or diets containing 100 mg/kg b.w. RES (test group 1), 20 mg/kg b.w. I-3-C (test group 2) or both RES and I-3-C at the same dosages (test group 3).

Increase of antioxidant capacity and reducing ability (FRAP assay) of plasma and liver cytosols was observed in test groups 1 (RES) and 2 (I-3-C) and was more pronounced in test group 3 (RES and I-3-C). There was no effect of RES on activities of detoxification enzymes and antioxidant enzymes while I-3-C induced increase of activities of quinone reductase (195%), heme oxygenase (120%), glutathione transferase (165%) and UDP-glucuronosyltransferase (144%). Similar increase of activities was observed in test group 3 (RES and I-3-C). Ex vivo NADPH-induced lipid peroxidation of liver microsomes was suppressed by 35, 39 and 60% in test groups 1, 2 and 3, respectively. Activities in liver of ethoxyresorufin-O-dealkylase (CYP1A1), methoxyresorufin-O-dealkylase (CYP1A2), pentoxyresorufin-O-dealkylase (CYP2B) and testosterone 6 β -hydroxylase (CYP3A) did not change significantly with addition of RES to diet, but increased 5.8, 10.6, 5.3 and 2.2 fold in rats given I-3-C and to a lesser extent – 3.2, 5.4, 2.9 and 1.8 fold in rats given I-3-C and RES. I-3-C resulted in increase of quantity of CYP1A1 mRNA, CYP1A2 mRNA and CYP3A mRNA (test group 2), which was partly reduced by simultaneous addition of RES (test group 3). The obtained in vivo data confirms results of numerous studies in vitro indicating ability of RES to hinder activation of various transcriptional factors, including Ah-receptor and to inhibit selectively gene expression of cytochrome P-450 isoenzymes.

THE ANTIDEPRESSANT-LIKE EFFECTS OF POMEGRANATE WHOLE FRUIT EXTRACT AND ITS COMPONENTS IN MICE

Mahsa Hadipour Jahromy, Shahrzad Khakpour, Jinuse Khorgami

Tehran Medical Branch, Islamic Azad University, Tehran, Iran

The putative antidepressant-like effects of pomegranate whole fruit hydro-ethanolic extract (PWFE) and its fraction have been evaluated on the performance of male mice in the forced swimming test (FST). A single dose (15 ml/kg p.o.) of PWFE, in male mice provoked a significant reduction of the immobility time ($p < 0.01$). Such effect was also observed with short-term treatment (7 days) with single doses of 10 ($p < 0.05$) and 15 ($p < 0.01$) ml/kg/day of PWFE. Additionally, in a different set of experiments, repeated administration in a 24-h period (24, 12 and 1 h before swimming test) with doses of 10.0 ($p < 0.01$) ml/kg p.o., of PWFE and 10.0 ml/kg p.o., ($p < 0.05$) of ethyl acetate fraction, provoked significant reduction of the immobility time of male mice in the FST. Moreover, it was noted important differences in the onset of the antidepressant-like effect in the FST, depending on the modality of treatment with PWFE (acute, short-term or repeated). Both, efficacy and potency were higher when repeated administration of PWFE was used, and surprisingly the dose of 10 ml/kg (24, 12 and 1 h before swimming test) was as effective as Fluoxetine. In the same way, the short term administration (7 days) improved significantly efficacy and potency of the PWFE in comparison to a single dose treatment. The ethyl acetate fraction submitted to TLC demonstrated phenolic components. These results indicate an antidepressant-like profile of action for the hydro-ethanolic extract and the component(s) of the ethyl acetate fraction obtained from PWFE which deserve further investigation.

EVALUATION OF CARBOHYDRATES IN TREATED HAIRY ROOTS CULTURE OF *SILYBUM MARIANUM*

***Tahereh Hasanloo*¹, *Roshanak Sepehrifar*¹, *Hassan Rahnama*²**

¹Department of Physiology and Proteomics, Agricultural Biotechnology Research Institute of Iran, Karaj, Iran

²Department of Tissue Culture and Gene Transformation, Agricultural Biotechnology Research Institute of Iran, Karaj, Iran, Email: thasanloo@abrii.ac.ir

To obtain further evidences for the involvement of primary metabolites in production of silymarin, the most potent antihepatotoxic from dried fruits of *Silybum marianum*. The content of different carbohydrates (sucrose, fructose and glucose) were assayed in hairy roots culture of *S. marianum* treated or non- treated with yeast extract. Hairy roots were induced for production of silymarin using *Agrobacterium rhizogenes* (strain AR15834) [1, 2]. The hairy roots were propagated in liquid Murashige and Skoog medium [3]. The biosynthesis of silymarin in treated hairy root cultures reached a maximum of 0.47 mg g⁻¹ DW, 72h after culture. The highest content of sucrose was achieved after 24h (0.35 μM g⁻¹ DW) that was higher than non- treated hairy roots (0.2 μM g⁻¹ DW) and the fructose and glucose content in treated hairy roots. The maximum contents of fructose and glucose reached to 0.39 and 0.23 μmol g⁻¹ DW after 48h YE treatment, which were higher than that of control. It is feasible that elicitation modulates the expression of primary metabolism molecules and regulates the levels of secondary metabolites.

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LIPID RAFTS AND POLYPHENOLS IN HUMAN HEALTH AND DISEASE

Raimo Hiltunen, Damien Dorman

Division of Pharmaceutical Biology, Faculty of Pharmacy, University of Helsinki,
P.O. Box 56 (Viikinkaari 5E), FIN-00014, Finland

Cellular membranes are amphipathic lipid bilayers containing a variety of biological molecules that participate in an array of biological processes, *viz.* cell adhesion, ion channel conductance, cell signalling, etc. Singer-Nicolson's fluid mosaic model proposed that membranes can be considered as a two-dimensional liquid through which lipids and proteins can freely diffuse [1]. However, that lateral motion was not necessarily free flowing but restricted. Karnovsky et al. proposed the concept of lipid domains, whereby lipids may have non-homogeneity in their lateral distribution and are thus organised into microdomains that may have functional and structural significance [2]. These platforms of sphingolipid- and cholesterol-rich domains containing a variety of signalling and transporter proteins are more ordered and tightly packed than their surroundings. In 1997, these domains were formally coined "lipid rafts" [3]. Different types of lipid raft have been identified, distinguished according to their protein-lipid composition.

As the roles of these domains has emerged, the most identified function appears to be to provide an environment for signalling molecules and receptors, *e.g.* doubly acylated tyrosine kinase Src family members, G-proteins, angiotensin II, eNOS, death complex receptor etc. As activation number of these proteins are associated with a variety of diseases, these lipid rafts may act as an interesting target for pharmacological approaches in the cure and prevention of specific diseases, *e.g.* Alzheimer's disease, autoimmune diseases, cardiovascular diseases, cancer, etc. Plant polyphenolic compounds (PolyP) may be a novel source of pharmacologically active substances.

PolyP are compounds ubiquitously found throughout the plant kingdom and constitute a significant component of our diet. Though principally known as powerful antioxidants, PolyP appear to be rather promiscuous in their biological activities, demonstrating anti-aging, antibacterial, anti-cancer, anti-inflammatory, cell metabolism regulation, neuroprotective and pro/anti-apoptotic properties amongst others. The antioxidant action of PolyP may play a role, however, they may exert their effect via other mechanisms *viz.* interactions with membranes [5] and, more specifically, lipid rafts. PolyP are known to interact with biological membranes and affect the formation or disruption of lipid rafts [6]. Thus, as signalling and transport proteins which play a role in these disease appear to be dependent upon the integrity of lipid rafts, polyphenols may exert a beneficial effect on these diseases through this mechanism.

In this presentation, the history and composition of lipid rafts will be reviewed as well as their roles in cellular signalling, surface receptor clustering/aggregation, etc. Thereafter, the effects of PolyP on the structure and function of lipid rafts will be discussed and their lipid raft-affecting role in the prevention of human disease will be explored with the aid of appropriate examples.



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CAN A TRIPLE HERBAL COMBINATION BE AN EFFECTIVE ALTERNATIVE TO BENZODIAZEPINES?

Isabelle Hörner¹, Christiane Staiger¹, Tankred Wegener², Marion Tschaikein¹

¹Merck Selbstmedikation GmbH, Darmstadt, Germany

²Consulting HMP, Weinheim, Germany

Benzodiazepines (BZD) are frequently prescribed hypnotics, but long-term intake may lead to the development of tolerance and dependency. During and after BZD withdrawal, patients seek for an effective and well tolerated alternative. Herbal drugs are popular due to their favorable profile of benefit-risk-ratio. This study investigated whether the triple herbal combination Kytta-Sedativum[®] Dragees (valerian root, hops strobiles, passionflower herb, Merck Selbstmedikation GmbH) is an alternative to BZD under everyday conditions.

The prospective, multicenter, observational cohort study recorded the safety and tolerability of the triple herbal combination in patients with non-organic sleep disorders. Five physicians collected the data of a total of 107 patients during three visits. Data on drug safety and efficacy was recorded during a phase of BZD withdrawal (approx. 2 weeks), followed by therapy with the study medication (for another approx. 4 weeks). Prior to the start, the study received a positive opinion by the Ethics Committee Münster.

At all examination visits, all clinical symptoms presented a marked improvement over the initial findings. However, the parameter sleep quality inquired from the patients changed only slightly (5.2%) during the first two weeks but improved markedly (by 31.4%) only after subsequent single-agent therapy with the herbal drug. 67.9% of the patients realized an improved sleep quality by the end of treatment. Particular improvement was recognisable for the clinical symptoms "pronounced tiredness" (by 65.8%) and "general unrest" (by 53.4%). No ADR occurred in any patient. The physicians rated the tolerability in 99.1% of the cases as "very good" or "good".

The results confirmed the positive benefit-risk-ratio of Kytta-Sedativum[®] Dragees. Patients under BZD withdrawal benefited from accompanying and subsequent therapy with the triple herbal combination (valerian root, hops strobiles, passionflower herb).

ANTICANCER EFFECTS OF LIGNANS FROM IN VITRO CULTURES OF *LINUM SERBICUM* ON HUMAN TUMOR CELL LINES

I. Ionkova, I. Antonova, G. Momekov

Faculty of Pharmacy, Medical University of Sofia, Dunav Str. 2, 1000 Sofia, Bulgaria

Some of the most effective cancer treatments to date are natural products or compounds derived from plant products. Podophyllotoxin (PTOX) is a lignan, which is used as educt for the semisynthesis of etoposide and teniposide. Both antineoplastic drugs are of importance for the therapy and in the future will be a high demand for the commercial drug and its precursors for the treatment of leukaemia.

As part of our ongoing program on the investigation of Bulgarian *Linum* species, extracts of *L. serbicum*, endemic species in the Balkan area, belong to the Section *Syllinum* were examined. We have established several callus and suspension cultures as described in [1] and checked for the occurrence of lignans. Aritetralin lignans 6-methoxypodophyllotoxin (6MPTOX) and β -peltatin were identified in the cultures. The both compounds, isolated for the first time from the in vitro cultures of this plant were identified by HPLC, UV and LC-MS. The main lignan in the suspension was 6MPTOX in both free and bound forms (as glycosides). The contents of 6MPTOX in suspension is 2.07 mg/g dry weight accompanied by β -peltatin 0.15 mg /g dry weight, an intermediate in 6MPTOX biosynthesis [2].

The antiproliferative action of the extracts was tested against malignant cell lines (the chronic myeloid leukemia – derived cell lines K-562 and LAMA-84, the Hodgkin lymphoma-derived HD-MY-Z and the human urinary bladder carcinoma-derived EJ cells) with etoposide as a positive control. The tested extracts reduced the viability of tumor cells in a concentration-dependent manner, whereby their relative potency was comparable or even superior to that of the referent drug etoposide. The extract from *L. serbicum* showed a moderate cytotoxicity to all tested cell lines with IC₅₀ in the range from 0.025 to 0.713 μ g/ml.

Acknowledgements: Financial support from Ministry of Education and Science, Sofia, Bulgaria (D002-104/08) is acknowledged.

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1-DEOXYNOJIRIMYCIN CONTENT AND ALFA-GLUCOSIDASE INHIBITORY ACTIVITY IN *SILKWORM* POWDER AS A CAM COMPONENT IN JAPAN

K. Yatsunami^{1, 2}, H. Tamiya³, K. Murata^{2, 4}, T. Kamei^{2, 4, 5}

¹Tamagawa University, Tokyo 194-8610, Japan

²Shimane Institute of Health Science, Izumo 693-0022, Japan

³BOMBYX Medicine Co.Ltd., Osaka 540-0011, Japan

⁴Kanazawa University Graduate School of Medical Science, Kanazawa 920-0934, Japan

⁵European University Viadrina Frankfurt (Oder), 15230 Frankfurt (Oder), Germany

Silkworm powder, containing 1-deoxynojirimycin (DNJ) and possessing alfa-glucosidase inhibitory activity, is expected to be used as a complementary and alternative medicine (CAM) component in Japan. *Silkworm* DNJ is known to have an antiviral effect against the human parainfluenza virus type 3. In this study, we investigated the biological function of the *Silkworm* powder as an alfa-glucosidase inhibitor. The *silkworm* powder (produced in South Korea) and mulberry powder (produced in Japan) were extracted with 75% ethanol. After derivatization with 9-fluorenylmethyl chloroformate (FMOC-Cl), the density of DNJ-FMOC in the extracts was measured with HPLC. Then, using 4-Nitrophenyl-alfa-D-glucopyranoside as substrate, the alfa-glucosidase inhibitory activity of both the *silkworm* and mulberry powders for pig liver crude enzyme was measured. The density of DNJ in *silkworm* powder (ranging from 0.39% to 0.58%) was higher than in mulberry powder (ranging from 0.08% to 0.12%). The alfa-glucosidase inhibitory activity of *silkworm* powder exceeded the inhibitory activity of both mulberry powder and commercial green tea powder. Therefore, these results suggested that the utilization of *silkworm* powder as an anti-diabetic component for CAM in Japan could be expected in the near future.

MULTI-TARGET ACTION OF A HERBAL MEDICINE IN DYSPEPTIC HEARTBURN

**Heba Abdel-Aziz¹, Olaf Kelber², Bettina R. Vinson², Samuel N. Okpanyi²,
Mario H. Müller³, Mohamed T. Khayyal⁴**

¹Faculty of Pharmacy, Ahram Canadian University, Cairo, Egypt

²Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany

³Department of Surgery, Ludwig-Maximilians University, Hospital Grosshadern, Munich, Germany

⁴Faculty of Pharmacy, Cairo University, Egypt

Heartburn is a bothering gastroesophageal symptom seriously impairing quality of life [1]. Recent results of gastroenterological research have shown, that, other than often supposed, enhanced acidity is involved only in a small proportion of patients with heartburn. Instead of this an impaired accommodation of the gastric volume, a reduced gastric transport, a reduced tone of the lower oesophageal sphincter leading to gastroesophageal reflux, or an increased susceptibility of the oesophageal mucosa to the attack of gastric acid. Randomized controlled clinical trials have shown the efficacy of the herbal medicine STW 5 (Iberogast) in dyspeptic heartburn [2, 3], and a systematic review has identified the following potential mechanisms of action:

A - Enhancement of muscular tone in the lower oesophageal sphincter, adding to the inhibition of gastric reflux into the esophagus [4].

B - Relaxation of fundus and corpus, allowing for a better adaptation of the gastric volume to ingested food, so reducing intragastric pressure [5, 6]

C - Prokinetic effect in gastric antrum, so improving gastric function. An onset of this effect within 10 min after oral intake of STW 5 has been shown [5, 6].

D - Protection of the oesophageal mucosa against acidic reflux, as inhibition of mucosal inflammation in an experimental model of acidic reflux has shown [7].

E - Inhibition of increased acid secretion, potentially reducing the exposition of esophageal mucosa to acid. [8].

Due to this multi-target action [9] STW 5 can even be a more rational treatment option for dyspeptic heartburn than antacids or proton pump inhibitors (PPIs).

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EFFECT OF PLANT EXTRACTS AND THEIR COMBINATION ON HISTAMINE-STIMULATED INTESTINAL TISSUE PREPARATIONS PRODUCING ROS

Katrin Wald¹, Olaf Kelber², Dieter Weiser², Helmut Heinle¹

¹Institute of Physiology, University of Tuebingen, Germany

²Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany

STW 5 (Iberogast[®]) is a fixed combination of nine hydro-ethanolic extracts, eight prepared from herbal drugs (*Angelica archangelica* root, *Matricaria chamomilla* flowers, *Carum carvi* fruits, *Chelidonium majus* herb, *Melissa officinalis* leaf, *Glycyrrhiza glabra* root, *Silybum marianum* fruits, *Mentha piperita* leaf) and one fresh plant extract (*Iberis amara* totalis), with clinically proven efficacy in the treatment of irritable bowel syndrome (IBS) and functional dyspepsia (FD) [1].

The pathogenesis of IBS and FD is still under discussion, but inflammation, and in this context reactive oxygen species (ROS), seem to promote the symptoms, which are e.g. altered bowel habits like obstipation or diarrhoea, pain and other digestive problems [2, 3]. In order to simulate oxidative stress in the gut, we showed, using luminol-enhanced chemiluminescence, that ROS production in mucosal preparations of mouse small intestine can be induced by stimulation with histamine.

We then investigated the effects of STW 5 and its single extracts on the ROS production. The extracts and their combination can reduce this increased ROS production dose-dependently; *Mentha piperita* leaf extract showed the strongest quenching effect, whereas *Chelidonium majus* herb had the weakest effect.

Further experiments will show whether the observed antioxidative effects of the herbal extracts and their combination are due to synergistic activities, and how the single chemical compounds present in the extracts contribute to their action.

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STW 1 OR STANDARD NSAIDS IN BACK PAIN SYNDROME AND GONARTHROSIS: RETROSPECTIVE STUDY IN A MEDICAL SPECIALIST PRACTICE

Martin Adler¹, Jürgen Müller², Olaf Kelber², Dieter Weiser²

¹Specialist for General, Naturopathic, Homeopathic, Environmental and Nutritional Medicine, Siegen, Germany

²Scientific Department, Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany

A large number of clinical studies [1] and also metaanalyses [2, 3] have demonstrated the efficacy of STW 1 (Phytodolor[®]), a fixed herbal combination phytomedicine containing extracts from *Populus tremula*, *Fraxinus excelsior* and *Solidago virgaurea*, exerting a multi-target effect in the therapy of painful diseases of the support and locomotor system.

For documenting the use of STW 1 in a specialist medical practice, a retrospective study was conducted. Using a detailed case report form, data from 300 patients have been included into this retrospective study. Analysis of variance with repeated measures was conducted (SPSS 15.0). 63% of the patients had a back pain syndrome, 28% gonarthrosis. 72% of them have been treated with STW 1 (4x30 drops), 14% with diclofenac (75 mg) and 14% with ibuprofen (600 mg). It could be shown, that symptoms of patients treated with STW 1 improved significantly. This was the case for subjective symptoms, as pressure induced pain and impaired motility, as well as for objective parameters (walking distance, manual force, ascending stairs). The efficacy of STW 1 (4x30 drops) was comparable to or even tended to be better that of diclofenac (75 mg) and ibuprofen (600 mg). The improvement of symptoms continued over the whole observational period (up to 18 month). The same was the case with CRP.

Tolerability of STW 1 was distinctly and significantly better than that of diclofenac and ibuprofen, especially regarding gastrointestinal symptoms. With STW 1, no drug interactions or habituation were observed.

These data demonstrate that the phytomedicinal approach, and especially STW 1 (Phytodolor[®]), has significant advantages over standard NSAIDs in clinical practice, so encouraging a broad use of this phytomedicine.

Acknowledgements: We thank Diana Long, Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany, for the data base handling.

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PROTECTIVE EFFECTS OF THE *ALTHAEA* ROOT EXTRACT STW 42 AND *ALTHAEA* POLYSACCHARIDES ON HUMAN ORAL MUCOSA CELLS

**F. Janina Zippel¹, Alexandra Deters¹, Niels Hellenbrand¹, Olaf Kelber²,
Dieter Weiser², Andreas Hensel¹**

¹Institute of Pharmaceutical Biology and Phytochemistry, University of Muenster, Germany

²Scientific Department, Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany

Althea officinalis L. root extract is successfully used for the treatment of oral or pharyngeal irritation and associated dry cough, especially in children of all ages, including infants. This has been documented in an observational study [1] with Phytohusstil[®] (active ingredient: STW 42, aqueous *A. officinalis* extract, 1:19.5-23.5). As its effect has been often assessed as being merely symptomatic, e.g. by the HMPC in its recently published monograph draft [2], a study has been conducted with the aim to identify mechanisms of action which point to an active, causal therapeutic effect in mucosal cells, beside its well characterized protective physical effect by formation of a bioadhesive polysaccharide layer [3].

The mode of action of STW 42 and of raw polysaccharides from the extract (RPS) was tested on a human nasopharynx mucosal cell line (KB-cells) and primary human dermal fibroblasts under *in vitro* conditions. In KB-cells, RPS (1-10 µg/mL) significantly increased cell vitality, measured as reduction of the tetrazolium salt WST-1, while STW 42 had no significant effect. Cellular proliferation, measured by BrdU-incorporation ELISA, was significantly stimulated by STW 42 (10 µg/L), while RPS had this effect only at 100 µg/mL. By laser scanning microscopy it was shown that the high molecular RPS, labelled by fluorescein 5-isothiocyanat (FITC), was internalised by endosomal transport into KB-cells, but not into fibroblasts, after incubation of the cells for 14 h.

These results allow the conclusion, that the aqueous *A. officinalis* root extract STW 42 has an active influence on the cell physiology of epithelial mucosal cells. *A. officinalis* polysaccharides can even be internalised by mucosal cells. This points to a not merely demulcent, but actively protective action, stimulating mucosal regeneration, so having a causal therapeutic effect in mucosal irritation and resulting dry cough.

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PHYTOCHEMICAL AND PHARMACOGNOSTICAL STUDY OF *OENOTHERA BIENNIS* L., GROWN IN MOSCOW AREA

A.S. Khomik¹, V.V. Vandishev¹, G.I. Klimakhin², S.N. Suslina¹

¹Department of General Pharmaceutical and Biomedical Technology; Department of Botany, Plant Physiology, Plant Patology and Biotechnology, Russian University of Peoples Friendship, 6, Miklukho-Maklaya str., 117198, Moscow, Russia

²All-Russian Research Institute of Medicinal and Aromatic Plants, 7, Grina str., 117216, Moscow, Russia

Oenothera biennis L. (Onagraceae family), is the grassy plant that is wide extended in Europe, central Russia and the Far East. The agricultural technology of *Oenothera biennis* cultivation in Moscow suburbs was developed in All-Russian Research Institute of Medicinal and Aromatic Plants. The oil, obtained from seeds of this plant, is a significant source of γ -linolenic acid. The phytochemical and pharmacognostical investigation of seeds and other parts of *Oenothera* was the aim of the present work. The seeds and grass, grown up in Moscow Region were used for analysis. Thin-Layer chromatography (TLC), ultraviolet (UV) spectrophotometry and qualitative reactions were carried out for phytochemical research of raw materials. The anatomic features of plant raw materials were investigated by the use of the passing light microscopy with a Nikon Eclipse 90i, and the scanning electronic microscopy with a Jeol JSM-6490 LV. It was established that extract has been received from degreased seeds after fat oil isolation, is enriched by a polyphenolic complex. The presence of (+)-catechine as a minor component was found out by TLC. Single intensive maximum of UV absorption at the wave length of 280 nm was observed in a solution of an extract for the region from 220 nm till 500 nm. By light and scanning electron microscopy diagnostic signs of *Oenothera biennis* seeds and leaves anatomic structure were defined. The presence of monocelled, simple warty trichomes, epidermis structure of leaves, and the architecture of microscopic section and a surface structure of seeds proves the real authenticity of raw materials. The main conclusion is that *Oenothera biennis*, cultivated in the conditions of Moscow suburbs, can be a perspective source of valuable oil and polyphenolic substances.



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IDENTIFYING BIOACTIVES IN THE FOOD CHAIN

Claus Kilpert

DSM Nutritional Products, P.O. Box 2676, Bldg 203/135b, 4002 Basel, Switzerland

Functional food is an emerging market. Nutraceuticals as food supplements should have a long-term effect in preventing a wide range of medical conditions, but many products lack efficacy and cannot deliver what they promise. DSM Nutritional Products as the world's leading vitamins supplier follows a scientific and target-based discovery approach to find and develop new products for this field as it was presented in earlier Phytopharm conference 07.

A permanent challenge is to improve the highly interdisciplinary process by provision of the relevant information (ethno-nutrition purposes, known metabolites and their bio-profiles, marketing aspects). We have to select the most promising samples from food sources for follow up based on that. The trend is to speed up the process by feeding the screening systems with smart qualified sets instead of huge libraries. The DNP edible plant extract library represents a unique asset in the industry. In order to maximize it's utility and ensure it's efficient use in projects a number of possibilities for improvement are proposed and will be presented.

SO-CHEONG-RYONG-TANG (SCRT), A HERBAL MEDICINE, MODULATES INFLAMMATORY CELL INFILTRATION VIA REGULATION OF IL-17 AND GM-CSF IN ALLERGIC ASTHMA

Hyung Woo Kim¹, Jae Seung Kang², Su In Cho¹, Wang Jae Lee²

¹Division of Pharmacology, School of Oriental medicine, Pusan National University, Republic of Korea

²Department of Anatomy and Tumor Immunity Medical Research Center, Seoul National University College of Medicine, Republic of Korea

So-Chung-Ryong-Tang (SCRT), an herbal medicine, has been used for the control of respiratory disease in East Asian countries. However, its therapeutic mechanisms, especially an inhibitory effect on inflammatory cell infiltration and airway remodelling in allergic asthma are unclear. We investigated the influence of SCRT on levels of IL-17, GM-CSF, IL-4, and IFN- γ in bronchoalveolar lavage fluid (BALF), OVA-specific IgE in serum, and histopathological changes in allergen-induced asthma. In our results, SCRT decreased levels of IL-17 and GM-CSF in BALF (fig. 1). IL-4, a Th2-driven cytokine, was also decreased by SCRT, but INF- γ , a Th1-driven cytokine, was not changed (fig. 2).

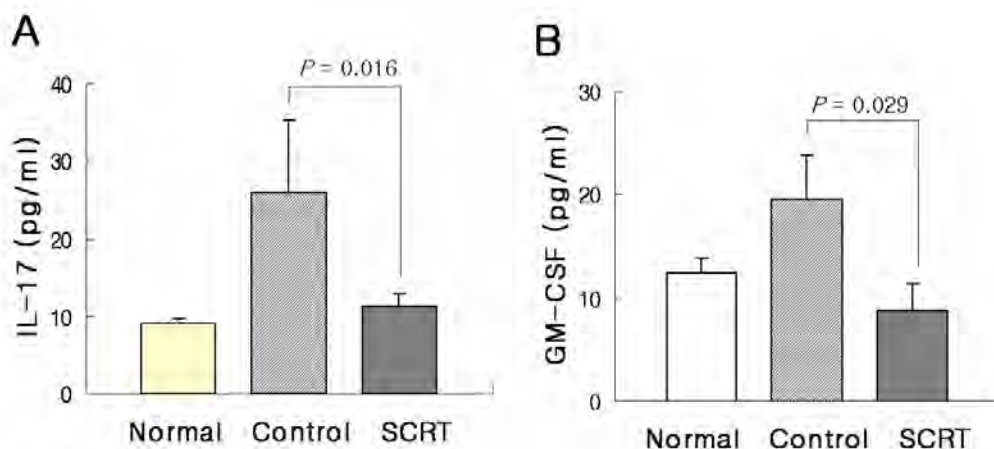


Figure 1. Effects of SCRT on production levels of IL-17 and GM-CSF in BALF

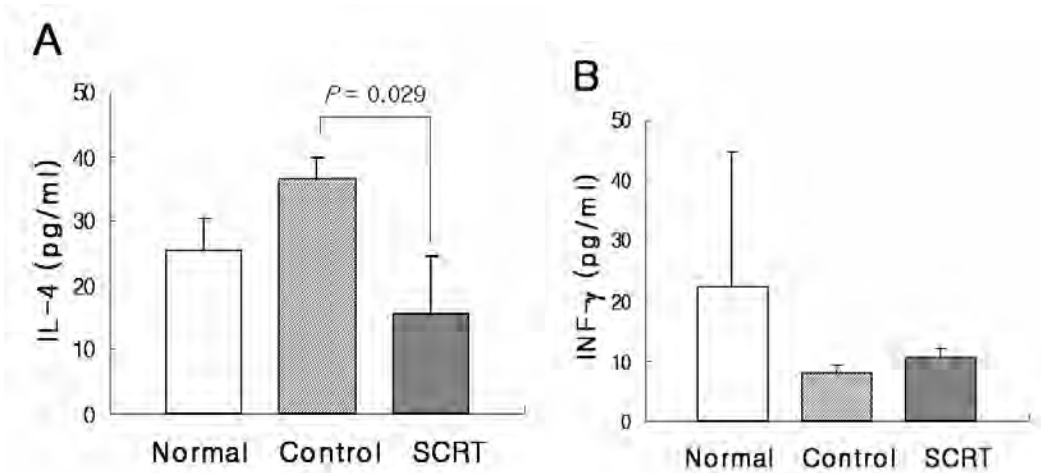


Figure 2. Effects of SCRT on production levels of IL-4 and IFN-γ in BALF

Levels of OVA-specific IgE in serum were also decreased by SCRT (fig. 3).

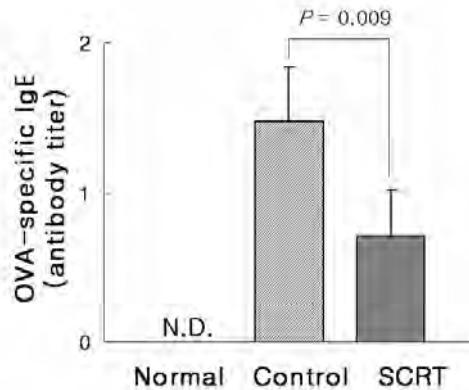


Figure 3. Effects of SCRT on levels of OVA-specific IgE in serum

With SCRT treatment, histopathological findings showed reduced tendency of inflammatory cell infiltration, and prevention from airway remodeling such as epithelial hyperplasia. In conclusion, we firstly demonstrated that regulation of IL-17 and GM-CSF production may be one of mechanism contributed to reduction of inflammatory cell infiltration and prevention from airway remodeling.

AN IMPORTANT THYME SPECIES FOR SEMI-ARID CLIMATIC CONDITIONS OF TURKEY: *THYMBRA SPICATA* VAR. *SPICATA* L.

Süleyman Kizil¹, Onur Ymanoğlu¹, Menşure Özgüven²

¹Department of Field Crops, Agriculture Faculty, Dicle University 21280, Diyarbakır, Turkey, kizils@dicle.edu.tr; tel: +90 412 2488509; fax: +90 412 2488153

²Department of Field Crops, Agriculture Faculty, Çukurova University 01330, Balcali-Adana, Turkey

Thymbra spicata L. var. *spicata* is naturally found as wild in Thracian, Aegean, Mediterranean coastal and Southeastern Anatolia areas of Turkey. *Thymbra* (Labiatae) is represented in Turkey by four taxa belonging to two species (*T. spicata* var. *spicata* and *T. spicata* var. *intricata*). *T. spicata* var. *spicata* is perennial plant that prefers dry sunny hillsides and high dry meadows in warmer and semi-arid climatic conditions Mediterranean and Southeastern Anatolia, Turkey. In the Southeast Anatolia, *T. spicata* var. *spicata* is known as "Zahter".

This study aimed to use *T. spicata* plants under natural conditions in various parts of Southeast Anatolia and Mediterranean regions of Turkey, and determine of variations among its characteristics based on localities and use the seeds for using further breeding studies. It was observed that *T. spicata* plants grow at altitude of 121 m to 1249 m. Essential oils from aerial parts of *T. spicata* were obtained by hydrodistillation. Their components were identified by gas chromatography/mass spectrometry. Essential oils of collected samples in different locations ranged 1.56 to 3.27%. These samples showed carvacrol as dominant chemotype with 72.67-94.22% content. It was concluded that wild populations of *T. spicata* in Turkey are represented by the carvacrol-containing chemotypes, whereas, *Thymbra spicata* L. var. *intricata* endemic to Turkey is represented by both carvacrol and thymol-containing chemotypes. In this study, all investigated locations showed carvacrol containing chemotype.

HIPPOPHAE RHAMNOIDES L. – INVESTIGATION EXPERIENCE AND FUTURE APPLICATION

V.M. Kosman

Interregional center Adaptogen, St-Petersburg, Russia

Sea buckthorn (*Hippophae rhamnoides* L.) is one of the famous and long time used medicinal plant known from an ancient time till present. The natural habitat of this two house bushes or small tree is China, Mongolia and Russia. This plant is widely cultivated in various parts of the world, including Canada, Northern Europe, and Russia. Selection was dimensioned to fruit size, yield, nutraceutical value, oil content and thorns absence. Berries are a source of various biologically active substances such as carotenes, tocopherols, ascorbic acid, fatty acids, sterols etc.

Sea buckthorn has applications in cosmetic, pharmacy and food industries. Whole fresh and frozen berries are used for manufacturing of various food products – jam, jelly, compote, sauce, juice, liquor, candy, tea, biscuits. Oil, extracts are used as component of capsules or tabulated forms in food supplements with vitamin, antioxidant properties. Extracts and oil are used in cosmetics in creams, tooth paste, tonics, and shampoo. Berries are included in Russian State Pharmacopoeia and Chinese Pharmacopoeia. In China oil from whole berries, oil from seed in capsules and tablets are used as drugs, in Russia - oil in capsules and in aerosols or sprays (Olasol, Hyposol). These drugs are recommended as anti-inflammatory agents to stimulate reparative and regenerative processes for external (skin diseases, burns treatment) and internal application (gastric ulcer, stomach and intestine diseases). The most valuable medicinal product is oil obtained by various technologies: cold pressure, diffusion extraction with fatty oils, extraction with organic solvents, CO₂ extraction. The first way gives the smaller yield but a much higher quality of the oil, especially for medicinal purposes.

It is perspective to create new kinds of drugs, food supplements for human and animal application on a base of natural (cold pressure) Hippophae oil and its byproducts to make medicinal usage of this plant more wide and effective.

STANDARDIZATION OF THE TABLETS WITH *ECHINACEA ANGUSTIFOLIA* AND *SALVIA OFFICINALIS* DRY EXTRACTS

V.M. Kosman¹, S.I. Guseva², O.N. Pozharitskaya^{1, 3}, A.N. Shikov^{1, 2, 3}

¹Interregional center Adaptogen, Saint-Petersburg, Russia

²Saint-Petersburg State Medicine Academy named after I.I.Mechnikov, Russia

³Saint-Petersburg Institute of Pharmacy, Russia

Combinations of a few different plant extracts with various pharmacological properties in one composition make it possible to obtain new effective and nontoxic drugs. Such tablets of *Echinacea angustifolia* herb dry extract (Euromed, Spain), *Salvia officinalis* leaves dry extract (Cognis Iberia, Spain), *Salvia officinalis* flowering weed essential oil and ascorbic acid are proposed as drug with anti-inflammatory and immunostimulation activity.

The aim of the present work was to develop parameters and methods for standardization of this drug, evaluate the parameters values and validate these methods.

The following main tests were proposed for standardization: identification and titrimetric assay of ascorbic acid after reaction with sodium 2,6-dichlorophenolindophenol solution [1], identification tujone and cineole as principal compounds of *Salvia* essential oil by TLC [2], identification the principal phenolic compound of *Salvia* (luteolin glucoside, caffeic and rosmarinic acids) and *Echinacea* extracts (caftaric, caffeic and cichoric acids) and assay of total phenolic compounds by HPLC.

The quantification limits were estimated after analytical results obtained for several tablets batches. Limit for ascorbic acid content should be 46.25-53.75 mg/tablet, total phenolic compounds – not less than 0.5 mg/tablet.

The methods were validated according to the ICH guidelines on the validation of analytical methods and [3]. The validity of chromatographic assays was established through a study of Specificity, Robustness, Linearity, Repeatability and Accuracy. The satisfactory results were obtained for all validation parameters.

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QUANTITATIVE ¹H-NMR DETERMINATION OF STACHYDRINE IN *LEONURUS JAPONICUS* (PH.SIN.)

***K. Kuchta*¹, *J. Ortwein*², *D. Brief*², *H.W. Rauwald*¹**

¹Department of Pharmaceutical Biology, Leipzig University, Johannisallee 21–23, D-04103 Leipzig

²Department of Pharmaceutical Chemistry, Leipzig University, Brüderstraße 34, D-04103 Leipzig

The aerial parts of *Leonurus japonicus* Houtt. as well as its seeds have been used in TCM against abnormal menstruation, postpartum abdominal pain, uterine bleeding, blood circulation disorders, oedema, eczema, and purulent abscess since at least 1800 years [1, 2, 3]. In recent years, its use by European TCM therapists has increased so far that trials for the cultivation of the herb in Germany were started and successfully completed [4]. In Ph.Sin. [3] the "alkaloids" of the tee preparation are regarded as the active constituents and in summary quantified photometrically after Reinecke's complexation as the betaine stachydrine (N-dimethyl-L-proline). Unfortunately, even well experienced phytochemical laboratories in Europe have failed to achieve any reproducible results with this Ph.Sin. method up to now (4).

In recent years, quantitative nuclear magnetic resonance (qNMR) has been developed into a viable means of phytochemical analysis (5, 6). In the present study, a reproducible qNMR method for the specific quantification of stachydrine in seeds and aerial parts of *L. japonicus* has been established using the N-methyl protons in cis-position to the carbonyl group as markers and maleic acid as internal standard. The measurements revealed stachydrine contents between 0.18 and 0.21% in the seeds. With one exception at 0.18%, all aerial part drug samples ranked above the minimum content of 0.40% according to Ph.Sin. (3) with the highest amount measured at 1.01% thus enabling regular quality control of this important TCM drug.

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A STUDY OF AMINOACID CONTENT OF SOME PLANTS FROM FABACEAE AND ASTERACEAE FAMILIES

O.A. Kyslychenko, U.V. Karpiuk, O.M. Yakymenko

The National University of Pharmacy, Kharkov, Ukraine

Search for plants with a rich chemical composition is of great importance for drug-developers and manufacturers nowadays. The plants of Fabaceae and Asteraceae families are believed to have a good potential to be used in modern pharmaceutical industry. The aim of our research was to make a study of amino acids of the *Glycine hispida* grass at its blooming stage and of *Achillea ptarmica* L. leaves and flowers.

A qualitative composition was studied by paper chromatography with the authentic amino acids sample-carrying solvents. The T339M Mikrotechna–Praha amino acid analyzer was used to study amino acids composition at the next stage of the research. The compounds under study were identified by comparison of the sample retention time with the retention time of prepared compounds standards in mixes and individual forms. The content of each component was calculated as a ratio of an appropriate peak area to the sum of all peak areas. 17 free and 17 bound amino acids were detected in the *Glycine hispida* grass of the Feya species; while the *Glycine hispida* grass of the Podilska species contained 17 bound and 14 free amino acids, 9 of which are irreplaceable including threonine, valine, methionine, isoleucine, leucine, phenylalanine, histidine, lysine and arginine.

The content of irreplaceable amino acids in a mix is one of the indices of the biological value which should be about 45-50%. The total quantity of free and bound irreplaceable amino acids in the Feya and Podilska species soy grass was 48.5% and 60% correspondingly. 18 amino acids, 9 of which are irreplaceable (just like in the soy grass) were found in the *Achillea ptarmica* L. leaves and flowers. The total quantity of free and bound irreplaceable amino acids made up 59% and 52% in the *Achillea ptarmica* L. leaves and flowers correspondingly. Thus, we can arrive at the conclusion that the soy grass of the Feya and Podilska species and the *Achillea ptarmica* L. leaves and flowers have a high biological value.

Our findings have proved that the soy grass in its blooming stage and *Achillea ptarmica* L. leaves and flowers are rich in amino acids and can be used to make medical preparations, biologically active food supplements and special purpose foodstuffs.

DEVELOPMENT OF A HERBAL SPECIES FOR TREATMENT OF GASTROENTERIC DISEASES

O.A. Kyslychenko, Ye.V. Gladukh, O.S. Kukhtenko, L.I. Shulga, Iu.S. Kolisnyk

The National University of Pharmacy, Kharkov, Ukraine

Treatment of gastroenteric diseases is a very important problem of modern therapy and requires new efficient and safe drugs. Plant-based drugs are known to be less toxic than synthetic anti-inflammatory drugs and can be used over a long period of time without any side effects. Therefore herbal species have good prospects for intensive use in the future and should be the subject further studies in medicine related areas.

The aim of our work was to create and to study herbal species combined from different species of medicinal herbal raw material based on their pharmacological properties.

Modern technological, instrumental, chemical and biological methods recommended by European Pharmacopoea and Ukrainian State Pharmacopoea were used to develop herbal species composition and to control their quality.

Theoretical and experimental research has shown the expediency of further pharmacological research of the developed species as a remedy for prevention and treatment of gastroenteric diseases. This species contains yarrow grass, St.-John's-worth grass, calendula flowers, peppermint leaves and chamomile flowers.

Authenticity and high quality indicators of the herbal species have been determined including micro- and macroscopic identification of the herbal species, characteristics of UV-spectrum and numerical indicators such as moisture, moisture absorption voluminous and bulk density, poriness, porosity, wash-out quotient. A draft of normative analytical documentation for the herbal species has been developed.

Extraction parametres of biologically active substances in infusion from the herbal species and its components have been studied. Raw material particle sizes and an optimal method of an herbal species water extract preparation have been established. An impact of ground raw material particle sizes on the extract output has been studied. That helped us to develop an effective technology of the plant species.



NEW APPROACHES TO THE STANDARDIZATION OF RAW MATERIALS IN ACCORDANCE WITH REQUIREMENTS OF STATE PHARMACOPOEIA OF UKRAINE

O.A. Kyslychenko, V.A. Georgiyants, I.N. Vladymyrova, U.S. Prokopenko

The National University of Pharmacy, Kharkov, Ukraine

Creation of single state legislative base on quality control of both medicinal raw materials and prepared dosage forms on their basis is an important task for today. As Ukraine took a course on integration in to European Union, State Pharmacopoeia of Ukraine (SPhU) is harmonized with European Pharmacopoeia (EP). An actual task on this stage was development and introduction of monographs on medicinal raw materials. The Ukraine is first postsoviet country, which published the SPhU in 2001.

The main criteria of introduction of medicinal raw materials in to SPhU are wide usage, presence of raw material, folk and scientific medicine at the market of Ukraine, presence of preparations, containing this medicinal raw materials, registered in Ukraine, presence of monographs on raw material in different pharmacopoeias (State Pharmacopoeia of USSR XI, EP, British pharmacopoeia, Deutsches Arzneibuch, Russian pharmacopoeia, USP NF etc.).

So, the purpose of our work was development of the list of medical plants for introduction of monographs in SphU.

The medicinal natural extracts: Icelandic moss (*Cetraria islandica* L.) raw material is standardized by EP monograph "Iceland moss". The main test is based on chromatography for caffeic acid and anethol. Common Fumitory (*Fumaria officinalis* L.) is included in the EP of "Fumitory" and Deutsches Arzneibuch of "Fumariae herbae", raw material is standardized on quantitative content of alkaloids in a count on fumarin; a Celandine greater (*Chelidonium majus* L.) is by officinalis raw material of State Pharmacopoeia of USSR XI "Herba Chelidonii" and EP "Greater celandine", raw material of which is standardization quantitative content of alkaloids in a count on chelidonin were researched according to methodics described in EP 2008. As a result projects of the monographs were developed. Conclusion: thus, coming from conception of harmonization of legislative base on control of quality of preparations and medicinal raw materials with requirements EP, development of monographs on the indicated types of medical plants is the actual task of SPhU.

GS-MS ANALYSIS OF THE ESSENTIAL OIL FROM *CORIANDRUM SATIVUM* L. FRUITS

V.S. Kyslychenko¹, I.I. Terninko², O.I. Neshcheret¹, V.A. Khanin¹

¹The National University of Pharmacy, Kharkov, Ukraine

²Lugansk State Medical University, Lugansk, Ukraine

Coriandrum sativum L. is an annual grassy plant from Apiaceae family that comes from Eastern area of the Mediterranean and is cultivated in Ukraine as the source of essential oils. It is also used as spicy-aromatic culture in food industry and as medicinal and odour plant for treatment different diseases in pharmaceutical and in perfumery industry. It is known, that the content of essential oil in *Coriandrum sativum* fruits may be from 0.7 till 7.5% and depends on ecological conditions of growing.

The aim of our research was to study the composition of essential oils from *Coriandrum sativum* fruits gathered in different climate and geographical conditions (Ukraine and Israel).

The essential oils were obtained from reduced fruits by hydrodistillation. The chemical composition was determined by GC/MS analysis, performed on Hewlett-Packard gas chromatograph HP-6890 with mass-selective detector HP-5972. Identification of individual compounds was made by comparison of their mass spectra with their internal reference mass spectra electronic libraries Nist 02 and Wiley 138k.

The essential oil content in *Coriandrum sativum* fruits that were gathered in Ukraine was about 1.7%, that were gathered in Israel – 7%. In the essential oil from fruits that were gathered in Israel 20 compounds were determined. Linalool was the principal compound in this sample (78.06%). In the essential oil from fruits that were gathered in Ukraine 16 compounds were determined. Linalool also was the principal one (68.41%).

These data proved that the content and composition of the essential oil depends on plant growing conditions. The quantity of the essential oil from *Coriandrum sativum* fruits that were gathered in mild and hot climate conditions increases from 1.7 to 7%, the quantity of active substances increases correspondingly.

A PHYTOCHEMICAL STUDY OF FLOWERS AND LEAVES OF FOUR ORCHIS SPECIES

V.S. Kyslychenko, I.V. Yaroshenko, N.Ye. Burda, I.A. Zhuravel

The National University of Pharmacy, Kharkov, Ukraine

Medicinal plants of genus *Orchis*, family Orchidaceae are interesting for phytochemical studies since they belong to rare and disappearing species. The *Orchis* bulb mucus contains 30% of starch, 1% of sugars and cellulose. Salep is used in treating various intestinal diseases, cough, as a detoxicant as well as anti-inflammatory, and coating, binding, general tonic means, to improve the function of sexual glands for men. The plants from the family Orchidaceae including the plants of genus *Orchis* are under protection in many countries of the world including Ukraine, Germany, the Czech Republic, Russia. Since the use of the underground plant parts cause plant death a study of the *Orchis* species grass which chemical composition isn't sufficiently studied is of great interest.

The flowers and leaves of *Orchis militaris* L., *Orchis maculata* L. (*Dactylorhiza maculata* L.), *Orchis provincialis* Balb., *Orchis sphaerica* M.B., (*Traunsteinera sphaerica* (M.B.) Schlechter) were the objects of our research. These species are more often spread in Ukraine, in the Caucasus and in Turkey.

It is common knowledge that the *Orchis militaris* L. ground part is used to treat furunculosis and panaris while *Orchis maculata* L. is characterized by antibacterial activity.

Biologically active compounds were studied by means of qualitative reactions and chromatography the following compounds were detected in the flowers and leaves:

- a) organic acids including ferulic, chlorogenic, gallic, malic, oxalic, succinic, citric, benzoic, ascorbic;
- b) amino acids: cysteine, methionine, alanine, valine, asparagic acid, ornithine, glutamic acid, phenylalanine, leucine, arginine, serine, tryptophan, asparagine, histidine, ursolic acid;
- c) flavonoids including glycosides of quercetin, isoquercitin, kaempferol, and other polyphenol compounds.

The obtained data make us believe that *O. militaris* L., *O. maculata* L., *O. provincialis* Balb., *O. sphaerica* flowers and leaves are sources of biologically active compounds and may be used for developing of new phytodrugs.

THE PHYTOCHEMICAL STUDY OF *LAWSONIA INERMIS* L.

V.S. Kyslychenko¹, I.A. Zhuravel¹, M.O. Alekseeva¹, V.V. Kozhukhar²

¹The National University of Pharmacy, Kharkov, Ukraine

²The Poltavian University of Consumative Cooperation of Ukraine, Poltava, Ukraine

Perennial evergreen bushes *Lawsonia inermis* L. from Lythraceae family is cultivated in Egypt, India, tropics of America, Middle East countries. Leaves, caulescents, fruits and essential oil from henna flowers are widely used in cosmetics and traditional medicine. On the other hand, they are not used in officinal medicine because their chemical content hasn't been studied enough.

During last years scientists from many countries have found different pharmacological effects of henna, such as anti-microbe, anti-inflammatory, analgesic and anti-fever action of ethanol extracts. Lipophylic extracts have shown nootropic and anti-convulsion activity. Water extracts and suspensions from lawsonia leaves and cortex have hepatoprotective, antioxidant, anti-microbe anti-fungal and sun-protective effects. Henna leaves have cytotoxic, tuberculostatic activity and can also inhibit some enzymes. Lawson and ethanol extracts from henna leaves inhibited tripsyn. It was also found the anti-parasitic action, especially for trypanosomes. Henna leaves have shown modulator effect in experiments with mice with different tumors. Chloroform extracts from lawsonia leaves have cytotoxic effect on some kinds of carcinoma. Leaves, cortex and seeds of *Lawsonia inermis* L. have cytotoxic activity on mollusks. Lawsonia is almost non-toxic, side effects haven't been found. Reserves of raw material of *Lawsonia inermis* L. in the world are great and this plant can be cultivated in Ukraine. Everything written above can explain the great interest to the further studying of this plant.

We have made the phytochemical research of leaves and caulescents of *Lawsonia inermis* L. Flavonoids, coumarines, hydroxycinnamomic acids, tannins, organic acids, triterpenoid substances, carbohydrates, ascorbinic acid have been found. The total content of phenolic compounds is 4-5%, flavonoids – 0.7-1.0%, hydroxycinnamomic acids – 1.9-2.0%. These obtained results prove the perspective of further studying of leaves and caulescents of *Lawsonia inermis* L. for working out new drugs and cosmetics creation.

SALVIANOLIC ACID B PROTECTS ENDOTHELIAL CELLS FROM OXIDANT-MEDIATED DAMAGE

Xue-Jun Li

Department of Pharmacology, School of Basic Medical Sciences, Peking University, Beijing 100083, P.R. China

Salvianolic acid B (Sal B) is considered as one of the most active anti-oxidant and the major pharmacological component of the herb, *Salvia miltiorrhiza*. Its beneficial effects including hepatoprotection, elicitation of endothelium-dependent vasodilation, lowering of blood pressure in hypertension, inhibition of HIV-1 replication and suppressing inflammatory cytokine-stimulated endothelial adhesiveness to human monocytic cells by its strong antioxidant activities.

Our study demonstrated the protective effects of Sal B on hydrogen peroxide (H₂O₂)-induced injury in human umbilical vein endothelial cells (HUVECs). Treatment with H₂O₂ significantly decreased the cell viability and increased the lactate dehydrogenase (LDH) leakage that is an apoptotic feature. Pretreatment with Sal B prevented significantly from H₂O₂-induced cell apoptosis and other damages in a concentration-dependent manner. The mechanisms of Sal B protection was studied with two-dimensional gel electrophoresis (2-DE) coupled to hybrid quadrupole time-of-flight mass spectrometry (Q-TOF) mass spectrometer. Data base searching implicated glucose-regulated protein 78 (GRP78), a central regulator for ER stress, was up-regulated in Sal B-exposed HUVECs. After exposure to Sal B, the level of activating transcription factor 4 (ATF4) was raised, with a transient phosphorylation of the α subunit of eukaryotic translation initiation factor (eIF2 α). Knock-down of GRP78 by siRNA significantly reduced protective effects of Sal B. These results suggest that Sal B-induced GRP78 upregulation via phosphorylation of eIF2 α and resultant translation of ATF4. And up-regulation of ER chaperones induced by Sal B may play an important role in protecting human endothelial cells from oxidative stress-induced cellular damage.

STUDY OF ANTIBACTERIAL AND TOXIC PROPERTIES OF SOME APHYLLOPHOROID FUNGI (PRELIMINARY RESULTS)

E.A. Luginina

Russian Research Institute of Game Management and Fur Farming, Engels st, 79, Kirov, Russia, 610000, +7(8332)353715 e-mail: e.luginina@gmail.com

The following species of aphylloroid fungi are studied for antibacterial activity and toxicity: *Hericium ramosum*, *Laetiporus sulphureus*, *Flammulina velutipes*, *Hydnum rufescens*, *Coltricia perennis*, *Albatrellus ovinus*, *Fomitopsis pinicola*, *Piptoporus betulinus*.

Antibacterial properties were investigated by disc-diffusion method using cultures of *Bacillus cereus* ATCC10720, *Staphylococcus aureus* ATCC25178, *Escherichia coli* 803, *Pseudomonas aeruginosa* PA26, *Yersinia pestis* EV. Significant antibacterial effect was marked for *F. pinicola* (zone of growth delay of *B.cereus* 2-2.5mm, *E.coli* 1.5mm) and *P. betulina* (*B. cereus* – 2.5-3.0 mm, *S. aureus* – 1.5 mm, *E. coli* – 2.0 mm, *Y. pestis* – 1.0 mm). No lethal cases of white mice were marked after peroral injection of water decoctions during 7 days. There was definite worsening (refusal to food, slow reactions for outer irritants) for *A. ovinus* decoction. Experiments to evaluate the influence of water decoctions on "luminous" bacteria *E. coli* SG139 were done. After 2 minutes from decoction injection of *F. velutipes* and *P. betulinus* the definite luminescence oppression. This is considered to be connected with peptid coagulation.

Biotesting was done with seeds of rye ("Iren"), leaf mustard and white haricot. As a result it was defined that *F. velutipes* is absolutely toxic to plants. *F. pinicola* and *P. betulinus* did not impede of rye and mustard seeds' germination and their fixation on substratum; haricot seeds did not form sprouts.

Thus, preliminary investigation of antibacterial and toxic properties of some aphylloroid fungi was done. The most strongly marked antibacterial properties were revealed for *F. pinicola* and *P. betulinus*. The most toxic for animals is *A. ovinus*, *F. pinicola* and *P. betulinus* - for bacteria, *F. velutipes* – for plants. However received data need further studies and analyses.

STUDY OF ADAPTOGENIC ACTIVITY OF COMPLEX HERBAL COMPOSITION

M.A. Lazukina, M.N. Makarova, V.G. Makarov, Y.A. Gushchin, O.N. Pozharitskaya, A.N. Shikov

St-Petersburg Institute of Pharmacy, Russia

It is known, that stress is a characteristic feature of the modern life. Stress has several aspects, of which the following are essential: adaptive reaction of the organism, insufficient adaptive reaction, pathologically dependent adaptive reaction to stress, premature aging of the organism, induced by nervous, mental and metabolic exhaustion. Complex herbal adaptogen (EA) was developed for stress protection. Its composition included *Rhodiola rosea* L., *Melissa officinalis* L., *Tilia cordata* Mill. et *Tilia platyphyllos* Scop., *Thymus serpyllum* L., *Epilobium angustifolium* L., *Leonurus cardiaca* L., *Melilotus officinalis* Pall., *Crataegi*, *Humulus lupulus* L.

The purpose of the study was an evaluation of adaptogenic activity of EA phytopreparation in the model of sleep deprivation on mice. Female and male mice line C57Bl/6 were used in experiment.

Surviving rate after sleep deprivation and a classical triad of Selye's (the mass coefficients of the adrenal glands and thymus, and the number of destructions in gastric mucosa were determined) were evaluated. After the deprivation mice were subjected to the physiological tests: "open field" and "zoosocial interaction" tests. EA was administrated intragastrically by gavage daily in dose 3.0 ml/kg. Control group received placebo (berry mors). Significant anti stress effect was observed in group treated by EA. Surviving rate of animals was increased in 10% after sleep deprivation. Expressiveness of triad of Selye's both in females and males was less, than in animals of control group (tabl.).

Indices of Selye's triad in mice

| Group | Coefficient of adrenals mass (rank of indices) | Coefficient of thymus mass (rank of indices) | Pauls' index (rank of indices) |
|---|--|--|--------------------------------|
| Indices of Selye's triad in male mice | | | |
| Control (n=7) | 0,045±0,004 | 0,150±0,018 | 45,6 |
| EA (n=8) | 0,032±0,001* | 0,197±0,001* | 5,5 |
| Indices of Selye's triad in female mice | | | |
| Control (n=7) | 0,035±0,003 | 0,120±0,017 | 34,2 |
| EA (n=8) | 0,025±0,003* | 0,170±0,016* | 14,7 |

* — Significantly different from control group (p<0.05)

EA effectively normalized psychoemotional state of animals that was confirmed by the absence of motivational duality and vocalization (fear) in the zoosocial interaction test. The data allow recommending EA phytopreparation for further study, including clinical.

EFFICACY OF PHYTO PREPARATIONS IN EXPERIMENTAL MODEL OF CERVICAL LYMPHADENITIS IN RATS

M.N. Makarova M.N., S.V. Tesakova, S.I. Guseva, K.L. Kryshen, T.V. Abrashova, V.G. Makarov

Saint-Petersburg Institute of Pharmacy, Russia

Research and development of anti-inflammatory and immunostimulator preparations for the treatment of respiratory infections is of special interest nowadays. The purpose of this study was to compare anti-inflammatory effects of the new complex herbal preparation "Salvia immune-active" (SI) and the known herbal product "Doctor Taiss salvia extract with Vit C" (ST) in the experimental model of cervical lymphadenitis in rats. The new preparation SI contains Salvia extract and oil, ascorbic acid and Echinacea extract.

The experimental model of the cervical lymphadenitis is very important and useful for the study of anti-inflammatory effects of new preparations. To develop this model, three inflammatory agents (LPS, γ -carragenan and formalin) were injected into the tissue of rat lymph node (LN). The most adequate inflammatory reaction was irritated by the γ -carragenan solution.

The difference in the weight of injured and intact LN, the level of C-reactive protein (CRP), the level of tumor necrosis factor alfa (TNF- α) and interferon- γ (INF- γ) in the blood were evaluated. Morphological changes in LN were registered.

In the experiment, female Wistar rats (250-300 g each; n=15 per group) were orally administrated with preparations (SI dose 0.56 g/kg and ST dose 1.2 g/kg) 10 days prior to the injection of carrageenan and 3 days afterwards. Two groups of animals were used for the positive and negative control. The rats were euthanized on the 3rd day after carrageenan injection.

The obtained results show that therapeutical doses of SI and ST (0.56 g/kg and 1.2 g/kg respectively) have the similar antiedematous effect and both decrease the level CRP and TNF- α in the blood in 1.6 and 1.8 times comparing to control respectively. At the same time, SI shows more expressed decrease in the level of INF- γ than the known preparation ST. Moreover, the morphological study revealed the ability of SI to stimulate the proliferation of B-lymphocytes in the tissue of LN.

Thus, the therapeutical doses of the new complex herbal preparation SI and the known ST have the similar anti-inflammatory effect, but SI demonstrates immunostimulatory activity due to the Echinacea extract. In conclusion, the new preparation with Salvia and Echinacea extracts can be recommended for the treatment and preventive maintenance of chronic tonsillitis.

ADJUVATION EFFECT ON TETRAPEPTIDE ACTIVITY UNDER CONDITIONS OF ASEPTIC INFLAMMATION AND IMMUNOSUPPRESSION WITH CYCLOPHOSPHANE

K.L. Kryshen¹, A.A. Acapkina¹, V.G. Makarov¹, M.N. Makarova¹, V.K. Osipovich²

¹Saint-Petersburg Institute of Pharmacy, Russia

²Access Biosciences, Saint-Petersburg, Russia

Determination of the short peptide role in the regulation of physiological functions is one of the important discoveries of XX century. The properties of low-weight short synthetic peptides may vary depending on modifications of functional groups of amino acids and stereoisomers. The properties also change in the presence of adjuvant.

The aim of the present study was the evaluation of the adjuvation effect on immunomodulator and anti-inflammatory properties of the short peptides. Peritoneal macrophages of BALB/c mice were investigated under conditions of immunosuppression with cyclophosphane and aseptic inflammation induced by *E. Coli* lipopolysaccharide (LPS). Immunosuppression was carried out with cyclophosphane 10 mg/kg (4 times every 2nd day). The aseptic inflammation was stimulated by the single intraperitoneal injection of 30 mg/kg LPS.

The effects of tetrapeptide and its adjuvated form by repeated intramuscular injection in the dose 100 µg/kg were compared. The amount of leucocytes in peritoneal exudate, macrophage activity with the neutral red reaction, macrophage secretory activity and free radicals formation were evaluated.

The results of the experiments indicate that tetrapeptide reveals proinflammatory effect: its application caused increase in the neutrophilic and macrophage infiltration of the inflammation zone. Decrease of phagocytosis in 4 times compared to control by macrophages was observed, no other changes were found. The modification of the tetrapeptide with ajuvant caused the opposite changes in cell infiltration. Moreover, the adjuvated tetrapeptide stimulated superoxide anion radicals formation in 46% and phagocytosis in 2.4 times compared to control group.

Thus, the adjuvation caused total reversion of the compound's effect. The modification turned pro-inflammatory and anti-inflammatory ones. The obtained data are good basis for further studies and search the new way of the peptides modification and application.

EFFECTS OF TWO DIFFERENT MEDICINAL FORMS OF ASCORBIC ACID ON URINE SAMPLES COMPOSITION

T.V. Abrashova, A.P. Sokolova, O.I. Avdeeva, I.V. Karachinskaya, M.N. Makarova

St-Petersburg Institute of Pharmacy, Russia

In control application of high doses of ascorbic acid is an often occurring mistake in treatment and prevention of influenza and cold. Long time application of vitamin C can cause CNS activation, irritation of intestinal mucosa, suppression of pancreatic function, negative effect on kidneys, increase in thrombogenesis, adrenal cortex function stimulation, and increase of blood pressure. WHO declared in 2002 that 500 mg/day is maximal therapeutic dose of ascorbic acid. However, its common practice to prescribe vitamin C in high doses like 2 g/day that can lead to mentioned negative effects.

The purpose of this study was to carry out comparative evaluation of effects of prolonged application of ascorbic acid in two medicinal forms - effervescent tablets and sachets. 60 male and 60 female Wistar rats were used in the experiment. Dose of vitamin C equivalent to 20 therapeutic doses (20-TD – 1600 mg/kg, equivalent to 20 g for human) was administered. Urine samples volume were collected during 4 hours on 7th, 14th, 21st, 28th day of study. Biochemical study was performed using URISCAN dipsticks and sludge microscopy.

Ascorbic acid in the dose 20 TD caused considerable changes of urine composition.

Decrease in volume of urine was observed in all test groups at all time points more than by 50% in comparison with intact animals.

In animals treated with ascorbic acid in sachets sharp decrease of urine pH was found (to $5,6 \pm 0,24$); struvite (magnesium phosphate ammonium) was increased in two times comparing to intact group.

Animals treated with vitamin C in effervescent tablets had pH shift to alkaline reaction (up to $8,9 \pm 0,5$); struvite amount was increased in 4 times comparing to intact group, probably due to the high dose of sodium carbohydrate in tablets.

Gender did not influence found changes. Dynamic of effect was similar for rats of both sex. Application of ascorbic acid in sachets affected urine sample content less than use of effervescent tablets. pH shift to acidic reaction is preferable. Furthermore, acidic pH is less favourable for pathogenic microflora growth than alkaline one. However, long time administration of high doses of ascorbic acid should be under control of laboratory indices, especially renal function.

EVALUATION FOR THE SAFETY OF NEW HERBAL DRUG WITH SAGE AND ECHINACEA EXTRACTS BY METHOD OF DOSE RANGE FINDING

O.I. Avdeeva, S.I. Guseva, M.N. Makarova, S.Yu. Stefanov, T.V. Abrashova, I.V. Karachinskaya

St-Petersburg Institute of Pharmacy, Russia

Research on adequate methods of toxicity evaluation of potentially non-hazardous medications is of current interest. The purpose of this study was to evaluate safety of new herbal medical drug with sage and echinacea extracts (HSE), by method of dose range finding (DRF).

General toxicity studies (evaluation DRF and subchronic toxicity) of new HSE had revealed that in case of acute administration as well as long-term application of highest dose for 4 weeks no toxic effect on the warm-blooded laboratory animals was produced. Planning and carrying-out of the study was performed in accordance with MHSD RF (the Ministry of Health and Social Development of the Russian Federation) as well as international standards of preclinical studies – GLP.

Studies were carried out in three stages.

1. Evaluation of momentary non-lethal dose (MNLD).

Single intragastrical administration of 10 ml/kg of 60% suspension of HSE (dose of 6.0 g/kg) does not cause any biological effect on the organism. Biological effect was evaluated by changes in the body weight 7 days after the administration. Experiment did not reveal any statistically significant differences in studied parameters between the experimental and control groups.

2. Evaluation of maximal tolerable repeated dose (MTDR).

Intragastric injection of testing drug for 14 days in MNLD did not cause any statistically significant differences in studied parameters between the experimental and control groups of rats. Presence of the biologic effect was controlled by the weight changes, dynamics of food and water consumption and organs mass coefficients. MTDR was taken as MNLD equivalent.

3. Study of subchronic toxicity of MTDR.

Study of subchronic toxicity of medication during daily intragastric injection for 28 days in a dose of 6.0 g/kg showed that studied medication does not cause any statistically significant differences in body weight dynamics, biochemical and hematological indices between the experimental and control groups. Results of pathomorphologic and histological studies proved that medication has not irritating effect on the esophageal, gastric, and intestinal mucosa as well as does not cause destructive or focal sclerotic changes in hepatic parenchyma and stroma. In all tests changes in indices in experimental group in comparison with control group were not statistically significant. Daily observation of animal's general condition in the course of HSE medication toxicity studies, behavioural reactions in hands and in open area as well as zoosocial behaviour studies had shown that administration of studied medication in a broad range of doses (1.2-6.0 g/kg intragastrically) does not affect the general condition, tentative-research activity and emotional status of experimental animals.

Thus, results of the toxicity study of HSE drug suggest its safety and adequacy of applied by DRF method for toxicity evaluation.

IMMUNOTOXIC AND IMMUNOMODULATOR EFFECTS OF COMPLEX HERBAL PREPARATION

K.L. Kryshen, A.A. Acapkina, S.I. Guseva S.I., M.N. Makarova, V.G. Makarov

Saint-Petersburg Institute of Pharmacy, Russia

Development of effective anti-inflammatory and immunostimulator preparations for treatment of respiratory infections is very important. In order to achieve balanced effect on all the components of the immune system, complex non-toxic preparation are required. The purpose of this study was to determine immunotoxic and immunomodulator effects of the new complex herbal preparation, containing *Salvia* plant extract and oil, *Echinacea* extract, and ascorbic acid.

In order to evaluate immunomodulator effect, herbal preparation was tested in therapeutic doses (TD, 1.27 g/kg), TD/3 (0.42 g/kg) and TDx3 (3.82 g/kg). Ten times higher dose (12.7 g/kg) was used for the study of possible immunotoxicity effects.

Effects of the preparation on the humoral, cellular and nonspecific immune response were studied. The T-lymphocytes blast-transformation reaction and delayed-type hypersensitivity reaction were used for the evaluation of the cellular immune response. The hemagglutination reaction with sheep erythrocytes and the blast-transformation reaction were used in the study of antibody production by B-lymphocytes and their proliferative activity, respectively. The neutral red reaction was used to study phagocytosis activity of peritoneal macrophages. In addition, T-helpers functional activity was evaluate in the study of the cellular immune response.

The obtained results show that the preparation stimulates the development of humoral and cellular immune response. In therapeutical doses antibody titre was in 2 times higher comparing to control. Study of T-helpers functional activity show decrease of the IL-4 level and increase the IFN γ level. Besides, the studied phyto preparation stimulates the macrophage activity (68% in therapeutical dose comparing to control). Delayed-type hypersensitivity reaction show that the anti-inflammatory effect of the preparation was revealed in the suppression of pro-inflammatory cytokine production and formation of T-lymphocyte clone test.

Thus, the studied phyto preparation in therapeutic doses and in a ten time higher dose do not lead to immunotoxic effects, more over it produces immunostimulator and anti-inflammatory effects.

POTENTIAL ANTI-AMYLASE, ANTI-GLUCOSIDASE AND ANTI-OXIDANT ACTIVITY OF CARVACROL ISOLATED FROM *ZATARIA MULTIFLORA* LEAVES

***Farzaneh Malekshahi*¹, *Reshad Masoumi*¹, *Maedeh Hosseinpour*¹,
*Babak Yari Kamrani*², *Sohrab Yari*³**

¹Department of Clinical Pharmacy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

²Department of Phytochemistry, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Evin, Tehran, Iran

³Department of Internal Medicine, Razi Medical School, Kermanshah, Iran

Herbal medicine has been used for many years by different cultures around the world for the treatment of diabetes. In this study, we examined in vitro the α -glucosidase, α -amylase and antioxidant activity of carvacrol (the main component) isolated from *Zataria multiflora* essential oil. The essential oil of air-dried samples was obtained by hydrodistillation. Enzyme inhibitory activities of carvacrol against rat intestinal α -glucosidase and porcine pancreatic α -amylase were done by standard methods. 2,2-diphenyl-1-picrylhydrazil (DPPH) was used to determine the anti-oxidant activity (RSC) by measuring the scavenging activity of examined essential oil. Carvacrol possessed the strong anti-amylase activity (AI index value = 2.1 ± 0.5 ; ~ 71% of inhibition) and also possessed the moderate α -glucosidase inhibitory activity (a-GI index value = 1.19 ± 0.03 ; ~ 35% of inhibition). Effect of carvacrol on the in vitro free radical (DPPH) was 161.7 (mg/ml) while BHT was used as a positive control 19.8 (mg/ml). Strong anti-oxidant activity and good α -amylase inhibitory activity among moderate α -glucosidase activity of essential oil of dried *Z. multiflora* would be useful as an oral treatment which can reduce not only the postprandial glucose level, but also the total amount of free radicals in patients with type 2 diabetes. This preliminary observation will provide the basis for further clinical investigation of *Z. multiflora* as a medicinal supplement that contributes towards the treatment of diabetes mellitus, reducing the risk and prevention of late diabetic complications.

Acknowledgement: Mr. A.S. Nasreldin Heidari

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ESSENTIAL OIL COMPOSITIONS OF DIFFERENT ACCESSIONS OF *CORIANDRUM SATIVUM* L. FROM IRAN

**Samad Nejad Ebrahimi¹, Javad Hadian¹, Farzaneh Malekshahi²,
Yousef Yari Kamrani¹**

¹Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran

²Department of Clinical Pharmacy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

Coriander (*Coriandrum sativum* L.) has been cultivated for a long time in different parts of Iran. It has been used as a folk medicine for the relief of anxiety and insomnia in Iran. The chemical profiles of different accessions were analyzed by means of GC-MS. The essential oil content of the dried seeds was varied from 0.1 to 0.36%. Thirty-Four different compounds were identified in essential oil of all accessions. Linalool (40.9-79.9%), neryl acetate (2.3-14.2%), γ -terpinene (0.1-13.6%) and α -pinene (1.2-7.1%) were identified as main components in the oil of Coriander accessions. Almost all studied accessions contain more than 60% linalool showing high quality of Coriander seeds produced in Iran and suitability of the accessions for use as initial genetic materials for breeding of homogenous and talent Coriander cultivars.

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DOPAMINERGIC ACTION OF AN EXTRACT OF GREEN COFFEE BEANS (*COFFEA ARABICA*)

F.R. Mendes, E.A. Carlini

CEBRID, Department of Psychobiology, Federal University of São Paulo, Brazil

Epidemiological data point to an inverse correlation between coffee intake and Parkinson's disease (PD). These observations are supported by experimental studies which indicate that caffeine, acting as an adenosinergic antagonist, plays a protective role in PD, since the dopaminergic system in the striatum is under adenosinergic modulation. The objective of this work was to assess whether an extract of coffee (EC) beans, *Coffea arabica*, containing 5% of caffeine, interferes in the dopaminergic system, modifying the responses induced by haloperidol (antagonist) and apomorphine (agonist).

Swiss mice (n=10) received acute administrations of EC at the doses of 50 to 800 mg/kg (orally) and haloperidol (5mg/kg, ip) after 30 min. After another period of 30 min, we recorded the time of catatonia (s) of each animal for a period of 10 min. In a second experiment, Wistar rats (n=8-9) received EC at the same doses and apomorphine (3 mg/kg, ip) after 30 min. The animals were then placed in wire cages and their degree of stereotypy was evaluated according to Barros et al. (1989) [1].

EC reduced the catatonia time of the mice (mean±) at all the doses used, being more intense at 400 mg/kg (control 445±81, EC-50 179±72*, EC-200 57±20*, EC-400 7±4*, EC-800 73±52* - Anova / Duncan, $p<0.05$). EC in small doses also potentialized the stereotypy induced by apomorphine in rats (M±SE) after 30 minutes of observation (control 1.8±0.3; EC-50 3.0±0.5*; EC-200 3.1±0.2*; EC-400 2.6±0.3; EC-800 2.6±0.3 - Anova / Duncan, $p<0.05$).

Our results show that EC presents a stimulant effect on the dopaminergic system, although the mode of action must be elucidated.

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QUALITY OF CLODBERRY (*RUBUS CHAMAEMORUS* L.) AS AFFECTED BY FEMALE PARENT, MALE PARENT AND TEMPERATURE

Inger Martinussen¹, ***Gordon McDougall***³, ***Susan Verdall***³, ***Derek Stewart***³,
Eivind Uleberg¹, ***Olavi Junttila***²

¹Norwegian Institute for Agricultural and Environmental Research, Bioforsk Nord Holt, Box 2284, N-9269 Tromsø, Norway, e-mail: inger.martinussen@bioforsk.no

²Institute of Biology, University of Tromsø, N-9037 Tromsø, Norway

³Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, UK

Cloudberrys (*Rubus chamaemorus* L.) contain several phenolic compounds, but ellagitannis/ellagic acids are the dominating ones, and the antioxidative effects of cloudberry are mainly due to ellagitannins. Generally, antioxidative activities and chemical composition of berries are affected both by the genotype and the environment. The aim of this study was to investigate the role of temperature and the genotypes, used in the crossings on fruit quality. Since different genotypes might respond differently to different temperatures, possible interactions between genotype and temperature were also investigated. Plants were grown under controlled temperature treatments (9, 12, 15, 18°C; variation ± 0.5 C) in 24-h photoperiod. Ripe berries were harvested daily, frozen individually at -100°C and stored at this temperature until chemical analyses. There is a significant effect of female parent on both total phenols and total anthocyanins in the berries. Temperature affected the level of total anthocyanins, while there was no significant effect on the level of total phenols. The results indicate that there is a need for breeding material with a special adaptation to different climatic conditions.

HERBS IN THE BARENTS REGION – A NATURAL RESOURCE THAT IMPROVES HEALTH AND CREATES BUSINESS (COLLABORATION OF THE NORTHERN EUROPE COUNTRIES ON ADAPTOGENIC PLANTS)

***I. Martinussen*¹, *V. Volodin*², *S. Volodina*², *I. Poletaeva*², *D. Bacharov*³, *R. Belyaeva*⁴, *M. Uusitalo*⁵, *A. Heinonen*⁵, *B. Galambosi*⁶, *Z. Galambosi*⁶, *M. Thomsen*⁷, *G. Rothe*⁷, *E. Fjelldal*⁸, *P. Aspholm*⁸**

¹Bioforsk Nord Holt, Tromsø, Norway

²Institute of Biology, Komi SC, UrD, RAS, Syktyvkar, Russia

³Syktyvkar State University, Syktyvkar, Russia

⁴Research and Technological Institute of Agroindustrial Complex of Republic of Komi, Syktyvkar, Russia

⁵MTT, Rovaniemi, Finland

⁶MTT, Mikkeli, Finland

⁷Bioforsk Ost Kise, Norway

⁸Bioforsk Svanhovd, Norway

At present the development of a modern life-style in the Barents region has become not only a medical, but a social and economic problem as well. To prevent organism from unfavorable factors of the North and life-style diseases much attention is paid now to the application of adaptogenic plants. "Herbs in the Barents region – a natural resource that improves health and creates business" is a title of the joint project of Norwegian, Finnish and Russian scientists on the adaptogenic plants. The project main goal is to create job opportunities in the Barents region based on the natural resources of adaptogenic herbs. This shall be done by the development of cultivation techniques for the adaptogenic plants *Rhodiola rosea*, *Rhaponticum carthamoides* and *Serratula coronata*. The industry will develop new herb products that will improve health conditions in the region. In order to promote adaptogenic products made from local plant raw material we have investigated the effect of the climate and geographical location on the level of active substances. Gene banks of plant species studied are established in all countries-participants. Experiments conducted in phytotrone showed that all three plant species like long days (northern conditions) and the levels of adaptogen's substances are highest at the low and intermediate temperatures. Cultivation techniques are developed. To ensure the best quality of the raw material the methods of harvesting, drying and storing of plants are optimized. New methods for extraction from fresh raw material of *R. rosea* are developed. Fields at growers are established. The project was financing by Interreg IIIA, Norwegian Barents Secretary and agricultural offices of the counties Troms and Finnmark.

PORTUGUESE PROPOLIS: THE EFFECT OF COLLECTION TIME AND LOCALIZATION ON ENZYME ANTIOXIDANT ACTIVITIES

A.V. Oliveira¹, A.L. Ferreira¹, S. Nunes¹, S.A. Dandlen¹, A. Cavaco¹, M.D. Antunes¹, M.L. Faleiro^{1,2}, M.G. Miguel¹

¹Universidade do Algarve, FCT, Campus de Gambelas 8005-139 Faro, Portugal

²Universidade do Algarve, IBB-Centro de Biomedicina Molecular e Estrutural, Campus de Gambelas 8005-139 Faro, Portugal

Propolis extracts are used as a folk medicine from ancient times. Nowadays, it was found to have a wide range of biological activities, namely antibacterial, anti-inflammatory, anti-oxidative, hepatoprotective effects and anti-tumoral activities. These pharmacological and antioxidant actions are probably due the presence of antioxidant compounds e.g. phenolic constituents, especially flavonoids and phenolic acids.

In this work, enzymatic antioxidant activities, namely: superoxide-dismutase (SOD), catalase (CAT), and total protein of propolis samples harvested at two different times (winter and spring) from several locations of the Algarve region (B.N. Arrodeios, B.N. Pé da Serra, B.S. Ameijoafra and Transição Norte) were determined.

In respect with protein contents, major differences were not observed for samples harvested at winter and spring time, except for samples from B.S. Ameijoafra. In this case, a decrease occurred from the sample collected at winter to sample collected at Spring.

Concerning antioxidant enzymatic results, SOD activity was collection time dependent. SOD activity decreased drastically from winter to spring for B.N. Arrodeios and B.S. Ameijoafra samples. In contrast, samples collected at B. N. Pé da Serra and Transição Norte registered a significant increase.

SOD generates hydrogen peroxide and oxygen molecules. These hydrogen peroxide molecules can be converted into water molecules and oxygen by the action of enzymes such as, catalase, guaiacol peroxidase and glutathione peroxidase. So, the samples with higher SOD activities should have higher CAT activities also. Such was not observed, which may suggest that the hydrogen peroxide was eliminated by other antioxidant enzymes.

Acknowledgment: This study was partially funded by *Cruz Alta Agricultura, Lda.* Loulé.

PORTUGUESE PROPOLIS: COLLECTION TIME, LOCAL AND TYPE OF EXTRACT ON THE PHENOL CONTENT AND ANTIOXIDANT ACTIVITY

S. Nunes¹, S.A. Dandlen¹, M.D. Antunes¹, Ana M. Cavaco¹, H. Guia², M.G. Miguel¹

¹FCT, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

²Cruz Alta Agricultura Lda., Ameixeirinhas, 8100 Salir, Portugal

Propolis is composed of 30% wax, 50% resin and vegetable balsam, 10% essential and aromatic oils, 5% pollen, and other substances [1]. Antibacterial, antiviral, antifungal, anti-parasite, anti-inflammatory, anticancer, antioxidant and anti-hepatotoxic are some of the propolis biological activities reported in recent articles [1, 2]. These activities depend on propolis chemical composition and therefore on the local flora of bees habitat and also on the following industrial handling and extraction methods. Then, if one intends to use this natural product for medicinal purposes, all its chemical, biological and cytotoxic properties must be previously and thoroughly evaluated. In the present work total phenols, flavonoid content and antioxidant activity measured by the TEAC (Trolox Equivalent Antioxidant Capacity) method of three types of extracts (methanolic, hydroalcoholic and aqueous) of propolis collected at several places of three main regions of Algarve (Mountain, Transition and Maqui) during winter and spring, were determined and compared.

The methanolic and aqueous extracts were obtained according to [3]. The hydroalcoholic extracts were obtained according to [4]. Phenols and flavonoids were determined as reported by [5, 6], respectively. Antioxidant activity was determined according to [7]. Hydroalcoholic and methanolic extracts have shown the highest phenol and flavonoids content, in spite of the collection time and local. Hydroalcoholic extracts of propolis from Mountain and Maqui had higher levels of phenols in spring than in winter, with some exceptions. The collection time of samples at the transition area did not show significant differences in total phenols. Generally, a slight increase of flavonoids was observed in hydroalcoholic from winter to spring, in Mountain and Transition extracts as well as in some collected in the Maqui area. Concerning methanolic extracts, phenol and flavonoids content remained constant along the seasons, independently of the collection site. In general, Maqui samples exhibited a higher capacity for scavenging free radicals and the antioxidant activity of the aqueous extracts was significantly lower than the other extracts, along the seasons. In winter, hydroalcoholic extracts presented a higher antioxidant activity than the methanolic ones. In spring, hydroalcoholic extracts from Maqui and Mountain revealed to be more effective as antioxidants than those from the Transition area. Concerning the methanolic extracts, those from Maqui were generally the most active.

Acknowledgements: Partially funded by Cruz Alta Agricultura, Lda Portugal) and CDCTPV.

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EFFECT OF LOW TEMPERATURE STEAM TREATMENT ON CHEMICAL CONSTITUENTS OF INDIAN HERBS

M.G. Rowan, J. Mitchelsova

University of Bath, United Kingdom

Steam treatment is commonly used to bring the microbial load in dried plant materials intended for human consumption to acceptable levels and is currently the only option of treatment for certified Organic products in the UK. This process often causes darkening of the product. More information is required on the impact of steam pasteurisation on plant constituents, especially for Indian plants. Four species were selected to encompass a variety of types of chemical constituents.

Andrographis paniculata Nees (aerial parts), *Bacopa monnieri* (L.) Pernel (aerial parts), *Piper longum* L. (fruit) and *Zingiber officinale* Roscoe (rhizome) were examined. For each species a methanolic extract of steam-pasteurised material and a control sample from the same batch prior to steam treatment were compared by HP-TLC against appropriate reference standards.

TLC profiles of the two aerial materials, *Andrographis paniculata* (Indian name: Kalmegh) and *Bacopa monnieri* (Brahmi), showed a slight decrease in polar flavonoids and a slight increase in intensity of the less polar bands. A slight decrease in andrographolide content was noted for *A. paniculata* whilst there was no significant change in bacoside A content of *B. monnieri*.

In *Piper longum* (Pippali) the concentration of piperine (alkaloid) in the sample was slightly smaller after steam pasteurisation but the overall TLC profiles of treated and control samples were the same.

The TLC profile of *Zingiber officinale* (Sunthi) showed a very slight increase in intensity of the shogaol band and a significant decrease in volatile oil content after steam treatment.

Overall, the steam treatment had little effect on chemical content of the herbs and these data may potentially be of value in addressing the concerns of consumers.

Acknowledgement to Technology Strategy Board and Pukka Herbs Ltd. for support via KTP programme no. 1622.

IS SUMAC (RHUS CORIARIA) USEFUL FOR DIABETES? AN INVESTIGATION INTO ANTIDIABETIC PROPERTIES AND MECHANISMS OF ACTION

S. Montasser Kouhsari, S. Mohammadi, A. Monavar Feshani, N. Andalib

Department of Cellular and Molecular Biology, School of Biology, University College of science, University of Tehran, P.O. Box 14155-6455, Tehran, Iran

Herbal drugs have always been a common recourse of the medications because of their effectiveness, fewer side effects and relatively low cost. The extracts of *Rhus coriaria* fruits are reported to have different medical properties, such as antifungal, anti-inflammatory, antimalaria, antimicrobial, antitumorogenic, antiviral and cytotoxic effects.

To determine the antidiabetic effects of *R. coriaria* ethanolic extract and their mechanisms, in the first phase, we investigated its effects on intestinal α -glucosidase using a spectrophotometric method (Nishioka, Kawabata, & Aoyama, 1998). Then, the plant extract was administered orally to alloxan-induced diabetic rats for three weeks. After the period of treatment, blood glucose, LDL/HDL, TC, TG, and VLDL were measured by enzymatic assays. Also the activity of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) were measured using commercial kits. Moreover, the mRNA level of INS gene in pancreas and GLUT-4 gene in heart were measured by RT-PCR technique in control and diabetic rats.

We observed that the extract inhibited maltase and sucrase activity by 44% and 27% respectively. Also our results showed that the ethanolic extracts of *Rhus coriaria* fruits lessened significantly the blood glucose and increase the blood serum HDL. In addition, the insulin mRNA level was mildly enhanced in diabetic rats treated with extract in comparison with diabetic control rats. The extract significantly raised erythrocyte SOD and CAT activity while it didn't effect on erythrocyte GPX.

We can conclude that Iranian sumac has effective components which can be utilized as a useful medicine for diabetic patients.

VACCINIUM ARCTOSTAPHYLOS, A COMMON HERBAL MEDICINE IN IRAN: MOLECULAR AND BIOCHEMICAL STUDY OF ITS ANTIDIABETIC EFFECTS

S. Montasser Kouhsari, A. Monavar Feshani, S. Mohammadi, N. Andalib

Department of Cellular and Molecular Biology, School of Biology, University College of science, University of Tehran, P.O. Box 14155-6455, Tehran, Iran

The use of herbal medicines as complementary or alternative approaches to existing medicaments is increasing worldwide. Fruit of Iranian blueberry (*Vaccinium arctostaphylos* L.) are traditionally used for improving of health status of diabetic patients. The purpose of this study was to define its antidiabetic effects and involved mechanisms which mediate its effects.

We examined the effect of three weeks oral administration of ethanolic extract of plant in four groups of rats (normal and diabetic controls, diabetic treated with plant extract and diabetic treated with Acarbose) on various biochemical parameters such as postprandial blood glucose (1, 3, 5, 8, 24 hours after a single dose administration of plant extract, and at the end of 1, 2 and 3 weeks following three weeks daily administration of it), oral glucose tolerance, TC, TG, HDL, superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) using commercial kits. Also the relative mRNA levels of INS, in the pancreas, and GLUT-4, in the heart, were assessed by RT-PCR. Moreover we examined the inhibitory effect of ethanolic extract against rat intestinal α -glucosidase by using a spectrophotometric method (Nishioka, Kawabata, & Aoyama, 1998).

The obtained results showed a mild decrease in the blood glucose within 1 to 3 hours and also a significant decrease at the end of three weeks of administration of ethanolic extract. The plant extract also decreased markedly triglyceride in blood serum. Also We observed that its extract had noticeable antioxidant effect which elevated GPX, SOD and CAT activities by 32, 57 and 49%, respectively, as compared to diabetic control group. Furthermore, in-vitro Study displayed 47 and 56% inhibitory effects of plant extract on activity of maltase and sucrase, respectively. Finally we observed that the ethanolic extract of *V. arctostaphylos* increased the expression of GLUT-4 and INS genes in treated Wistar rats. For the first time, findings of this study allow us to establish scientifically Iranian blueberry as a potent antidiabetic agent with various beneficial effects which are mediated by different mechanisms. Further investigation is needed for detecting active component of *V. arctostaphylos*.

BIOCHEMICAL AND MOLECULAR STUDY OF CARUM CARVI SEEDS ETHANOLIC EXTRACT IN DIABETIC MALE WISTAR RATS

Shideh Montasser Kouhsari¹, Andisheh Imani Khoshkhou²

¹Department of Cellular and Molecular Biology, School of Biology, University College of science, University of Tehran, P.O. Box 14155-6455, Tehran, Iran

²Department of Biology, Faculty of Science, Tehran Payame Noor University, Iran

Carum carvi is from Umbelliferae family and it has numerous medical benefits, mainly anti-bronchial, diuretic, antiscorvy and antiasthmatic properties. In this research, the effect of ethanolic extract of Carum carvi seeds in control and Alloxan monohydrate induced diabetic male wistar rats (190-200g) was compared with that of three antidiabetic drugs: Metformin, Glibenclamide and Acarbose. 18 male wistar rats were divided in 6 groups of 3 rats. Diabetic rats were obtained by subcutaneous injection of 100mg/kg body weight, Alloxan monohydrate. Blood glucose was measured, by enzymatic assay (glucose oxidase). The effect of ethanolic plant extract on blood glucose was measured and compared with that of antidiabetic common drugs, Metformin, Glibenclamide and Acarbose. For measuring the insulin hormone mRNA levels in pancreas, using insulin primer, the RT-PCR assay was done in control and diabetic treated rats.

The obtained results indicated that ethanolic extract of Carum carvi seeds reduces significantly blood glucose in diabetic rats by 39.6% and 27.3% at 8 hours and at the end of 3 weeks of treatment, respectively, in comparison with diabetic group. C.carvi ethanolic extract effect in short term is comparable with that of Acarbose but Metformin and Glibenclamide decrease blood glucose in 24 hours. The expression of insulin gene showed no difference between control and diabetic treated rats.

PROCESSED RICE WITH BROWN RICE EXTRACT REGULATES THE LEVEL OF THE SERUM CHOLESTEROL

K. Murata^{1, 2}, ***T. Kamei***^{1, 2, 3}, ***Y. Toriumi***⁴, ***I. Fukumoto***⁵, ***M. Yoshida***⁵

¹Shimane Institute of Health Science, Izumo 693-0022, Japan

²Kanazawa University Graduate School of Medical Science, Kanazawa 920-0934, Japan

³European University Viadrina Frankfurt (Oder), 15230 Frankfurt (Oder), Germany

⁴Department of Pediatrics, Shimane University, Izumo 693-8501, Japan

⁵Alpha Foods Co. Ltd., Izumo 699-0722, Japan

Brown rice and rice with germs are rich in functional components such as γ -oryzanol. γ -Oryzanol has been reported to decrease the serum cholesterol level. In the present study, we examined the effects of processed rice enriched with brown rice extracts (test diet) on the serum cholesterol level. In the study, 53 subjects (volunteers) took the test diet 3 times a day for 30 days and blood samples were taken before and after the test diet administration period. Then 45 subjects were included in the study and 52 were evaluated for the safety of the test diet. Compared with the initial baseline level (222.8 \pm 54.2 mg/dl), the total cholesterol (TC) level decreased significantly after 30 days of test diet administration (216.3 \pm 48.6 mg/dl, p <0.05). Triglycerides (TG), HDL-cholesterol (HDL-C) and LDL-cholesterol (LDL-C) levels did not change significantly during the 30 days when compared with the baseline. In sub-analyses, the subjects with a baseline TC level over 200 mg/dl showed significantly lower TC levels on day 30 (TC level of baseline 248.6 \pm 46.7 mg/dl reduced significantly to 235.9 \pm 46.7 mg/dl), but normal subjects did not show any significant differences. Therefore, it is suggested that the test diet-enriched functional components have a possibility to adjust the serum cholesterol level, but only in people with already high TC levels. A diet rich in brown rice extracts could be beneficial as a functional food to lower cholesterol.

ESSENTIAL OILS OF AROMATIC MEDICINAL PLANTS, WHICH CAN BE USED IN FOOD PREPARATION AS ANTIOXIDANTS

L. Nedorostova¹, E. Petitolas², M.A. Vian², F. Chemať², K. Urbanova³, I. Valterova³,
L. Kokoska⁴, P. Kloucek¹, M. Stolcova¹

¹Faculty of Agrobiolgy, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, 165 21 Praha 6-Suchdol, Czech Republic

²Université d'Avignon et des Pays de Vaucluse, UMR A 408 INRA-Université d'Avignon Sécurité et Qualité des Produits d'Origine Végétale, 33, rue Louis Pasteur, 84029 Avignon cedex I, France

³Institute of Organic Chemistry and Biochemistry, Academy of Sciences of Czech Republic, Flemingovo square 2, 166 10, Prague, Czech Republic

⁴Institute of Tropics and Subtropics, Czech University of Life Sciences Prague, Kamýcká 129, 165 21 Praha 6-Suchdol, Czech Republic

It has been known for a long time that the free radicals existent in the environment can give rise to many diseases, such as cancer, atherosclerosis, and many more [1]. Antioxidants protect food from oxidation processes and can also help in the human organism to counteract diseases caused by free radicals [2]. Synthetic phenolics, such as butylated hydroxyanisole (BHA), are widely used as antioxidants in food lipids [3], can have negative effects to human health [4]. The aim of this study was to evaluate the antioxidant properties of nine essential oils obtained from various spices and herbs by hydro-distillation and characterized by GC-MS analyses. All EO samples, together with carvacrol, citral, linalol, thymol (main components of the tested Eos) and synthetic antioxidant BHA were tested by 1,1-diphenyl-2-picrylhydrazil (DPPH) method. Carvacrol and thymol were highly antioxidant active. BHA was found to have scavenging effects on DPPH in 6 µg/ml. The essentials oils were found to have scavenging effects on DPPH in the range of 0.5-8.45 µg/ml. The best results were shown by *Ocimum basilicum* var. "Grant Verte", followed by *Ocimum basilicum* var. hybriden "Magic Blue" > *Ocimum basilicum* var. hybriden "Magic White" > *Thymus vulgaris* > *Satureja hortensis* > *Thymus serpyllum* > *Satureja montana* > *Ocimum basilicum* var. "Red" > BHA > *Thymus citriodorus*. In summery, we observed the effects of EOs tested in this study were similar and/or superior to that of commercial antioxidant BHA, which suggest their possible use as effective antioxidant natural products.

Acknowledgements: Ministry of Education of the Czech republic MSM 6046070901

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GENETIC RESOURCES OF SOME ESSENTIAL OIL PLANTS IN SOUTHERN TURKEY

Mensure Ozguven

Department of Field Crops, Faculty of Agriculture, University of Cukurova 01330 Adana, Turkey, mensur@cu.edu.tr, tel: +90 322 3386451; fax: +90 322 3386381

In this Research essential oil plants such as *Mentha*, *Melissa*, *Thymus*, *Salvia*, *Rosmarinus* and *Thymbra* species are searched in the Cukurova Region, Tarsus mountains at north, Amanos mountains and Antakya at south, Göksu deltoid at west and K.Maras and Ahirdagi at east, and 109 plant materials which have different origins were collected and their essential oil rates and components were analyzed. Most of medicinal plants show wide genetic variation due to their wild plant characteristic thus, valuable genotype selection is being possible. Main selection criteria are pharmaceutical substances with high rate and their components. Plant material collected after first selection in natural environment was propagated, field trials were conducted in two different ecological conditions representing plain and mountainous zones, and then this material was maintained in a collection garden to be used in selection and breeding studies, and also to make up a gene reservation (ex-situ).

EVIDENCE-BASED EFFICACY OF ADAPTOGENS IN FATIGUE, AND MOLECULAR MECHANISMS RELATED TO THEIR STRESS-PROTECTIVE ACTIVITY

Alexander Panossian, Georg Wikman

Swedish Herbal Institute Research and Development, Spårvägen 2, SE-432 96 Åskloster, Sweden

The aim of this review is to assess the level of scientific evidence presented by clinical trials of adaptogens in fatigue, and to provide a rationale at the molecular level for verified effects. Strong scientific evidence is available for *Rhodiola rosea* SHR-5 extract, which improved attention, cognitive function and mental performance in fatigue and in chronic fatigue syndrome. Good scientific evidence has been documented in trials in which *Schisandra chinensis* and *Eleutherococcus senticosus* increased endurance and mental performance in patients with mild fatigue and weakness. Based on their efficacy in clinical studies, adaptogens can be defined as a pharmacological group of herbal preparations that increase tolerance to mental exhaustion and enhance attention and mental endurance in situations of decreased performance. The beneficial stress-protective effect of adaptogens is related to regulation of homeostasis via several mechanisms of action associated with the hypothalamic-pituitary-adrenal axis and the control of key mediators of stress response such as molecular chaperons (e.g. Hsp70), stress-activated c-Jun N-terminal protein kinase (JNK1), Forkhead Box O transcription factor DAF-16, cortisol and nitric oxide (NO). The key point of action of phytoadaptogens appears to be their up-regulating and stress-mimetic effects on the "stress-sensor" protein Hsp70, which plays an important role in cell survival and apoptosis. Hsp70 inhibits the expression of NO synthase II gene and interacts with glucocorticoid receptors directly and via the JNK pathway, thus affecting the levels of circulating cortisol and NO. Prevention of stress-induced increase in NO, and the associated decrease in ATP production, results in increased performance and endurance. Adaptogen-induced up-regulation of Hsp70 triggers stress-induced JNK-1 and DAF-16-mediated pathways regulating the resistance to stress and resulting in enhanced mental and physical performance and increase in longevity.

HPLC – MS ANALYSIS OF SOME MISTLETOE EXTRACTS

Camelia I. Peev¹, Laurian Vlase², Cristina Dehelean¹, Stefana Feflea¹

¹University of Medicine and Pharmacy Victor Babes, Faculty of Pharmacy, Eftimie Murgu Square no.2, 300041, Timisoara, Romania

²University of Medicine and Pharmacy Iuliu Hatieganu, Faculty of Pharmacy, Cluj Napoca, Romania

European mistletoe, *Viscum album* L. (*Loranthaceae* family) is a hemi-parasitic plant; the pharmaceutical herbal product is represented by *Visci folium cum stipites*. Due to the phytochemical composition of lectins, viscotoxins and amines, among others, it is known for its pharmacologic activity as antitumoral, immunostimulating agent, with anti-inflammatory and hypotensive effects.

Polyphenols are known in therapy for their antioxidant effect, their capacity to reduce the noxious effect of the free radicals in the living organisms; the anti-inflammatory activity, the induction of apoptosis in cancer cells, and as topical protective agents against the deleterious effect of UVB radiation.

The aim of this preliminary study was to evaluate the polyphenolic composition of three types of liquid European Mistletoe extracts: in methanol, in ethanol (70%) and in alkaline water, all realized in a vegetal product : solvent-proportion of 1:9. Both nonhydrolyzed and hydrolyzed samples were analyzed. The experiment was carried out using an Agilent 1100 HPLC Series system (Agilent, USA); 19 polyphenol standards (Extrasynthese) were used.

The nonhydrolyzed sample of methanolic mistletoe extract contained: chlorogenic acid 35.36 µg/ml, p-coumaric acid 1.82 µg/ml, sinapic acid 1.36 µg/ml, isoquercitrin 1.89 µg/ml, that of ethanolic extract: chlorogenic acid 34.52 µg/ml, sinapic acid 0.98 µg/ml, isoquercitrin 1.61 µg/ml. While for the hydrolyzed samples, high concentration of sinapic acid was found (> 22.0 µg/ml). In the aqueous extract only qualitative detection of the following compounds was possible according to MS data: caffeic acid, chlorogenic acid, p-coumaric acid, ferulic acid, sinapic acid and isoquercitrin.

GINSENG RADIX- ANALYSIS OF POLYPHENOLS AND PHYTOSTEROLS

***Camelia I. Peev*¹, *Laurian Vlase*², *Cristina Dehelean*¹, *Corina Tiulea*¹**

¹University of Medicine and Pharmacy Victor Babes, Faculty of Pharmacy, Eftimie Murgu Square no.2, 300041, Timisoara, Romania

²University of Medicine and Pharmacy Iuliu Hatieganu, Faculty of Pharmacy, Cluj Napoca, Romania

Ginseng, *Panax ginseng* Meyer (*Araliaceae*) is a plant original from eastern Asia. The vegetal product is represented by Ginseng radix (6th European Pharmacopoeia) and it contains: triterpenic saponins (ginsenosides), vitamins, phytosterols, etc.

Ginseng is an anti-aging remedy, general tonic, adaptogen and an imunostimulating agent. It is frequently used for the preparation of pharmaceutical products intended for internal and external administration, as well as in cosmetology.

We performed a HPLC-MS analysis of polyphenols (for both hydrolized and nonhydrolized samples) and of phytosterols (for stigmasterol, sitosterol) of the ginseng root (Galke Company). Two types of liquid herbal extracts (vegetable products 10%) were analyzed: an alcoholic extract prepared according to German Homeopathy Pharmacopoeia (2002 HAB) and a methanol extract, realized by maceration for 10 days. The experiment was carried out using an Agilent 1100 Series HPLC system (Agilent, USA) ; 18 polyphenol standards (Extrasynthese) were used.

Polyphenols were found in greater concentration in the ethanol extract: chlorogenic acid 14.93 µg/ml, p-coumaric acid 10.69 µg/ml and ferulic acid 43.97 µg/ml, in the nonhydrolyzed sample, while in the hydrolyzed sample gentisic acid, caffeic acid and synapic acid have also been outlined. The highest concentration of phytosterols was found in the ethanol extract: 3.4 µg/ml stigmasterol and sitosterol 7.9 µg/ml.

EFFECTS OF *PANAX GINSENG* EXTRACT (G115) AND THE GINSENOSES ON ANGIOTENSIN-CONVERTING ENZYME ACTIVITY

Ingrid A-L Persson, Karin Persson

Department of Medical and Health Sciences, Division of Drug Research/Pharmacology, Faculty of Health Sciences, Linköping University, Linköping, Sweden

Root and/or rhizome from *Panax ginseng* L (Araliaceae) is frequently used as an adaptogen. Adaptogens are referred to as natural plant products that are proposed to balance the homeostasis of the body i.e. by increase of mental and physical performance [1]. The aim of this study was to investigate effects of *Panax ginseng* extract G115 and its triterpene saponins, the ginsenosides on angiotensin-converting enzyme (ACE) activity. Cultured endothelial cells from human umbilical veins (HUVEC) [2] were incubated with *Panax ginseng* extract G115 and the ginsenosides Rb1, Rb2, Re, Rd, Re, Rf and Rg1. After incubation ACE activity was analysed with a commercial radioenzymatic assay [3]. After 10 minutes incubation with *Panax ginseng* extract G115, a significant and dose-dependent inhibition of ACE activity in HUVEC was seen with 5 and 10 mg/ml ($p < 0.01$) compared to PBS control [3]. After 10 minutes incubation with the ginsenoside Re, a significant inhibition ($p < 0.05$) of ACE activity was seen, while the ginsenosides Rb2 ($p < 0.01$), Re ($p < 0.001$) and Rd ($p < 0.01$) significantly increased ACE activity and the ginsenosides Rb1, Rf and Rg1 showed no significant effect on ACE activity compared to DMSO control. The inhibitory effect of *Panax ginseng* extract on ACE activity do not seem to be associated with the ginsenosides but rather with other compounds present in *Panax ginseng*.

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STUDY OF GENOTOXIC PROPERTIES OF TAXIFOLIN AND QUERCETIN BY THE COMET ASSAY

K.L. Pligina, I.A. Rodina, T.V. Shevchenko, N.P. Syrota, V.P. Tikhonov

DIOD Company, JSC Plant of Ecological Equipment and Ecological Nutrition, Moscow, Russia

The wide spectrum of pharmacological effects of bioflavonoids causes its popularity as natural antioxidants in medicine, food supplements and functional food. Although genotoxicity and oncogenic effects of quercetin in *in vivo* and *in vitro* are still been discussed in the literature.

The purpose is comparison of the effect of Quercetin and Taxifolin on the DNA structure of human uterine cervix carcinoma cells (Hela) *in vitro* by the Comet assay (single-cell gel electrophoresis).

Quercetin (USA, 96%) and Taxifolin (DIOD Company, 98%) were used in 4 different concentration – 0.5, 5.0, 50 and 500 µg/ml (on active substance basis). Hela cells were incubated with the samples at 37°C within 30 min, then they were immobilized into agarose gel in order to assay by the alkaline variant of the Comet-test. The slides were lyzed, alkaline denatured and subjected to electrophoresis (8°C, 20 min, 2.0 V/cm). Hela cells, incubated with PBS, were used as the control to estimate spontaneous damages. The received preparations were colored by ethidium bromide and visualised by fluorescent microscopy. The obtained images were captured by a digital photo camera and processed by the special software in order to calculate the % of DNA in a comet tail (TDNA %).

The number of spontaneous DNA damages was 1.03±0.29% TDNA in the control sample. Taxifolin did not show genotoxicity in all concentrations: 0.5, 5.0, 50 and 500 µg/ml – 0.70±0.23%, 1.45±0.37%, 1.71±0.51% and 1.24±0.40% TDNA respectively. But Quercetin exerted the evident doze-dependent damaging action in relation to DNA of Hela cells, starting with the concentration of 5.0 µg/ml (0.5, 5.0, 50, 500 µg/ml – 2.06±0.40%, 3.15±0.89%, 3.97±0.91%** and 6.47±0.98%** of TDNA respectively, ** - p < 0.001).

Taxifolin did not show its genotoxicity over all the researched concentrations. Quercetin exerted its doze-dependent damaging action in relation to DNA of Hela culture *in vitro*.

SOME ASPECTS OF STANDARDIZATION OF ADAPTOGENS

O.N. Pozharitskaya, A.N. Shikov, S.A. Ivanova, V.M. Kosman, V.G. Makarov

St-Petersburg Institute of Pharmacy, St-Petersburg, Russia

Current Good Manufacturing Practices (cGMPs) for dietary supplements [1] have just recently become effective. After a long period of discussion and anticipation, the dietary supplement industry is now expected to implement and utilize a rigorous quality management system to ensure safety, quality, and consistency of products. Because quality can not be tested into a product, it is important that quality is already an attribute of the raw material of any production process. For "botanicals" and "herbal drugs" which can be fresh, or processed plants, extracts, or other preparations, the identity is one of the central elements of quality. cGMP regulation therefore requires identity testing for each raw material that enters a process. Appropriate and scientifically sound methods should be applied. Data generated with these methods must be secure, transparent, and traceable. The only problem seems to be where to get all the required methods. Analysis of botanicals and herbal drugs is a great challenge because they are highly complex mixtures of compounds covering a broad range of substance classes and exhibit natural variability. Tools and acceptance criteria developed in an attempt to comply with cGMP for synthetic pharmaceuticals have only limited applicability to these challenges. It seems necessary to explore new approaches, which are fit for the purpose.

The purpose of this work was to formulate a concept for standardization of the "appropriateness" of different chromatographic methods for identification and quantification of its active principles of herbal drugs adaptogens.

Comprehensive quality assessment methods for herbal adaptogens were developed. In contrast to the conventional quality assessment standard of herbal drugs [2], the present improved methods adopts chromatographic (HPTLC and HPLC) fingerprinting of total extracts of the herb. These methods provides more chemical information that can be used for the identification of the crude drug as well as for the quantification of pharmacologically active marker compounds that are directly associated with the quality of the herbal medicine.

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GASTROPROTECTIVE EFFECT OF A POPULAR SPICE ASSAFOETIDA "*FERULA ASAFOETIDA*" USED IN ARAB TRADITIONAL MEDICINE

Syed Rafatullah, Saleh Alqasoumi, Ibrahim Al-Mofleh

Department of Pharmacognosy and Medicinal, Aromatic and Poisonous Plants, Research Center, College of Pharmacy, P.O. Box 2457, King Saud University, Riyadh 11451, Saudi Arabia

To validate the claims of Unani, Ayurvedic and Arabian traditional medicine practitioners on the gastric antiulcer properties of a popular spice Assafoetida "*Ferula asafetida*" on experimentally-induced gastric secretion and ulceration in albino rats.

Different group of animals were used in each experiment. Gastric acid secretion studies were undertaken using pylorus-ligated Shay rats using 6 animals in each group, the suspension was given intraperitoneally. In another experiment, gastric lesions were induced by noxious chemicals including ethanol, strong alkalis and indomethacin, the assafoetida suspension was administered orally. The levels of gastric wall mucus (GWM) and non-protein sulfhydryls (NP-SH) were measured in the glandular stomach of rats following the ethanol administration in separate group of rats. The gastric tissue was also examined histologically. The suspension was used in two doses (250 and 500 mg/kg body weight) in all experiments.

In pylorus-ligated Shay rats, aqueous suspension of assafoetida significantly and dose-dependently reduced the basal gastric acid secretion, titratable acidity and ruminal ulceration. Asafoetida suspension significantly attenuated gastric ulceration induced by necrotizing agents (80% ethanol, 0.2 M NaOH, 25% NaCl) and indomethacin. The antiulcer effect was further confirmed histologically. On the other hand, the pretreatment of rats with suspension significantly replenished GWM and NP-SH levels.

Aqueous suspension of assafoetida possesses antisecretory, cytoprotective, and antiulcer activities against experimentally-induced gastric lesions. The antiulcer effect is possibly through prostaglandin-mediated and/or through its anti-secretory and antioxidant properties.

PROOF OF ENDOMETRIAL SAFETY OF THE SPECIAL *CIMICIFUGA RACEMOSA* EXTRACT CR BNO 1055

Karel Rauš, MD¹, Christoph Gorkow, DVM²

¹Department of Obstetrics and Gynecology, Charles University Teaching Hospital, Prague, Czech Republic

²Bionorica AG, Neumarkt, Germany

To investigate endometrial safety by assessment of endometrial biopsies, and to investigate tolerability and efficacy concerning climacteric complaints as well as possible influence on bone turnover of the special *Cimicifuga racemosa* extract CR BNO 1055.

400 postmenopausal women suffering from symptoms related to estrogen deficiency were enrolled into a prospective, open-label, multinational, multicentre study. Treatment duration (daily dosage corresponding to 40 mg of herbal drug) was 52 weeks. For probability determination of the incidence of endometrial hyperplasia and/or more serious adverse endometrial outcome the point estimator and upper limit of 95% confidence were calculated. Descriptive statistics was used to assess the secondary endpoints.

Endometrial safety of the special *Cimicifuga racemosa* extract CR BNO 1055 has been proven since no case of hyperplasia or more serious adverse endometrial outcome occurred (point estimate: 0.0; upper limit of confidence interval: 0.011). Endometrial thickness measured by endovaginal ultrasonography did not increase over the course of treatment. The 4-week weighted score of hot flushes showed a significant decrease of 80.7%. As for the bone turnover markers, the β -CrossLaps (marker of bone degradation) decreased significantly (change from baseline 25.67%) indicating an antiresorptive activity of the special *Cimicifuga racemosa* extract CR BNO 1055. Unlike treatment with estrogens, osteocalcin (marker of bone formation) remained nearly unchanged. Mammography revealed no increase of breast density. The drop-out rate was below 10%. The overall tolerability was good.

The absence of signs of endometrial proliferation, improvement of climacteric complaints, the beneficial effects on bone turnover as well as just a few gynecologic-organ related adverse events are reported for the first time after a treatment period of one year. Due to the improved risk/benefit ratio the *Cimicifuga racemosa* special extract BNO 1055 can be assumed a safe alternative for treatment of climacteric complaints.

"PHYTONEERING" - KEY TO MODERN PHYTOTHERAPY

Bernd Roether

Bionorica AG, Kerschensteinerstraße 11-15, D-92318 Neumarkt, Germany

Bionorica AG has developed a new term for its forward-looking focus on the investigation and development of herbal medicines: phytoneering. The new and unique feature of this concept: Nature and modern technology not as opposites, but as complementary partners. Phytoneering means intensive research for innovative, high-quality and effective herbal medicines.

Treatment of a wide variety of diseases using herbal medicinal products (phytotherapeutics) is steadily gaining in importance today. Effective substances with few side effects, created by nature, are the preferred alternative to synthetic substances accepted by patients more and more. The use of medicinal plants for treatment of a whole range of complaints has been passed down for centuries in some cases. At Bionorica AG, this knowledge and current data on botanical agents form the basis of modern phytotherapy.

The use of innovative technical and pharmaceutical processes plays a leading role in the investigation and production of herbal medicinal products. Bionorica AG has given the resulting corporate concept a name: phytoneering. This concept signifies a unique link between nature and plants (phyto-) on the one side, and science and technology on the other (engineering). At the same time, it represents the company's international orientation, which makes the highest quality standards for herbal medicines possible in the first place.

Manufacturing: Only the Best Is Good Enough.

An essential element of the phytoneering concept is the ongoing optimisation of all manufacturing processes. At Bionorica, this begins with the choice of seeds, and continues through cultivation of the plants, gentle extraction of the active substances from the plant parts, and production of the preparations. To this end, Bionorica itself and all its suppliers work according to GMP (good manufacturing practice) guidelines. Every stage of production is based on cutting-edge scientific knowledge and technical methods. Production as a whole consists of processes that are optimally adapted to the special requirements of herbal substances, some of which have been developed and patented by Bionorica itself. Constant quality control measures accompany and document the manufacturing process seamlessly - from plant cultivation to the release of the drug product for delivery to pharmacies. The balanced and reproducible composition of the relevant active substances/substance group(s) in the final product is the result of this optimised high-tech production process - a central element of phytoneering.



PHYTOPHARM 2009

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REGULATORY ASPECTS OF HERBAL MEDICINAL PRODUCTS IN EUROPE

René Roth-Ehrang, Thomas Kersten

Finzelberg GmbH, D-56626 Andernach, Germany

The European Community pursues harmonisation also in terms of marketing authorisation procedures for medicinal products. The regulatory framework is based on a two-stage system with a centralised and a decentralised procedure.

A Centralised Procedure is compulsory for human medicines derived from biotechnology processes but also for certain indications such as diabetes. Of course this could also open prospects for Herbal Medicinal Products.

In a Decentralised Procedure (DCP) applicants may apply for simultaneous marketing authorisations in more than one EU Member State, provided that the medicinal product has not been authorised in the community at time of application.

If the marketing authorisation was first applied and granted in only one EU Member State, identical marketing authorisations can be sought in further EU Member States by a Mutual Recognition Procedure (MRP).

With regards to the fact that there are many preparations in several Member States of the European Union with a long tradition and high safety but whose efficacy can only hardly be proven according to the current requirements to clinical or bibliographic evidence, a simplified registration procedure was established in 2004.

For these Traditional Herbal Medicinal Products the efficacy must be plausible on the basis of longstanding use and experience. This means a use of at least 30 years. Products from outside the European Union must prove a use of at least 15 years within Europe.

All different types of registration procedures require the same comprehensive documentation to prove the quality of the active ingredients and the product.

Despite the EU efforts of harmonisation one problem remains unsolved: Similar composed products are not classified to uniform criteria. A Herbal Medicinal Product in one country can be legally sold as foodstuff in an other country.

PESTICIDE CONTENT OF RAW MATERIAL MEDICINAL PLANTS IN PHARMACEUTICAL MARKET

I.V. Gravel, E.A. Ivanova, I.A. Samylina

Sechenov's Moscow Medical Academy, Moscow, Russia

The problem of environmental pollution by anthropogenous substances is deserved a special attention. Pesticides are one of the most toxic for living organisms among environmental contaminants. According to previous data pesticides are capable of being accumulated by medicinal plants and can pass into medicine [1].

The aim of the work was to compare the pesticide content in raw material of medicinal plants which come to the pharmaceutical market from different countries.

Since 1940's organochlorine pesticides (OCP) had been widely applied in agriculture, but because of their toxicity and ability to be accumulated in environment, since 1970's application of OCP was significantly reduced in most countries. The analysis of raw material collected in Poland in 1980's revealed high content of some OCP (DDT, HCH and other) in elder flowers, absinth herb, coltsfoot leaves, dandelion herb and roots. Moreover, 30-50% of the samples contained DDT above 1.0 mg/kg. In Europe in the middle of 1990's it is observed the reduction of the contents of OCP in medicinal plants by 30%. Nowadays DDT concentration of raw materials, marketed through drug shops system in Russia are 0.0052-0.0078 mg/kg.

Since the end of the XX century organophosphate pesticides (OPP) have been widely used in medicinal plant cultivation. Recent researches of the quality of raw material collected in Egypt indicated that 72% of samples were polluted by OPP (dimethoate, malathion, profenofos) in content 0.5-310 mg/kg. Maximum concentration was detected in mint leaves and anise seeds [2, 3].

Thus the analysis of data of pesticide content of raw material medicinal plants showed that they have been polluted by various classes of pesticides.

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OPEN COMPARATIVE STUDY IN PATIENTS WITH OSTEOARTHRITIS (BY ALTMAN CRITERIA R 1990) OF THE KNEE I-III STAGES (BY THE KELLGEREN CRITERIA). ALL PATIENTS WITH NSAIDs INTOLERANCE

Evgeniya Shikh

Institute of Clinical Pharmacology, Moscow Medical Academy, Russia

Aims: to research efficiency and safety of application of Zinaxin with glucosamin in patients with osteoarthritis and with NSAIDs intolerance.

We included patients with OA (by the Altman criteria R 1990) of the knee I-III stages (by the Kellgren criteria). Age is 45-70 yrs, men and women. Patients were with pain during motion ≥ 40 mm by 100-mm VAS scale. Patients didn't receive other Chondroitin sulfate and Glucosamin sulfate (Structum, Dona, Artra). The group consisted of 27 patients with NSAIDs intolerance: gastrointestinal hemorrhage in anamnesis-5 patients, recidivating ulcers caused NSAIDs -9, bronchialis obstruction in anamnesis- 6, relative overdose of digoxin (increase of free fraction – Nimesulid(Nimesil)-3.

Before treatment there were 0 patients without pain (0-25 points), 11 (44%) with mild pain (25-50 points), 14 (56%) suffered from severe but tolerable pain (50-75 points). After 2 weeks of treatment 2 (8%) patients didn't have any pain, 16 (64%) were with mild pain and 7 (28%) were with severe but tolerable pain. After 3 weeks of treatment the following changes occurred: 5 (20%) of patients were without pain, 16 (64%) were with mild pain and 4 (16%) had severe but tolerable pain. Statistically reliable decrease of pain by WOMAC were identified by the end of 2 week of the therapy (from 11.3 ± 1.7 to 5.9 ± 1.2). It was detected statistically significantly decrease of stiffness by WOMAC (from 3.5 ± 0.5 to $2.2 \pm 0.6^*$) and activities by WOMAC (from 26.9 ± 8.2 to $14.9 \pm 5.6^*$). During the third week of therapy we observe further statistically reliable positive dynamics for all knee parameters.

We can clearly see clinical effectiveness of treatment by Zinaxin with glucosamin (2 caps twice a day) after 2nd-3rd weeks. Zinaxin with glucosamin doesn't cause an irritating effect on the digestive tract, doesn't induce bronchialis obstruction in patients with Aspirin-sensitive Asthma and doesn't lead to relative overdose of Digoxin.

Zinaxin with glucosamin can be used in therapy of joint's syndrome for patients with NSAIDs intolerance.

ADAPTOGENS - FROM HISTORY TO FUTURE

Alexander N. Shikov, Olga N. Pozharitskaya, Valery G. Makarov

Interregional Center "Adaptogen", 47/5, Piskarevsky prosp., 195067, St-Petersburg, Russia

The term adaptogen was introduced in 1947 by N.V. Lazarev to describe any substance which, when chronically administered, can increase an organism's ability to cope with atypical physical or chemical insult. Adaptogens should possess: nonspecific activity; normalizing effect independent from the nature of the pathology and be non toxic; not influence normal physiological functions greater than required [1]. Since 1997, the term "adaptogen" has been used as a functional term by Russian health-regulatory authorities, and in 1998 this term was allowed as a functional claim for certain products by the U.S. Food and Drug Administration. There are already over 1700 studies (mainly pharmacological and clinical) published in Russia concerning extracts or individual compounds from the adaptogenic plants. However review of PubMed data base shows that term adaptogen referred only 183 times starts from 1978.

In 1960 to 1980, after a large number of pharmacologic and clinical studies, 8 plants were considered to be adaptogens in USSR officinal medicine: *Aralia mandshurica*, *Echinopanax elatum*, *Eleutherococcus senticosus*, *Panax ginseng*, *Rhaponticum carthamoides*, *Rhodiola rosea*, *Sterculia platanifolia*, and *Schisandra chinensis*. Since the introduction of the concept of adaptogen, several plants have been investigated. These were used as tonics in folk medicine, and were found to fulfill the criteria laid down by Brekhman and Dardymov and therefore may be qualified as candidates to adaptogens.

According to chemical structure of active ingredients, adaptogens can be divided into the three groups of: those that contain phenolic compounds such as phenylpropanoids, phenylethane derivatives, and lignans, whose structural resemblance to catecholamines could suggest an effect on the sympathoadrenal system and possibly imply an effect in the early stages of the stress response; those that contain tetracyclic triterpenes, such as ginsenosides, cucurbitacin R diglucoside, which structurally resemble the specific corticosteroids that inactivate the stress system to protect against overreaction to stressors; and oxylipins - unsaturated trihydroxy or epoxy fatty acids structurally similar to leukotrienes and lipoxines.

Adaptogens have different pharmacological effects. Extracts of plant adaptogens have been shown to increase the resistance of a variety of organisms against the negative effect of different stress conditions and to increase cellular defense against reactive oxygen species in different cell types. In addition, it has been shown that adaptogens significantly increase longevity and stress resistance both in *C. elegans* and in *D. melanogaster*, probably by alleviating oxidative stress [2-4]. Clinical evidence has suggested that a standardized extract of *R. rosea* has antifatigue, antistress, antioxidant and immune-enhancing effects, whereas a fixed combination of the 3 adaptogens has been shown to increase the quality of life [5].

Adaptogens industrially produced in Russia at the moment as standardized extracts in tablet and liquid forms as medicines having stimulating, restorative, and antistress effects. Adaptogens hold great promise for optimum wellness and prevention of chronic illness

due to their ability to enhance our resistance to a variety of adverse influences. Our adaptive capability is critical, not only in our ability to resist disease, but also in our ability to thrive and be full of zest and zeal. And the future of adaptogenic formulations is colossal.

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INNOVATIVE METHODS IN RESEARCH AND QUALITY CONTROL OF HMPS

Günther Stecher¹, Günther Bonn²

¹Bionorica research GmbH, Mitterweg 24, 6020 Innsbruck, Austria

²Institute of Analytical Chemistry and Radiochemistry, University of Innsbruck, Innrain 52a, 6020 Innsbruck, Austria

Herbal medicinal products (HMPs) are widely used and enjoy high acceptance in patients. From the analytical point of view the fundamental difference between a synthetic chemical drug and a HMP lies in the complexity of the sample. Whereas the chemical drug consists, beside some excipients, of one single, active pharmaceutical ingredient (API), the HMP represents a highly complex mixture of compounds. Due to this complexity it is essential to characterize and determine relevant ingredients in the medicinal plant, but also in the produced and commercialized extract.

The actual talk will give an overview about standard extraction and separation techniques available for the routine analytical laboratory. On basis of this overview different strategies and novel and innovative developments and approaches will be presented: In fact, examples will concentrate on extraction techniques like microwave assisted extraction and Aq-solv extraction, on purification methodologies and on novel separation tools. Characterization of plant extracts via matrix enhanced laser desorption mass spectrometry (MELDI-MS) and capillary liquid chromatography-mass spectrometry (cap-LC-MS) will be shown. In this context fundamental differences, but also synergies are discussed.

Finally the critical evaluation and resulting implementation of novel and innovative analytical techniques represents a fundamental contribution to Bionorica's phytoneering concept.

THE "-OMIC-"TECHNOLOGIES TO INVESTIGATE SYNERGY EFFECTS***G. Ulrich-Merzenich, F. Hartbrod, D. Panek, H. Zeitler, H. Vetter***Universitätsklinikum Bonn der Rheinischen Friedrich-Wilhelms-Universität Bonn,
Wilhelmstr. 35-37, D-53111 Bonn, Germany

Drug development has been based on the use of one single compound to hit one target in order to encounter a specific disease. However, based on investigations with the "omic"-technologies we have seen that one compound does not hit one but several targets. In addition it has been rationalized that multifactorial diseases require a multitargeting [1, 2]. Thus, combination therapies of synthetic drugs have become common in the treatment of diseases like cancer, cardiovascular diseases or rheumatic diseases. But the term "synergy" is rarely used and the combinatory mechanism of actions are seldom completely understood. At the same time complex interactions as the ones of phytopharmaceuticals were regarded as unpredictable. Today, high-throughput technologies in form of microarray based gene/protein expression analyses can relate complex mixtures to complex effects and may therefore be suitable to assess molecular mechanisms of action and synergistic effects of phytopharmaceuticals.

We investigated the gene/ protein expression profiles of standardized salicin containing plant extracts [Willow bark (WB), *Populus tremulus* (PT)], and compared those expression profiles with the ones of quercetin (Q) and of nonsteroidal antiinflammatory drugs [acetylsalicylic acid (ASS) and diclofenac (D)] in two cell types- in human chondrocytes and fibroblasts in order to see whether salicin is the primary antiinflammatory and immunomodulatory compound.

Synchronized cells were stimulated for 6 hrs with either WB (30 µg/ml, 50 µg/ml salicin), PT (2 µg/ml salicin), Q (5.10 µM), ASS (30 µg/ml) and D (50 µg/ml). Gene expression profiles were acquired by gene microarrays (Agilent Human Genome Microarray, Piquor® Pathology Microarray). Protein expression of cell lysates were examined by antibody microarrays (RayBio®, 23 cytokines, 10 matrixmetalloproteinases (MMP)) (n=3).

Interexperimental correlation- and clusteranalyses of data revealed specific RNA expression profiles for each substance. In chondrocytes the RNA expression profile of WB was closer to Q than to ASS and D. Selected protein expression profiling revealed a modulation of IL-5, IL-6, IL-7, IL-10; MCP-1, MCP-3, Groa, GCSF, GMCSF, MIG, RANTES, MMMP-2, MMP-3, MMP-8 for WB (30µg/ml) and additionally IL1a, TNF-α, TNF-β, TGF-β, MCP-2 for WB (50µg/ml); Q modulated IL-2, IL-7, IL-10, IL-13, IL-15, MCP-3; MIG, RANTES, MMP-2, MMP-3, MMP-8, MMP-9, MMP-10 and TIMP-4 supporting gene-expression data. In fibroblasts protein expression of WT was close to Q, whereas the one of PT was closer to ASS.

Not only salicylates, but also polyphenols have an immunomodulating effect in chondrocytes and fibroblasts and may contribute to the overall effect of willow bark. However, in vivo gene- and proteinexpression profiling, considering bioavailability, are essential to substantiate the clinical significance of these findings. Microarray expression profiling will develop our knowledge on the functional mechanisms of complex phytopreparations and our understanding of synergism.



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MICROELEMENTAL COMPOSITION OF *SYRINGA VULGARIS* L. LEAVES

V.V. Velma, V.S. Kyslychenko, A.I. Popik, V.V. Korol

The National University of Pharmacy, Kharkov, Ukraine

Syringa vulgaris L. (lilac, family Oleaceae) is a bush or a tree cultivated as decorating and ornamental plant. In folk medicine the flowers and leaves of the plant are widely used (especially as compresses made from the infusions) against the joints diseases, festering wounds, neuralgias.

The aim of the present work was to study mineral composition of *Syringa vulgaris* leaves. *Syringa vulgaris* leaves were collected in May-June, 2008 in the Kharkov area (Ukraine), dried and served as objects for the research. Their mineral composition was determined by atomic absorption spectrometry (device KAC-120, BO "Elektron", Sumy) with atomisation in an air - acetylene flame.

16 macro- and microelements were revealed in lilac leaves (tab.), 9 of them are essential for a human body (Fe, Si, Mn, Pb, Ag, Ni, Mo, Cu and Zn). Such elements as K, Ca, Fe, P, Mg, Mn and Al were dominated in *Syringa vulgaris* leaves.

The results of the elemental analysis of *Syringa vulgaris* leaves

| № | Element | Contents, mg/100 g | № | Element | Contents, mg/100 g |
|---|---------|--------------------|----|---------|--------------------|
| 1 | Fe | 100 | 9 | K | 3330 |
| 2 | Si | 440 | 10 | Ni | 0.45 |
| 3 | P | 170 | 11 | Ca | 1000 |
| 4 | Mn | 120 | 12 | Mo | <0.03 |
| 5 | Al | 130 | 13 | Cu | 2 |
| 6 | Pb | 0.30 | 14 | Na | 70 |
| 7 | Ag | <0.003 | 15 | Zn | 10 |
| 8 | Mg | 550 | 16 | Sr | 2 |

As a result of the present the qualitative and quantitative characteristic of macro and micro-elements composition of *Syringa vulgaris* leaves was done.

VITIS VINIFERA AND MALUS DOMESTICA – LONG-TERM SOURCES FOR DIETIC FOOD SUPPLEMENTS

V.V. Velma, V.Yu. Kuznetsova, E.N. Novosel

The National University of Pharmacy, Kharkov, Ukraine

Creation of new drugs and biologically active food supplements explains interest on a base of fruit and berry species such as Cultured Grape, *Vitis vinifera* (family Vitaceae), and home apple – *Malus domestica* (family Rosaceae) is perspective because of sufficient raw material resources and possibility to a complex use of these plants. Grape and apple fruit are widely used in food industry. Grape and apple industrial wastes are apple and grape husks. About 80% of them are later used as fertilizers and as substances for producing apple pectin. The interest in these husks for the pharmaceutical industry can be explained by several reasons: one of which is that the qualitative and quantities composition of the biologically active compounds of the husks makes it possible to use them as a source for new drugs and biologically active food supplements. Also the potential amount of grape and apple husks makes it possible to classify them as industrial raw material.

"Endoterol" (Sanofi-Winthrop, France) is a drug contains a purified extract of grape seeds with a standard content of procianidin oligomers (50 mg and 150 mg). European companies produce a number of biologically active food supplements that contain apple pectin "Pectin with vitamin C" ("Cammi medical" Bulgaria), "Florafiber" ("Salus-Haus", Germany) etc.

We have done a comprehensive phytochemical study of apple and grape husks. In addition, it has been identified that grape husk contains antocians in a high quantity, mostly presented by glycosides: 3-O-cyanide glucoside, 3-O-malvidin glucoside, 3-O-delphinidin glucoside that are responsible for antioxidant activity. In addition, apple husks are rich in pectin substances that are used as enterosorbents. The results obtained shown that apple and grape husks are perspective for creating phytodrugs and biologically active food supplements.

DEVELOPMENT OF A NEW FOOD SUPPLEMENT – VITARGEN

V.V. Velma, V.S. Kyslychenko, Z.I. Omelchenko, O.M. Koshevoy

The National University of Pharmacy, Kharkov, Ukraine

Majority of the biologically active substances (BAS) vital for our organism are consumed with food. The population diet structure is going through some changes leading to a disbalance between some main diet components. But even a well-balanced diet doesn't provide an organism with all BAS it requires. An unfavorable ecological and economic situation calls for a need to search the means that will increase our organism resistance to a harmful environmental impact.

The goal of our research was to develop a composition of a biologically active food supplement aimed for increasing organism's resistance to unfavorable environmental conditions.

Vitargen was developed in the National Pharmaceutical University. It contains water-alcohol extracts of dog rose fruits, knotgrass, elder flowers, silkworm eggs, ethyl alcohol, bee glue, purified water. Vitargen was received by a component blending method with a further blend settling and filtration. Its pharmacological effect is produced by the complex of substances that serve as ingredients in its composition.

A developed product is recommended to increase men's potency and women's libido. Other Indications: fatigue, physical and emotional loads, stresses, asthenia, chronic diseases of gastroenteric tract as well as a preventive means against chemical, radiation and temperature factors. Contraindications: inborn or acquired alcohol intolerance, acute diseases of liver and kidneys, heart arithmia, serious forms of ischaemic heart disease, arterial hypertension, acute and chronic poisoning with chemical compounds, alcoholism, pregnancy, breast feeding, Not to be used by children before 16 and drivers.

Vitargen has anti-stress, antitoxic, and immunostimulating effects. It improves intellectual and physical activities. State registration certificate N 1086, dated July 11, 2006.

BOTANICALS AND NMR-BASED METABOLOMICS: A PERFECT HOLISTIC MATCH

R. Verpoorte, Y.H. Choi, H.K. Choi

Department of Pharmacognosy, Section Metabolomics, Institute of Biology Leiden,
PO Box 9502, 2300RA Leiden, The Netherlands, Email: verpoort@lacdr.leidenuniv.nl

An estimated 40,000-70,000 plant species are used as medicine worldwide. Some of these are extensively studied and their use is supported by clear clinical evidence. But in fact most have not been studied in any detail, and little is known about their activity, mode of action and possible active compounds. This poses two major problems, one is the acceptance of botanicals which are not yet evidence based, the other is quality control. In fact acceptance is hampered by the fact that no methods for proper quality control are available if no active compound(s) is/are known.

With globalization the use of botanicals is clearly increasing. Pharmacognosy has thus a major task in developing medicinal plants into evidence-based medicines. This will include both mentioned aspects: evidence for activity and quality control. In the past decades drug development has gone from *in-vivo* testing into molecular based assays (High Throughput Screening, HTS) for finding new leads. Certainly by HTS one may find active compounds in medicinal plants, but synergy and pro-drugs will certainly not be found in such an approach. We, pharmacognosists, should thus rethink our approaches for proving activity of medicinal plants. This is where systems biology and metabolomics do offer interesting options. It means going back to *in-vivo* pharmacology in combination with the "-omics" technologies to measure the response of a test organism on treatment with the medicinal plant, and metabolomics to phytochemically characterize the medicinal plant. By chemometric methods, such as multivariate analysis, links can be made between compounds present in the plant and activities observed in the model organism. That means that not only active compounds, but also synergy and pro-drugs can be found. This approach will also be the basis for quality control, as by metabolomics in combination with multivariate analysis one can define the required profile for activity. Particularly NMR-based metabolomics has a great potential for both quality control and identification of compounds related to activity.

A NEW REFINED EXTRACT FROM PINK ROCK ROSE (ECCE60) HELPS TO SUPPORT HUMAN IMMUNE DEFENSE

Bernd Walbroel¹, Björn Feistel¹, Kurt Appel²

¹Finzelberg GmbH & Co. KG, Koblenzer Strasse 48-56, D-56626 Andernach, Germany

²Vivacell Biotechnology GmbH, Ferdinand-Porsche-Str. 5, D-79211 Denzlingen, Germany

Several species of the genus *Cistus* (Cistaceae) have been reported to exhibit a variety of pharmacological activities including anti-inflammatory and anti-viral potential [1]. In-vitro pharmacological activities of *Cistus* extracts were previously shown to be in strong correlation with the samples' total phenol content [2]. In order to obtain an extract enriched in phenolic compounds, a new manufacture process via resin columns was established, resulting in 60% polyphenols in dry extract (Patent application, Finzelberg GmbH & Co. KG). This extract from Herba *Cistus creticus* L. subsp. *eriocephalus* (Viv.) Greuter Burdet (ECce60) shows its specific anti-inflammatory potential in-vitro [3] and was further investigated herein.

Compared to common aqueous or aqueous-ethanolic extracts from Herba *Cisti ssp.* with rd. 20% Polyphenols (ORAC value 1500 µmol Trolox units/g) the new refined extract with min. 60% Polyphenols shows a more than 3 fold higher antioxidative capacity of 5100 µmol Trolox units/g. The same tendency shows an antiviral test on neuraminidase activity. ECce60 inhibits dose depended the Neuraminidase activity, tested from viral influenza in the same manner like Zanamivir®.

The new refined *Cistus* preparation ECce60 combines the main product characteristics (anti-oxidative, anti-inflammatory, anti-viral) which products need to support the natural immune system against flu and cold or cough viruses.

References:

1. Pomponio, R. et al. (2003) J Chromatogr A 990:215-223
2. Taila, S. et al. (2008) J Pharm Pharmacol 60:A62 - 63.
3. Obolskiy, D. (2008) Phytochemical and in vitro NF-kappa B inhibitory / Antioxidant Profiling of *Cistus creticus* L subsp. *eriocephalus* (Viv.) Greuter Burdet; MSc dissertation, The School of Pharmacy, University of London.

RUSSIAN TARRAGON (*ARTEMISIA DRACUNCULOIDES L.*) EXTRACTS AND THEIR ANTIDIABETIC POTENTIALS

Bernd Walbroel¹, Björn Feistel¹, Ivo Pischel²

¹Finzelberg GmbH & Co. KG, Koblenzer Strasse 48-56, D-56626 Andernach, Germany

²PhytoLab GmbH & Co. KG, Dutehdorfer Strasse 5-7, D-91487 Vestenbergsgreuth, Germany

A long tradition refers to Russian Tarragon (*Artemisia dracunculoides L.=Artemisia dracunculus L. var. inodora*), used since centuries in Russia and middle Asia as an herbal medicine as digestive, diuretic and antipyretic drug. The classical application forms were aqueous decocts or aquos-ethanolic tinctures. Nowadays also aqueous dry extracts as well aqueous-ethanolic dry extracts exist, safeguarding without harmful components of the essential oil like Estragol or Methyleugenol, therefore generally recognized as safe (GRAS).

New possibilities for the use as an antidiabetic drug were firstly described by Ribnicky et al. [1] for an Extract from 60% ethanol. In our new study it was found, that the oral administration of aqueous Russian tarragon extracts showed a change of blood glucose levels within a well established animal model using an oral glucose tolerance test (OGTT), which is well transferable to human post-prandial lowering of blood glucose that occurs after meals. The results for an aqueous extract are clearly improved over the formerly described 60%-ethanolic extracts. We can show that the aqueous Russian Tarragon extract helps to dear glucose from the blood and reduces post-prandial glucose peaks, problematized for Diabetes mellitus type 2. Therefore it could help to reduce undesired glycoxylation reaction with vital molecular body structures or metabolites in the blood serum or tissues. In addition, it was found that the extract does not have an effect on the basal blood glucose levels, a fact which can be interpreted as crucial positive safety aspect related to missing of a hypoglycemia status after oral administration of the said extract by fasting individuals.

Reference:

1. D.M. Ribnicky, A. Poulev, M. Watford, W.T. Cefalu, I. Raskin: Antihyperglycemic activity of Taralin™, an ethanolic extract of *Artemisia dracunculus L.*, Phytomedicine 13 (2006) 550-557.

THE NOMENCLATURE ANALYSIS OF HOMOEOPATHIC AND HERBAL MEDICINAL PRODUCTS IN THE RUSSIAN FEDERATION

D.A. Yavorsky, T.P. Lagutkina

Department of Pharmacy Economics and Management, Peoples Friendship University of Russia, 6, Miklukho-Maklaya str., 117198, Moscow, Russia

For the last few years, the number of the medicinal products registered in the Russian Federation has increased, and the pharmaceutical market became one of the most fast-growing and highly profitable sectors of a national economy. This situation shows the actuality of registered medicinal products nomenclature analysis as base for manufacture or import planning and formations of assortment of the pharmacy organizations.

The aim of this work is the nomenclature analysis of officinal and homoeopathic herbal medicinal products (HMP) for period from 1995 till 2008 year. According to the State register of medicine, for the specified period the number of all registered medicine has grown from 8071 to 13143. The number of registered officinal HMP has grown in 2 times - from 775 to 1741. The number of registered homoeopathic HM has grown in 25 times - from 33 to 834.

For the last 13 years the share of HMP among all registered medicine has increased from 9.6% to 13.2%. The share of homoeopathic HMP from level of 0.41% has grown to 4.5%, "having borrowed" these percent other groups of medicine. The quantity fast growth of homoeopathic HMP is connected with more intensive registration Russian medicine which number has increased in 30 times - from 19 to 589. The quantity of foreign homoeopathic HMP for this period of time has increased in 17 times - from 14 to 245. The same peculiarity is observed in registration of officinal HMP. For last 13 years the quantity of registered HMP in Russian, has increased almost in 2.5 times - from 609 to 1509. The quantity of registered foreign HMP has changed slightly - from 166 to 232.

Thus, assortment of officinal and homoeopathic HMP in the Russian pharmaceutical market of late years constantly increased. On rates of annual growth of quantity registered officinal HMP and, especially, a homoeopathic HMP, advance all other groups of medicines.

TANNINS FROM *ELEAGNUS ORIENTALIS* L. LEAVES

S.P. Zavadsky¹, E.A. Abizov¹, O.N. Tolkachev², S.D. Maltsev³

¹Moscow Sechenov Medical Academy, Moscow, Russia

²All-Russian Research Institute of Medical and Aromatical Plants, Russian Academy of Agricultural Sciences, Moscow, Russia

³Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Moscow, Russia

The aim of the work was isolation, detection, qualitative and quantitative analysis of tannins in *Eleagnus orientalis* L. leaves. This plant is potential raw material for astringing action drugs creation.

E. orientalis leaves were harvested in Samara and Belgorod regions of Russia and also in Yerevan (Armenia) in the end of July and beginning of August. The herbal air-dried raw material were disintegrated up to particles size less than 1 mm. was proceeded according to Russian State Pharmacopoeia X and XI procedures and by using NMR, UV-spectroscopy and TLC were used for qualitative analysis of individual catecholamines (wet spray developer was 1% vanillin in sulfuric acid solution) and other compounds. ¹³C-NMR spectra were obtained by using pulsed multifunctional spectrophotometer Bruker AC-200 (Germany). UV-spectra of samples were registered at Hitachi EP-3T spectrophotometer (Japan). Permanganatometric titration method was used for quantitative determination of tannins.

Seven phenolic compounds were isolated and identified *E. orientalis* leaves: gallic acid, catechin, epicatechin, hypophenin, strictinin, isostrictinin and casuarinin. Casuarinin was dominated substance among galloelagotannins in ¹³C-NMR spectra. Quantitative data were calculated to casuarinin. The mean value of tannins content was 34±5%. The maximum yield of casuarinin were obtained for the leaves accepted from Yerevan (37.9%).

As a result of comparative study of *E. orientalis* leaves growing in Armenia and cultivated in Russia it was shown that this plant raw material can be used as a source of natural tannins. The results of the study may be used for validation and possibility of use these leaves for production of astringent agents.

**MORPHOLOGO-ANATOMIC AND HYSTOCHEMICAL STUDY OF *ELEAGNUS* L.
SPECIES INTRODUCED IN EUROPEAN PART OF RUSSIA**

E.A. Abizov, S.P. Zavadsky

Moscow Sechenov Medical Academy, Moscow, Russia

Aims of the work was the morphologo-anatomic study of *Eleagnus angustifolia* L., *E. orientalis* L., *E. oxycarpa* Schlecht., *E. multiflora* Thunb., *E. umbellata* Thunb., *E. pungens* Thunb. and *E. argentea* Pursch. These plants are perspective to use as a raw material for drugs with astringing, wound-healing, anti-bacterial and psychostimulatory action.

Comparative-morphologic, morphologo-metric, microscopic and histochemical methods were used for detection of specific features of young generative plants of seven varieties of *Eleagnus* cultivated indoor and outdoor in central non-chernozem part of Russia.

The morphologo-anatomic features of underground organs, stems, leafstalks, leaves, flowers, fruits and seeds of young generative plants were studied. External features such as: size, shape, colour, features of leaf margin, surface "relief", venation pattern, length, leafstalk cross-section were determined. Diagnostic characteristics including ground, parenchyma, mechanical, conductive tissues cell-composition, colour and microchemical nature of their content, size, shape, type of stomata were determined as the result of the anatomical study of vegetative and generative organs. Palisade and spongy cell tissue parenchyma disposition and a number of their layers in transversal sections also were studied. The type of *Eleagnus* leaves was estimated after observing of the mesophyll position. The type of bundles and vessels and a character of disposition of these tissues in plant organs were also characterized.

As a result diagnostic characteristics of underground and above-ground parts of seven species of *Eleagnus* introduced in European part of Russia were studied. Inter-specific peculiarities were determined by morphologo-anatomic and histochemical methods. The data of the study may be used for formation normative documentation on crude drugs of *Eleagnus*.

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