

## NEUROPHYSIOLOGICAL CHARACTERIZATION OF PLANT-DERIVED DRUGS IN RATS AND HUMANS

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Natural compounds and plant-derived products play an ever-increasing role not only in medical therapy but also in medical prophylaxis using food supplements and functional food. The topic of this presentation will try to bridge the gap between animal studies and clinical trials using an electrophysiological approach validated for years in testing pharmaceutical preparations of chemically synthesized compounds. Common parameter of analysis in rat and humans is the local field potential as it is recorded from the depth of the rat brain and from the scalp of the human brain. These potentials — when recorded in the presence of drugs — are called electropharmacograms. In the rat, field potentials are recorded from implanted steel electrodes and transmitted via a telemetric system wireless to the secondary amplifiers and further transmitted to evaluation units by means of a glass fibre. The experimental design consists in recording a pre-drug base line for 45 minutes before administration of the natural ingredients or plant extracts. Recording continues for the next 5 hours. Effects are described in % change from pre-drug values with respect to 6 frequency ranges for each of the four brain areas: frontal cortex, hippocampus, striatum and reticular formation = 24 variables. Extracts from decaffeinated green tea, Schisandra, Ginkgo, Ginseng, St. John's Wort, Radix Rhodiola, Valeriana, Camilla, Guarana, Passiflora, Kava-Kava were compared to the actions of caffeine, quercetin, theanine and theogallin. All extracts and single ingredients produced electrical changes which could be differentiated from each other by feed-

ing the data into a discriminant analysis, where the first three discriminant axes were coded into spatial x, y and z axes. The next three discriminant axes were coded into an additive colour mixture of the colours green, red and blue. Using this method products with a similar effect on the brain provide a similar spatial location like seen for theanine, quercetin, St. John's Wort and give similar colours. In humans a similar approach is performed with data derived from EEG recordings. Development of a 16-channel EEG brain mapping system allowed on-line real time depiction of all frequency changes within one chart. Data from a lozenge containing four plant derived extracts (lemon balm, oats, lavender oil and hops), a drink containing ginkgo and ginseng as well as a decaffeinated green tea extract and a tablet containing passiflora were tested within a similar experimental design in comparison to placebo. After base line recording, administration of the extracts was followed by hourly recordings under conditions of eyes open as well as in the presence of different mental challenges. The data from the electropharmacograms (17 electrode positions and 6 frequency ranges = 102 variables) were fed into discriminant analysis. It could be shown that the actions of the four preparations were entirely different from placebo. This proved the extremely high sensitivity of the method and the consistency of the observed effects. The data document that field potentials from rats and humans are valid parameters for the description of effects of natural compounds and herbal extracts on the brain.

## ANTIOXIDANT ACTIVITY AND PHENOLIC PROFILE OF SERBIAN HONEY WITH DRIED PLUMS

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Honey serves as a good source of natural antioxidants, which are effective in reducing the risk occurrence of heart disease, cancer, cataracts, different immune-system decline, etc. Dried plums contain higher levels of polyphenols and show stronger antioxidant activity than most other fruits. The aim of this research was to investigate the contribution of addition (20–40% mass) of dried plums to phenolic and flavonoid contents and radical scavenging activity (RSA) of lime honey — Serbian polyfloral honey with appellation of origin. Total phenolic

(TP) and flavonoid contents (TF) were determined spectrophotometrically after 10 days after plums addition. Also, RSA on superoxide anion ( $O_2^{\cdot-}$ ) and hydroxyl radicals ( $\cdot OH$ ) were investigated by electron spin resonance spectroscopy. TP and TF in lime honey (27.4 mg/100g and 9.8 mg/100g, respectively) was increased after addition of dried plums in a dose dependent manner. Highest TP (61.4 mg/100g) and TF (38.5 mg/100g) were determined in honey sample with 40% of dried plums. RSA of lime honey ( $EC_{50} O_2^{\cdot-} = 55.5$  mg/ml;

EC<sub>50</sub>·OH=9.0 mg/ml) increased with the addition of 20% (EC<sub>50</sub>O<sub>2</sub>·=27.4 mg/ml; EC<sub>50</sub>·OH=2.7 mg/ml), 30% (EC<sub>50</sub>O<sub>2</sub>·=28.0 mg/ml; EC<sub>50</sub>·OH=3.2 mg/ml) and 40% (EC<sub>50</sub>O<sub>2</sub>·=28.8 mg/ml; EC<sub>50</sub>·OH=7.9 mg/ml) of dried

plums. The obtained results indicate that the honey with dried plums is a new product with high antioxidant activity and their inclusion in the diet may be recommended to complement other polyphenol sources.

## RESEARCH OF EXTRACTION CONDITIONS OF PEPPERMINT AND BOGBEAN RAW MATERIALS AS THE ACTIVE COMPOUNDS OF HERBAL MEDICAL PRODUCT "TRIVALUMEN FORTE"

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Peppermint and bogbean leaves use for preparation of the active substance of the herbal medical product "Trivalumen". The therapeutic role of these components is antispasmodic and choleric action of phenolic compounds and secretolytic action of iridoids. The work's purpose was determination of the optimal extraction conditions of these raw materials in process of herbal medical product "Trivalumen Forte". Raw material was extracted separately by different solvents (93%, 70%, 40% ethanol and water) till the total DER 1:10. Sampling was carried out at interval DER 1:1 for each experiment. In the peppermint and bogbean extracts the amount of total flavonoids was determined by spectrophotometry. The composition of flavonoid fraction of the peppermint extracts and presence of loganin in the bogbean extracts was identified by TLC. The efficacy of extraction process was

assessed using experimental data. The dependency of extraction dynamic of total flavonoids and extractable substances were represented vs. extractant polarity and DER. The optimal conditions of peppermint extract for herbal medical product "Trivalumen Forte" was extraction of raw material by 40% ethanol at DER 1:7–8. The extract contained not less than 9% of total of flavonoids in equivalents of gesperidin (dry extract) and composition of this extract include rutin, giperoside, quercetin, chlorogenic and caffeic acids. Yield of extractable substances was not less than 20%. For bogbean extract the optimal extraction conditions of raw material was by 40% ethanol at DER 1:6–7. This extract contained not less than 2.5% of total flavonoids in equivalents of rutin (dry extract) and characterized by the presence of loganin. Yield of extractable matters was not less than 30%.

## THE METODOLOGICAL APPROACH TO THE IRIDOID ANALYSIS IN HERBAL RAW MATERIALS

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Iridoids are a large group of cyclopentapyran monoterpenoids widely distributed in nature. Iridoids are represented mainly in dicotyledonous plant families namely Plantaginaceae, Lamiaceae, Asteraceae, Gentianaceae, Rubiaceae, Oleaceae. Medicinal plants containing iridoids have a long history of use in the official and folk medicine as bitter tonics, choleric, anti-inflammatory and antimicrobial agents, remedies for wounds and skin disorders. Recent studies have shown antioxidant, neuro-, hepato-, cardioprotective and adaptogenic properties of iridoids. A number of iridoids-containing medicinal plants are included in National Pharmacopoeias of leading countries of the world. In Russian Federation adequate and maximum acceptable intake levels for iridoids (aucubin, harpagoside, oleuropein, asperulosidic deacetylasperulo-

sidic acid) were established by «Unified sanitary-epidemiological and hygienic requirements for goods subject to sanitary and epidemiological surveillance (monitoring)». Standardization of iridoids containing herbal materials is hindered by lack of reliable methods for determination of specific indicative iridoids such as aucubin and catalpol for genus *Plantago* and *Veronica*, harpagide, leonuride for *Leonurus* (Lamiaceae), oleuropein for *Olea* (Oleaceae), loganin for *Menyanthus* (Menyanthaceae), asperulosidic and deacetylasperulosidic acid for *Morinda* (Rubiaceae), etc. A quantitative HPLC–DAD/ESI-TOF-MS method was developed for the simultaneous determination of iridoids mentioned above. Optimal chromatographic conditions were achieved using gradient elution with 0.1% aqueous formic acid and methanol. Analytes were identified