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Abstracts

7th Edition of International Conference on

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Eleni Anastasopoulou et al., Am J Ethnomed 2019, Volume 6
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Comparative chemical analyses of seven endemic Greek *Citrus* hybrids

Eleni Anastasopoulou, Konstantia Graikou and **Ioanna Chinou**

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In the framework of our studies on Greek *Citrus* hybrids, we report here the chemical analyses of the oils from cold pressed peels of one grapefruit [*Citrus paradisi* Cv. Star Ruby x *Citrus aurantium*], four oranges [*Citrus* sinensis cv. Newhall x Citrumelo *C. sinensis* Cv. Newhall x trifoliata, *C. sinensis* Cv. Valencia Porou x citrumelo, *C. sinensis* Cv. Valencia Porou x trifoliata], one pomelo [*C. grandis* Cv. Cuban shaddock x trifoliata] and one mandarin [*Citrus reticulata* Cv. Tangelo Minneola x trifoliata]. All volatiles have been analyzed through GCMS and forty five constituents were identified: Terpenes (94%-99%), among which monoterpenes (88-98%) with limonene as the most abundant (80-95%). It is noteworthy that the detection of the sesquiterpene nootkatone (3.76%) in the essential oil of *Citrus paradise*, which is well known to inhibit acetylcholinesterase, which is the most expensive and valuable component of all Citrus. The essential oils of all six among studied *Citrus* leaves showed a different chemical profile in comparison with their peels content, as sabinene is considered (0.6%-78.5%) as the main component, which is in accordance with literature, while only in mandarin (*Citrus reticulata*) was absent. Moreover, alkanes are identified in high percentages in all *Citrus* samples. Furthermore, six polymethoxyflavones were isolated and structurally determined by NMR spectroscopy from the peels of *C. sinensis* Cv. Valencia Porou x citrumelo and *Citrus reticulata* espectively. Their structures were identified as: 3',4',5,6,7-pentamethoxyflavone (sinensetin),3',4',5,6,7,8-hexamethoxyflavone(nobiletin),3',4',3,5,6,7-hexamethoxyflavone,4',5,6,7-tetramethoxyflavone, 3,5,6,7,8,3',4'-heptamethoxyflavone(3-methoxy-nobiletin) and4',5,6,7,8-pentamethoxyflavone (tangeretin), together with the fatty acid linoleic acid. All the above isolated polymethoxyflavones are among appreciated chemotoxonomic markers in *Citrus* genus showing also strong bioactivities (antioxidant, anti-inflammatory, antibacterial, cytotoxic).

Recent Publications

1. Miyazawa M, Okuno Y, Fukuyama M, Nakamura S and Kosaka H (1999) Antimutagenic activity of polymethoxyflavonoids from *Citrus aurantium*. Journal of Agricultural and Food Chemistry 47(12):5239–5244.
2. Furusawa M, Hashimo T, Noma Y and Asakawa Y (2005) Highly efficient production of nootkatone, the grapefruit aroma from valencene, by biotransformation. Chemical and Pharmaceutical Bulletin 53(11):1513–1514.
3. Gosslau A, Chen K Y, Ho C T and Li S (2014) Anti-Inflammatory effects of characterized orange peel extracts enriched with bioactive polymethoxyflavones. Food Science and Human Wellness 3(1):26–35.
4. Borah N, Gunawardana S, Torres H, Mc Donnell S and Van Slambrouck S (2017) 5,6,7,3',4',5'-Hexamethoxyflavone inhibits growth of triple-negative breast cancer cells via suppression of MAPK and Akt signaling pathways and arresting cell cycle. International Journal of Oncology 51(6):1685–1693.

5. **Lai H C, Wu M J, Chen P Y, Sheu T T, Chiu S P, Lin M H, Ho C T and Yen J H (2011) Neurotrophic effect of *Citrus* 5-Hydroxy-3,6,7,8,3',4'-hexamethoxyflavone: promotion of neurite outgrowth via cAMP/PKA/CREB pathway in PC12 Cells. PLoS One 6(11):e28280.**

Biography

Eleni Anastasopoulou has graduated from the Faculty of Agriculture, at the Department of Food Science and Technology, Aristotle University of Thessaloniki (GR). Throughout her studies, she appreciated the value of natural products, therefore during her research thesis; she optimized a green extraction method for the recovery of polyphenols from olive leaf using cyclodextrins and glycerin as co-solvents. Furthermore, she participated in the creation of an innovative yoghurt product, on the Greek competition Ecotrophelia 2016. Finally, she continued her Postgraduate studies in the Div. of Pharmacognosy and Chemistry of Natural Products, Dept. of Pharmacy at National and Kapodistrian University of Athens. In the framework of her dissertation on essential oils, she had the opportunity to learn the analytical methods on the isolation, development, production and control of bioactive natural products.

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Nenad Stojiljković et al., Am J Ethnomed 2019, Volume 6
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Nanoliposome encapsulated lycopene ameliorates methotrexate-induced hepatotoxicity

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Introduction: Nano liposomes have the potential to increase bioavailability, stability, improve time-controlled drug releasing, enable cell specific targeting and decrease adverse effects of drugs. In this study, we evaluated the potential protective effect of lycopene, a potent antioxidant carotenoid, given in free and encapsulated form in methotrexate induced hepatotoxicity in rats.

Methods: Experiments were performed on 48 male Wistar rats divided into eight groups of 6 animals, treated daily by an intraperitoneal injection. MTX group received methotrexate in a single dose (20 mg/kg) on the first day; other experimental groups received the same dose of methotrexate and empty nanoliposomes (10 mL/kg) (MTX-NL-group), lycopene (6 mg/kg) (MTX-LYC-group) and encapsulated lycopene (6 mg/kg) (MTX-ENL-group), for 10 days. The remaining four groups served as controls and received for 10 days: corn oil (0.2 mL/day) (C-group), empty nanoliposomes (10 mL/kg) (NL-group), lycopene (6 mg/kg) (LYC-group) and encapsulated lycopene (6 mg/kg) (ENL-group). Quantitative evaluation of structural and functional changes of liver was performed by histopathological (HE staining) and biochemical serum analyses and determination of oxidative stress parameters.

Results: Methotrexate induced severe functional and morphological alterations of liver with conspicuous disorganization of hepatic cords. Hepatocytes diffusely exhibited apoptosis and degeneration with vacuolation of the cytoplasm. Portal veins and sinusoid capillaries showed congestion. Marked inflammatory infiltrate was present in the portal tract (Figure 1E). Pathohistological findings were followed by AST and ALT increase and disturbances of tissue antioxidant status. Application of both forms of lycopene ameliorated changes in serum AST and ALT and oxidative damage markers and markedly reversed structural changes of liver tissue induced by methotrexate. Animals that received nanoliposome encapsulated lycopene showed higher degree of recovery then those treated with free lycopene in Figure 1.

Discussion: Encapsulated lycopene was shown to possess stronger antioxidant activity which could be possibly related to its position in the lipid bilayer and its higher stability in nanoliposomes which might prolong the presence of lycopene in circulation. Treatment with nanoliposome-encapsulated lycopene compared to free lycopene has an advantage since it has more efficiently reduced methotrexate induced hepatotoxicity.

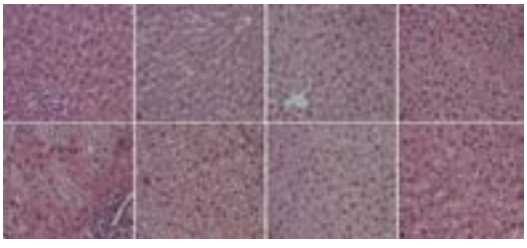

Figure 1: Histological evaluation of liver tissue (HE, 400x) in: (A) C-group; (B) NL-group; (C) LYC group; (D) ENL group; (E) MTX group; (F) MTX-NL group; (G) MTX-LYC group and (H) MTX-ENL group

Recent Publications

1. Stojiljkovic N, Ilic S, Jakovljevic V, Stojanovic N, Stojnev S, Kocic H, Stojanovic M and Kocic G (2018) The encapsulation of lycopene in nanoliposomes enhances its protective potential in methotrexate-induced kidney injury model. Oxidative Medicine and Cellular Longevity 2627917.
2. Taskin B, Erdoğan M A, Yiğittürk G, Günenç D and Erbaş O (2017) Antifibrotic effect of lactulose on a methotrexate-induced liver injury model. Gastroenterology Research and Practice 7942531:1-5.
3. Abu El-Saad A M, Ibrahim M M, A A Hazani A A and El-Gaaly G A (2015) Lycopene attenuates dichlorvos-induced oxidative damage and hepatotoxicity in rats. Hum Experiment Toxicology 35(6):654-65.
4. Tan C, Xue J, Abbas S, Feng B, Zhang X and Xia S (2014) Liposome as a delivery system for carotenoids: comparative antioxidant activity of carotenoids as measured by ferric reducing antioxidant power, DPPH assay and lipid peroxidation. J Agric Food Chem 62: 6726–6735.
5. Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo S W, Zarghami N, Hanifehpour Y, Samiei M, Kouhi M and Nejati-Koshk Ki (2013) Liposome: classification, preparation, and applications. Nanosc Res Lett 8(1):102–111.

Biography

Nenad Stojiljkovic, Associate Professor at the Department of physiology, Faculty of Medicine, University of Nis is doing the experiments in the fields of experimental medicine, especially in the field of experimental nephrology and hepatology. His main work is related to the clarification of mechanism of action of different hepato/nerphoprotecitve agents (antibiotics, cytostatic drugs, heavy metals, etc.) in rats. Also, he is work is aiming to discover new hepato/nephroprotecive agents that can be used in every day clinical practice, such as naturally occurring and/or synthetic antioxidant agents. His current work involves encapsulation of carotenoid like antioxidants in nanoliposomes and evaluation of their potential in preventing anticancer drugs-induced liver and kidney damage.

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Soleya Dagnon et al., Am J Ethnomed 2019, Volume 6
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Mass spectrometric elucidation of polyphenol constituents in *Vernonia amygdalina* Del.

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Vernonia amygdalina **Del. (VA), family Asteraceae is a well-known shrub in tropical countries because of its nutritive and medicinal properties. The antioxidant and anticancer therapeutic effects of VA were related to polyphenol content. For this purpose, mass spectrometric investigation was conducted aiming at characterizing the polyphenol composition of Vernonia amygdalina. Previous publications on the polyphenol constituents in VA raise some questions on the component composition caused by inadequacy of the data. It seems necessary to prove the presence of luteolin 7-O-glycosides and the aglycone luteolin. Monocaffeoyl-, dicaffeoyl- and tricaffeoylquinic acids, as well as, feruloyldicaffeoylquinic acid were also reported. In this study the polyphenols were extracted using methanol in an ultrasound bath for 40 min at room temperature (25°C). The extract was evaporated to dryness, resolved in water and divided into chloroform fraction and ethyl acetate fraction. The compounds in both the fractions were characterized by HPLC-PDA. Structural elucidation was performed on Orbitrap UHPLC mass spectrometer. Two flavone aglycones luteolin and apigenin were identified in the chloroform fraction. In the ethyl acetate fraction six flavone glycosides luteolin 7-O-glucoside, luteolin 7-O-glucuronide, luteolin 4'-O-glucoside, apigenin 7-O-rutinoside, apigenin 7-O-glucoside and apigenin 7-O-glucuronide were found, as well as mono and dicaffeoylquinic acids and feruloylquinic acid-O-glycoside. The caffeoylquinic acids (chlorogenic 1,5-3,5 and 4,5-dicaffeoylquinic acids), luteolin 7-O-glucoside, luteolin 7-O-glucuronide and luteolin are the main compounds composing the polyphenol complex of VA. The largest percentage of them was due to 1,5 and 3,5 dicaffeoylquinic acids. Furthermore, the presence of luteolin 4'-O-glucoside, apigenin 7-O-rutinoside and feruloylquinic acid-O-glycoside as minor constituents in *Vernonia amygdalina* is reported for the first time.**

t_R	Name	[M-H]-	MS/MS
3.74	neo-Chlorogenic acid	353	191, 179, 161, 135
5.78	Chlorogenic acid	353	191, 179
6.95	4-Caffeoylquinic acid	353	191
10.96	Lut-7-O-glucuronide	461	285,199,175,151,133,107
11.09	Lut-7-O-glucoside	447	285,175,151,133,107
11.51	1,4-di-CQA	515	353, 191, 179, 173, 161, 135
11.79	3,4-di-CQA	515	515, 353, 191, 179, 173, 135
11.94	1,5-di-CQA	515	353, 191, 179, 161, 135
11.97	Apg-7-O-rutinoside	577	269,151,117
12.38	Apg-7-O-glucoside	431	431, 269, 151, 107
12.45	Apg-7-O-glucuronide	445	269, 175, 151, 149, 117
12.64	3,5-di-CQA	515	353, 191, 179, 161, 135
13.11	Lut-4'-O-glucoside	447	447, 285, 151, 133, 107
14.01	4,5-di-CQA	515	353, 191, 179, 173, 161, 135
14.99	FQA-glucoside	529	529, 367, 179, 161, 135, 133
15.68	Lut	285	285, 217, 199, 175, 151, 133, 121, 107
17.55	Apg	269	269, 151, 149, 117, 107

Table 1. Polyphenol constituents in *V. amygdalina* Del. Legend: Lut-Luteolin, Apg-Apigenin, di-CQA-Dicaffeoylquinic acid, FQA-Feruloylquinic acid

Recent Publications

1. Imafidon C, Akomolafe R, Sanusi A, Ogundipe O, Olukiran O and Ayowole O (2015) Polyphenol-rich extract of *Vernonia amygdalina* (del.) leaves ameliorated cadmium-induced alterations in feeding pattern and urine volume of male Wistar rats. Journal of Intercultural Ethnopharmacology 4(4):284–292.
2. Johnson C E, Lin L, Harnly J M, Oladeinde F O, Kinyua A M, Michelin R and Bronner Y (2011) Identification of the phenolic components of *Vernonia amygdalina* and Russelia equisetiformis. Journal of Natural Products 4:57–64.
3. Ong K W, Hsu A, Song L, Huang D and Tan B K H (2011) Polyphenols-rich *Vernonia amygdalina* shows anti-diabetic effects in streptozotocin-induced diabetic rats. Journal of Ethnopharmacology 133(2):598–607.
4. Ola S S, Catia G, Marzia I, Francesco F V, Afolabi A A and Nadia M (2009) HPLC/DAD/MS characterisation and analysis of flavonoids and cynnamoil derivatives in four Nigerian green-leafy vegetables. Food Chemistry 115(4):1568-1574.
5. Igile G O, Oleszek W, Jurzysta M, Burda S, Fafunso M and Fasanmade A A (1994) Flavonoids from *Vernonia amygdalina* and their antioxidant activities. [Erratum to document cited in CA121:251243]. Journal of Agricultural and Food Chemistry 42(11):2445–2448.

Biography

Soleya Dagnon has her expertise in evaluation and chromatography profiling of polyphenols in plants. Her analytical and chromatography evaluation model based on deep cognition of chemical and spectral properties of compounds and their chromatographic behavior creates confidence in the information needed for improving the knowledge. She has built this model after many years of experience in chromatography, method evaluation and teaching both in research and education institutions. At the Plovdiv University, she is teaching Modern Chromatographic Methods and Chromatographic Methods in Pharmaceutical Analysis.

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Antimicrobial and antimalarial properties of secondary metabolites of an endophytic fungus isolated from *Azadirachta indica (meliaceae)*

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[2]Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria

Background information: Natural products of endophytic fungi have generated significant interest in drug discovery programmes due to their immense potential to contribute to the discovery of new biologically active molecules.

Purpose: This study was carried out to investigate the antimicrobial and antimalarial properties of secondary metabolites of an endophytic fungus isolated from leaves of A. indica.

Method: Endophytic fungal isolation, solid sate fermentation in rice medium; and extraction of secondary metabolites were carried out using standard methods. The fungal extract was screened for antimicrobial and antimalarial activities using the agar well diffusion method and Peters' 4-day suppressive test respectively. The extract was also subjected to HPLC analysis to identify its constituents.

Results: At 1 mg/mL, the fungal extract inhibited the growth of Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli and Candida albicans with inhibition zone diameters of 6, 4, 5, and 4 mm respectively. Also, at doses of 150 and 50 mg/kg/day, the extract displayed a dose dependent suppression of Plasmodium berghei by 89 and 83% respectively. HPLC analysis of the extract revealed the presence of several biologically important compounds including protocatechuic acid, ruspolinone, dimethyl gallate, indole-3-carbaldehyde, 4-methoxy benzaldehyde, 2-carboxymethyl-3-n-hexylmaleic acid anhydride, pestalotioprolide F and p-hydroxy-phenyl-acetic acid.

Conclusion: The results of this study reveal the potentials possessed by endophytic fungi of A. indica as sources of biologically active compounds with pharmaceutical importance.

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Chemistry and medicinal properties of *Erythrina brucei* (A medicinal plant of Ethiopia)

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Erythrian brucei is a tree grown only in Ethiopia. It grows up to the height of 15-20 m long with colorful flowers. In different parts of the country the root and stem bark of this plant is used to treat ear infection and other diseases. Previous studies revealed that the flower and seed of *E. brucei* contain biologically active alkaloids; however, there was no report for the nonalkaloidal constituents of this plant. Recently, however we did a comprehensive phytochemical investigation on root bark, stem bark and twigs of the plant concentrated on nonalkaloidal compounds. In the study, we isolated biologically significant compounds and come up with two new compounds which were not isolated so far from the nature. The identities of the compounds were established based on spectrometric and spectroscopic analysis including MS, 1D and 2D NMR. Resazurin based antibacterial minimum inhibitor concentration (MIC) and DPPH antioxidant assay were carried out on some selected compounds using standard references chloramphenicol and trolox respectively. The tested compounds showed promising antibacterial activity up to (IC50 = 0.0625 mg/mL) and antioxidant activity (IC50 = 1.1µg/mL) comparable to standard references.

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Methanolic extract leaf of *Scabiosa atropurpurea* promote an anticancer activity *in vitro* and demonstrate significant impact on tumor growth *in vivo*

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Scabiosa atropurpurea **(S.atrp) was extracted with methanol then the chemical composition was identified by HPLC MS/MS (High Performance Liquid Chromatography Mass spectrometry). The current study aims to investigate the multidrug resistance (MDR) of colorectal cancer cells which is a multidimensional problem involving several mechanisms and targets. This study demonstrates that Methanolic extract from S.atrop, which contains flavonoids, phenolic acids and saponins has the ability to inhibit cell proliferation and reverse resistance of some colorectal cancer cells (Caco-2, HCT-116 and CT26) to the cisplatin chemotherapy (CDDP), by induction of apoptosis. This extract promoted G2/M and S arrest at 48h and it also overcame P- gp/MDR1 and MRP1 activities in a concentration-dependent. Moreover, it inhibited the activity of the drug modifying enzyme GST. Besides, this study evaluated the antitumoral effect of this extract. BALB/c mice were subcutaneously inoculated with CT-26 cells, then treated intra-peritoneally after 7 days with the methanolic extract for 21 days. Our results showed that S.atrop could significantly inhibit the tumor growth and possess a synergistic antitumoral effect with the CDDP . This effect caused a significant reduction in the serum levels of aspartate transaminase, alanine transaminase and creatinine in comparison to the positive control. These results suggest that the methanolic extract of S.atrop might contain phytoconstituents endowed with antitumoral properties, which could protect against the colorectal cancer.**

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Clinical trial of herbal treatment gene *Eden-Vir/Novirin* in oral herpes

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Our previous articles showed that suppressive or preventive treatment with the herbal gene-*Eden-VIR/Novirin* reduced the number and duration of genital herpes outbreaks with no adverse effects. These studies also revealed that the herbal gene-*Eden-VIR/Novirin* is mostly superior to acyclovir, valacyclovir and famciclovir drugs in genital herpes. This study tested the effect of gene *Eden-VIR/Novirin* in oral herpes also called cold sores and fever blisters.

Methods: **The framework of the study was a retrospective chart review. The study included 68 participants. The participants took 1 to 4 capsules per day over a period of 2 to 36 months. The study included two Food and drug administration recommended controls: baseline and a no treatment.**

Results: **Gene *Eden-VIR/Novirin* was effective in 89.3% of participants. The treatment reduced the mean number of outbreaks per year from 6.0 and 3.6 in the control groups to 2.0 in the treatment group (P<.0001 and P=.07, respectively). Gene-*Eden-VIR/Novirin* reduced the mean duration of outbreaks from 9.8 and 5.8 days in the control groups to 3.2 days in the treatment group (P<.0001 and P=.02, respectively). There were no reports of adverse experiences. Gene-*Eden-VIR/Novirin* was compared to acyclovir and valacyclovir in 6 tests. In all tests, Gene *Eden-VIR/Novirin* showed higher efficacy. Gene *Eden-VIR/Novirin* also showed superior safety.**

Conclusions: **This clinical study showed that suppressive or preventive treatment with the herbal gene *Eden-VIR/Novirin* reduced the number and duration of outbreaks in oral herpes without any adverse effects. The study also showed that the herbal gene *Eden-VIR/Novirin* had better clinical effects than acyclovir and valacyclovir, the leading drugs in the category. Based on these results, we recommend using the herbal gene *Eden-VIR/Novirin* as preventive treatment for oral herpes and specifically as an alternative to the acyclovir and valacyclovir drugs**

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Microbiological property evaluation of natural essential oils used in green cosmetics

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Essential oils are also having long been known to have antimicrobial properties and these properties have been reviewed in the past studies as having strong antimicrobial effects. Wilkins and Board also reported that more than 1340 plants are known to be potential sources of antimicrobial compounds. Furthermore, antimicrobial activity of plant extracts is frequently due to the essential oil fraction or to sulfur containing compounds in the aqueous phase. These compounds are also responsible for the characteristic aroma and flavor of the spices. The antimicrobial activity of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies. This study was designed to investigate antimicrobial properties of natural essential oils including; citronella, cinnamon, palmarosa, niaouli, clove, lavender, bergamot, lemon, grapefruit, petitgrain, peppermint, tea tree and eucalyptus from different oil sources. Data's taken from the disk diffusion assay indicate that cinnamon, niaouli, lavender, petitgrain and the tea tree oils have the most intense antibacterial effects on related microorganisms at the concentration 100% (direct usage). Endpoints confirm results reported in the past studies including the MIC assays on the related natural oils. Overall, natural oils have been used safely for many decades on the cosmetic formulations and this experiment emphasizes one more time the importance of the natural oils usage in industry. After all these endpoints, we can summarize that essential oils are gaining popularity within the supported antimicrobial results. As personal care and naturopathic remedies continue to provide alternative solutions for people desiring to take charge of their own health care, it is important to have a firm understanding of essential oils.

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Some biological active phenolic compounds from *Polygonatum obtusifolium*

Lili Gvazava

TSMU - Iovel Kutateladze Institute of Pharmacochemistry, Georgia

In continuation of the search for biologically active compounds in plants of the Georgian flora, we studied the chemical composition of *Polygonatum* L. (Convallariaceae). Species of the genus *Polygonatum* L. are spread in Caucasus. In traditional medicine they are used as a haemostatic, anti-inflammatory and wound healing activity. Raw materials (air-dried ground roots) were extracted with MeOH (80%, 3x) for 1h at reflux. The solvent was distilled off. The remaining aqueous phase was worked up with hexane to remove lipophilic substances and then extracted successively with CHCl3, EtOAc and n-BuOH. After the solvents were removed and the EtOAc and BuOH extracts that contained three stilbenes were combined and chromatographed over a column of sephadex LH-20 with elution by H_2O:MeOH with increasing two alcohol content. This produced eight fractions and the fractions containing stilbenes were combined and rechromatographed over an analogous column with elution by EtOH to afford two pure compounds 1 (0.078 g, 0.019% calculated per raw material mass) and 2 (0.110 g, 0.027%). Compound 1: C15H14O5, [M]+ 274, oily liquid. IR spectrum (KBr, vmax, cm-1): 3360 (OH), 3005, 2850, 1580, 1520, 1440 (aromatic), 1150 (C–O), 860, 845 (substituted rings), 680 (cis-bonded CH=CH). A comparison of the spectral data with the literature identified 1 as cis-3,5,3′,5′ -tetrahydroxy-4-methoxystilbene. Compound 2: C14H15O5, [M]+ 274, colorless crystals, Mp 167–169°C. IR spectrum (KBr, vmax, cm-1): 3270 (OH), 3005, 2840, 1620, 1595, 1520, 1430 (aromatic), 1160 (C–O), 980 (trans-bonded CH=CH), 835, 675 (substituted rings). A comparison of the spectral data with the literature identified 2 as trans-3,5,3′,5′ -tetrahydroxy-4-methoxystilbene. Compound 3: C9H6O3, [M]+ 162, colorless prisms or needles, Mp 234–235°C. UV spectrum (EtOH, max, nm): 320, 255. It was identified as umbelliferone (7-hydroxycoumarin). Compound 4: C10H8O4, [M]+ 192, colorless or yellowish crystals, Mp 202–204°C. UV spect¬rum (MeOH, max, nm): 233, 255, 295, 347. It was identified as scopoletin (6-methoxy-7-hydroxy¬cou¬marin). All compounds were isolated for the first time from *P. obtusifolium*.

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Ethnopharmacological profile of traditional plants used in Morocco by cancer patients as herbal therapeutics

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Cancer is one of the major causes of mortality throughout the world. In Morocco, cancer patients are increasing, which indicate that by the year 2020 it is predicted that cancer will be causing seven out of 10 deaths. To reduce this high mortality rate, medical research now turns to the discovery of new molecules that will help to develop natural anticancer drugs. The current study was designed as a prospective randomized investigation on different plants used by the patients of a center for cancer treatment in Morocco. This study was based on a sample of 600 patients from the Cancer Institution in Marrakech in a period from September 2017 to march 2018. Among the investigated cases, 201 patients (39%) were identified to regularly use medical plants and 160 patients among them use plants along with medical treatment. While 152 patients used traditional medicine before using conventional modern drugs. Fifty-five plants have been cited during this study, of which *Aristolochia longa, Trigonella foenum-graecum, Cassia absus* and *Nigella sativa* are the mostly used medicinal plants. Cross-references of all plants identified in the current scientific literature have revealed species that have not been documented for cancer treatment to date. Particular interest has been given to the use of plants for the treatment of cancer in children and adults. This data shows that phytotherapy has always been practiced in Morocco.

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Enzyme inhibitory activities of *Nymphaea alba* fruit extracts

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Statement of the Problem: In the developing countries the diabetes and also the hyperuricemia can cause many problems for people's health and has shown an increasing trend among the urban population. The studies published before reported that the alcoholic extracts of *Nymphaea alba* from the Danube Delta Biosphere Reserve have bioactive compounds like polyphenols and flavonoids. The *Nymphaea alba* fruit extracts were investigated further to determine their therapeutic potential to inhibit key enzymes involved in carbohydrate metabolism or in the catabolism of purines.

Methodology & Theoretical Orientation: The evaluation of enzyme inhibitory activity of alcoholic extracts of *Nymphaea alba* fruits was carried out against α-amylase and xanthine oxidase. It is known that polyphenols readily complex with protein and as a result, there is an inhibition of enzyme activity. Enzyme inhibitory activities of the extracts from *Nymphaea alba* fruits were assayed in vitro.

Findings: The results indicate that the evaluated alcoholic extracts were exerted higher inhibitory activity against both α-amylase and xanthine oxidase enzymes, having the highest xanthine oxidase inhibitory activity (IC50 = 160 µg/mL), and also α-amylase having highest inhibitory activity (IC50 = 4 mg/mL).

Conclusion & Significance: The obtained results suggest that the *Nymphaea alba* fruit extracts have the major components inhibiting the α-amylase and xanthine oxidase activity in vitro.

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Glycyrrhizic acid as a multifunctional drug carrier: A modern insight on the ancient drug

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Glycyrrhizic acid is the main active component of Licorice root which has been known in traditional Chinese and Japanese medicine since ancient times. In these cultures glycyrrhizic acid (GA) is one of the most frequently used drugs. However, only in 21st century a novel unusual property of GA to enhance the activity of other drugs has been discovered. In this report author will describe briefly the experimental evidences of wide spectrum of own biological activity of glycyrrhizic acid as well as discuss the possible mechanisms of the ability of GA to enhance the activity of other drugs. We have shown that due to its amphiphilic nature GA is able to form self-associates in aqueous and non-aqueous media, as well as water soluble complexes with a wide range of lipophilic drugs. We focus our attention on physicochemical studies of the molecular mechanisms of GA activity as a drug delivery system (DDS). In our opinion, the most intriguing feature of glycyrrhizic acid which might be the key factor in its therapeutic activity is the ability of GA to incorporate into the lipid bilayer and to change various physical and functional properties of cell membrane, for example to increase the membrane fluidity and permeability. The ability of biomolecules derived from medicinal plants to change the properties of cell membranes is of great significance from both fundamental and practical points of view.

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Mechanisms behind hypoglycemic actions of butanol fraction of *Azadirachta indica* in a type 2 diabetes rat model

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The cost and side effects of oral hypoglycemic agents for the treatment and management of type 2 diabetes (T2D) has led to increase the use of natural medicines, particularly from medicinal plants. Hence the validation of the folkloric and use of these medicinal plants. In the present study, the mechanisms behind the antidiabetic effects of the butanol fraction of *Azardirachta indica (A. indica)* (BFAI) were evaluated. T2D was induced by feeding 10% fructose solution ad libitum for two weeks followed by an intraperitoneal injection of streptozotocin (40 mg/kg body weight) and the animals were treated with a low dose (150 mg/kg) and a high dose (300 mg/kg) of BFAI for 4 weeks as a single oral dose daily. Body weight and blood glucose were determined every week. Oral glucose tolerance test was performed in the last week of treatment. Insulin homeostasis and liver glycogen concentration were determined after 4 weeks of oral administration. Both doses of the fractions showed significant improvement of body weight, reduced blood glucose, stimulated insulin secretions, improved pancreatic β- cell function (HOMA-β), decreased insulin resistance (HOMA-IR) and increased liver glycogen concentration compared to untreated diabetic rats, when high dose had a better activity. GC-MS analysis of the fraction revealed the presence of polyphenols. The results of this study suggest that, the polyphenols present in the fraction may be responsible for the antidiabetic effects which have been achieved via decreasing insulin resistance, modulating β-cell function, as well as by inhibiting of α-amylase and α-glucosidase activity.

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Abortive effect of the aqueous extract of germinated seeds of *Trigonella foenum graecum* L. in mice

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Trigonella foenum graecum **L. is one of the oldest traditional medicinal plants. Fenugreek belongs to the family of Fabaceae and is used as an herb (dried or fresh leaves), spice (seeds), vegetable (fresh leaves). This study was undertaken to test the side effect of fenugreek (*Trigonella foenum graecum* L.) germinated in pregnant female albino mice, fetal development and evaluation of locomotive development.**

Material & Methods: **A freeze dried aqueous extract of sprouted fenugreek seeds are administered to mated female mice throughout pregnancy at doses of 200, 500, 800, 1000 mg/kg/day. Females were examined for parameters of reproductive performance. The fetuses have been weighed and their behavior is evaluated. A high-performance liquid chromatography (HPLC) technique was used to identify the major phenolic compounds in the aqueous extract of germinated seeds of Fenugreek.**

Results: **In pregnant mice, there were no obvious symptoms of toxicity, deaths related to the lyophilized aqueous extract of sprouted fenugreek seeds. But we noted the presence of increased number of abortions in treated mice compared to controls. The main phenolic compounds identified in the lyophilized extract of germinated fenugreek seeds were gallic acid, caffeic acid, syringic acid, tyrosol, and rutin. The germinated seeds of fenugreek showed that their aqueous extract had an antioxidant effect.**

Conclusions: **This study is the first study showing the abortive effect of the aqueous extract of germinated *Trigonella foenum-graecum* seeds. Our finding suggests that prenatal mouse exposure to high doses of lyophilized aqueous extract of germinated fenugreek seeds may lead to toxic effects on mouse reproduction.**

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Chemical study, antioxidant analysis and evaluation of the larvicidal potential against *Aedes aegypti* larvae of essential oil of *Ocimum basilicum* Linn.

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The purpose of this research was to accomplish chemical study, antioxidant analysis and evaluation of the larvicidal potential against *Aedes aegypti* larvae of essential oil from the leaves of *O. basilicum* Linn. The research was carried out in the Pharmacognosy and Phytochemistry Laboratory, Department of Biological and Health Sciences, Federal University of Amapá (UNIFAP), between July 2013 and March 2014. Arthropoda Laboratory, Department of Biological and Health Sciences, Federal University of Amapá (UNIFAP) between September 2013 and March 2014. The essential oil was obtained by hydrodistillation; the identification and quantification of components was achieved with the use of GC-MS analysis. The antioxidant activity was evaluated by the method of sequestration of DPPH. The essential oil was tested in the third larval state of the development of the mosquito *Aedes aegypti*. The third larval instar were exposed to different concentrations of the oil (500, 400, 300, 200 and 130 ppm) in triplicates. Chromatographic analysis identified that the major constituents found in essential oil of *O. basilicum* were limonene (13%), 1,8-cineole (15%), linalool (20%) and methyl chavicol (45%). In trials of free radicals sequestration, the essential oil showed (AA%) 67.35±1.11 in the highest concentration and inhibitory concentration, IC50 value of 61.517 mg/mL. The essential oil of *O. basilicum* showed larvicidal potential with CL50 of 67.22 ppm. A more detailed study should be done to verify the larvicidal potential and biological mechanism of action, as several authors claimed that the constituent of essential oils affect the nervous system of the mosquito *Aedes aegypti* and the action mechanism is not yet fully elucidated. New studies demand the development of tests using samples of lower concentrations to verify the degree of toxicity in other animal species, including man and preparation of formulations that may function as a natural alternative to combat mosquito larvae.

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Cannabis a new beginning!

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Cannabis is probably the most researched plant in the world at the moment and it hasn't even got a monograph in a pharmacopoeia. The last time it had a monograph was in the British Pharmaceutical Codex of 1934 and this was for a tincture which is an alcoholic dilution of an extract made by immersing cannabis leaves and seeds in alcohol for a while . It was available on prescription until 1949. While the monograph cannabis contained both THC and CBD there are hybrids now which can produce very high THC with low CBD such as Dutch Skunk which has 18% THC and similar hybrids with THC below 0.2% and CBD contents of up to 24% . In USA cannabis is a recreational drug contains both THC and CBD whilst in U.K. cannabis with more than 0.2% THC is illegal but cannabis with only CBD is regulated as a foodstuff under EU rules. There are many novel dose forms of CBD , I have seen CBD oil in various strengths and sublingual sprays which deliver between 10 and one hundred mg per actuation , I tasted gummy bear children's sweets impregnated with 12Mg CBD and tablets and capsules of various strengths and I believe a skin patch exists. The serious growers of cannabis are mainly in the USA in Oregon and California .But there are growers in Slovakia ,Poland Switzerland and Germany , I am working with other groups growing cannabis in New York ,Korea , Mozambique, Vietnam , Laos and U.K.There are two cannabis products registered and these are Epidolex and Sativex from GW Pharmaceuticals , one treats MS and the other Epilepsy and both work in the cannabinoid receptors.There is great scope for future licensing of cannabis derived medicines as people use CBD for pain, rheumatism ,anxiety, depression and even cancer.They also use it just to feel good , so how many medicines do that ? Tom Chapman is a Pharmacist who qualified when Pharmacognosy involved walking the Yorkshire Dales picking Male Fern (Dryopteris filix-mas) and an oral examination in Edinburgh on 10 different plants Tom has served on the British Pharmacopoeia herbal Committee and studied Traditional Chinese medicines and undertaken registration of THR traditional herbal remedies.At one point he was the largest producer of registered herbal medicines in the U.K. He edited the Herbal Extraction Manual produced by EU (DG7) and taught extraction of herbs in Africa for the Commonwealth secretariat. He designed and commissioned a herbal manufacturing plant for the King of Bhutan.He remains registered as a pharmacist and a QP and actively advises on cannabis.

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Cuban propolis and nemorosone in combination with chemotherapeutic agents induce a synergistic cytotoxic effect in drug-resistant human colon carcinoma cells

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Propolis is a resinous product collected by honeybees from the buds and exudates of various plant sources. This natural product has been used empirically as a traditional remedy in folk medicine for centuries. Several studies have confirmed that propolis mono-therapy can inhibit the growth of different carcinoma cells due to its chemical composition. At present, there are few studies about the possible use of this natural product in the treatment of chemo-resistant tumors. Combination experiments were carried out in order to study the ability of Cuban propolis extracts (CP) and nemorosone, its main phytocomponent, to increase the antiproliferative efficacy of traditional cytotoxic chemotherapic drugs doxorubicin (Dox) and oxaliplatin (Oxa) in human colon carcinoma cells (LoVo), doxorubicin resistant cells (LoVo Dox) and oxaliplatin resistant cells (LoVo Oxa). The effects of combination therapy were investigated in vitro, evaluating cell viability at 24, 48 and 72 hrs by MTT assay. Synergism/additivity/antagonism effect was assessed by isobologram/combination index analysis. Molecular mechanism of combination treatment was examined assessing LDH release, Annexin/PI assay, ROS production, mitochondrial membrane potential ($\Delta\Psi m$) disturbance and ATP intracellular levels. Our results revealed that combination treatment resulted in a significantly synergistic antiproliferative and cytotoxic effect at 72 hrs with respect to monotherapy, in particular in drug-resistant cell lines. Furthermore, combined treatment induced apoptosis cell death with a marked disruption of $\Delta\Psi m$, decrease in ATP production and induction of ROS. These findings indicate that combination of CP or nemorosone with classical chemotherapic drugs may represent an intriguing treatment option for drug-resistant human colon carcinoma.

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Recent advances in nutritional medicine

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In reality, nutrition matters far more than calories, protein, fat and carbohydrates, etc. Recent studies in the USA found nutrition has the relationship with mood and has the characteristic of anti-inflammation and even has the function to lower blood pressure such as DASH diet. Nutritional medicine is a science based medicine and sees each person as an integrated whole. It looks for the underlying causes, which, although often partly genetic, are usually very much, tied up with nutritional, lifestyle and environmental factors. It provides a holistic outlook on how nutrition impacts health, the relationship between diet and disease, as well as a focused understanding of how nutritional, dietary, lifestyle and environmental factors impact wellbeing through the lifespan. Nutritional medicine combines food as medicine traditions and dietary therapy with recent scientific advances in nutritional biochemistry and therapeutics, which are now used by naturopathic, conventional and holistic practitioners alike in USA.