



IPPC 2017

International Phytocosmetics & Phytotherapy Congress (IPPC2017)

15-17 May 2017, Patras, Greece

ABSTRACT 1

Nefertiti's eyes – cosmetics of the past for present times

Alain Touwaide

ABSTRACT

From Antiquity to present time, the make-up of Nefertiti's eyes has seduced generations of viewers. A fresh laboratory analysis led to the identification of the substances used to compose the eyeliner, suggesting that it was not only a cosmetic, but also a preventative device aimed to protect eyes against ophthalmic infections. This conclusion opens new avenues for a renewed examination of ancient make-up and perfumery: they were probably not limited to aesthetics, but might have also a preventative if not curative function, creating a barrier to protect the body against external pathogens or, in the case of infections, to treat them with substances externally applied on infected parts. The lecture will present and discuss selected formulas for cosmetics, perfumes and products of bodily hygiene described in the medical and cosmetic literature of Antiquity, the Middle Ages, Byzantium and the Renaissance, with a special focus on the plants and the other substances they were made of, the preventative or therapeutic properties they were credited with in ancient materia medica literature, and the validation of such properties by contemporary laboratory analysis when available. The lecture will prospect how a transdisciplinary collaboration between historical studies and cosmetology can benefit both, and open new development for the repurposing of past uses of natural substances.

Keywords: perfume, Byzantium, power, St Nicolas, holiness

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ABSTRACT 2

Looking beyond seed to skin integrity: A new sustainable and eco-friendly natural peeling agent from Quinoa husk

Amit Tewari, Fabien Cabirol, Jinhzu Xu, Isabelle Terrisse, Simon Trouille, Brice Bonnet, Christèle Ribaud, Yégor Domanov, Anissa Azouaoui, Christian Tran, Caroline Fraise, Catherine Bredoux, Corinne Ferraris, Hèléne Mercat, Nisrine Zaaraoui, Jean-Jacques Schoonjans, Anaide Agha, Arthur Croizier, Christian Oresajo, Catherine Marion

ABSTRACT

Chenopodium quinoa Willd, commonly known as Quinoa, is a well-known major and common food ingredient in the Andean communities. It is also an alternative food source in other regions of the world, with regard to its higher nutritional value than that of most common cereals. The outer husk of the grain, which is removed before consumption and discarded, is particularly rich in saponins and other bioactive compounds. We aimed to identify a novel, gentle cosmetically applicable exfoliation extract, Quinoa Husk Extract (QHE) from quinoa husks. QHE appears to be an alternative a new skin friendly desquamating agent, usable in cosmetic products, as a complement to dermatological peeling. Such a sharing beauty with all approach illustrates our aims at searching new and effective cosmetic ingredients of natural origin with accessible cost and minimal environmental impact(s).

Keywords: skin physiology/structure, skin barrier, cell culture, exfoliation, chenopodium quinoa Willd.

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ABSTRACT 3

Advanced formulation strategies for phytocosmetic/ phytotherapeutic active ingredients with the application of nanotechnology

Sophia Antimisiaris

ABSTRACT

In the last years a number of advanced nanotechnologies have been developed for the administration of drugs that provide possibilities to control their release rate, as well as their bio-distribution, and additionally preserve their stability. Between the various types of nano-medicines for drug delivery, our laboratory has expertise in the design and development of liposomal formulations, which have the advantage of being composed of completely biocompatible ingredients. Some examples of the applications of liposomal formulations for the targeted delivery of drugs, from our recent projects will be given. The advantages of liposomes as carriers for drugs can also be applied as solutions for the formulation of various types of natural origin active ingredients. Specific examples of such potential applications will be presented.

Keywords: active ingredients, phytocosmetics, phytotherapeutics, liposomes, nanotechnology, nanoparticles, perfume, Byzantium, power, St Nicolas, holiness

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ABSTRACT 4

Preservation crisis: Is green preservation a valuable option?

Andrea Mitarotonda

ABSTRACT

In recent times cosmetic preservatives have been under the spot light. Not only has bad press been affecting the reputation of safe ingredients, but also Regulators are reviewing their status. It is estimated that approximately 20 entries of Annex V of the European Cosmetic Regulation EC/1223/2009 are under investigation. As a consequence the number of preservatives available, or potentially available, to cosmetic formulators has been significantly decreasing for the last few years. At the same time, it can be seen that the use of claims such as preservative-free and paraben-free has been growing in the cosmetic industry. Apart from marketing and legal implications of such claims, this shows a growing interest in a greener way of preserving cosmetic products. And indeed nature has plenty to offer to formulators in order to improve the overall preservation profile of a wide variety of products. In this lecture the author will give an overview on the current status of preservatives in the industry highlighting which ones are in particular proving to be more frequently used and which ones are being phased out. An overview on natural substances with anti-microbial effect will also be given as well as on those substances that can be found in nature but their synthetic versions are normally used. The Author will also discuss the importance of researching substances used in other industries (e.g. food) or substances that have no evident anti-microbial functionality but that can contribute to boost the overall preservation profile of cosmetic products. Additionally, some case studies will be presented showing the use of so called multifunctionals as well as the importance of pH to maximise performances. The lecture also includes a brief review of the chemistry of essential oils and how they can be efficiently used to help preserve cosmetic products.

Keywords: preservation, multifunctionals, essential oils, green preservation

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ABSTRACT 5

Assessment of commercially available calendula and sage extracts for incorporation in cosmetic products

Afrodite Apostolopoulou, Panos Koumitzelis, Konstantinos Avgoustakis, Fotini Lamari and Sophia Hatziantoniou

ABSTRACT

Background: Greek plant extracts have rich content in antioxidant compounds. However, the commercial exploitation of these useful materials is not fully realized. Calendula and sage extracts are two ingredients that are extensively used for a large number of cosmetic or pharmaceutical products. The aim of this work was to assess the content of phenolic compounds and the antioxidant activity of Greek commercial calendula and sage extracts and to compare them with two commercially available extracts of other geographical origin.

Methods: Hydroglycerin extracts from Greek Calendula (*Calendula officinalis*, Asteraceae) or Greek Sage (*Salvia officinalis* L., Lamiaceae) collected in Greece (Itea, Komotini) and compared to two commercial extracts (Organic Calendula & Sage Hydroglycerin) of other origin. Total phenolic and flavonoid content were determined colorimetrically (Folin-Ciocalteu and ferric chloride assays). The results were expressed as gallic acid (GAE) and quercetin (QE) equivalents, respectively. The free radical scavenging activity was evaluated (expressed as percentage of inhibition (%), IC₅₀ and IC₉₀ of DPPH free radical).

Results and Discussion: Determination of the total phenolic content: The total phenolic content was 5.28±0.23 for Greek Calendula and 21.02±1.19 mg GAE/100g dry extract for Greek Sage, while their commercial analogues of different origin contained 2.52±0.36 and 3.80±0.51 mg GAE/100g dry extract, respectively. Determination of the flavonoids content: The flavonoid content of Greek Calendula was 1.81±0.07 and of Greek Sage 3.51±0.16 mg QE/100g dry extract, while in the respective extracts of different origin, the content was 0.78±0.03 and 1.22±0.05 mg QE/100g dry extract, respectively. Evaluation of antioxidant activity of Greek calendula extracts: IC₅₀ value was calculated at 0.406 %v/v and IC₉₀ was 1.333% v/v. This antioxidant activity was estimated higher than that exhibited by the corresponding commercial extract. Sage extracts in general demonstrated higher antioxidant activity; Greek sage reached IC₉₀ at the lowest concentration tested (0.333% v/v), which was three-fold lesser than the sample of different geographic origin.

Conclusions: The two commercially available Greek Calendula and Sage extracts were richer in phenolic and flavonoid contents and demonstrated higher antioxidant activity compared to extracts from other origin. This difference may be attributed to different genotypes and chemotypes, cultivation conditions and drying and isolation procedures.

Keywords: Plant extracts, antioxidant, flavonoid content, phenolic contents, cosmetics, DPPH

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ABSTRACT 6

Development and breeding of plants as a source of natural ingredients for phytotherapy and phytocosmetics

Nativ Dudai

ABSTRACT

Background: Medicinal and aromatic plants (MAP) breeding for phytotherapy and the cosmetics industry is characterized especially by the relatively great importance of values such as the secondary metabolites content and composition, aroma, flavor, or bioactivity. However, the MAP consumer market commonly rejects GM methods.

Methods: The conventional breeding process is based on the selection of the best individuals with the most ideal combination of traits to be the parents of the next generations. The main tools to achieve this goal are: diversity and variation in the source germplasm and cross-breeding in order to either instill new traits into an existing variety or to achieve hybrid vigor (Heterosis).

Results and Discussion: To achieve variation, an important task preliminary to the breeding process is creation of a wide germplasm collection. Plant material of the target crop is collected systematically in order to represent its natural diversity. One excellent way to enhance the genetic variation is artificial crossing of distant genetic sources and then either self-pollination of the hybrids or open pollination in the experimental field where all of the genetic types are grown together. In this way we can get new combinations of traits that could never be found in nature. Moreover, in some cases inter-specific crossing can be done, which may combine values desired by the consumer with those desired by the producer such as resistances or suitability to environmental conditions in the target cultivation area.

Conclusions: The goal of the industry is the breeding of uniform cultivars of MAP with optimal secondary metabolites content and composition for modern industrial production. MAP breeding focuses on an array of high value traits such as content and composition of secondary metabolites, aroma, flavor, and bioactivity.

Keywords: aromatic plants, breeding, biodiversity, essential oil, domestication

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ABSTRACT 7

Potential actives from native argentine and south american medicinal plants in phytocosmetics

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ABSTRACT

Background: We have been working in a research project entitled "Traditional Medicinal Plants as Source for Phytocosmetic and Dermatological Products" whose main objective is to produce standardized extracts with proven biological properties and to evaluate their end products under clinical studies. For this purpose, we performed a survey of native plants of Argentina and South America used in the traditional medicine for the treatment of skin ailments, bruises and inflammation. Based on different criteria, we selected thirteen different species: *Passiflora caerulea* (Pc), *Gentianella achalensis* (Gach), *Gleditsia amorphoides* (Gam), *Achyrocline satureioides* (As), *Zuccagnia punctata* (Zp), *Baccharis spicata* (Bs), *Tipuana tipu* (Tt), *Chorisia speciosa* (ChS), *Erythrina crista-galli* (Ecg), *Buddleja globosa* (Bgl), *Solidago chilensis* (Sch), *Smallanthus sonchifolius* (Ss) and *Punica granatum* (Pg). These species were previously studied on their phytochemical composition and their toxicological and biological activities.

Results: The TLC analysis of ethanolic extract (E80) Tt-E80, Chs-E80, Ecg-E80, Bgl-E80, Sch-EE80, Pg-EE80 and propilen glycolic extracts (PPG): Pc-PPG, Gach-PPG, were performed in CS1, CS2 and CS3; while Zp-E80, Zp-PPG, Bs-E80 and Gam-E80 were performed using CS3. As-E80 was analyzed in CS4, whereas Gach-PPG was also analyzed using CS4 and CS5. SS-E80 was tested using CS4 and CS6. Through TLC analysis, phenolic compounds, mainly flavonoids were determined as the main components of the Pc-PPG, Gach-PPG, Chs-E80, Ecg-E80, Bgl-E80, Sch-E80, Pg-E80 and SS-E80. On the other hand, the TLC analysis of Zp-E80, Bs-E80, Gach-PPG and Gam-E80 allowed the detection of terpenes. The average total phenolic content determined by the Folin Ciocalteu assay of the ethanolic extracts was: *T. tipuana* 0.19 g/100ml, *C. speciosa* 0.03 g/100ml, *B. spicata* 0.28 g/100ml, *B. globosa* 0.53 g/100ml, *E. crista-galli* 0.03 g/100ml and *S. chilensis* 0.22 g/100ml. The DPPH• radical scavenging activity (%) was investigated in the ethanolic extracts (80%) at two concentrations (0.1 and 1 mg/ml). The highest activity (at 0.1 and 1 mg/ml, respectively) was observed with: *C. amorphoides*: n.d. and 79.91%, *Z. punctata*: 79.67% and 74.95%, *B. spicata*: 81.48% and 77.98%, *T. tipu*: 67.90% and 69.31%, *B. globosa*: 76.07% and 71.66%; and for *P. granatum*: 12.05% and 79.84%, respectively. The development of the cosmetic formulations is performed by postgraduate students from Universidad de Belgrano, Universidad de Buenos Aires and Asociación Argentina de Químicos Cosméticos (AAQC), an associated collaborator in the project.

Keywords: phytocosmetics, argentine medicinal plants, *Zuccagnia punctata*, *Gentianella multicaulis*, *Buddleja globosa*, *Tipuana tipu*

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ABSTRACT 8

Use of natural cosmetics in history of Egypt

Mona Hetta

ABSTRACT

Cosmetics from natural sources are of great interest to the consumer in all the markets for their functional benefits. Ancient Egyptians used natural cosmetics or phytocosmetics, not only for aesthetic purposes but also a religious one. They consider beauty as a sign of holiness, which was proved by the presence of cosmetic palettes buried in their tombs. Natural cosmetics for Ancient Egyptians are considered, not only as agents for maintaining better body appearance and fashion (as eye makeup, facial makeup, paint or dye for nails and hair, etc.), but also of medicinal benefits for the civilizations lived in bad or harsh conditions (as treatment of ocular infections, remedy of burns, healing effect, skin care, etc.). Egypt is the earliest civilization on the earth. It is the birthplace of the western civilization. It is the home of the commonly used beauty agents. From Egypt, many sciences spread across the Europe. Few studies and poor statistic data deal with the current state of phytocosmetics in Egypt in the industry and the market. As a conclusion, cosmetics are important over thousands of years, for the life of different classes of Egyptians: royalty, aristocracy and middle class, as fashion products and gifted by the gods.

Keywords: ancient Egyptians, phytocosmetics, beauty, therapy

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ABSTRACT 9

In Silico structural analysis of peroxidase in *Theobroma cacao* – a potential alternative of *Raphanus sativus* peroxidase

Rodrigo Vargas and Ana Lucia Valle

ABSTRACT

Background: *Raphanus sativus* peroxidase (RSP) is extensively used for its antioxidant properties, great stability, and commercial applications in immunochemistry. However, the extraction process yields low active protein, thus, there is a need to search for alternative peroxidases that could be used in natural antioxidant products and enzyme for cosmetic uses. *Theobroma cacao* peroxidase (TCP) may provide with similar or better catalytic properties. We here aim to perform *in silico* studies of their structures

Methods: The peroxidase sequence of *T. cacao* was obtained from UniprotKB-A0A061ET, a search was performed on PDB, ModBase and ModIIB to render the 3D structure. Swiss-Model homology modeling was performed, using as template 4A5G-PDB corresponding to *R. sativus* with Software ProMod3 Version 1.0.1. A PDB file, was then created, and the software UCSF Chimera was used to obtain the percentage of identity and root- square-mean deviation (RSMD). An alignment with the Smith-Waterman algorithm was performed with the existing databases in NCBI.

Results and Discussion: The homology (QMEAN -3.24 and 0.65 QMQE) and the identity (RMSD of 0.33) values are consistent with a small, conserved structure of the class III peroxidases. Both peroxidases share similarities in their glycosylation sites and the central position of the Hemo group especially in the Residue His 170 that is present equally in both structures coordinating forces of van der Waals with the iron. However, we found significant differences at the level of Arg 38 and His 42, which are key in the stabilization of the complex formed by the heme group in its catalytic function.

Conclusions: The degree of similarity found warrants further research to verify if the *in vitro* activity of this enzyme is similar to or superior to RCP.

Keywords: *in silico*, cosmetics, peroxidase, *Theobroma cacao*, *Raphanus sativus*

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ABSTRACT 10

Biotechnological approaches for conservation of some Indian medicinal plants

Sunil Dutta Purohit

ABSTRACT

Biotechnology has emerged as an important tool to solve several problems in plant sciences which are otherwise not possible to be addressed with the help of conventional methods. Plant biotechnology has several applications including molecular characterization of the diversity of plant species, micropropagation and *ex situ* conservation. All the above methods can help in developing a suitable strategy for conservation of threatened plant species. Aravallis in south-east Rajasthan have remained a resource rich area dominated by variety of plant species which are both ecologically and economically important. Over-exploitation and poor regeneration in some of these species account for their rapid disappearance from their natural habitats. Among different threatened plant species *Chlorophytum borivillianum*, *Boswellia serrata*, *Feronia limonia* and *Celastrus paniculatus* are most important. Molecular characterization of these species using RAPD and ISSR markers has helped in our understanding of their diversity and the manner in which their populations interact. Basic methods of plant tissue culture can augment our efforts not only in large-scale production of these plants but also in their conservation. Cryopreservation and slow growth are the methods wherein isolated plant parts can be stored as long term cultures and can be preserved for their subsequent utilization. The present paper would address these three strategies which could be useful in conserving these species *in situ* as well as *ex situ*.

Keywords: biotechnology, conservation, ex-situ, molecular markers, micropropagation

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ABSTRACT 11

Equibiotics: a new type of plant drugs for the treatment of common infectious diseases equilibrating local microbiota

Xavier Lozoya and Juan Agüero

ABSTRACT

Recent studies of the human microbiome have revealed the role played by microbes in the maintenance of health by an existing relationship and functional coexistence with the cells of different tissues of the human body. The microbiota is the functional unit composed of multiple interdependent microorganisms developing molecular relationships of mutual benefit with specific cell tissues in the skin, the gastrointestinal and respiratory systems and the genitourinary tract. In the mucosa all along the digestive tract, useful microorganisms find the appropriate environment for its reproduction and survival creating specific ecosystems; the local microbiota are characteristically for the oral cavity, the stomach and intestines and determine the healthy digestive, nutritional and metabolic functions of the human body. Thus, at present, the interpretation of any dysfunction by pathogenic microbial colonization of digestive tract can be considered as the rupture of certain microbiotic equilibrium in the injured or infected area. On the other hand, a big group of products has emerged as preventive and therapeutically resources developed from complex extracts of medicinal plants. Their low toxicity makes them products with wide safety and their therapeutic efficacy, not always easy to demonstrate by clinical studies, has turned them into natural resources actually in study for the prevention of several common dysfunctional ailments and for the maintenance of health. Within this group of products, the researchers of Phytomedicamenta SA, in Mexico, developed the so-called Equibiotics, products made with plant extracts that, having a potent anti-infectious activity on pathogenic strains, have an important participation in the preservation and strengthening of the balance of the microbiota in the places where they are applied. Diverse active principles of the equibiotics acting synergistically at very low concentrations, present modalities of combined actions. For example, by modifying the pH of the medium, inhibiting the adhesion process of pathogenic bacteria, reducing local inflammation and facilitating the proliferation of beneficial microbial groups. The present work describes the pre-clinical and clinical studies performed with the equibiotic HA-2E developed by Phytomedicamenta S.A., for the treatment of dyspepsia and chronic gastritis. HA-2E is a chemically standardized mixture of two plant extracts (leaves of *Psidium guajava*, and roots of *Coptis chinensis*), both extracts with reported antimicrobial, anti-inflammatory, antioxidant and spasmolytic properties. Our pre-clinical studies of this combination of extracts allowed determining its growth inhibitory effect on antibiotic-resistant strains of *Helicobacter pylori* cultivated *in vitro*. In a second assay, the effects of the mixture studied on cultures of AGS cells infected with *H. pylori* strains allowed establishing the bacterial anti-adherent effect of the product. Further, conventional animal and cellular toxicological studies performed with the formulation HA-2E established the safety of the product for clinical trials.

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Normal and dyspeptic volunteers and patients clinically and endoscopically diagnosed with gastritis by *Helicobacter pylori* studied in a double blind, controlled clinical trials received HA-2E orally during two weeks of treatment. No changes in diet and style of life of the participants were proposed. Together with the clinical parameters obtained by medical interviews, the microbiota of oral cavity (saliva), stomach (endoscopy samples of mucosa) and colon (feces) were determined before and after the treatments. Results obtained showed a remarkable positive effect in all cases. Participants reported disappearance of dyspepsia symptoms, gastric inflammation and consistence of stools together with a recovery of appetite and a relief sensation after treatment. Analyses of samples of patients with gastritis by *H. pylori* reported eradication of the bacteria with the treatment. The microbiota studies showed a clear tendency to recovery of diversity and richness of a benefic microbiota composition. The study intends to measure and explain the proposed equibiotic effect and its correlation with the multiple active components present in these type of plant extracts.

Keywords: equibiotics, *Psidium guajava*, *Coptis chinensis*, gastritis, *Helicobacter pylori*, plant extracts

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ABSTRACT 12

Variation of anti-oxidant constituents from the hardy Kiwi depending on after-ripening

JongHoon Ahn, Qing Liu, SeonBeom Kim, YangHee Jo, BangYeon Hwang
and Mik Yeong Lee

ABSTRACT

Background: The fruits of *Actinidia arguta*, called hardy kiwi, are cultivated in south parts of Korea. It is native fruit of Korea and has cold-resistance characteristic while kiwi fruit (*A. deliciosa*) does not have this property. It has been traditionally used as anti-diabetes and liver-protecting agent and reported to have anti-oxidant, anti-inflammatory and anti-proliferative effects. It is harvested in hard condition and consumed after-ripening due to its bitter taste. Therefore, it is valuable to investigate variation of bioactive contents and anti-oxidant effect through after-ripening period.

Methods: Hardy kiwi was divided into 10 groups depending on after-ripening day and extracted with 50% ethanol for the quantitation of phenolic contents, glucose contents and antioxidant activity. A phytochemical research of *A. arguta* was further performed to find out anti-oxidant constituents. Isolation of compounds were conducted using various chromatographic techniques and their structures could be determined based on spectroscopic methods including 1D, 2D NMR and MS spectrometry.

Results and discussion: After-ripening had little effect on the amounts of phenolics per dried fruit. As time passed, on the contrary, the content of glucose per dried fruit and the yield of extract increased. In terms of extract, the phenolic content and antioxidant activity were reduced during after-ripening, whereas the glucose content was increased. Further analysis showed the correlation between phenolic content and antioxidant activity of hardy kiwi. Two new phenolic compounds along with 12 phenolic compounds, four quinic acid derivatives and three flavonoids were isolated from the fruits of *A. arguta*. Among them, some phenylpropanoids and flavonoids showed significant effect on antioxidant activity.

Conclusions: We believe that phenolics are responsible for antioxidant activity of hardy kiwi and extract of hardy kiwi in hard condition showed strong antioxidant activity due to high phenolic content with low glucose content.

Keywords: Hardy kiwi, *Actinidia arguta*, after-ripening, phenolics, anti-oxidant

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ABSTRACT 13

***Costus spicatus* Swartz [Costaceae (K. Schum.) Nak.]: a promising source of bioactive compounds against Gram-negative bacteria**

Dionnata Martins Pedrosa, Ygor Ferreira Garcia da Costa, Carolina Feres-Netto, Nicolás de Castro Campos Pinto, Elita Scio, Orlando Vieira de Sousa and Maria Silvana Alves

ABSTRACT

Background: *Costus spicatus*, popularly known in Brazil as “cana-de-macaco” and traditionally used to treat gonorrhea, syphilis, nephritis, leukorrhea, ulcers and bladder infections, is a South American native species predominantly growing in wetlands. This medicinal plant has several secondary metabolites, including phenolic compounds, mainly flavonoids, which have been studied as potent antibacterial agents. This study aimed to investigate the total phenols and flavonoids, and the antibacterial activity of hexane (HE), ethyl acetate (EAE) and ethanol (EE) extracts of *C. spicatus* stems.

Methods: Total phenolic and flavonoid contents were determined by Folin-Ciocalteu and aluminum chloride colorimetric methods, respectively. Antibacterial activity was determined by the Minimal Inhibitory Concentration (MIC) using the micro-dilution method according to Clinical Laboratory Standards Institute (CLSI) guidelines followed by the Minimal Bactericidal Concentration (MBC), which allows to classify the bacteriostatic or bactericidal effect. *Escherichia coli* (ATCC® 25922), *Salmonella enterica* subsp. *enterica* serovar *Typhimurium* (ATCC® 13311), *Salmonella enterica* subsp. *enterica* serovar *Choleraesuis* (ATCC® 10708) and *Pseudomonas aeruginosa* (ATCC® 9027) were tested.

Results and Discussion: EAE was active against all strains tested, with MIC values of 5 mg/mL and bactericidal effect against *P. aeruginosa* (ATCC® 9027). EE exhibited MIC values of 5 mg/mL for *E. coli* (ATCC® 25922), *S. Typhimurium* (ATCC® 13311) and *S. Choleraesuis* (ATCC® 10708), with bacteriostatic effect against these strains. HE exhibited MIC values greater than 5 mg/mL. Extracts with MIC values lower than 8 mg/mL may serve as sources for compounds with therapeutic potency. The total phenolic content of extracts varied from 0.132 to 6.096 g/100 g, while flavonoids ranged from 0 to 5.46 g/100 g. Those compounds were detected at higher concentrations in EAE and EE, and were probably involved with the antibacterial effect.

Conclusions: In the context of global bacterial resistance, the present results highlighted the potential of *C. spicatus* as a promising source of bioactive compounds against Gram-negative bacterial strains, leading the prospection of new therapeutic approaches.

Keywords: *Costus spicatus*, phenolic compounds, flavonoids, Gram-negative bacteria, antibacterial activity, medicinal plant

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ABSTRACT 14

***Vernonia polyanthes* Less. (Asteraceae Bercht. & Presl): a potential natural source of antimicrobial agents**

Jônatas Rodrigues Florencio and Alves Maria Silvana

ABSTRACT

Background: *Vernonia polyanthes* Less. (Asteraceae Bercht. & Presl) is a Brazilian native plant species, popularly known as “assa-peixe”, and traditionally used to treat wounds, torsions, fractures, respiratory system disorders, and kidney problems. The current study aimed to investigate the antibacterial activity of *V. polyanthes* flowers (Vp-FRE) and leaf (Vp-LRE) rinse extracts in order to corroborate with its traditional use and prospecting new therapeutic options against bacterial infections.

Methods: Antibacterial activity of Vp-FRE and Vp-LRE was established by the Minimal Inhibitory Concentration (MIC) followed by the Minimal Bactericidal Concentration (MBC), which allows to classify the bacteriostatic or bactericidal effect. *Staphylococcus aureus* (ATCC® 6538/ATCC® 29213) *Escherichia coli* (ATCC® 10536/ATCC® 25922) *Salmonella Choleraesuis* (ATCC® 10708) *Salmonella thyphimurium* (ATCC® 13311) and *Pseudomonas aeruginosa* (ATCC® 9027/ATCC® 27853) were tested. Methicillin-resistant *Staphylococcus aureus* (MRSA) (1485279, 1605677, 1664534, 1688441, 1830466) *Salmonella* spp. (1266695, 1507708) and *Salmonella enteritidis* (1406591, 1418594, 1428206) clinical strains were also tested.

Results and Discussion: Vp-FRE revealed antibacterial activity against 9 of 18 strains tested, being more active against *S. aureus* (ATCC® 29213 and *S. aureus* 1605677) with MIC values of 1250 µg/mL and 156 µg/mL, respectively, and MBC of 1250 µg/mL and bacteriostatic effect for both strains. Vp-LRE was active against 6 of 18 strains tested, demonstrating more activity against *S. aureus* (1605677 and 1485279) with MIC values of 78 µg/mL and 625 µg/mL, in this order, MBC values of 1250 µg/mL and > 5000 µg/mL, respectively, and bacteriostatic effect. These values are consistent, even lower than MIC and MBC values range (500 to 8000 µg/mL) showed by other medicinal plants with antibacterial activity.

Conclusions: The present results suggest the potential of *V. polyanthes* as a promising source of antibacterial agents, contributing to the prospecting of new therapeutic options against the increasing bacterial resistance to currently available antibiotics.

Keywords: *Vernonia polyanthes*, Asteraceae, antibacterial Activity, antibacterial Agents, natural products, medicinal Plant

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ABSTRACT 15

Antioxidant activities and efficacy of 24 commercial extracts/compounds used in skin care formulation

Nik Nur Azwanida Zakaria, Okello Edward and Olusola Idowu

ABSTRACT

Background: The so called active ingredients in the skin care product formulations are purported to deliver the intended functions of the product. Active ingredients such as the antioxidants can only efficiently protect the skin if the activities were retained after mixing with the base matrices in the product formulation. This research was aimed to screen the antioxidant activities of 24 commercial extracts/compounds that are used in the skin care product formulation and investigate their efficacy in the final product formulation.

Methods: The antioxidant activities were evaluated using ABTS and DPPH assays. To test the efficacy of the extracts in formulations, selected extracts/compounds were mixed with cream base at 1%, 2% and 4% (extracts: cream) and allowed to settle overnight. The antioxidant activities of the extracts and cream mixtures were evaluated using the same methods. The comparison of the antioxidant activities to Trolox (vitamin E analogue) standard was either expressed as percentage inhibition (%) or IC₅₀ (µg/mL).

Results and Discussion: Six extracts/compounds exhibited very potent antioxidant activities with IC₅₀ values lower than Trolox in the ABTS assay. The extracts/compounds in increasing potency were bear-berry (6.30 ± 0.75 µg/mL), amla (3.60 ± 0.25 µg/mL), mangiferin (3.50 ± 0.28 µg/mL), tetrahydrocurcumoids (2.34 ± 0.10 µg/mL), pomegranate (1.65 ± 0.12 µg/mL), green tea with 90% EGCG (1.72 ± 0.03 µg/mL) and resveratrol (0.88 ± 0.05 µg/mL). In the DPPH assay, four samples had IC₅₀ value lower than Trolox, which were mangiferin (3.63 ± 0.01 µg/mL), tetrahydrocurcumoid (3.10 ± 0.23 µg/mL), pomegranate (1.59 ± 0.14 µg/mL) and green tea with 90% EGCG (1.20 ± 0.05 µg/mL). In the efficacy test, most of the tested extracts/compounds had a decrease in the antioxidant capacities after formulation with the base cream except for mangiferin extracts that showed a synergistic effect with the cream base.

Conclusions: This research demonstrated the potential interaction between active and vehicle compounds which may hinder or enhance the activities of the active ingredients in the final product. The outcome of the research has an impact in the cosmetic product formulation particularly in the quality control, chemistry and efficacy of the finished products.

Keywords: antioxidant, cosmetic, active ingredients, ABTS, DPPH, mangiferin

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ABSTRACT 16

Anti-*Candida albicans* effect of *Astronium urundeuva* loaded into a nanosystem using a rat model of vulvovaginal candidiasis

Bruna Vidal Bonifacio, Isadora Fantacini Masiero, Patrícia Bento da Silva, Leonardo Perez de Souza, Wagner Vilegas, Marlus Chorilli and Tais Maria Bauab

ABSTRACT

Background: The high incidence rates of infections caused by *Candida* species have stimulated the search for new natural sources of antimicrobials, such as medicinal plants. *Astronium urundeuva* (Allemão) Engl. (Anacardiaceae) (Au), an arboreal species widely distributed in South America, especially in dried environment of Cerrado, comprises antimicrobial property and was tested in order to evaluate the antifungal activity using an *in vivo* model of vulvovaginal candidiasis.

Methods: The extract was tested alone or loaded into two nanosystems and to improve bioadhesion, Poloxamer 407® was added in system 2. Female rats were immunosuppressed with cyclophosphamide and infected with *C. albicans*(SC5314). Formulations containing the unloaded or loaded extract were administered to the animals. During the treatment, vaginal load of *C. albicans* was evaluated through vaginal lavage. After the treatment period, animals were submitted to recurrent control of the infection to further identify the infectious state.

Results and Discussion: The positive and negative controls of infection remained constant throughout the experiment. We also could confirm that vehicle controls did not reduce infection. On day 4 of treatment, the animals treated with Au loaded into the nanosystems were not infected anymore, while free Au cured the animals after 6 days of treatment. The positive control just reduced the infection in the last day of treatment. No significant difference was observed between systems 1 and 2, which can be explained probably by the low concentration of Poloxamer 407® ($p < 0.05$). After the period of treatment we could detect a re-infection for the group treated with free Au and the positive control, which did not happen with animals treated with Au loaded into the systems.

Conclusions: Even though this experiment yielded promising results, the complete analysis on the eighth day of treatment showed that Au loaded into the systems are more effective than free extract.

Keywords: *Candida albicans*, *Astronium urundeuva*, vulvovaginal candidiasis, nanosystem, antifungal activity, plant extract

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ABSTRACT 17

Popular use of phytocosmetics in three districts from north-eastern Algeria

Amel Bouzabata

ABSTRACT

Background: With a net turnover of 181 billion, the cosmetic industry is a leading worldwide business with a very lucrative future. In Algeria, the cosmetic market is competitive, with a growth rate of 12% per year. Because of the toxicity of synthetic cosmetics, the industry is currently more concentrated on herbal cosmetics. The present study has been conducted to assess quantitatively the use of phytocosmetics, associated with use of herbal products for cosmetic application.

Methods: Data was collected from 300 users via face-to-face interviews using semi-structured questionnaire in three districts from North-eastern Algeria: Annaba, El Tarf and Skikda. Four key informants were evaluated including the form of cosmetic preparation, the frequency of use, medicinal species used in cosmetic applications, and the perception of the efficacy products. Each parameter was evaluated in each district.

Results and Discussion: In this study, the soap (67%) and a fixed oil (56%) were the preparation forms mostly used in phytocosmetics application. Five medicinal plants were recorded, with the highest citation frequency (FC) were: *Aloe vera* (L.) Burm.F. (0.56), *Chamaemelum nobile* (L.) All. (0.49) and *Lavandula angustifolia* Mill. (0.43). Moreover, it was found that 55% of respondents perceived the medium efficacy of phytocosmetics, v/s synthetic cosmetics. Eleven traditional recipes have been described in phytocosmetics applications.

Conclusions: This study is able to record the available information on use of phytocosmetic. However, further research should be conducted in Algeria, to explore the toxicity of these products.

Keywords: Use, phytocosmetic, traditional, Algeria, toxicity

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ABSTRACT 18

Valorisation of grape pomace and seeds: A comparison between UAE and Naviglio extracts

Ilaria Burlini, Massimo Tacchini, Alessandro Grandini, Immacolata Maresca, Alessandra Guerrini, Tatiana Bernardi, Alessandro Massi and Gianni Sacchett

ABSTRACT

Background: Food waste accounts for more than 50% of total waste production. Nine million tons of winery industrial wastes, known as grape pomace, are produced every year in the world, counting about 20% w/w of total grape use for vinification. Recycling biomass from agro-food chain waste could be a way of valorisation of internal resources and it represents an eco-friendly solution for the grape pomace valorisation.

Methods: Hydro-alcoholic extractions of red and white grape (*Vitis vinifera* L.) pomace and seeds, were performed using ultrasound and Naviglio®. In addition, with the aim of removing sugars, a selective extraction was performed (Wagner, 2009). Qualitative analyses were performed with 1H-NMR and HP-TLC, including HP-TLC Bioautographic assay, used also to investigate the antioxidant activity. In order to evaluate grape pomace phenolic content, quantitative analysis on total polyphenol content was performed with Folin-Ciocalteu method.

Results and Discussion: In a preliminary HP-TLC analysis, both ultrasound assisted extractions (UAE) and Naviglio® extracts showed the best fingerprinting using 50% ethanolic solution as extraction solvent. Assuming that all the extracts did not exhibit a rich profile, UAE lyophilized extracts showed the highest yields (29, 27%) and Naviglio® lyophilized extracts showed the richest phytoextract in HP-TLC evaluation. HP-TLC Bioautographic assay demonstrated radical scavenger activity of the extracts, for both ABTS and DPPH assays, between Rf 0 and 0.5. Another active fraction, undetectable with the UV visualizer, was found at Rf 0.65. In general UAE showed higher total phenolic content (white seeds extracts exhibited the best yield, 446.74±10.74 mgGAE/g of dried extract) than Naviglio®. Comparing the red grape pomace with the white one, the former showed higher polyphenol content than the latter (189.11±5.95 mgGAE/g of dried extract; 116.11±5.46 mgGAE/g of dried extract). Finally extraction with ethyl acetate, aimed to obtain a sugar-free fraction. 1H-NMR analyses were performed in order to better describe the fingerprinting of our extracts.

Conclusions: Even if phenolic composition of grape pomace and its radical scavenging activity have been widely studied in literature, we performed the first comparison between a conventional extraction method (UAE) and Naviglio® on grape pomace and seeds. Further studies will focus on the complete chemical characterisation and the bioactivities evaluation in terms of antimicrobial and anti-cholinesterase activities.

Keywords: Grape, pomace, extraction, phenols, ultrasound, Naviglioactivity, plant extract

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ABSTRACT 19

Sub-critical water extraction of bioactive compounds from *Vanda coerulea* Griff ex. Lindl (Orchidaceae)

Veronika Cakova, Xavier Vitrac, Caroline Vitrac, Frédéric Bonté, Julien Delecolle, Dimitri Heintz and Annelise Lobstein

ABSTRACT

Background: Phytochemical analysis of crude hydroethanolic (90:10, v:v) stem extract of *Vanda coerulea* led us to the isolation of bioactive phenanthrene derivatives, namely imbricatin, methoxycoelonin and gigantol, and their precursor eucomic acid. Indeed, the three phenanthrene derivatives displayed antioxidant and skin anti-inflammatory activities and were also able to prevent the release of MMP-1, 2 and 9 involved in dermal matrix breakdown. Therefore, *V. coerulea* extract has been used as a natural ingredient for skin antiaging topical preparations.

Methods: In order to develop new sustainable procedure for the extraction of cosmetic ingredients, sub-critical water extraction at several temperatures and constant pressure (50 bar) has been studied to extract bioactive compounds from *V. coerulea* stems, and to demonstrate the ability of sub-critical water to extract phenolic compounds. The obtained extracts were compared to classical hydroethanolic extractions. The four compounds of interest have been quantified in each obtained extract, using UPLC-ESI-MS/MS.

Results and Discussion: Significant differences in UPLC profiles between hydroethanolic and sub-critical water extracts were observed. Results indicated high selectivity of the sub-critical water toward eucomic acid, which was the most concentrated compound in all extracts. Comparable amounts were observed in hydroethanolic and sub-critical water extracts at 150 and 175°C. At 200°C, eucomic acid was 1.5 times more enriched. Even if imbricatin, methoxycoelonin and gigantol were better enriched in hydroethanolic extracts, they were also present in sub-critical water extracts, in 1.5 to 3 times lesser quantity. The best results were obtained at 150°C.

Conclusions: Our study shows the ability of sub-critical water to extract phenolic compounds, which are usually weakly soluble in water at ambient temperature and pressure. This technique can be an alternative green procedure without use of co-solvents to extract bioactive ingredients for cosmetic industry.

Keywords: *Vanda coerulea*, phenanthrene derivatives, sub-critical water, sustainable extraction

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ABSTRACT 20

Sustainable alternatives to microbeads in cosmetic products: A case study

Paolo Camattari and Patrick O'Byrne

ABSTRACT

In recent years the presence of plastics in the oceans has reached alarming levels, giving rise to patches of plastic islands growing bigger and bigger in size. Many countries have reacted to this situation by passing laws and acts limiting (or even banning) the use of microbeads in rinse-off products, one of the contributors of the plastic soup from the cosmetics industry. The presentation is a case study of how we in Oriflame addressed this issue from a formulation perspective, and how we successfully re-developed surfactant based products with a higher sustainable and environment-friendly impact.

Keywords: microbeads, cosmetics, sustainability, surfactants, formulation, development

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ABSTRACT 21

Madeira Moneywort (*Sibthorpia peregrina*) phenolic content and *in vitro* biological activities: A relation to verbascoside content

Vitor Spinola and Castilho Paula

ABSTRACT

Background: Madeira moneywort (*Sibthorpia peregrina* L.; Scrophulariaceae) is an endemic herb from Madeira archipelago (Portugal) with traditional applications. Previous studies on other Scrophulariaceae species have documented verbascoside a phenylethanoid glycoside (PhEG), as the most active compound, known for several beneficial properties. This work aimed to assess the phytochemical profile of *S. peregrina* and its *in vitro* antioxidant and digestive enzyme inhibitory activities.

Methods: Methanolic extract was investigated for its phytochemical content by HPLC/DAD/ESI-MSn. Main phenolic components were also quantified. *In vitro* antioxidant potential and inhibitory enzyme (α -amylase and α -, β -glucosidases and lipase) effects were determined.

Results and Discussion: A total of 56 compounds were characterized (polyphenols, terpenoids, saccharides, organic and fatty acids). The main class of compounds was PhEGs (95.23 mg g⁻¹ of dry extract), verbascoside and its derivatives being the most abundant compounds (up to 98.85% of the total phenolic content). Lower contents of verbascoside were reported for *S. europaea*, *Scrophularia ningpoensis* (0.98-60.00 mg g⁻¹ DE)(Taskova *et al.*, 2006). Analyzed extracts were able to scavenge free-radicals (ABTS., DPPH, nitric oxide and superoxide) and effectively inhibited enzymes responsible for the metabolism of carbohydrates and lipids.

Conclusions: *S. peregrina* showed good perspectives to be exploited as potential ingredient of food supplements and herbal medicinal products for the management of diabetes and obesity complications and other chronic pathologies associated with oxidative stress. Due to the many reported biological properties of verbascoside, *S. peregrina* emerges as a new source for this compound and its derivatives.

Keywords: *Sibthorpia*, verbascosides, antioxidant, enzyme inhibitor, HPLC-DAD-MS, phytochemical profile

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ABSTRACT 22

Phytochemical screening, antioxidant and antimicrobial activity of *Echium nervosum*

Paula Castilho, Filipe Olim, Katherine Aguirre and Pedro Ideia

ABSTRACT

Background: *Echium nervosum* (Boraginaceae) is an endemic plant from the Macaronesia region, cultivated mainly for its ornamental value and its capacity to attract honeybees, thus promoting the production of honey. Other *Echium* subspecies have been studied for the contents of their seeds in polyunsaturated fatty acid, especially the high commercial valued ALA and GLA. In the present study, the fatty acid composition of seed oil was determined and leaf extracts were characterized and evaluated as antioxidants and antimicrobial agents.

Methods: Seed oil was obtained by hexane extraction, trans-esterified with methanol and the resulting FAME were characterized and quantified by GCMS and GC-FID, respectively. Dry leaves were defatted with hexane and extracted successively with chloroform and methanol. Total phenolic were determined by the Folin Ciocalteu method, total flavonoids were determined by Allothman (2009) colorimetric assay. Antioxidant activity was determined by DPPH and FRAP methods. *In vivo* toxicity was assayed using *Artemia salina* and antimicrobial activity was assessed over 10 micro-organisms.

Results and Discussion: Seed oil (ca. 18%) contains a very small amount of saturated fatty acids with α -linolenic acid (ALA) as major component (> 45%). Stearidonic acid (18:4-3) (SDA) accounts for 14%. Phytochemical screening showed the presence of fatty acids, terpenes, steroids, carotenoids and in the chloroform extract and anthraquinones, flavonols, saponins and catechins in the methanol extract. Both extracts gave a faint coloration (positive result) in the Dragendorff test for alkaloids but only leaves dried by lyophilization. Toxicity over *Artemia salina* (20% mortality) was only observed for the chloroform extract at very high concentration (1000 g/ml). Methanol extract exhibited moderate antioxidant capacity in both assays and was effective against filamentous fungi and yeasts (not bacteria). Chloroform extract was active against Gram (-) and Gram (+) bacteria strains but not against fungi.

Conclusions: *Echium* seed oil has a unique ratio of 3 to 6 fatty acids, unlike any other plant. *E. nervosum* has high contents of SDA compared to other *Echium* species, representing a good source of this anti-inflammatory substance. The leaves are good sources of antimicrobial compounds. A detailed phytochemical profile should be obtained and alkaloids identified and quantified.

Keywords: *Echium nervosum*, bioactive extracts, antimicrobial, antioxidant, seed oil, stearidonic acid

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ABSTRACT 23

Immobilization of natural anthocyanins into solid matrices and evaluation of their color stability

Pedro Ideia, Núria Fernandes, Joana Pinto, Paula Castilho and Catarina Rosado

ABSTRACT

Background: Anthocyanins are a class of natural pigments that are interesting alternatives to artificial colorants. They degrade easily, so they need to be stabilized to be included in commercial products. Adsorption into porous solids is a possible form of stabilization. Color variation is an important indicator of quality assessment in food and cosmetics industries and it is monitored by UV/Vis spectroscopy in liquids or by reflectance colorimetric methods in solids. This work, evaluates the immobilization of anthocyanins into five solid matrices.

Methods: Anthocyanin-rich extracts were obtained from *Myrica faya*, *Rubus grandifolia* and *Vaccinium cylindraceum* berries; the extracts were characterized by LCMS-MS and anthocyanins were quantified in ESI-(+) mode and immobilized on 5 sorbents: 3 clays, 1 talc and one synthetic resin (non-functionalized Lewatit). Adsorption efficiency was measured by UV/vis quantification of anthocyanin in solution before and after contact with adsorbent. Stability of complex pigment/adsorbent was determined by colorimetric analysis (CIELab) evaluation parameters such as pH, temperature, radiation exposure and shelf life.

Results and Discussion: *M. faya* and *R. grandifolia* showed only cyaniding glucosides while *V. cylindraceum* presented 10 glucoside conjugates of delphinidin, cyanidin, petunidin, malvidin and peonidin. Adsorption parameters were optimized: initial concentration 5g/L, time of contact 20 minutes, pH 4.5, at room temperature. Complexes were submitted to processes of accelerated degradation and stability was evaluated by color variations, as CIELAB parameters. Results are presented as L*, a* and b* and as color swatches using Adobe Photoshop software.

Conclusions: Adsorption at equilibrium ranged from 2 to 43 mg of anthocyanin/g of adsorbent, the best the resin and the worst the talc. The 3 clays showed similar results, about 20 mg/g. The resin, which adsorbs the highest quantity, form the least stable complex. Clays proved to be the most adequate adsorbents.

Keywords: anthocyanins, stability, adsorption, clays, CIELab, red berries

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ABSTRACT 24

Anti-chlamydial activity of *Hydrocotyle bonariensis* LAM.

Alejandra Catalano, Carolina Entrocassi, Adriana Ouviaña, Paula Lopez and Marcelo Rodriguez Fermepin

ABSTRACT

Background: *Hydrocotyle bonariensis* Lam. (Araliaceae), known as “paraguaitas”, is a plant growing in South America. Leaves are used as poultices to heal wounds, inflammatory processes and skin rashes. Leaves infusions and flowering tops are used as diuretic, emmenagogue and antiseptic. *Chlamydia trachomatis* causes the most prevalent sexually transmitted bacterial infections and treatment failures are observed. The purpose of this study was to detect *in vitro* anti-chlamydial activity of different extracts from *H. bonariensis* and evaluate the phytochemical composition of the active extract.

Methods: 10% P/V infusion, methanolic extract and CH₂Cl₂ extract obtained from aerial parts of *H. bonariensis*; *Chlamydia pneumoniae* strain and five strains of *Chlamydia trachomatis* were used in five different conditions; immunofluorescent staining after incubation was used to determine the reduction in the number of inclusions. 1H NMR spectra were recorded at 25 °C on a 600 MHz Bruker DMX-600 spectrometer (Bruker, Karlsruhe, Germany) with a proton NMR frequency of 600.13 MHz.

Results and Discussion: Anti-chlamydial assays: the CH₂Cl₂ extract showed inhibitory effect over *C. pneumoniae* as much as *C. trachomatis* independently of its genotype or infection site. The greatest inhibition (90%-100%) was observed when added immediately after inoculation, during the growth stage of inclusion, independently of the assayed strain. The 10% P/V infusion and the methanolic extract from *H. bonariensis* did not show activity. 1H NMR study: characteristic signals of the fatty acid moiety of lipids or cerebrosides, volatile phenolics, phytosterols, methyl triterpenes, formic acid and glucose moiety of the cerebrosides were observed in the 1H NMR spectra of the CH₂Cl₂ extract. **Conclusions:** The highest anti-chlamydial activity was shown by the CH₂Cl₂ extract. The inhibitory effect was observed once the inclusion had been achieved by the EBs. This could be attributed to its interference with cell metabolic pathways related with the development of the chlamydial inclusion. The 1H NMR analysis is the first study of *H. bonariensis* compounds with antichlamydial activity.

Keywords: *Hydrocotyle bonariensis*, anti-chlamydial activity, *Chlamydia trachomatis*, *Chlamydia pneumoniae*

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ABSTRACT 25

Quality control of extracts of *Arctium lappa* L. (Asteraceae)

Alejandra Catalano, Ingrid Cufre, Verónica Tarcaya, Laura cogoi, Paula Lopez and Adriana Broussalis

ABSTRACT

Background: In recent years, there has been considerable interest in using botanical agents to prevent skin ageing. Extracts from different parts of *Arctium lappa* L. (Asteraceae), commonly known as burdock, have been reported to have biological activities and pharmacological functions, including anti-inflammatory, antioxidant, antimicrobial and anti-ageing effects. In this work, TLC and HPLC chromatographic systems for quality control of *A. lappa* extracts were developed.

Methods: TLC: ETOH:H₂O 60% V/V extract; rutin, rosmarinic acid and chlorogenic acid reference standards in MeOH; Stationary phase: Silica gel HF254; Mobile phase and Reagents: a) CH₂Cl₂:Acetic acid:MeOH:H₂O (15: 8: 3: 2); NPR reagent b) Toluene:Ethyl Acetate (6:4); H₂SO₄ Reagent; c) Ethyl Acetate:Formic acid:Acetic acid:H₂O (100:11:11:27); NPR reagent. HPLC: C18 column 150 x 4,6 mm; 5 μ. Mobile phase: PO₄HNa 5mM pH 5: MeOH (60:40); Temperature: 35 °C. Detection: 365 nm.

Results and Discussion: For the a) and b) TLC systems assayed, the extracts showed the chromatographic profile specified in pharmacopoeias. In the chromatograms obtained with the system c) it was observed an intense orange spot (Rf 0.38), coincident in Rf and colour with the reference substance rutin. Between the spots of the reference standard rosmarinic acid and chlorogenic acid three green-light blue spots (Rf 0.91, Rf 0.83, Rf 0.69) and a fluorescent light blue spot (Rf 0.95) were observed. Quantification method was specific, linear (R² > 0.99) and accurate (98.8%). Intra- assay precision presented a R.S.D < 2%.

Conclusions: TLC and HPLC methods described were adequate for the identification and quantification of the dried extract of *Arctium lappa* L.

Keywords: *Arctium lappa* L., quality control, *Arctium lappa* L. extract

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ABSTRACT 26

Effects on anti-depressant and motor co-ordination activities of linalool-type *Cinnamomum osmophloeum* leaf oil in mice

Hui-Ting Chang, Mei-Ling Chang, Chun-Ya Lin, Yan-Ting Chen, Chia-Chen Wu, Cheng-Kuen Ho and Shang-Tzen Chang

ABSTRACT

Background: *Cinnamomum* species (Lauraceae) are the woody species native to South and Southeast Asia, and widely used as food flavors and traditional medicines. *C. osmophloeum* Kanehira is an endemic species to Taiwan, and it has long been used as a medicinal plant. The study aims to evaluate the chemical constituents of linalool-type *C. osmophloeum* leaf oil, its anti-depressant effect in the mouse model of depression, and the effect on the motor co-ordination activity in mice.

Methods: *C. osmophloeum* leaves were hydrodistilled in a Clevenger apparatus to get the leaf essential oil. Constituents of leaf oil were analyzed by using Thermo Trace GC Ultra gas Chromatograph and Polaris Q MSD Mass Spectrometer. The presence of linalool was confirmed by the chiral GC analysis. Mice were orally treated with the linalool-type leaf oil. Anti-depressant effect was evaluated by the open field test and the forced swimming test; motor coordination activity was estimated by the rotarod test.

Results and Discussion: The major component of leaf oil is linalool (93%) analyzed by GC-MS. The linalool is confirmed to be S-(+)-linalool by the chiral GC analysis. In the open field test, leaf oil also increased the time spent in center zone by the treated mice; it provided the evidence that the leaf oil has the anti-depressant activity. The oral treatment on mice with linalool-type leaf oil decreased the immobility time and induced the anti-depressant effect in the forced swimming test. Motor co-ordination activity was evaluated by the rotarod test, mice treated with linalool-type leaf oil showed significant increase in the time spent on the rotating rod.

Conclusions: These results revealed the linalool-type *C. osmophloeum* leaf oil has the anti-depressant activity and the effect to enhance the motor coordination activity.

Keywords: antidepressant effect, *Cinnamomum osmophloeum*, linalool, motor co-ordination activity

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ABSTRACT 27

Anti-tyrosinase activity and anti-melanogenesis activity in zebrafish of Myricetin

Xiang-Zhe Huang, I-Hsuan Liu, Chi-Ya Huang, Shang-Tzen Chang and Hui-Ting Chang

ABSTRACT

Background: Many investigations reported that natural products including phenolics, flavonoids, coumarins, and alpha hydroxy acids exhibited the skin whitening effects. Myricetin is one of the flavonoids with the bioactivities, such as the anti-oxidant, anti-inflammatory, anti-cancer activities. The objectives of this study are to evaluate the anti-tyrosinase activity and melanogenesis inhibition activity in zebrafish (*Danio rerio*) of myricetin.

Methods: L-Dopa and L-tyrosine were used as substrates in the antityrosinase assay, respectively. After incubation, the absorbance at 475/485 nm of each well was measured. Zebrafish embryos (9 hpf) were placed into a 24-well plate, treated with the different dosages of specimens (in 1% DMSO), and incubated for 48 h. The melanin content of each zebrafish embryo was analyzed by the ImageJ software. Positive controls were arbutin and phenylthiourea (PTU, a melanin synthesis inhibitor).

Results and Discussion: Myricetin exhibited the mushroom tyrosinase inhibition activity in both substrate (L-tyrosine and L-Dopa) assays, with the IC₅₀ values of 102.23 and 94.94 μ M. The IC₅₀ value of positive control, arbutin, was 92.43 μ M when using L-tyrosine as the substrate. In the enzyme kinetic analyses, myricetin was the mix-type inhibitor against mushroom tyrosinase whether using L-tyrosine or L-Dopa as the substrate. Myricetin also showed the good performance in inhibition of melanin formation in zebrafish embryos. At a concentration of 200 μ M, myricetin and arbutin inhibited 43.27 and 47.49% of melanin production of zebrafish embryos, respectively.

Conclusions: According to the results, myricetin has good effects on the anti-tyrosinase activity and melanogenesis inhibition activity in zebrafish, and is of the potential to be used as the skin whitening agent.

Keywords: anti-melanogenesis activity, anti-tyrosinase activity, *Danio rerio*, melanin, myricetin

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ABSTRACT 28

a-Glucosidase inhibitory activity and toxicity of *Chrysophyllum cainito* extract

Hau Doan Van, Siriporn Riyajan and Nuannoi Chudapongse

ABSTRACT

Background: *Chrysophyllum cainito* L., commonly known as star apple, has been found widely distributed throughout the tropics, including Southeast Asia. It has been used in folk medicine around the world for the treatment of diabetes mellitus. However, scientific evidence supporting the anti-diabetic activity of *Chrysophyllum cainito* and its mechanism of action is very limited. The current study was to investigate the mechanism of anti-diabetic action and toxicity of the aqueous extract of *Chrysophyllum cainito* stem bark cultivated in Vietnam.

Methods: *In vitro* a-glucosidase inhibitory activity of the extract was tested. Toxicity of the extract was studied in human hepatocarcinoma HepG2 cells using MTT assay. The acute oral toxicity was also conducted in rats.

Results and Discussion: It was found that the extract possessed stronger inhibition on the enzyme a-glucosidase than the clinically used a-glucosidase inhibitor acarbose. The concentration of the extract that caused 50% cell death was about 50 times higher than the IC₅₀ on a-glucosidase inhibition. The results from the acute oral toxicity showed that the extract at the doses of 500, 1000, 2000 and 4000 mg/kg given as single dose induced no signs of toxicity or mortality after 14 days of the extract administration.

Conclusions: The data suggest that a possible mechanism underlying anti-diabetic activity of *Chrysophyllum cainito* extract is an inhibition of a-glucosidase enzyme. The *in vitro* and *in vivo* toxicity studies provide scientific evidence to corroborate the safety of this plant as an alternative anti-diabetic agent.

Keywords: *Chrysophyllum cainito*, a-glucosidase, anti-diabetic agent, toxicity

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ABSTRACT 29

Bryophytes as potential source of secondary metabolites with anti-proliferative and anti-microbial activities

Dezcsó Csupor, Martin Vollár, István Zupkó, Boglárka Csupor, Marianna Marschall, Péter Szucs and Edit Urbán

ABSTRACT

Background: Bryophytes have been used in the traditional medicine especially due to their antimicrobial activities. *In vitro* studies confirm the efficacy of certain species against several pathogens. Some bryophyte metabolites e.g. maytansinoids possess anticancer effect. The aim of our study was to screen Hungarian bryophytes for their anti-proliferative and anti-microbial effects.

Methods: Extracts were prepared with solvents of different polarities from each species. Anti-proliferative activities of these extracts were analyzed using MTT assay on HeLa, A2780 and T47D cell lines. Anti-microbial effects were examined by disc diffusion test.

Results and Discussion: From the 50 screened species, 6 exhibited remarkable antimicrobial activities and 15 had anti-proliferative effect on at least one of the tested cell lines.

Conclusions: As part of our studies we identified several species with prospective bioactivities. The aim of further studies will be the activity-guided isolation of biologically active secondary metabolites.

Keywords: Bryophyte, anti-microbial, anti-proliferative

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ABSTRACT 30

Cymbopogon nardus: anti-*Candida albicans* activity of the unloaded and loaded essential oil into nano-structured drug delivery system

Luciani Gaspar de Toledo, Patrícia Bento da Silva, Matheus Aparecido dos Santos Ramos, Marlus Chorilli, André Gonzaga dos Santos, Margarete Teresa Gottardo de Almeida and Taís Maria Bauab

ABSTRACT

Background: *Candida albicans* is a normal component of microbial flora, but it can induce severe infections in immuno-compromised patients. The therapy of fungal infections presents limitations. Thus, the search for new anti-fungal agents is necessary. *Cymbopogon nardus* (L.) Rendle is a plant popularly known as *Citronella*, and its essential oil shows anti-fungal action against *Candida* species. This work aimed to evaluate the anti-*C. albicans* activity of unloaded and loaded essential oil of *C. nardus* (EO) into a nanostructured drug delivery system.

Methods: The minimal inhibitory concentration (MIC) of the unloaded and loaded EO against *C. albicans* (ATCC 10231 and a clinical isolate) was determined by micro-dilution assay according to the protocol described by CLSI, with modifications. The nano-structured drug delivery system (lipid micro-emulsion) developed consisted of grape seed oil as the oil phase (10% w/w), polyoxyethylene (23) lauryl ether as the surfactant (10% w/w) and phosphate buffered saline (80% w/w) as aqueous phase prepared by sonication.

Results and Discussion: The unloaded OE showed an important antifungal activity with MIC values of 250 µg/mL (ATCC) and 500 µg/mL (clinical isolate). In addition, the use of the lipid micro-emulsion improved the anti-*C. albicans* action of the EO. The loaded EO exhibited MIC values of 15.6 µg/mL (ATCC) e 125 µg/mL (clinical isolate). The results observed in this study are relevant, as they corroborate with the results obtained by our research group, considering the lipid micro-emulsion containing the EO enhanced the activity against these strains (ATCC and clinical isolate).

Conclusions: The EO of *C. nardus* is an effective source against *C. albicans* strains. Moreover, the use of the lipid micro-emulsion in order to improve the activity of EO can be used as a promising anti-fungal agent in the control of candidiasis caused by *C. albicans*.

Keywords: *Cymbopogon nardus*, essential oil, *Candida albicans*, anti-fungal activity, nano-structured drug delivery system, micro-dilution

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ABSTRACT 31

Natural products: From benchtop to bedside, a translational approach

Alberto C. P. Dias

ABSTRACT

Extracts, infusions, or other types of preparations from medicinal plants have been used since ancient times for the treatment of several diseases, in what is commonly designated as “Folk Medicine”. In recent years, science has proven that some plant extracts, fractions or specific compounds may have an important role as drug sources with relevant properties. In this work, particular emphasis has been given to anti-microbial and healing properties of specific plants/compounds in *in vitro* as well as *in vivo* models. New approaches, including nanotechnology, have been addressed. Based on specific plants some particular cosmeceutical formulations were developed, used in clinical practice for skin problems, namely recalcitrant injuries, diabetic foot, sores and scars. The synergy of the properties of selected plant constituents, gave very positive results associated with a high degree of skin hydration, contributing to cell regeneration. In all cases, after repeated applications, significant improvements or complete treatment were observed, without tangible side effects. Formulations were revealed to be associated to a high degree of skin hydration, cellular skin regeneration, and anti-microbial activity. The results demonstrated, at least, a similar effectiveness compared to the conventional treatments. Based on scientific knowledge it was possible to produce cosmeceuticals of vegetable origin, with high quality and established bioactivities.

Keywords: cosmeceutical formulations, recalcitrant injuries, sores and scars, anti-microbial activity

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ABSTRACT 32

***Baccharis dracunculifolia* decreases nociception, depressive-like behaviour and supraspinal activated microglia in rats with experimental monoarthritis**

Inês M. Laranjeira, Elisabete Apolinário, Diana Amorim, Ademar A. Silva-Filho, Filipa Pinto-Ribeiro and Alberto C.P. Dias

ABSTRACT

Background: In arthritic disorders, inflammation and progressive degeneration of joints leads to the development of persistent pain and comorbid emotional impairments. Arthritis-induced peripheral sensitization leads to increased release of nociceptive molecules by primary afferents that activate neuronal cells in the spinal cord and supraspinal pain modulatory areas such as the amygdala (AMY) and the periaqueductal grey matter (PAG). *Baccharis dracunculifolia* (Bd) is a medicinal plant from Brazilian flora, popularly known as "Alecrim do Campo", used as anti-inflammatory and anti-nociceptive.

Methods: Adult 8-weeks old ovariectomized female rats (*Rattus norvegicus*, vr. *Albinus*, Wistar) weighting 210 ± 17 g were divided in four groups (n=6 per group): (i) SHAM, (ii) ARTH, (iii) ARTH treated with *B. dracunculifolia* (50mg/kg), and (iv) ARTH treated with *B. dracunculifolia* (100 mg/kg).

Results and Discussion: Mechanical hyperalgesia in ARTH animals was assessed using the pressure application measurement apparatus, anhedonia using the sucrose preference test and learned helplessness using the forced swimming test. Activated microglia was stained with IBA-1 and quantified in a subset of brain slides containing the target areas, the amygdala and the periaqueductal grey matter. A three-week oral treatment with Bd extract reversed ARTH-induced mechanical hyperalgesia and partly reversed depressive-like behaviour. Concomitantly, Bd treatment decreased the number of activated microglia in the AMY and PAG of ARTH animals.

Conclusions: *Baccharis dracunculifolia* decreases nociception, depressive-like behaviour and supraspinal activated microglia in rats with experimental monoarthritis.

Keywords: *Baccharis dracunculifolia*, monoarthritis, anti-inflammatory, anti-nociceptive, microglia

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ABSTRACT 33

Analysis of mountain tea volatiles *via* distillation and ultrasound-assisted extraction after enzymatic or acidic pre-treatment

Virginia Dimaki, Gregoris Iatrou and Fotini Lamari

ABSTRACT

Background: *Sideritis* L. (Lamiaceae), growing mainly in the Mediterranean region, comprises of more than 150 species worldwide. The majority of the taxa are widely known as mountain tea and have been consumed as herbal tea since antiquity not only for its exquisite flavor and odor, but also for its medicinal properties. Apart from its flavonoids, its terpenes (mono-, sesqui- and diterpenes), components of the essential oil and the extracts, contribute to their anti-microbial, anti-inflammatory, analgesic, anti-ulcer, cytotoxic and anti-tumor activities.

Methods: *Sideritis clandestina* ssp. *peloponnesiaca*, an endemic taxon in northern Peloponnesus, was collected from mountain Chelmos. Isolation of its essential oil via hydrodistillation of its leaves and flowers gave a low yield (<0.12%); about 65 components, mostly monoterpenes, were identified via GC-MS. To increase the yield and miniaturize the procedure, maceration (MAC) and ultrasound-assisted extraction (UAE) were studied, as well as the effect of sample pretreatment with cellulase, hemicellulase and pectinase or just acidic medium for 75 min at 37°C.

Results and Discussion: MAC and UAE provide chemo-profiling of the main volatile compounds (about 20) avoiding alterations induced by exposure to high temperature, but UAE outweighs MAC, since it has low demands on time, solvent and plant material (3 g) and higher yields. Both enzymatic and acidic treatments prior to hydrodistillation or UAE were more efficient in comparison to the respective techniques without pretreatment; however, the results of the enzyme-assisted procedures were not significantly different from their respective controls (acidic pre-treatment). Isolation of the essential oil of cultivated *Sideritis raeseri* with acidic pretreatment before hydrodistillation revealed an increment on the yield around 27%; α -terpinene, α -pinene, d-cadinene and α -terpinene were its main components. A further phytochemical investigation on small amounts of leaves and flowers was successfully conducted with acidic pre-treatment *via* UAE.

Conclusions: In conclusion, acidic treatment prior to ultrasound extraction or hydrodistillation in citrate buffer, pH 4.8, can enhance the yield and it is recommended for the analysis of *Sideritis* volatiles and subsequently for other aromatic plants.

Keywords: mountain tea, *Sideritis*, volatiles, enzyme-assisted extraction, ultrasonic-assisted extraction, acidic pretreatment

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ABSTRACT 34

Stability and retention of lavender essential oil in conventional emulsions and nano-emulsions: A comparative study

Konstantina Flekka, Virginia Dimaki, Pavlos Klepetsani, Elena Mourelatou, Konstantinos Avgoustaki, Fotini Lamari and Sophia Hatziantoniou

ABSTRACT

Background: Essential oils are mixtures of large number of components and are widely used in cosmetic and pharmaceutical products. Their volatility causes decrease of their concentration in the final product and reduction of products performance. Lavender essential oil (Lav) in particular has proven to possess valuable action when used topically. Nano-emulsions (NE) are vehicles for enhancement of stability of bioactive ingredients. The aim of this work was to investigate the impact of the carrier on the stability of the lavender essential oil.

Methods: Conventional (CE) and nano-emulsions incorporating Lav, were prepared (n=3). Their physico-chemical characteristics and stability assessed by monitoring particle size changes using Dynamic or Static Light Scattering, after (i) centrifugation, (ii) accelerated ageing (three cycles of heating/cooling: 45°C/25°C) and (iii) storing in various conditions (25°, 4° and 45°C). The incorporation efficacy in CE and in NE was evaluated at preparation day and monitored over time by GS/MS, using eucalyptol as external and octane as internal standard.

Results and Discussion: The size of the dispersed phase droplets of CE was $17.46 \pm 1.48 \mu\text{m}$ and the Uniformity 0.27. NE droplets were $97.54 \pm 3.65 \text{nm}$ (Pdl 0.22). The zeta potential ($-60.5 \pm 2.25 \text{mV}$, width 9.45) was indicative of good long term stability. Both CE and NE remained stable for 30 days at 25°C and 60 days at 4°C and passed successfully the centrifugation and accelerated ageing tests. The incorporation of lavender did not change significantly the size of the dispersed droplets but affected the physical stability of the samples. Both CE Lav and NE Lav were stable at 4°C for 60 days. The concentration of lavender incorporated in both CE and NE, as measured after preparation, was 39.53 and 57.95 mg/ml, respectively. At 30 days, the content dropped to 22.15 mg/ml for the CE and 51.60 mg/ml for NE, while at 60 days the lavender content was found 3.8 mg/ml and 39.16 mg/ml, respectively.

Conclusions: The results revealed that the Lav incorporation enhances the stability of both NE and CE. Nano-emulsions exhibited higher stability as they retained the incorporated Lav in higher percentages and for longer time than conventional emulsions.

Keywords: essential oils, nano-emulsion, emulsion, stability

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ABSTRACT 35

TASCMAR Project: Tools and strategies to access the original bioactive compounds through cultivation of marine invertebrates and associated symbionts

Nikolas Fokialakis, Ioannis Trougkos, Konstantinos Gardikis, Evangelos Topakas and Jamal Ouazzani

ABSTRACT

TASCMAR is the acronym of an H2020 EU-funded project, joining the forces of five academic institutions, seven commercial partners and one NGO, and sets out to find new, efficient and sustainable ways of discovering marine-derived molecules and applying them in the field of pharmaceuticals, cosmeceuticals and nutraceuticals with a particular focus on the theme of anti-ageing. The project will also engage innovative cultivation technologies for marine invertebrates and associated symbionts. The project will focus on the potential of marine invertebrates and their associated symbionts located in the under-investigated mesophotic zone (between 30 and 100 meters depth) and aims to end with the industrial application of bioactive compounds and the development of innovative cultivation equipment. Seven expeditions dedicated to mesophotic coral ecosystems and temperate mesophotic ecosystems will be carried out, with particular emphasis placed on respecting biodiversity, resource preservation and ethical principles as laid out in local, regional, national, European and international rules and policies. Innovative approaches for the cultivation and extraction of marine organisms from lab to pilot-scale, will be used and marine dedicated cultivation and extraction equipment will be built and validated. These unique improvements will ensure the sustainable supply of biomass and promote the production of high added value bioactive marine compounds. An integrated, holistic technological metabolomic approach will be applied, in conjunction with bioactivity profiling, as filtering and bio-prioritisation tools. State-of-the-art analytical instrumentation and in-house databases will be employed for the dereplication and characterization of valuable compounds. A panel of libraries (marine organisms, extracts, pure metabolites and biocatalysts) will be constructed and exploited throughout the project. A focused panel of *in vitro*, cell-based, *in ovo* and *in vivo* bioassays for discovering metabolites with anti-ageing activity will frame the entire work-flow and will reveal the lead compounds. In working to achieve these objectives, TASCMAR aims to promote fruitful and long-term collaboration between research institutions and industry in the field of novel and bioactive marine derived molecules and industrial biomaterials and will contribute to the implementation of the European Commission Blue Growth Strategy for unlocking the potential of seas and oceans to support sustainable growth.

Keywords: natural products, marine organisms, microorganisms, anti-ageing, cosmeceutical

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ABSTRACT 36

Actinobacteria of global biodiversity as a source of bioactive metabolites for the discovery and development of novel cosmeceutical agents

Katerina Georgousaki, Nikolaos Katsinas, Laure-Anne Peyrat, Nikolaos Tsafantakis, Sentiljana Gumeni, Celso Almeida, Ioannis Trougakos and Nikolas Fokialakis

ABSTRACT

In the frame of MICROSMETICS EU project more than 110 potential candidate actinobacteria strains of global biodiversity were selected to be studied. In total 614 extracts were produced and a broad spectrum of cell-free bioassays have been incorporated for the evaluation of their anti-ageing activity, including analyses for anti-oxidant, skin-protecting and skin-whitening bioactivity. Among the initial 614 extracts, 12 actinobacteria strains were selected as promising bioactive products and have been further evaluated in cell-based assays for their skin whitening (bleaching) activity (*i.e.* tyrosinase inhibition) in mouse melanocytes (B16F10 cell line), as well as in normal human skin fibroblasts for their capacity to activate proteostasis ensuring anti-ageing mechanisms, namely the ubiquitin-proteasome and autophagy-lysosomal systems. The 4 most bioactive strains CA-129531, CA-129255, CA-126581 and CA-218259 have been selected for dereplication, cultivation in larger scale and bioguided fractionation and identification of bioactive compounds. The dereplication was performed using UPLC-HRMS while the isolation of the compounds contained in those extracts was performed using both classical chromatographic methods, as well as more innovative techniques, like HPLC and FCPC, respectively. The full set of spectroscopic data (HRMS and NMR) was recorded for all isolated compounds in order to unambiguously elucidate their structure. All the isolated compounds were evaluated for their bioactivity. The evaluation of those specific strains in cell-free and mammalian cell based assays emphasized the anti-ageing effect of several metabolites isolated from the selected strains. Special attention was also given to isolated hydroxamic acid derivatives due to the remarkable skin-whitening activity they demonstrated both in cell-free and cell-based assays. Therefore, these extracts can be considered as potential candidates for industrial development and can serve as a proof of concept that microbial ingredients can have successful applications in cosmeceutical industry.

Keywords: cosmeceuticals, actinobacteria, natural products, anti-ageing

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ABSTRACT 37

Novel cosmeceuticals and food supplements from exploitation of aromatic plants™ by-products

Nikolas Fokialakis and Nektarios Aligiannis

ABSTRACT

EXANDAS is the acronym of a European research project which aims to apply emerging and cutting edge technologies in the field of Natural Products in order to fully and efficiently exploit the therapeutic potential of medicinal and aromatic plants processing waste and by-products. The consortium, consisting of six academic and six SMEs partners, will join forces and exchange know-how through an extended secondments scheme to advance Research & Innovation. The scientific concept of EXANDAS involves the exploitation of aromatic plants™ by-products for the development of novel cosmeceuticals and food supplements. The cornerstone of EXANDAS project is the development of novel processes based on eco-friendly technologies for the efficient extraction, purification and transformation of active ingredients, as well as the complete chemical characterization and biological evaluation of produced extracts and pure compounds that can be commercially exploited. Optimization and scaling up of these procedures, as well as formulation using emerging technologies will lead to the development of novel final products. In more details, by-products and wastes from the industrial exploitation of mastic gum, rose, mountain tea, lavender, geranium and sweet basil will be selected. Improved techniques such as ASE, CPC, MAE etc. will be used to isolate, purify, and structurally characterize the active constituents that will be further investigated with the aim to be exploited commercially. A broad-spectrum of bioassays will be incorporated for the evaluation of antioxidant, anti-inflammatory, anti-microbial and anti-ageing properties activity of all derived extracts and products. Using the experience of the academic partners in phytochemistry and natural product chemistry, as well as the practical experience of the SMEs in large-scale processing of plant material and development of innovative final products, transfer of scientific knowledge, best practices and know-how will take place. The abovementioned objectives will be implemented through an extended and balanced scheme of researchers' exchanges, in both directions and via a mutual scientific project developed on the needs and interests of both industrial and academic sectors, exploiting the existing complementary expertise. Overall, the implementation of EXANDAS aspires to develop a successful and sustainable international and inter-sectoral collaboration model, which will contribute to the innovation potential of Europe for the most effective exploitation of natural resources and the development of novel cosmeceuticals and food supplements.

Keywords: cosmeceuticals, plants, by-products, anti-ageing, anti-microbial, anti-inflammatory

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ABSTRACT 38

Assessment of anti diabetic and hypolipidemic activity of the methanolic extract of *Panicum sumatrense* seeds

Kattamanchi Gnananath

ABSTRACT

Background: *Panicum sumatrense* (Synonym *Panicum miliare* Auct. Lam. Popularly known as little millet belongs to the family Poaceae. Now-a-days there is increasing interest in the health benefits of foods. People have begun to look beyond the basic nutritional benefits of food to prevent diseases and enhance health. Millets provide an opportunity to improve the health of people, reduce healthcare costs and economic support in rural, tribal and hilly communities.

Methods: In the current study alloxan monohydrate was used to induce diabetes in rats. All the tests were carried for 28 days to evaluate *in vivo* hypoglycemic activity and hypolipidemic activity in methanolic extracts of *Panicum sumatrense* seeds at 200mg/kg, 400mg/kg per body weight in both normal and hyperglycemic rats. Parameters like blood glucose levels and body weight and different biochemical parameters like serum triglycerides, cholesterol, HDL and LDL were also tested.

Results and Discussion: At the end of the 28th day, methanolic extracts of *Panicum sumatrense* significantly lowered the blood glucose (190.3±0.30 mg/dL) ($P>0.01$), whereas in case of body weight, it remained constant throughout the study. For hypolipidemic activity, biochemical parameters like serum triglycerides, cholesterol, HDL, LDL levels were found to be 161.5±17.33 mg/dL ($P<0.01$), 72±9.54 mg/dL ($P<0.05$), 62.7±8.51 mg/dL ($P<0.01$), 61±6.58 mg/dL ($P<0.001$), respectively. All the biochemical parameters were significantly lowered when compared with pathological control.

Conclusions: Polyphenols which are present in the seeds of *Panicum sumatrense* might be responsible for the activity and they may be further utilized as source of nutraceutical.

Keywords: *Panicum sumatrense*, millets, anti-diabetic, hypolipidemic activity

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ABSTRACT 39

Exploring Greek texts on cosmetics in imperial Rome

Constantinos Grivas

ABSTRACT

We have investigated the possible Greek terms associated with cosmetic use and especially the presence of the word cosmetic (*kosmetikon*) itself. Examining the available Greek texts and writers of the Roman period, we focused on the most important ones, like Dioscorides *De materia medica* and Galen works on compound medicines (mainly *De compositione medicamentorum secundum locos*, *De compositione medicamentorum per genera* and *De antidotis*). Categorizing the cosmetic recipes according to use (for example hair or skin recipes) and listing their ingredients offered us the opportunity to compare between them. As a conclusion, we could point to the most interesting writers and texts for further investigation, identify and evaluate several common ingredients in cosmetic recipes and propose some of them as highly interesting in terms of modern usage.

Keywords: ancient Greek medicine, phytocosmetics, ethnobotany, phytomedicine, galen, dioscorides

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ABSTRACT 40

Nanotechnology and innovative delivery systems in cosmetics

Sophia Hatziantoniou

ABSTRACT

Nanotechnology has found a wide range of applications in life sciences. Nanoparticles have been used in therapeutics mainly as carriers of bioactive molecules as well as in imaging, as shading agents. This new technology is also applied in cosmetic products. Because of their ability to provide novel improved characteristics, several nano-carriers, either biodegradable or not, have been used in cosmetic products. Such nanoparticles as liposomes, solid lipid nanoparticles, nano-structured lipid carriers, nano-emulsions, metal and metal oxide nano-particles are few of the new technological platforms deriving from nanotechnology that may be found in several cosmetics. However, the use of nanotechnology on cosmetics has raised concerns on their safety. The interaction of nanoparticles with the skin and nanotoxicity are issues that are currently studied globally. The regulatory status of these products is currently under discussion. New scientific evidences are expected from the competent authorities in order to implement them to the relevant legislation.

Keywords: nanotechnology, nanoparticles, nanotoxicity, innovative delivery systems, regulatory, cosmetics

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ABSTRACT 41

An optimized *Lythrum salicaria* extract for the cosmetic industry

Florence Henry, Isabelle Bonnet, David Bothorel, Florence Trombini and Valérie Andre-Frei

ABSTRACT

Background: Purple loose strife is a herbal remedy that has been used for centuries in traditional European medicine. Indeed, blooming *Lythrum salicaria* contains high concentrations of polyphenols and tannins, that exhibit multiple health benefits. In this work we wanted to adapt this well-known plant from the European pharmacopeia to the industry of cosmetics.

Methods: Regarding chemical composition, *Lythrum salicaria* is a rich source of polyphenols including ellagitannins (hydrolysable tannins), flavonoids, phenolic acids and anthocyanins. Among C-glucosidic ellagitannins, the presence of castalagin and vescalagin was established. An extraction process has been purposely designed to increase castalagin and vescalagin content.

Results and Discussion: Because vescalagin and castalagin are described to have anti-inflammatory activities known to be in favor of skin health, optimized extraction process was designed in order to obtain high amount of polyphenol content and particularly of these 2 ellagitannins. The first step is a hydro-alcoholic extraction of *Lythrum salicaria* plants. Then, a second step based on a separation and concentration process, allows us to obtain *Lythrum salicaria* as a powder without any preservative, with a titrated content of vescalagin and castalagin (sum of 0.8-1.2%). *Lythrum salicaria* extract and pure vescalagin and castalagin were used in a performance *in vitro* test. The hydroalcoholic extract of *Lythrum salicaria* has been proven to tighten the dermis and to thin the hypodermis by inhibiting the lipid storage into adipocytes. Vescalagin and castalagin, its two biomarkers, demonstrate their contributing efficacy by inhibiting the lipogenesis.

Conclusions: The optimization of the extraction process of *Lythrum salicaria* permitted to obtain a hydro-alcoholic extract titrated in vescalagin and castalagin. This improved extract has demonstrated *in vitro* its beneficial properties for the skin. Vescalagin and castalagin, its two biomarkers, revealed their efficacy by inhibiting the lipid storage into adipocyte useful for body reshaping cosmetic product.

Keywords: *Lythrum salicaria*, castalagin, vescalagin, ellagitannin

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ABSTRACT 42

Oligosaccharides from flax seed to rejuvenate the skin

Philippe Moussou, Christine Jeanmaire and Florence Henry

ABSTRACT

Background: Flax is a multipurpose resource whose by-products and every parts are valorized in industry. In health and food industries as well as in ancient medicine (Ayurveda), flaxseed is considered as a healthy food and a source of healthy fat, antioxidants, dietary fiber, soluble polysaccharides, secondary metabolites, vitamins and minerals. Based on this knowledge, a hydrolyzed flax seed extract concentrated in oligosaccharides has been developed to emphasize its beneficial properties for dermocosmetic application.

Methods: The oligosaccharides are obtained through a first step of specific extraction of soluble polysaccharides located in flaxseed hull of *Linum usitatissimum* L.(France), followed by a second step of controlled hydrolysis. This hydrolyzed extract is dried after separation and concentration steps, to obtain a 100% hydrolyzed linseed extract powder, concentrated in oligosaccharides, without any preservative.

Results and Discussion: To better characterize our extract, we evaluated a weight average of oligosaccharides between 20000 and 30000 g/mol, (mainly xylose, galactose, glucose and rhamnose monomers). Two oligosaccharides fractions have also been purified by successive ultrafiltrations: a 15-50 kDa fraction, with an average of 31000 g/mol, and a 5-15 kDa fraction, with an average of 5000 g/mol. Thanks to *in vitro* tests such as dermal cell migration, collagen and proteoglycan production, these two oligosaccharidic fractions were identified as biomarkers of the hydrolyzed flax extract. We demonstrated here that this flax seed extract rich in oligosaccharides is a multi-functional rejuvenating ingredient able to regenerate the dermis by activating the fibroblasts, restructuring the extracellular matrix and regenerating the epidermis (thickness and differentiation).

Conclusions: Within flax, soluble polysaccharides located in the seed hull are not valorized compounds despite their obvious interest especially for the cosmetic industry. Based on the demonstrated activities on multiple and key skin components, our flax seed extract concentrated in oligosaccharides can be used to rejuvenate, strengthen the skin and to fight against age signs.

Keywords: *Linum usitatissimum*, oligosaccharide, ayurveda, seed

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ABSTRACT 43

Inhibitory constituents of nitric oxide production from *Siegesbeckia pubescens*

Hari Jang, Jun Gu Kim, Thi Phuong Linh Le, Hye Ryoung Hong, Mi Kyeong Lee and Bang Yeon Hwang

ABSTRACT

Background: *Siegesbeckia pubescens* Makino (Compositae) is an annual herb widely distributed in Korea, China, and Japan. The aerial parts of this plant have been used as traditional medicine to treat rheumatic arthritis, asthma, hypertension, and malaria. Previous phytochemical investigations show the genus *Siegesbeckia* is a rich source of sesquiterpenoids, ent-kaurane and ent-pimarane type diterpenoids.

Methods: The MeOH extract of the aerial parts of *S. pubescens* was partitioned with n-hexane, CH₂Cl₂, EtOAc, and water, successively. The methylene chloride and ethyl acetate soluble fraction were separated using several column chromatography including MPLC and preparative HPLC. The structures of the isolated compounds were determined by 1D-(¹H and ¹³C-NMR), 2D- (HSQC and HMBC) NMR, and HR-ESI-MS data. Additionally, all of isolates were evaluated for their inhibitory effects on lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW 264.7 cells.

Results and Discussion: A new ent-pimarane type diterpenoid and two new sesquiterpenoids along with eighteen known compounds were isolated from the methylene chloride and ethyl acetate soluble fraction of the aerial parts of *S. pubescens*. All of isolates were evaluated for their inhibitory effects on LPS-induced NO production in RAW 264.7 cells. Compounds 1, 2, 13, and 14 inhibited the NO production with IC₅₀ values ranging from 3.9 to 16.8 μmol while aminoguanidine, a positive control, showed an IC₅₀ value of 20.3 μmol.

Conclusions: Three new terpenoids along with eighteen known compounds were isolated from the aerial parts of *S. pubescens*. Their structures were elucidated on the basis of 1D-, 2D-NMR, and HR-ESI-MS data. Compounds 1, 2, 13, and 14 showed dose-dependent inhibitory effects on NO production. Accordingly, *S. pubescens* worthy of further investigation for its potential as anti-inflammatory agent.

Keywords: *Siegesbeckia pubescens*, ent-pimarane type diterpenoid, sesquiterpenoid, nitric oxide

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ABSTRACT 44

Chemical constituents isolated from the *Cudrania tricuspidata*

Yang Hee Jo, Seon Beom Kim, Qing Liu, Bang Yeon Hwang and Mi Kyeong Lee

ABSTRACT

Background: *Cudrania tricuspidata* (Moraceae) is a deciduous tree which is indigenous to the East Asia area, mainly in South Korea. Different parts of *C. tricuspidata* such as roots, fruits, leaves have been reported for its medicinal uses, roots for gonorrhoea, hepatitis and inflammation, leaves for mumps, eczema and tuberculosis, and fruits for contusion. Previous studies have shown that this plant contains xanthenes and flavonoids with prenyl moieties as major constituents.

Methods: For the comparison of constituents of different parts of *C. tricuspidata*, the roots, stem barks, leaves and fruits were subjected, respectively, to various chromatographic analysis. The structures of isolated compounds were determined by various spectroscopic methods including 1D-NMR (1H and 13C), 2D-NMR (HSQC, HMBC and NOESY) and HR-ESI-MS.

Results and Discussion: Our extensive chemical investigation on the roots, stem barks, leaves and fruits of *C. tricuspidata* resulted in the isolation of seventeen new compounds, together with more than 100 various known compounds. Interestingly, chemical constituents showed the differential patterns depending on plants parts; Xanthenes and flavonoids from roots and stem barks, flavonoids and isoflavonoids from fruits, and isoflavonoids from leaves. Furthermore, chemical constituents of *C. tricuspidata* possess prenyl moieties in many cases, however, the number, position and structure of prenyl moieties varied in different parts of this plant. These compounds also showed diverse biological activities. Our study suggested anti-proliferative and anti-inflammatory activity of xanthenes, anti-obesity activity of flavonoid and isoflavonoid of *C. tricuspidata*.

Conclusions: Our study showed the characteristic chemical constituents and biological activity of different parts of *C. tricuspidata*. All the parts of *C. tricuspidata* have potential for further development in many purposes.

Keywords: *Cudrania tricuspidata*, xanthone, flavonoid, isoflavonoid, anti-inflammatory, anti-obesity

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ABSTRACT 45

Efficacy, claims and borderline cosmetic products

Yiannis Kapetanstratakis

ABSTRACT

Background: According to the Regulation 1223/2009 on cosmetic products, all cosmetic products that circulate in the European market must be accompanied by a product information File. This file includes all the necessary information regarding the product use, safety, presentation, efficacy and others.

Methods: European Union has established guidelines regarding the justification of claims and the responsible advertisement and communication on cosmetic products (Regulation No 655/2013 and Guidelines to this Regulation). Both publications focus on the way the products are presenting their efficacy and the justification of the claims they use in their advertising. These should not be misleading and should be fully justified through scientific studies (instrumental, sensorial etc).

Results and Discussion: Industry has the need to establish when and if a product based on the claims used can be considered a cosmetic or not. The clear determination of the scope of application of Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30th November 2009 on cosmetic products is crucial for the proper implementation of the Cosmetics Regulation and its correct interpretation and enforcement by national competent authorities of the Member States. European Commission has published a Manual on products that is not clear if they should be considered cosmetics or not (Borderline Products) in order to assess both Industry and National Authorities.

Conclusions: European Union has always been sensitive regarding the unfair business-to-consumer commercial practices in the internal market (Directive 2005/29/EC) and now they imply this to the cosmetic products.

Keywords: efficacy, claims, borderline products, cosmetics

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ABSTRACT 46

Chemical constituents from the tuber of *Bletilla striata*

Jun Gu Kim, Hari Jang, Thi Phuong Linh Linh, Hye Ryoung Hong, Mi Kyeong Lee and Bang Yeon Hwang

ABSTRACT

Background: *Bletilla striata* Reichb. fil is a medicinal plant in the genus *Bletilla* which is one of the widespread members of the Orchidaceae family in North-eastern Asia. Its dried tubers have been used as a traditional medicine for the treatment of tuberculosis and hemorrhage of the stomach or lung. Previous phytochemical studies on this plant have been reported that it contained flavonoids, lignans, stilbenes, bibenzyls, and phenanthrene derivatives.

Methods: In the course of our research program for the discovery of bioactive constituents from medicinal plants, the tuber of *B. striata* was extracted with MeOH, and sequentially partitioned with n-hexane, CH₂Cl₂, EtOAc, and water. The ethylacetate-soluble fraction of the methanol extract of the *B. striata* was further investigated by using various chromatographic methods.

Results and Discussion: A new bibenzyl derivative along with ten known compounds was isolated from the ethylacetate-soluble fraction of the *B. striata*. Their chemical structures were elucidated on the basis of spectroscopic data interpretation, especially 1D- and 2D NMR, HR-ESI-MS and circular dichroism.

Conclusions: Eleven compounds including one novel bibenzyl derivative were isolated from the ethylacetate-soluble fraction of the methanol extract of the *B. striata* by using various chromatographic methods.

Keywords: *B. striata*, bibenzyl derivatives, chromatography, 1D-, 2D-NMR

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ABSTRACT 47

Characterization of *Crocus Taxa* in Greece: *in vitro* and *in vivo* antioxidant studies of *C. sativus*

Fotini Lamari

ABSTRACT

The genus *Crocus* includes eighty-eight perennial species worldwide and twenty-three in Greece. *C. sativus* is the one triploid species cultivated for the high value of its styles, which constitute the spice saffron. Beyond its culinary uses, saffron has been in constant use as a drug for over 3000 years. Its coloring and medicinal properties are mainly attributed to its glycosylated apocarotenoids, the crocins, which are glucosyl esters of crocetin. Crocetin is determined in human blood plasma in micromolar concentrations after drinking a cup of saffron tea. Chemical characterization of wild greek taxa has been performed in our laboratory. GC MS analysis led to the detection of about 20 volatile constituents in styles of *C. sativus*, *C. cartwrightianus*, *C. reocreticus* and *C. laevigatus*, although none was detected in *C. nivalis*. Safranal is the major volatile component in *C. sativus*, whereas its percentage is kept low in wild taxa. *C. sativus* has the highest crocin content and *C. laevigatus* the lowest one (only 5% of that of *C. sativus*). The crocin profile between *C. cartwrightianus* and *C. oreocreticus* is not significantly different, whereas in *C. nivalis*, LC-MS analysis suggests the presence of new crocins. The antioxidant capacity of saffron in simple *in vitro* assays is not as high as that of polyphenol-rich plant extracts. However, its efficacy *in vivo* is superior. Results showed that saffron-treated mice exhibited significant improvement in learning and memory, accompanied by reduced lipid peroxidation products, higher total brain antioxidant activity and reduced caspase-3 activity. In order to investigate the potential preventive effects of saffron against the neurotoxicity induced by aflatoxin B1 (AFB1), male Balb-c mice received AFB1, saffron infusion or saffron infusion plus AFB1. Besides demonstrating for the first time that AFB1 administration caused memory impairment in adult mice accompanied by significant elevation of cerebral lipid peroxidation and decrease of reduced glutathione content, we showed that pre-treatment with saffron infusion prevented memory decline triggered by AFB1 and prevented the induction of cerebral oxidative stress. *In vitro* we compared the effect of saffron, crocetin and safranal on H₂O₂-induced toxicity in human neuroblastoma SH-SY5Y cells. Both saffron and crocetin provided strong protection in rescuing cell viability, repressing reactive oxygen species production and decreasing caspase-3 activation. Altogether, these data suggest that the medicinal properties of saffron are in part attributed to the significant antioxidant protection it confers, and the metabolite crocetin is a powerful *in vivo* mediator of those effects.

Keywords: saffron, *Crocus*, bioactives, learning-memory, neurotoxicity, analysis

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ABSTRACT 48

Ethnobotanical survey on the use of traditional medicine for the treatment of osteoarthritis in Oran, Algeria

Abderrahmene Lardjam, Rim Mazid, Aicha Sadaoui, Walid Khitri, Ali Khalfa, Noureddine Djebli and Houari Toumi

ABSTRACT

Background: Osteoarthritis is a very common rheumatic disease that is associated with a couple of disabilities and chronic pains. Though, curative treatment are not available. Recently, more and more patients are using complementary medicines and particularly phytotherapy. The aim of the present study is to determine the frequency of phytotherapy in patients with osteoarthritis.

Methods: Two ethnobotanical surveys were conducted in patients and herbalists, all data were processed and analysed by using SPSS (Statistical Package for the Social Sciences, IBM Corporation;Chicago, IL) and Excel software program.

Results and Discussion: The first survey was carried out on 314 osteoarthritis, at the level of the Rheumatology Department Oran-Algeria, and showed that 38% of the studied population uses medicinal plants, about 54 species and 43 recipes have been recorded, belonging to 27 botanical families. However, the most used part is the fruit and the majority of remedies are prepared as oil. The second survey was conducted on 52 herbalists in the region of Oran, 52 plant species (plants alone, 15 recipes) have been identified belonging to 27 botanical families and 37 commercial products. The most used part is the leaf and the most recommended mode of preparation is the powder form.

Conclusions: Phytotherapy is still a practice used by osteoarthritis patients, both surveys have helped identify important medicinal plants used by the local population and recommended by herbalists in order to treat osteoarthritis. These plants can provide a convenient solution to the problems of osteoarthritis and being a therapeutic prospect in order to improve the disease management.

Keywords: ethnobotanical survey, patients, herbalists, medicinal plants, osteoarthritis, Oran, Algeria

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ABSTRACT 49

Network pharmacognosy as a new paradigm in the post-genomic era—application to the development of a new anti-ageing natural extract

Michele Leonardi, Christina Österlund, Emma Sjölander, Lene Visdal-Johnsen and Susanne Fabre

ABSTRACT

Background: Skin-ageing is a complex process not only influenced by inherited features but also by several environmental factors such as UV, pollutants and lifestyles. Due to the complexity of the interaction and roles between the different cells involved in the skin-ageing processes, the exact definition of biomarkers remains the major research challenge in cosmetics. In the last decade, network approaches have transformed the traditional approaches to biological systems.

Methods: Based on the assumption described above, we are proposing and introducing a new concept and methodology: the Network Pharmacognosy. Network Pharmacognosy involves unbiased large-scale network-based analyses of diverse data describing omics interactions. This method exploits our understanding of the network connectivity and signalling system dynamics to help identify optimal targets using natural compounds within cosmetics research and development. In addition, this approach in combination with the large activity database represented by the natural compounds, enabled the development of new discovery platforms.

Results and Discussion: In this presentation we report the application of the Network Pharmacognosy for the determination of target biomarkers, *in silico* chemical translation and development of a new natural anti-ageing extract. The determination of the biomarkers was performed in three steps: Big-Data analysis (by NPL search in the scientific literature), Pathways and Network creation and finally the determination of the targets by dynamic analysis of the generated network. For the translation and determination of the natural compounds and corresponding active extracts on selected target, a structure activity relationship (SAR) study combined with Big-Data analysis have been used. Processes listed above have led to the identification of four potential natural extracts. Results obtained by targeted *in vitro* biochemical and cellular activity screening have led to the selection of the final extract ready for formulation work and *in vivo* clinical trials.

Conclusions: The use of Network Pharmacognosy combined with a Target-Customized Analytical and screening platform, proved to be a successful methodology for the rapid and rational determination of active natural compounds combined with a reduction of the risks during the development process.

Keywords: Network Pharmacognosy, skin-ageing, natural extract, big data, cellular activity

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ABSTRACT 50

APIVITA: research and innovation on natural Products: From Athens labs to Tokyo market

Sophia Letsiou

ABSTRACT

Background: APIVITA has been creating natural, effective and holistic products to promote health and beauty since 1979. A pivita story started 37 years ago in a pharmacy in Athens.

Methods: APIVITA, besides a philosophy driven company, is also a research and innovation driven company. The story of APIVITA innovation dates back to 1972 through the incorporation of bioactive extracts, essential oils and bee products in cosmetic formulas. Active collaboration with the academia through research programs is an on-going process for APIVITA that dates since the 1990s in collaboration with the Pharmacy School of Athens and with the Agricultural University.

Results and Discussion: APIVITA, nowadays, is more active than ever in research programs. Currently we are running 7 research programs that are either thoroughly or partly funded by the EU and Greek state. In two of these programs APIVITA is also the project coordinator while in the rest it usually is the leader of the Industrial Research workpackages. APIVITA is also running 3 more privately funded research programs while there are numerous other synergies with the academia, through Master and PhD supervision. APIVITA has recently been awarded by three HORIZON 2020 programs. This is a major success and recognition for the innovation efforts of the company as the last framework of the EU is probably far more competitive than the previous ones. It promises more breakthroughs, discoveries and world-firsts by taking great ideas from the lab to the market.

Conclusions: In total at this moment we are collaborating with more than 70 academic partners, in 22 countries. This ensures us that we are always leading innovation and we are one step beyond the state of the art in the field of natural products, thus promoting by the best practice way our mission and philosophy as a cosmos sustainable company.

Keywords: APIVITA, research, innovation

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ABSTRACT 51

Perfumes and perfumers in ancient Greece

Angeliki Liveri

ABSTRACT

Background: This paper presents information about perfumes and perfumers of Ancient Greece. Names, components/ingredients and preparation methods will be discussed. The focus of the research/presentation is on figures of ancient Greek religion and mythology (e.g. Aphrodite, Charites, Hores, Beautiful Helen, Adonis) but also on historical persons (e.g. Megallos, Peron, Kleopatra) whom I refer to as perfumers and creators (*evretai*) of perfumes and cosmetics or their patrons. Perfumes, cosmetics and perfumery centers vary depending on the historical period. Their preparation methods increasingly improved.

Methods: References in written sources will be compared to and combined with archeological findings (e.g. perfume vases, their remains, representations of the mythic or historical perfumers).

Results and Discussion: This paper attempts to classify perfumes and perfumers as to regions of Ancient Greece, and shows their relationship with plants, processing knowledge, trade, and other relating factors.

Conclusions: Thus, we can observe the development of the Ancient Greek Perfumery from the pre-history to the Hellenistic period.

Keywords: perfumes, perfumers, cosmetics, ancient Greece, ancient Greek mythology, ancient Greek religion

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ABSTRACT 52

Synthesis of novel hybrid peptides with potential antioxidant activity

Athina Lykoura, Eleana Chioni, Anastasios Routsis, Nikolaos Assimomytis, Eftichia Kritsi, Panagiotis Zoumpoulakis and Vassiliki Magafa

ABSTRACT

Background: Natural antioxidants have attracted attention in recent years. Specifically, antioxidant peptides have directly been related to the prevention and treatment of various chronic degenerative diseases, which are partly attributed to oxidative stress and the reactive oxygen species (ROS). Despite the interest, antioxidant peptides have not fully been explored. It is well known that many peptides, which derived from various food proteins, exhibit high antioxidant activity. Rice proteins are also known as one of the most valuable plant proteins.

Methods: The analogues were synthesized by Fmoc/But solid phase methodology, utilizing 2-chlorotrityl chloride resin as solid support. Crude peptides were purified by gel filtration chromatography (GFC) on Sephadex G-15 using 15% acetic acid as eluent. The identification of the analogues was performed by electro-spray mass spectrometry (ES-MS). The reducing power was determined by measuring the ability to reduce ferric iron to ferrous iron (FRAP assay). The test was carried out in triplicate and the reducing power was measured at 595 nm.

Results and Discussion: Peptides from rice residue protein hydrolyzed, were synthetically produced and examined. The aim of the study is the synthesis and evaluation of hybrid peptides with potential antioxidant activity. The hybrid peptides were composed by interfering in the amino acid sequence of the natural antioxidant substances such as caffeic acid, cinnamic acid, coumarin-3-carboxylic acid. The stepwise synthesis of the peptide analogues was achieved without problems, even with the addition of natural substances at the N-terminus. Solid phase synthesis yields ranged within 70-85%, when calculated on the amount of amino acid initially coupled to the resin. The purity of peptides determined by analytical HPLC was higher than 97%. The antioxidant activity of the samples was examined using the FRAP assay and was expressed as $\mu\text{mol FeSO}_4$ per mg peptide. Furthermore, structure elucidation and conformational analysis of the synthesized peptides were performed through NMR spectroscopy and *in silico* studies. The results indicate that peptides containing amino acid residues with intrinsic antioxidant activity exhibit enhanced antioxidant activity. The introduction of natural antioxidants at the N-terminus of such peptides affects slightly their potency.

Conclusions: In the present study, hybrid peptides containing natural antioxidants at the N-terminus were synthesized in good yield and high purity. The antioxidant activity of them was investigated by FRAP assay.

Keywords: antioxidant hybrid peptides, FRAP assay, Fmoc/But solid phase methodology

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ABSTRACT 53

***Rubus adenotrichus* fruit extracts: phytochemical characterization and antioxidant power valuation for dermocosmetic formulations**

German Madrigal Redondo, Rolando Vargas Zuniga, Gustavo Carazo Berrocal, Nils Ramirez Arguedas, Lidiette Fonseca Gonzalez and Jorge Campos Fernandez

ABSTRACT

Background: There are more than 700 species of the genus *Rubus*, popularly known as Mora (tropical highland blackberry). In Costa Rica, the species *Rubus adenotrichus* has been characterized by its high content of antioxidant substances, becoming one of the most cultivated species and that may have characteristics to be used in cosmetics or medicine.

Methods: Ripe fruits of *Rubus adenotrichus* were fractionated with solvents of low, medium and high polarity, followed by a phytochemical screening, the antioxidant properties were evaluated of the H-ORAC and DPPH test; the concentration of total phenols by Folin ciocalteau and antocinainins by differential pH was determined. Finally, the physico-chemical properties of the aqueous extract, such as pH, specific gravity, Brix grades, conductivity, and osmolarity were determined and an absorption spectrum from 260 to 700 nm was obtained.

Results and Discussion: Phenolic compounds were found, as condensed and non-condensed tannins, anthocyanins, flavonoids, terpenes, and alkaloids as major phytochemical groups, a high antioxidant power measured in H-ORAC 311 ± 7.63 g/mol Trolox Equivalent/g of dry fruit, and EC 50 118.46 mg/L for the aqueous extract; the total phenols found were 20.85 ± 0.27 mg/g of dry sample of gallic acid equivalents, which makes the *Rubus adenotrichus* fruit an excellent component for bacteriostatic, anti-ageing, anti-wrinkle, nourishing and moisturizing formulations, and the astrigent effect also allows its application to small superficial wounds on the skin. Likewise, the acidic pH of 3.55 ± 0.1 is beneficial for maintaining the cutaneous acid mantle and thereby favoring the normal flora of the skin, but may be a problem for the formulation of carbomer-based gels or the incorporation of preservatives.

Conclusions: The aqueous extract is hyperosmotic and has a high electrical conductivity due to the presence of electrolytes and a considerable amount of sugars. Finally, the 4% w/w aqueous extract shows an absorption of ultraviolet radiation of 25% in the wavelengths from 260 to 400 nm, by which it also could be useful for formulating compositions for sun protection.

Keywords: *Rubus adenotrichus*, blackberry, antioxidant, phytochemical screening, phytocosmetic

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ABSTRACT 54

Skin whitening effects of Royal Jelly extract: an *in vitro* approach

Natalia Mente, Konstantinos Gardikis, Sophia Hatziantoniou, Konstantinos Avgoustakis and Sophia Letsiou

ABSTRACT

Background: Royal Jelly is a honey bee secretion from the salivary glands of the mandible and the hypopharynx glands of worker honeybees, and its purpose is to induce the superior growth and development of the queen bee. The aim of the present study is to evaluate *in vitro* the effects of royal jelly extract in primary normal human epidermal keratinocytes (NHEK), so as to investigate the potential applications of it in cosmetics.

Methods: In order to gain an insight into the molecular mechanisms of royal jelly extract bioactivity, we studied the transcript accumulation of genes involved in melanogenesis process. Transcript accumulation of genes was determined using an in-house RT-qPCR method. NHEK cells were purchased from Lonza Clonetics™. Cells were incubated for 48h with royal jelly extract. Cytotoxicity was assessed by MTT assay.

Results and Discussion: Keratinocytes incubated with royal jelly extract showed a significant increase in cell viability. Whitening effects of royal jelly extract on NHEK were confirmed by the regulation of several transcripts (TYR MC1R TNFα PGE2,) involved in melanogenesis pathway

Conclusions: The findings of this study indicate that royal jelly extract possesses strong skin whitening properties and provide new insights into the beneficial role of natural bioactive compounds in cosmetic formulations protecting our skin.

Keywords: Royal jelly extract, primary keratinocytes, tyrosinase, whitening agent, skin pigmentation

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ABSTRACT 55

Sustainable quality: The hidden part of the iceberg, suggestions on how to develop produce and measure the right quality of cosmetic products with added value for all the stakeholders

Elio Corrado Mignini

ABSTRACT

This speech is dedicated to quality as a strategic tool used to create competitive advantage to the cosmetic companies, their suppliers and partners and to all the involved customers. Quality is intended as a holistic entity, which embraces many different characteristics: products performance, consumers and workers safety, environmental sustainability, faithfulness of the enterprises, durability of the products, ethics of the business, aesthetics and sensory pleasantness of the products. Consumers are the king of this process, as they assign success or failure to every product. Quality and sustainability are abused terms; this speech will discuss on how to create the right organisation and system to get the best compromise to satisfy all the stake-holders. Quality and sustainability are not absolute values as they are differently appreciated by the different stakes; in addition, quality is composed of tangible and intangible variables, which make it difficult to objectively measure the absolute level. But in order to raise the iceberg, you must find a way to measure where you currently are in order to comply with one of the most important principle of quality: continuously improve in a sustainable way.

Keywords: quality management, sustainability, ethics, standards, KPI

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ABSTRACT 56

In vivo assessment of safety and efficacy of polysaccharides from photosynthetic microorganisms in cosmetics formulations

Ana Lucía Morocho-Jácome, Marcello Bresaola, Maria Valeria Velasco, João Carlos Carvalho, Luis Rodrigues, Catarina Rosado and André Baby

ABSTRACT

Background: Polysaccharides (PS) show high biological activities, such as antibacterial, anti-oxidative, among others. Cyanobacteria produce PS and exopolysaccharides (EPS) during the stationary phase or if the cells suffer environmental stress during cultivation. *Arthrospira platensis* produces a PS named spirulan and other EPS with bioactivity, namely antiviral and antibacterial effects. Studies in the literature with the microalgae *Ankistrodesmus braunii* are limited to a few reports about cultivation. Additionally, there are currently no reports about putative cosmetics effects assessed with *in vivo* measurements.

Methods: In this preliminary study, a cutaneous compatibility assay was performed in 3 male and female volunteers. The volunteers were 28-35 years old with skin phototypes of II-IV. Five sites were marked in the volar forearms of the volunteers, one for each formulation. Basal measurements of hydration, erythema and color were performed with a Corneometer® and a Mexameter. After application of each formulation, measurements were repeated after 30, 45, 60 and 90 minutes.

Results and Discussion: PS was extracted from *A. braunii* biomass but Bold™ media did not present EPS. However *A. platensis* releases EPS in media cultivation and also produces PS in biomass. Skin hydration was maintained during the experiments after the application of cosmetic formulations with PS from *A. braunii* biomass. EPS obtained from *A. platensis* cultivation provided slightly higher hydration values, but only during the first 60 minutes. Erythema values measured after cosmetic application was not different from basal measurements. Conversely, color could be affected by cosmetics formulations, principally at the beginning of the experiments. No significant changes in erythema or skin color were detected in any of the tested formulations. These preliminary results suggest a good cutaneous compatibility and some potential as moisturizing agents.

Conclusions: The cultivation methods enabled the extraction of PS and EPS from two photosynthetic microorganisms. PS from *A. braunii* provide better hydration when used in 1% hydrogel. All the formulations had a good cutaneous compatibility. PS and EPS derived from *A. braunii* and *A. platensis*, respectively, could potentially be incorporated in cosmetic formulations.

Keywords: cosmetic formulation, cyanobacteriae, *in vivo* assessment, microalgae, polysaccharides

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ABSTRACT 57

Cosmetic claims substantiation: why and how?

Fernanda Motta

ABSTRACT

Anti-ageing product claims have evolved over the last decades thanks to the advances in research and technology driven by a highly competitive marketplace. This paper will give an overview on the process of cosmetic claims creation, the different types of claims and the likely body of evidence necessary for its substantiation.

Keywords: cosmetic claims, claims substantiation, ingredient claims

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ABSTRACT 58

Feasibility of phenolic acids rich fraction from *Gynura procumbens* as potential anti-hyperlipidemic agent

Vikneswaran Murugaiyah, Sultan Ayesh Mohammed Saghir, Kisantini Murugesu, Mohd. Zaini Asmawi and Amirin Sadikun

ABSTRACT

Background: *Gynura procumbens* is a popular medicinal plant used as a folk medicine in South-east Asia to treat kidney diseases, diabetes mellitus and hyper-lipidemia.

Methods: The present study aims to investigate the anti-hyperlipidemic potential of phenolic acids rich fraction (PARF) from *G. procumbens* in chemically-induced acute and high fat diet-induced chronic hyperlipidemic rats.

Results and Discussion: Ethanolic extract of *G. procumbens* leaves exhibited significant reductions in total cholesterol (TC) and triglycerides (TG) levels ($P < 0.01$ and $P < 0.001$, respectively) of poloxamer 407-induced rats compared to hyper-lipidemic control after 58 h of treatment. Upon bioactivity guided fractionation the anti-hyperlipidemic activity was found to be concentrated in the PARF, which significantly reduced the TC and TG levels ($P < 0.001$). HPLC analysis revealed that 3,5-dicaffeoylquinic acid; 4,5-dicaffeoylquinic acid and chlorogenic acid are the major compounds in the PARF. Likewise, chlorogenic acid (60 mg/kg) exhibited significant reductions in TC and TG levels of hyper-lipidemic rats ($P < 0.001$). Both chlorogenic acid and PARF significantly reduced LDL, VLDL and atherogenic index ($P < 0.01$), while PARF increased the HDL ($P < 0.01$) compared to hyper-lipidemic control. Both were found to be not cytotoxic against normal and cancer cell lines. In addition, LD50 of orally administered PARF was more than 5,000 mg/kg. Further investigation in high fat diet-induced chronic hyper-lipidemic rats revealed that chronic administration of PARF dose-dependently restored the increase in lipids parameters.

Conclusions: In summary, the phenolic acids rich fraction of *G. procumbens* leaves showed promising anti-hyperlipidemic effect in both chemically- and diet-induced hyper-lipidemic rats that warrants further elucidation on its mechanisms of action.

Keywords: anti-hyperlipidemic, *Gynura procumbens*, phenolic acids, chlorogenic acid, poloxamer-407, high fat diet

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ABSTRACT 59

Morpho-anatomical characters of three species popularly used in Guatemala as calahuala

Deivy Mazariegos Pérez, María De los Angeles Abarca Nerio and María Eugenia Paredes Sánchez

ABSTRACT

Background: Calahuala is considered among the most important medicinal plants in Guatemala, because of its volume and value marketed. Traditionally it is used for anemia, arthritis, stomach cancer, renal pain and for its depurative, anti-age and anti-inflammatory properties, being the species most used under that name *Phlebodium decumanum*, *pseudoaureum* and *Serpocaulon triseriale*.

Methods: A descriptive study was performed, in order to establish quality control parameters of fronds and rhizomes of three Calahuala species, collected from the experimental fields of the Agronomy Faculty of San Carlos University of Guatemala. Organoleptic and morpho-anatomical characteristics were analyzed in fresh and dry drug through freehand cross sections, weak dissociation and diaphanized leaf. Secondary metabolites were identified by histochemical and phytochemical tests, moisture and total ashes were done in order to guarantee the quality of plant material used.

Results and Discussion: The studied species show similarities and differences, all show creeping and fleshy rhizome nevertheless *P. pseudoaureum* has shiny reddish-brown scales, and *P. decumanum* shows similar color but is covered with hairs; *Serpocaulon triseriale* is glabrous and greenish. The fronds exhibit some differences in sori distribution and type of pinnae. Among the most important microscopical features are the shape of the midrib and vascular bundles, the shape of the meristemes in rhizomes and types of thickenings in the wall of tracheids; *decumanum* shows helical thickening, *pseudoaureum* reticulated and *S. triseriale* scalariform ones. The secondary metabolites found were: saponins, mucilage, catechol, atropine and papaverine. Similar to those reported in other evergreen species, starches are present in fronds parenchyma cells.

Conclusions: There are macroscopic and microscopic characteristics for distinction between the studied species and important data useful for its quality control; in addition the metabolites found can be related to some of the properties attributed to Calahuala ferns in traditional medicine.

Keywords: traditional medicine, quality control, *Phlebodium decumanum*, *Phlebodium pseudoaureum*, *Serpocaulon triseriale*

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ABSTRACT 60

Bioactive peptides in cosmeceuticals

Despoina Koussoulou, Yiannis Sarigiannis and Christos Petrou

ABSTRACT

Background: The cosmetic industry requires a variety of different ingredients for skin care products. These include peptides, antioxidants, anti-inflammatory agents of natural origin, and polysaccharides. As these products show drug-like effects they are commonly referred to as cosmeceuticals. Bioactive peptides are involved in many physiological and biological processes. Their broad acceptance as natural molecules, their relatively high stability and their well-defined actions have made them attractive for various skin applications such as antiaging or wound healing factors.

Methods: Information on the application of bioactive peptides in cosmetics and cosmeceuticals was collected *via* electronic search (using Pub Med, Scopus, Google Scholar and internet search tools) and library search for articles published in peer-reviewed journals from 1980 to 2016. We also reviewed the formal analytical data of marketed cosmetic products containing bioactive peptides.

Results and Discussion: Bioactive peptides have a broad application in cosmetics and cosmeceuticals. GHK tripeptide, carnosine and its derivatives (Carnosine Related Compounds, CRCs) are important carrier peptides and their antioxidant activity is extended to cosmetology where they take their full advantage as counteracting ageing factors. Tetrapeptide GQPR, is found to be combined with the GHK tripeptide in different ratios in skin care and cosmeceutical products and offer remodeling and oxidative protection of the skin. Signal-peptides VGVAPG, KTTKS, SDKP and RGD tripeptide may act as regenerative and reparative factors as well as antiaging agents. Synthetic analogs of the neurotransmitter affecting peptides botulinum toxin of *Clostridium botulinum* (octapeptide SNAP-8) and the myotoxin Waglerin-1 of the snake venom of *Trimeresurus wagleri* (hexapeptide Argireline) have been developed and used in dermatological products.

Conclusions: Cosmeceutical and cosmetic industry finds peptides as an attractive and promising class of bioactive compounds, therefore, we should research on intensify biological role and their wide distribution on biological targets.

Keywords: cosmeceuticals, bioactive peptides, GHK tripeptide, carnosine, signal-peptides

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ABSTRACT 61

Mixed lipid/block co-polymer nanovesicles for loading and controlled release of ibuprofen

Natassa Pippa, Dimitris Stellas, Athanasios Skandalis, Stergios Pispas and Costas Demetzos

ABSTRACT

Background: Ibuprofen (IBU) is a non-steroid anti-inflammatory drug (NSAID) which has been available as topical skin formulations for the relief of pain and inflammation in rheumatic disease and other musculoskeletal conditions. The aim of this study is to design and prepare advanced drug delivery systems for IBU with controlled release properties.

Methods: The nano-structures, formed by the co-assembly of low and high molecular weight amphiphiles, have the potential to be utilized as drug delivery platforms. We have utilized two lipids, L- α -phosphatidylcholine, hydrogenated (Soy)(HSPC) and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine(DPPC), and a poly(oligoethylene glycol acrylate)-b-poly(lauryl acrylate) (POEGA-PLA) block copolymer, at different molar ratios, in aqueous media. Light scattering, differential scanning calorimetry (DSC) and imaging techniques (cryo-TEM, AFM) were employed in order to elucidate the structure of the nanostructures, as well as the cooperativity between the components.

Results and Discussion: DSC experiments showed considerable interaction of the block co-polymer with the lipid bilayers and suggested an inhomogeneous distribution of the co-polymer chains and lateral phase separation of the components. Vesicle formation was observed in most cases by cryo-TEM with a chimeric membrane exhibiting kinks, in accordance to DSC data. A series of bio-compatibility experiments indicated good *in vitro* biological stability and low cytotoxicity *in vivo* of the novel nanocarriers. The drug incorporation efficiency (IE) was estimated to 80%, for conventional HSPC and DPPC liposomes and between 47 and 68% for mixed liposomes. We did not observe differences in IE at the higher molar ratio of the polymeric guest. On the contrary, the conventional liposomes did not release the encapsulated bioactive compound. The chimeric lipid/block co-polymer vesicles released the IBU. The 80% and the 50% of the encapsulated IBU was released from HSPC and DPPC chimeric lipid/block copolymer vesicles in 3 h, respectively. The different amount of the released IBU should be attributed to the different thermal and inherent organizational/morphological properties of the HSPC and DPPC/POEGA-PLA liposomal membranes.

Conclusions: IBU was incorporated into biocompatible nanostructures, due to its hydrophobic character and the IE values were sufficient. The release of the IBU was strongly influenced by the physicochemical and thermodynamic properties of membranes. These studies could be a road map for the development of future formulation incorporating IBU nanostructures for topical administration of this NSAIDs drug with controlled release properties.

Keywords: Ibuprofen, drug delivery, nanostructures, controlled release, liposomes, polymers

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ABSTRACT 62

Medicinal plants of ancient Greece: pharmaceutical forms and predicted therapeutic indications

Antonio Pugliese, Paola Colace, Marco Francesco Madrigrano and Annamaria Pugliese

ABSTRACT

Background: Calabria is a region in Southern Italy, known in antiquity as Bruttium. Within the Magna Grecia, a few centuries before Christ, and particularly in Calabria, the influence of Ancient Greeks has influenced some parts of the region (Ionian coast); that influence still remains particularly evident in the lexicon of Greek Calabria (today still spoken in the area called the 'Bovesia').

Methods: According to a contrastative criterion will be examined pharmacologic vocabulary and medical ancestry herbal present in the area of Calabria region influenced by Ancient Greeks, highlighting their ancestral origin. The authors analyzing ancient encyclopaedias reported the name of traditional herbal medicine, the use and the indication for the treatments.

Results and Discussion: These substances, expression of ethnomedicine, were employed, alone or in compounds in various pharmaceutical forms (infusions, decoctions, powders, extracts, tinctures, inhalation, etc) for the treatment of lesions of different nature and gravity, involving various organ or systems, such as skin, respiratory, digestive, neuromuscular, kidney, ear and eye as well as to treat dystonia and bacterial lesions in general.

Conclusions: A pharmacotherapy of botanical nature, enriched by oriental knowledge, present in the different Arabic manuscripts, and preserved in Greek and Latin tradition before then, allowed for a recovery of the pharmacological and therapeutic Greek and Roman wisdom.

Keywords: Calabria, medicinal plants, ancient Greeks

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ABSTRACT 63

Screening of micro-clones of *Chlorophytum borivilianum* (safed musli) for their genetic stability at different culture age using molecular and cytological markers

Neelu Joshi, Parul Mathur and Sunil Dutta Purohit

ABSTRACT

Plant regeneration through tissue culture is often associated with the occurrence of somaclonal variations due to a variety of factors and therefore, periodic monitoring of *in vitro* grown plantlets for genetic variability is necessary to ensure the production and supply of genetically uniform planting material. The present investigation was undertaken to evaluate the genetic stability of long term *in vitro* cultures of *Chlorophytum borivilianum*, a well explored Indian medicinal herb. Both cytological and molecular (RAPD) parameters revealed genetic stability in 2.5 years old cultures of three accessions (PBL-3, PBL-5, PBL-8) of *C. borivilianum*. The three accessions showed slight variation in their karyotypic formula. Chromosome number, morphology and ploidy level varied with culture passages in accession PBL-3 and PBL-5. PBL-5 maintained somatic chromosome number ($2n=28$) for upto 21st passage and showed aberrant chromosome number (25-26) at later passages. PBL-8 maintained stability in chromosome number only till third passage and showed aneuploidy (30-65) in subsequent passages. Accession PBL-5 maintained chromosome number of $2n=28$ till 30th passage indicating its suitability for scaling up production of *C. borivilianum*. For RAPD analysis, 22 out of 53 random primers produced clear, scorable bands in all the three accessions. Total number of amplicons obtained from these primers ranged from 120 to 149 among the accessions. RAPD profiles of micropropagules obtained from different culture passages of all the accessions showed monomorphic banding patterns indicating genetic stability.

Keywords: chromosomal aberration, genetic stability, karyotyping, molecular marker, polyploidy, somaclonal variation

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ABSTRACT 64

Effect of polyamines on *in vitro* growth and development in *C. paniculatus* - a threatened medicinal plant

Nikita Goswami and Sunil Dutta Purohit

ABSTRACT

Polyamines are small aliphatic amines found in all living organisms. They have been regarded as a new class of plant growth regulators. They are considered to be the regulators of growth in higher plants and have been implicated in range of developmental processes. In the present study effect of different concentrations of polyamines alone and in combination with BAP was studied on *in vitro* grown *Celastrus paniculatus* – a threatened medicinal plant of Aravallis. Shoots of *C. paniculatus* cultured on MS medium were treated with different concentrations of putrescine, spermidine and spermine. It was observed that the number and length of shoots decreased with increasing concentration of put and spm in combination with BAP. Therefore, put and spm at 0.1 mM produced maximum number and length of shoots when incorporated with 0.5 mg l⁻¹ of BAP. In case of spd number and length of shoots increased with increasing concentration of spd in the culture medium. Maximum number and length of shoots was obtained at 1.0 mM of spermidine in combination with 0.5 mg l⁻¹ of BAP. No multiplication was observed when put, spd and spm were added alone in the culture medium. Omitting BAP in all the above treatments caused an increase in size of leaf lamina and shoot elongation.

Keywords: polyamines, micropropagation, *Celastrus paniculatus*, growth regulators

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ABSTRACT 65

Molecular characterization of diversity in *Annona squamosa* : a high value tropical medicinal plant

Rakhi Nagori and Sunil Dutta Purohit

ABSTRACT

Annona squamosa L. commonly known as custard apple, is an important underutilized fruit species from tropical and subtropical parts of India with high potential as commercial horticultural crop. The aim of this study was to determine the genetic diversity among populations spread across districts of Udaipur, Rajsamand and Chittaurgarh falling within Aravallis in Rajasthan. In all, 21 populations were identified and subjected to molecular evaluation using RAPD and ISSR Markers. The cumulative analysis carried out on the basis of RAPD and ISSR data sets revealed 73.91% polymorphism. A total of 251 amplicons were produced using 19 RAPD and 18 ISSR primers. The pairwise distance matrix calculated by Dice's co-efficient showed a distance range of 0.67 to 0.95 when computed using cumulative data set. Characterization of genetic diversity within populations based on combined data set showed that percentage of polymorphism (PPB) ranged from 29.19 to 50.93. Nei's gene diversity (HE) values varied from 0.1002 to 0.1964 within a total genetic diversity (HT) of 0.2509. The average observed no. of alleles per locus (Na) was 1.3602 and Ne was 1.2400. The Shannon's index was 0.2002 (Hpop) and 0.3800 (Hsp) at population and species level respectively. The moderate gene flow value $N_m=0.59$ and significant coefficient of genetic differentiation $G_{ST}=0.458$ revealed low level of genetic diversity within population and highly significant genetic differentiation among populations of *Annona squamosa*. The UPGMA dendrogram resolved all the 21 populations representing 3 districts into various clusters according to their genetic distances. The principal component analysis [PCA] supported the UPGMA results and justified the relationship among populations.

Keywords: *Annona squamosa*, diversity, molecular markers, RAPD, ISSR

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ABSTRACT 66

Hancornia speciosa: antioxidant activity of the fruit extract and top gel

Rosana Gonçalves Rodrigues Das Dores, Juliana Cristina Dos Santos Almeida Bastos, Maira C Marques Fonseca and Tatiane Vieira Braga

ABSTRACT

Background: *Hancornia speciosa* Gomes known as mangaba is a native plant of Brazilian Cerrado and its fruits are used in culinary preparations. In ethnobotanical studies it has been found that, fruits are used for ulcers, tuberculosis and inflammatory disorders. These activities can be correlated to phenolic compounds (chlorogenic acid), flavonoids (rutin) and latex (mangaba milk). The aim of this study was to compare the values of antioxidant activity in mature fruit extract with the phyto gel developed to use as topical gel for wound healing.

Methods: Gels were prepared with CMC and HEC, mangaba extract (100mg/g-50mg/g) to obtain "the phyto gel". Antioxidant activity was done by DPPH. The treatments for analysis were mangaba dry extract, CMC and HEC phyto gel (50mg/100mg). The positive controls were BHA gel CMC, BHA gel HEC and CMC, BHA solution (5mg/10mg). The negative controls were DPPH solution, DPPH, CMC and HEC gels, constituting 17 treatments with four repetitions. The absorbance was measured in time zero (t₀) thirty minutes (t₃₀) at 520nm.

Results and Discussion: The results showed pH=5 for mangaba dry extract and for CMC phyto gel in both concentrations, and 5.5 for PGHEC100; 6.5 in PGHEC50. To standard and control gels were 6.5 in CMC and 7 for the others. The sensorial characters for mangaba extract were green slightly brown or dark orange color, with odor and flavor peculiar of mature fruits. Phyto gel was odorless and slightly brown. The phyto gel obtained with HEC gel showed clear appearance, transparent, consistent, homogeneous and with good ability to spread. The appearance of the CMC gel revealed low luminosity, homogeneity and very dense aspect. The larger AAT % in relation to controls BHAHEC10 and BHAHEC5 were in t₀ (CV=5,12%) for phyto gels 100 mg (for both) and PGHEC50 was superior to others in same concentration including the gels controls, mangaba dry extracts and BHA solution. In t₃₀ (CV=2,38%), none of the treatments was as equivalent to BHAsol (controls), but, the PGHEC100 (phyto gel) was so effective as to dry extracts and BHA gels (both).

Conclusions: AAT in mangaba fruit extract and HEC gel were equal in t₃₀, showed that there was no decrease in the activity of the extract in relation to the phyto gel. This result is very important for development of a phyto product used in healing wounds, because it will guarantee slower action and formation of protective film.

Keywords: phyto gel, mangaba, topical gel, healing, DPPH

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ABSTRACT 67

Development of liquid soap with barbatiman extract

Bruno Persoti Passos, Guilherme Batista Costa, Juliana C. Santos A. Bastos, Rosana Gonçalves Rodrigues Das Dores and Tatiane Vieira Braga

ABSTRACT

Background: *Stryphnodendron adstringens* (Mart) Coville, Barbatimão, Brazilian species, is used ethnopharmacologically in seat baths, against vaginosis, candidiasis and astringent due to the high concentration of tannins. The aim of this work was to develop an intimate liquid soap formulation with barbatimão extract (*S. adstringens*), evaluating the stability and the MIC against *Staphylococcus aureus*, *Escherichia coli* and *Proteus* spp.

Methods: The formulation was made with anionic surfactant (28%) and amphoteric (3%), super-thickener (3.4%), pH corrector (3.5%), active principle: Barbatiman Extract (2.5%), preservative (0.2%), co-solvent (1%), coadjuvant (0.1%), essence, colorant, thickener (qs) and vehicle (qs) 100%. The packaging was in high density polyethylene bottles. Stability tests were at room temperature (25°C), oven at 40°C and at 10°C for 30 days. The visual characteristics, viscosity, homogeneity, deposit formation, color, odor and pH were evaluated, in time 7, 15 and 30 days. CIM was made on MH agar and disk diffusion for *S. aureus*, *E. coli* and *Proteus* sp.

Results and Discussion: Oven stability, over time, maintained the color (brown), odor (chocolate), brightness (satisfactory) and pH (4) maintained until the final time. The viscosity decreased with respect to time zero (T0). In refrigeration, color, odor, gloss and pH remained constant, however, the viscosity increased from T7 (seven days), with the presence of deposit. At room temperature (22°C), all variables remained stable. MIC was positive for *S. aureus* and negative for other strains. Storage was adequate, protecting the formulation, without precipitation or phase division.

Conclusions: The formulation of the Barbatimão liquid soap presented a pleasant appearance and good acceptability. The soap was effective in inhibiting the growth of *Staphylococcus aureus*. The formulation was stable at room temperature (22 °C).

Keywords: medicinal plants, antibacterial action, healing, hygiene soap, manipulation

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ABSTRACT 68

Efficacy test of rosemary oil soap parabens free against bacteria and yeasts

Juliana C. Santos Almeida Bastos, Fernanda Santos Lima, Juliane Oliveira Freitas, Rosana Gonçalves Rodrigues Das Dores and Tatiane V. Braga

ABSTRACT

Background: Intimate soap is a formulation for the hygiene of the female intimate region, being essential for the maintenance of vaginal pH, avoiding the appearance of bad smell, irritation and microbial infections. Parabens are preservatives used in cosmetic formulations, but recent studies associate these compounds with cancer development. The aim of this work was to develop an intimate liquid soap formulation without parabens, evaluating the stability and MIC in relation to *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Candida* spp.

Methods: The formulation composition was anionic (30%) and amphoteric (1.5%), super-thickener(1.5%), thickener (0.5%), active principle: rosemary oil(0.5%), camphor (0.2%), menthol(0.3%), imidazole urea as preservative (0.2%), co-solvent (1%), pH corrector, thickener (q.s) and vehicle (qsp 100%). The stability tests were at room temperature (25°C), conventional oven at 40°C and 10°C in refrigeration, with daily evaluation for 30 days, in which were of the visual characteristics, viscosity, homogeneity, color, odor and pH. The CIM was made by disc diffusion front for *Escherichia coli* and *Pseudomonas aeruginosa* in MH agar and for *Candida albicans* and *Candida* spp. agar in Sabouraud.

Results and Discussion: In the stability test at 10°C and 25°C, the formulation kept the color (colorless), odor (refreshing), homogeneity (satisfactory) and pH (4.0) until the final time. At 40°C there was alteration of the coloration (yellow), and the other parameters were constant. The viscosity decreases at time zero (T0) in conventional oven, kept stable in ambient and increased in refrigeration. MIC was positive for all strains tested.

Conclusions: Intimate liquid soap with rosemary oil and paraben free was effective in inhibiting the growth of *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Candida* spp. The formulation was stable under refrigeration (10°C) and room temperature (25°C), being unstable in an oven (40°C).

Keywords: *Rosmarinus officinalis*, medicinal formulation, phytocosmetic, paraben free, *Candida*, natural cosmetic

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ABSTRACT 69

Development and characterization of new *Cassia grandis* Lf. extract nano-formulation

Ariadna Lafourcade, Jesus Rafael Rodriguez A., Hady Keita, Jose Carlos Tavares C. and Raimundo Nonato Picanço S.

ABSTRACT

Background: *Cassia grandis* is an important species with folk use with potential for the treatment of diabetic and anemia. Nano-dispersions are disperse systems of insoluble substances in a liquid medium that may be prepared with or without coating polymers, improving the absorption and the efficacy of many drugs. To our knowledge, no studies were carried out in order to achieve coating-polymer nanoformulations using the extract of *C. grandis*. Thus, on the present study it was developed a *C. grandis* nano-formulations.

Methods: The low energy solvent displacement method was used to prepare nano-formulations. It used three different polymers (Eudragit® L 100 55, PEG 4000 and Kollicoat® MAE 100P), as layer coating agents in nano-formulations. Characterization of nano-formulations was performed by Dynamic Light Scattering. It were measured particle size, polydispersity index and zeta-potential. It verified the stability of the optimal nano-formulations during 21 days.

Results and Discussion: From the three used polymers, the nano-dispersion obtained by using Kollicoat MAE 100P was the best. Thus, it obtained a nano-dispersion with a low particle size (90.22 ± 0.90 nm) and a monomodal distribution of particle size (polydispersity index equal to 0.189 ± 0.006). The zeta potential of the selected nanodispersion was -12.7 mV and was kept almost constant along the study. The nanoparticles showed to be resistant to the acid pH (from 1 to 5) and temperatures between 10-80 °C. All parameter remained stable along the study.

Conclusions: On this context, the present study contributes to nanobiotechnology development of a product with a great potential for the treatment in diabetes and anemia. It achieved a novel nano-delivery system with an important folk medicinal plant extract. This is an innovative nanobiotechnology phytopharmaceutical product.

Keywords: *Cassia grandis*, Kollicoat, zeta potential, polydispersity index, particle size, nano-dispersions

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ABSTRACT 70

Quantitative HPLC analysis of the eugenol content in leaf extracts from *Ocimum* species from north-eastern Brazil

Maria Goretti Silva, Jackelyne Monteiro and Ícaro Vieira

ABSTRACT

Background: Eugenol (1,4-allyl-2-methoxyphenol), a naturally occurring food flavoring agent, is an important compound due to its biological potential as antiseptic, bactericide, antibacterial, and as analgesic in dental preparations. The cost and availability of eugenol demand the search for potential sources of it and the genus *Ocimum* is reported as a promising source of eugenol.

Methods: This study presents the HPLC analysis of eugenol content in the methanol extract and essential oil from steam distillation of seven different *Ocimum* species: *O. americanum* L., *O. basilicum* L., *O. basilicum* var *purpurascens* Benth, *O. gratissimum* L., *O. micranthum* Willd, *O. selloi* Benth. and *O. tenuiflorum* L. grown in North-eastern Brazil. HPLC was performed using C18 column eluted with methanol:water, 85:15. The calibration curves presented $r=0.9972$ ($Y=8E-06X-5.8911$); $LQ=0.10 \mu\text{g/mL}$; $LD=0.03 \mu\text{g/mL}$ and RSD less than 2.0%.

Results and Discussion: The content of some metabolites present in plants may vary according to several parameters. The eugenol content in *O. gratissimum* varies with the collection time (0.3 to 98.0%) for the plant cultivated in the Brazilian north-east, while in *O. micranthum* and *O. selloi* under the same soil and climatic conditions, the content of eugenol was maintained without significant changes. In these *Ocimum* species, eugenol was detected in different yields: *Ocimum gratissimum* showed better yields of the two extraction techniques used, showing 1.67 and 58.97% in ME and EO, respectively. *O. tenuiflorum* and *O. micranthum* also exhibited high contents of eugenol. The content of eugenol in essential oil of *O. selloi* was below the detection limit.

Conclusions: The methodology employed for the quantification by HPLC of eugenol in *Ocimum* samples showed satisfactory results which confirmed those obtained by gas chromatography. The results indicate that these species are potential sources of eugenol for use in several applications.

Keywords: eugenol, *Ocimum*, HPLC, essential oil, methanol extract

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ABSTRACT 71

Anti-inflammatory and anticancer effect of essential oils from hairy and normal roots of *Leonurus sibiricus* L. and their chemical composition

Przemyslaw Sitarek, Ewa Skala, Danuta Kalemba, Adam Bialas, Dariusz Pytel, Halina Wysokinska and Tomasz Sliwinski

ABSTRACT

Background: The aim of this study was isolation and chemical composition of essential oils from hairy and normal roots of *Leonurus sibiricus* and evaluation of anti-inflammatory and anticancer effect of these essential oils.

Methods: The essential oils were analysed by GC-MS method. Anticancer effect was evaluated by MTT test. In turn, anti-inflammatory cytokines were tested in LPS-stimulated astrocytes by Elisa kit. Level of expression of genes were determined by RT-PCR method.

Results and Discussion: The major constituents in NR essential oil were β -selinene (9.9%), selina-4,7-diene (9.7%), (E)- β -caryophyllene (7.3%), myli-4(15)-ene (6.4%), guaia-1(10), 11-diene (5.9%). In HR essential oil the main constituents were (E)- β -caryophyllene (22.6%) and germacrene D (19.8%). Both essential oils showed cytotoxic activity against grade IV glioma cell lines (IC₅₀=400 μ g/mL). Decreased level of IL-1 β , IL-6, TNF- α and IFN in LPS-stimulated cells was observed.

Conclusions: This is the first report to examine composition of the essential oils and their anticancer and anti-inflammatory activities. These findings support the idea that essential oils may contribute to the reduction of inflammation and anticancer effect and may also be preventive against various diseases.

Keywords: : hairy roots of *Leonurus sibiricus*, essential oils, anticancer activity, gene expression, anti-inflammatory activity

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ABSTRACT 72

Antimicrobial, antioxidant, and anti-inflammatory activities of the essential oils of *Rhaponticum carthamoides* hairy roots and roots of soil-grown plants and their chemical composition

Ewa Skala, Przemyslaw Sitarek, Patrícia Rijo, Catarina Garcia, Danuta Kalemba, Halina Wysokinska and Tomasz Sliwinski

ABSTRACT

Background: The roots and rhizomes of *Rhaponticum carthamoides* (Willd.) Iljin (Asteraceae) have been used for centuries in the traditional medicine of Siberia for overstrain and weakness after illness. The objective of this study was isolation and chemical composition of *Rhaponticum carthamoides* essential oils of hairy roots (HR) and roots of soil-grown plants (SGR) and determine the antimicrobial, antioxidant and anti-inflammatory potential of these essential oils.

Methods: The essential oils were isolated by hydro-distillation and analyzed by GC-MS method. Antimicrobial activity was evaluated by determination of Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC). Anti-inflammatory activity in LPS-stimulated astrocytes was determined by Elisa kit and expression levels of anti-inflammatory cytokines were analysed by RT-PCR. Antioxidant properties were evaluated by ROS level.

Results and Discussion: The essential oils of *R. carthamoides* hairy roots and roots of soil-grown plants showed the differences in the qualitative and quantitative composition. The sesquiterpene hydrocarbons (55-62%) dominated in both essential oils. Cyperene, 13-norcypera-1(5),11(12)-diene and cadalene dominated in HR essential oil. The major compounds of SGR essential oil were aploxene, nardosina-1(10),11-diene and dauca-4(11), 8-diene. Both essential oils showed antibacterial activity especially against *Enterococcus faecalis* (ATCC 29212) and *Pseudomonas aeruginosa* (ATCC 27853) (MIC value = 125 µg/ml). HR and SGR essential oils also decreased the expression of IL-1 β , IL-6, and TNF- α and the ROS level in LPS-treatment astrocytes.

Conclusions: Our study suggests that *R. carthamoides* essential oil from hairy roots may be used as an effective natural antibacterial and antioxidant agent, and could be used for the treatment of some infectious and central nervous system diseases.

Keywords: *Rhaponticum carthamoides*, hairy roots, essential oil, antioxidant activity, antimicrobial activity, anti-inflammatory activity

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ABSTRACT 73

Plant Extract with anti cancer activity: *Hemidesmus indicus*– a case study

Massimo Tacchini, Ilaria Burlini, Guglielmo Paganetto, Giulia Greco, Carmela Fimognari, Gianni Sacchetti and Alessandra Guerrini

ABSTRACT

Background: The great interest that natural products draw is often based on their intrinsic complexity, that allows them to interact with numerous molecular targets. The decoction of the roots of *Hemidesmus indicus* (L.)R.Br.(Asclepiadaceae) is widely used in the Ayurvedic traditional medicine for the treatment of various diseases. The goal of this ongoing study is achieving simple fraction(s) or pure compound(s) responsible of the anticancer activity.

Methods: Starting from the formulation of two traditional preparations (decoction and hydro-alcoholic extracts), different polarity extractions were performed with the aim of increasing the bio-efficacy (cytotoxic activity towards cancer cells). The obtained extracts were chemically characterised by RP-HPLC-DAD, GC-MS and 1H-NMR following fully validated methods. In order to follow a bioassay-guided strategy, cytotoxicity tests were performed with several cancer cell lines, to evaluate the biological potential of *H.indicus*.

Results and Discussion: Since 2013 our results highlighted the great multi-target potential of *H.indicus* decoction in term of cytodifferentiation and apoptosis induction. In 2015, we showed the anti-angiogenic activity of this traditional preparation and the cytotoxic potential of another traditional preparation (hydro-alcoholic extract, HE) against eight cancer cell lines. HE, the most performing extract, was studied in order to identify fraction(s) or pure compound(s) responsible of the bioactivity. The resazurin tests performed after incubation of HE with CCRF-CEM and CEM-ADR5000, showed IC50 value dropping down after the elimination of sugars (from IC50=84, 85±3, 34 µg/ml to 69,70±1, 22 µg/ml against CEM-ADR5000), and even more after soxhlet extraction (IC50=5, 76±0, 01 µg/ml). The main chemical components of the soxhlet extract are vanillin derivatives. During the extraction process their concentration is increased, but they did not exhibit cytotoxic activity against leukaemic cells, neither tested alone, nor considered together.

Conclusions: *H. indicus* soxhlet extract proved to be an interesting phytocomplex showing remarkable antileukaemic activity, which fractionation will be the next step to pinpoint the molecule(s) responsible of this biological activity. Moreover, it would be interesting to verify if *H. indicus* extracts could exhibit activity also interacting with non-canonical cell death pathway, confirming its importance in prevention of cancer relapses and pharmacological resistance.

Keywords: plant extract, natural products, leukaemia, *Hemidesmus indicus*, chemical fingerprinting

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ABSTRACT 74

The contribution of *Colchicum autumnale* in the therapy of gout

Costas Tsiamis, Effie Poulakou-Rebelakou, Dimitrios Vasilopoulos and Chrisoula Hatzara

ABSTRACT

This study presents the use and the contribution of *Colchicum autumnale* in the treatment of gout through history. During the late Roman period the works of Dioscorides and Rufus are referring to rheumatic diseases. The Byzantine physicians, based on the ancient Greek texts, explained the causes of rheumatic diseases, described the symptomatology and proposed treatments of plant origin. The medical concept concerning the rheumatism's pathogenesis was that the disease is induced when viscous phlegm or bile flows in the ligaments of the joints. The Byzantine medical sources described the inflammatory arthritis, the chronic deforming polyarthritis, and gout. It seems that the rheumatic diseases were common to the Byzantine population. Moreover, according to the Byzantine historiography, fourteen emperors suffered from arthritis. In the core of the plant therapy against gout was the miraculous *Colchicum autumnale* or *Hermodactylus*, a well-known plant since the era of Dioscorides (*De Materia Medica*). The reputation of *Colchicum autumnale* will survive for centuries and it will be an important anti-rheumatic remedy in the European Pharmacopoeia. In our days, according to the guidelines for management of gout by the American College of Rheumatology and the European League Against Rheumatism, oral colchicine (originally extracted from plants of the genus *Colchicum*) is an appropriate first-line gout attack prophylaxis therapy. As it can be concluded, the case of *Colchicum autumnale* is an example of the impact of the ancient knowledge of phytotherapy in the modern therapeutics.

Keywords: phytotherapy, gout, *Colchicum autumnale*, colchicine, pharmacology, inflammation

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ABSTRACT 75

Characterization of volatile compounds, anti-inflammatory and antioxidant activities of two *Achillea* species from eastern macedonia and thrace (menoikio mountain)

Maria-Eleni Atskakani, Olga Tsiftoglou, Maria Lazanaki, Christos Gounaris, Dimitra Hadjipavlou-Litina and Diamanto Lazari

ABSTRACT

Background: Various species of the genus *Achillea* L. (Asteraceae) are traditionally used for wound healing, against diarrhoea and flatulence, as a diuretic, as emmenagog agents, and for abdominal pain.

Methods: In the present study, the essential oils obtained from leaves and inflorescences of *A. grandifolia* Friv. and *A. crithmifolia* Waldst. & Kit. growing wild on Mt Menoikio, NE Greece, were analyzed by Gas Chromatography-Mass Spectrometry.

Results and Discussion: The major compounds found in essential oils were (a) for *A. grandifolia* (inflorescences): sabinene (4.12%), a-terpinene (6.37%), 1,8-cineole (11.88%), cis-thujone (36.85%), trans-thujone (3.56%), camphor (9.96%), ascaridole (7.30%) and jasmone (3.39%), (b) for *A. grandifolia* (leaves): 1,8-cineole (19.97%), cis-thujone (50.77%), trans-thujone (5.49%), camphor (5.53%), borneol (3.58%) and a-terpineol (3.11%), (c) for *A. crithmifolia* (inflorescences): a-terpinene (2.59%), p-cymene (3.13%), 1,8-cineole (34.42%), artemisia ketone (4.88%), cis-thujone (7.21%), camphor (7.08%), borneol (4.63%) and terpinen-4-ol (5.29%), (d) for *A. crithmifolia* (leaves): p-cymene (3.27%), 1,8-cineole (38.3%), artemisia ketone (14.54%), borneol (8.34%), terpinen-4-ol (3.69%) and trans-crysanthenyl acetate (6.89%). Subsequently, the samples tested for the interaction of essential oils with DPPH, their soybean LOX inhibitory activity and their inhibition of lipid peroxidation. All of them, seemed to have worthy effectiveness but *A. grandifolia* (leaves) seems to be the most potential antioxidant through DPPH method and to have the highest percentage on inhibition of LOX, while *A. crithmifolia* (leaves) is the sample, which resulted the most remarkable inhibition of lipid peroxidation

Conclusions: Till now, the results show that this investigation deserves to be continued.

Keywords: *Achillea*, gas Chromatography-mass Spectrometry, essential oil, antioxidant, anti-inflammatory

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ABSTRACT 76

In the perfume laboratory of empress Zoe. Fragrances between earth and heaven in Byzantium

Marco Cilione, Alain Touwaide, Valentina Gazzaniga, Berenice Cavarra

ABSTRACT

The 11th-century Byzantine empress Zoe (ca. 978-1050) is believed to have been actively involved in the preparation of cosmetics and perfumes. Her creations, which required to burn costly substances in a way that was not much different from the alchemical practice, were not limited to the cure of the body and esthetics, as they aimed to generate an image of imperial rulers as young beings with a perfect physical appearance that suggested vitality and strength, and inspired respect and obedience. The effect and values of perfumes went beyond, however, as a connection of the empress with St Nicolas of Myra suggests. Zoe might have commissioned a fresco representing the saint in the St Marina church at Muro Leccese in Southern Italy. While St Nicolas emanated the scent of holiness which opposed the deleterious effects of bodily passions and corruption of the soul, Zoe's perfumes aimed to fight bodily degenerescence. As these intertwined aspects indicate, perfumes seem to have been powerful instruments of personal, political and religious communication deftly used among 11th-century Byzantine rulers.

Keywords: perfume, Byzantium, power, St Nicolas, holiness

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ABSTRACT 77

Current safety methodology assessment of herbal products used in cosmetics

Ana L. Valle

ABSTRACT

Phyto-ingredients contain hundreds to thousands of interrelated chemical compounds. Natural products show, in general, less toxic effects than synthetic products. However, relatively rare adverse reactions have been documented in the literature. Thus in some countries, there are no appropriate guidelines or regulatory standards. As for cosmetics, there is often a lack of GAP and GMP guidelines implementation. What is the current methodology for assessing natural products toxicity? For the safety assessment of cosmetic substances, the implementation of Scientific Committee on Consumer Safety Guidelines and according to 2003/15/EC 2, 1223/2009/EC, validated alternative methods, in particular *in vitro* replacement methods, becomes imperative. The physical and chemical identity, cell and tissue culture, *in silico* data, toxico-kinetics, mutagenicity and genotoxicity test show a remarkable advance in molecular biology and genomics. There is a need to integrate coordinated efforts between organizations to promote method development, identification of biomarkers, and mechanisms of action, using appropriate testing strategies, supported by scientific research to support regulatory decision-making risk assessment. The community needs to know that phyto-ingredients not only have potential benefits, but also may become a risk under certain circumstances.

Keywords: toxico-kinetics, safety assessment, biomarkers, phyto-ingredients

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ABSTRACT 78

Study of the effects of plant oils on uv filter performance

Dimitra Veskouki, Konstantinos Avgoustakis and Sophia Hatziantoniou

ABSTRACT

Background: Plant oils are widely used in cosmetic products due to their action as skin emollients. Sun care products provide protection against UV radiation due to their content of UV filters. Their protective performance is expressed by Sun Protection Factor (SPF) and is influenced by the presence of other ingredients such as plant oils. The aim of this work was the study of the effect of plant oils on the sun protection efficacy of the most commonly used UV filters.

Methods: Four organics filters were used: 4-methylbenzylidene camphor (MBC), Benzophenone-4 (BEN-4), Octocrylene (OCT), Ethylhexyl-methoxycinnamate (EMC) and the plant oils were from: Sweet-almond, Apricot, Argan, Avocado, Calendula, Evening-primrose, Grape-seed, Jojoba and Olive. The protection of UV radiation was measured *in vitro* using (i) Kaur & Saraf protocol, and (ii) Mbanga *et al.* protocol. Filters and oils alone or in mixtures were dissolved in ethyl-alcohol and the solutions were measured by UV-spectrometry. The calculation of SPF was made using Mansur equation.

Results and Discussion: The results according to the first protocol were not accurate since there is no requirement for exact concentration of the sample and the dilutions were made arbitrarily resulting in a wide range of concentrations that could be acceptable. According to the second protocol the calculation of SPF requires sample concentration of 0.02% w/v. The SPF of plant oils based on the second protocol ranged from 0.046 for the apricot oil, to 0.446 for the calendula oil. The SPF enhancement when used in combination with organic filters, was ranging depending on the combination of filter and the type and concentration of oil used. The maximum SPF enhancement (16.8 % ± 0.1 %) was caused by combining olive oil with BEN-4 in 16:1 (w/w) ratio. At lower plant oil to UV filter ratio (1:1 w/w) the maximum enhancement in SPF (7.97% ± 0.6 %) was caused by grape-seed oil and EMC mixture. Finally, the vegetable oils did not appear to significantly affect the critical wavelength of each filter.

Conclusions: Plant oils do not provide adequate protection of UV radiation when used alone on skin. When they are used in combination with UV filters each plant oil affects both the maximum absorption and SPF of the UV filter depending on the type and concentration of the oil and UV filter.

Keywords: plant oil, UV-filter, SPF, *in vitro*

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ABSTRACT 79

Quantitative ethnobotanical study of the medicinal plants used by herbalists in Oran (Algeria) for gastrointestinal disorders

Khitri Walid, Nessakh Bouchra, Mostefa Ben Hamida Souad, Lachgueur Nassima, Lardjam Abderrahmen and Khalfa Ali

ABSTRACT

Background: Gastrointestinal diseases are common worldwide. Many people use medicinal plants to cure and prevent different types of gastrointestinal disorders. Aim of the study was to compile information on plants used for the treatment of different gastrointestinal disorders in Oran (North west of Algeria).

Methods: The survey was carried out in a period of three months (December 2016 to February 2017). Thirty-four herbalists were interviewed using semi-structured interviews. The questionnaires were used to compile data about the herbalist as a holder of information and about medicinal plants. The plants were identified and deposited as voucher specimens. Plant importance was determined using quantitative ethnobotanical indices such as Use Value (UV), Fidelity Level (FL) and Informant Consensus Factor (ICF). The data were analyzed by SPSS Statistics 20.

Results and Discussion: Fifty-eight species in 23 families were identified. The most used plants (11 species) were grouped in Lamiaceae. The aerial parts were the most commonly used against gastrointestinal disorders, while infusion and decoction were the most common methods of traditional drug preparation. The doses of the plants for different treatments varied widely.

Conclusions: This documentation on medicinal plants and their uses shows the rich tradition in ethno-medicinal knowledge of the herbalist in Oran, Algeria. This study could open an avenue for pharmacological research works, or serve as reference for future quantitative ethnobotanical investigations.

Keywords: ethnobotanical study, gastrointestinal disorders, indices, Oran Algeria

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ABSTRACT 80

Identification control of spices: *Crocus sativus* L. and *Zingiber officinale* Roscoe

Khitri Walid, Lachgueur Nassima, Lardjam Aberrahmen, Khalfa Ali and Smati Dalila

ABSTRACT

Background: At present, more and more published studies demonstrate the virtues of spices, which are conditioned by their quality. In Algeria, spices occupy an essential place in our everyday life. The aim of the work was to take stock of the current situation of the quality of spices (*Crocus sativus* L. and *Zingiber officinale* Roscoe) sold at herbalists settled in Oran, Algeria.

Methods: The evaluation of the quality of *Crocus sativus* L and *Zingiber officinale* Roscoe was carried out through the study of organoleptic characteristics, chemical constituents and determination of loss on drying.

Results and Discussion: The results of the analyses were not corresponding with those fixed by the European Pharmacopoeia, 6th edition (Samples were not corresponding to the standards).

Conclusions: It is suggested to establish recommendations of best practice of culture, harvest, drying, preservation and the marketing of spices.

Keywords: identification control, *Crocus sativus* L, *Zingiber officinale* Roscoe

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ABSTRACT 81

The effect of gold nanoparticles, green synthesized by using *Curcuma xanthorrhiza* rhizome extract, on zebrafish embryos

Khairiza Lubisa, Jidapa Misikab Misika, Roongtip Iyara, Siriporn Riyajan, Oratai Weeranantanapan and Nuannoi Chudapongse

ABSTRACT

Background: Nowadays, green synthesis using plant extracts is a topic of intense interest for the development of gold nanoparticles (Au-NPs). Plants which belong to Zingiberaceae family, such as *Curcuma longa* and *C. pseudomontana*, have been used to synthesize and study the biological effects of Au-NPs. However, to our best knowledge, Au-NPs synthesized by using *C. xanthorrhiza* has never been reported.

Methods: In the present study, the green synthesis of the Au-NPs using *C. xanthorrhiza* rhizome extract was performed. Then, the obtained Au-NPs was characterized by UV-visible spectroscopy and X-ray diffraction (XRD) analysis. Its toxicity test was conducted in zebrafish (*Danio rerio*) embryos. Toxicological endpoints, mortality and hatching, were recorded.

Results and Discussion: The UV-visible spectroscopy revealed that the absorption peak of the synthesized Au-NPs from *C. xanthorrhiza* was 530 nm. The XRD analysis showed that the sample composed of crystalline gold. The characteristic peaks corresponding to (111), (200), (220), (311) of Au were located at = 38.19°, 44.39°, 64.58° and 77.58°, respectively. In addition, the calculated size of the nanoparticles was found to be around 10 nm. A concentration-dependent increase in mortality and hatching delay was observed in Au-NP-treated embryos. However, at 10 µM of the synthetic Au-NPs from *C. xanthorrhiza*, the results showed that there was no toxicity on treated-zebrafish embryos compared to the precursor HAuCl₄.

Conclusions: *C. xanthorrhiza* can be used for green synthesis of Au-NPs. At the concentration below 10 µM, the Au-NPs showed no toxicity on zebrafish embryos. These preliminary experiments provide useful information for the future studies of pharmacological effects of Au-NPs, such as anti-inflammatory, anti-bacterial and antioxidant activities.

Keywords: *Curcuma xanthorrhiza*, gold nanoparticle, X-ray diffraction, Zebrafish embryo

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ABSTRACT 82

A comparative study of epiphytic lichens mentioned by Ibn Sina and Ibn Al Baytar

Mustafa Yavuz

ABSTRACT

Background: Lichens are symbiotic organisms consisting in a mycobiont and at least a photobiont partner. Producing unique secondary metabolites, they have been used in medicine, pharmacy and dyeing from antiquity to modern times.

Methods: The purpose of this study is to investigate and compare the uses of lichens in the medicinal works of Ibn Sina and Ibn Al Baytar in Arabic, on the basis of the term used to describe epiphytic lichens.

Results and Discussion: In the eastern Medieval Islamic Community, Ibn Sina (d. 1037) created a system of medicine with three pillars including Aristotelian philosophy, Galenic medicine, and Dioscoridean pharmaco-botany in his *Qanun Tibb (Canon of Medicine)* which later became a mainstream work followed and commented on. In the western Medieval Islamic Community, the Andalusian physician Ibn Al Baytar (d. 1248) cited Dioscorides and Ibn Sina among many others in his encyclopaedic work which can be considered as an update of medicinal lore in the Islamic Community in his time.

Conclusions: In this study, two Arabic manuscripts kept in Suleymaniye Library were investigated by means of medicinal uses of lichens. It is found that, both physicians mention lichens in two different terms: one dedicated to saxicolous lichens and the other to epiphytic lichens, with more precise information on the latter.

Keywords: lichens, medieval medicine, Ibn Sina, Ibn Al Baytar

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ABSTRACT 83

Chemical composition of *Propolis* essential oils and biological activity of *Propolis* extracts from four different geographic regions of north Greece

Elena Zioga, Olga Tsiftoglou, Despoina Katsiamouri, Ioanna Karakasi, Smaro Peppas, Dimitra Hadjipavlou-Litina and Diamanto Lazari

ABSTRACT

Background: *Propolis* is widely used in folk medicine and cosmetology. Chemical composition of *Propolis* is highly variable and depends mainly on the local flora. Given the biodiversity of Greek flora, this implies the chances to find new bioactive compounds in *Propolis* samples from different parts of Greece. Consequently, the present study attempts to expand the knowledge of Greek *Propolis* chemodiversity and the potential biological activity of *Propolis* extracts.

Methods: *Propolis* samples from different locations of Northern Greece (Chalkidiki, Drama, Florina and Trikala) were hydrodistilled and their essential oils were analyzed by capillary gas chromatography (GC-MS). The water of each hydrodistillation was collected and the liquid-liquid extraction method was followed using solvents of increasing polarity (hexane, ethyl acetate, butanol and aquatic residue). The extracts were checked for their antioxidant activity using the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) and for their inhibitory activity towards soybean lipoxygenase, using linoleic acid as substrate.

Results: Ninety-one compounds were identified from the essential oils. The major components identified per region were: α -eudesmol for Chalkidiki and Trikala (13.38% and 10.98%), decanal for Florina (39.10%) and γ -cadinene for Drama (19.82%). The total profile of the volatile constituents reveals the predominance of sesquiterpenoids. Only four compounds were common among the investigated samples, showing that the chemical profile of the examined *Propolis* samples has notable differentiations, unlike similar studies. The majority of the extracts were found to interact with DPPH at significantly high percentages, indicating their notable radical scavenging ability in an iron-free system. The hexanic, the ethyl acetate and the butanolic extracts showed the highest results. Moreover, the butanolic extract of Drama and the ethyl acetate extract of Trikala, demonstrated a relatively high inhibitory activity on the soybean lipoxygenase.

Conclusions: The present research highlights the problem of *Propolis* standardization, since its composition may vary depending on the geographical origin of samples. On the other hand, many of the studied Greek *Propolis* extracts were rich in natural antioxidants and showed high inhibitory activity. Thus, it is possible they contain protective agents that could be applied against various degenerative diseases.

Keywords: Greek *Propolis*, essential oils, extracts, biological activity

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ABSTRACT 84

Plants in gynecology: ethnobotanical survey carried out in gynecology service of the hospital center at Oran, Algeria

Hanane Zitouni, Ibtissem Bekhti, Moufida Baira, Abderahmane Lardjam, Walid Khitri, Asma Memou and Houari Toumi

ABSTRACT

Background: In Algeria, as in all countries of the Maghreb, the use of traditional medicine is widespread, and several herbal remedies used individually or in combination are recommended to treat gynecological diseases. The aim of this work is to draw up an inventory of the medicinal plants used in gynecology and to describe the characteristics of this use.

Methods: This is a cross-sectional descriptive study, carried out from 1 January 2014 to 30 April 2014, using questionnaires; we first interviewed women consulting at the gynecology service of the EHUO: We interviewed 100 patients whose age-range varies between 16 and 65 years, and in a second time we extended our investigation to the medical and paramedical staff of the service.

Results and Discussion: We have identified 22 plant species belonging to 17 botanical families, these medicinal species are used against several pathologies, namely gynecological cancers, cysts, genital infections and of course for infertility. According to the results, we found that 80% of women use traditional medicine in the treatment of diseases, whereas a low proportion 20% do not use it and prefer conventional medicines. Whole plants is the most used with a rate of 23.33%, then fruits 20%, aerial parts 19%, seeds 16.66%, leaves 13.33%, stems 10%, bulbs 6.66% and finally the rhizome 6.45%.

Conclusions: Traditional medicine is a knowledge that must be conserved and exploited. This type of investigation will subsequently allow phytochemical and pharmacological studies of the listed plants, some of which prove to be very interesting and deserve in the future to be thoroughly researched, proving experimentally their therapeutic activities described by our interlocutors.

Keywords: traditional medicine, gynecology diseases, ethnobotanical survey, plants

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IPPC 2017

ABSTRACT 85

Preparation and control of a mouthwash prepared from a sage extract

Hanane Zitouni

ABSTRACT

Background: Thanks to the size of Algeria and the diversity of its local flora, traditional medicine includes a territorial pharmacopoeia, it constitutes an untapped indigenous wealth. *Salvia officinalis* L. called in Arabic swakelnbi, salma or merymeya, preferring the calcareous soils of the Mediterranean region, the sage is cultivated almost everywhere in Algeria. This species has choleric, emmenagogue, antispasmodic, antiseptic, antioxidant, antiperspirant and astringent properties. It is also used as an infusion and gargle as anti-inflammatory to treat sore throats and aphthae.

Methods: In order to preserve and promote this therapeutic heritage, the pharmaco-vigilance service of the EHU of Oran has launched an experiment mainly aimed at the use of Algerian natural sources and seeing the possibility of conditioning them in pharmaceutical forms respecting the standards of the pharmacopoeia. Our pharmaceutical form is a mouth-wash prepared from officinale sage alcoholic extract.

Results and Discussion: Our form has responded to the tests required by the European Pharmacopoeia, Sixth edition because our plant is inscribed there as well as the alcoholic extract.

Conclusions: Traditional medicine must be given the respect and place it deserves, WHO is willing to work with its partners to make traditional medicines widely available and accessible.

Keywords: sage, mouthwash, alcoholic extract

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ABSTRACT 86

Traditional medicine and cancer in Algeria

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ABSTRACT

Background: There are few studies in Algeria regarding the use of medicinal plants in cancer patients. Therefore, it seemed useful and important to draw up an inventory of the practice of traditional medicine in cancer patients in the city of Oran. Our objectives are: to list the medicinal plants used against cancer and to describe the characteristics of this use and the profile of the users.

Methods: This is a cross-sectional descriptive study carried out from 26 November 2015 to 10 January 2016, within the EHUO oncology services as well as the EHS Emir AEK Oran. The survey was conducted using a questionnaire for 384 patients. Data entry and statistical analysis were done on SPSS and Excel softwares.

Results and Discussion: The analysis of the questionnaire data identified 46 vegetal drugs belonging to 30 families used for traditional cancer treatment, which many have interactions with anticancer drugs. Our results show that 43% of the population studied are women with breast cancer, among the 384 cancer patients, 47.4% use at least one medicinal plant. The use of medicinal plants differs significantly with the sex with a $P = 0.003$ with a female predominance or a frequency of 75%. We also found that 39.4% of patients use medicinal plants in combination with their treatment and that 26.4% of patients can experience interactions following this association.

Conclusions: It is imperative that medicinal plants benefit, as well as drugs, of a system of vigilance and awareness which prevents the risks of toxicity or inefficacy.

Keywords: cancer, traditional medicinal plant, Algeria

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