

# POSTERS

Abstracts



6<sup>th</sup> Edition of International Conference on  
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Vedrana Čikeš Čulić et al., Am J Ethnomed 2018, Volume 5  
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## ANTICANCER ACTIVITY OF ALLIUM COMMUTATUM, ALLIUM SATIVUM, AND LEPIDIUM GRAMINIFOLIUM ON HUMAN CANCER CELL LINES

Vedrana Čikeš Čulić, Maja Katičić, Ana Polunić, Mirko Ruščić, Petra Brzović, Azra Đulović and Ivica Blažević

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The anticancer activity of volatile isolates from *Allium commutatum* Guss., *Allium sativum* L. (*Amaryllidaceae* family), and *Lepidium graminifolium* L. ssp. *graminifolium* (*Brassicaceae* family), analyzed by GC-MS, as well as the pure sulfur volatiles, were evaluated against two human tumor cell lines: glioblastoma cell line LN-229 and bladder cancer cell line UM-UC-3, using MTT assay. The major sulfur volatiles found in *A. commutatum* hydrodistillate from flower and *A. sativum* bulb originated from S-alk(en)yl cysteine sulfoxide degradation. The most abundant sulfur volatile in *A. commutatum* isolate was dipropyl trisulfide, and in *A. sativum* diallyl disulfide. *A. commutatum* distillate showed very weak cytotoxic effect on both cancer cell lines except at incubation time of 72 h on LN229 cell line (IC<sub>50</sub> 6.364 µg/mL), while dipropyl trisulfide showed much stronger cytotoxic effect: with IC<sub>50</sub> 12.34 and 10.35 µg/mL, and IC<sub>50</sub> 22.19 and 8.434 µg/mL for UM-UC-3 and LN229 cell line, respectively during incubation time of 48 and 72 h. Both *A. sativum* extract and distillate showed strong time and concentration-dependent cytotoxic activity on both cancer cell lines, with the best results at incubation time of 48 and 72 h. *A. sativum* extract had IC<sub>50</sub> 14.49 and 12.48 µg/mL,

and 40.84 and 10.41 µg/mL for UM-UC-3, and for LN229 cell lines, respectively, during incubation time of 48 and 72 h. *A. sativum* distillate showed very similar results: IC<sub>50</sub> 20.86 and 14.13 µg/mL, and 17.41 and 12.01 µg/mL for UM-UC-3, and for LN229 cell line, respectively. As expected, an active compound from *A. sativum* diallyl disulfide showed very strong anticancerogenic potential with IC<sub>50</sub> 22.3 and 19.07 µg/mL, and 44.69 and 8.85 µg/mL for UM-UC-3, and LN229 cell line, respectively. *L. graminifolium* extract and distillate sulfur volatiles originated from glucosinolates degradation i.e. 3-methoxybenzyl isothiocyanate, and benzyl isothiocyanate. They didn't show strong cytotoxic activity on UM-UC-2 cell lines. While there was effect on LN229 cell line of distillate at incubation time of 48 hours (IC<sub>50</sub> 53.92 µg/mL) and of extract at incubation time of 48 and 72 hours (IC<sub>50</sub> 30.71 and 54.37 µg/mL, respectively). On the contrary, benzyl isothiocyanate showed much stronger cytotoxic effect: with IC<sub>50</sub> 13.16 and 12.3 µg/mL, and IC<sub>50</sub> 6.48 and 12.29 µg/mL for UM-UC-3, and LN229 cell line, respectively during incubation time of 48 and 72 h.

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## TWO METABOLITES FROM THE MARINE-DERIVED FUNGUS *A. CHEVALIARI* ISOLATED FROM TURKISH COAST OF THE MEDITERRANEAN SEA

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The marine habitat is a rich source of bioactive natural compounds with pharmaceutical potential. Some of them are novel compound with novel mechanisms of action. Marine derived fungi isolated from marine species such as sponges, anemone, tunicate and etc. have become a focus of interest. Numerous studies about diverse and unique compounds of marine fungi and their biological activities including antimicrobial, anticancer, anti-inflammatory and antiviral properties have been reported.

As part of our ongoing chemical investigation of biologically active metabolites from marine fungi, two known compounds, echinuline and preechinulin have been isolated from the marine-derived fungus *A. chevaliari*. The structures of the metabolites were determined on the basis of mass spectroscopy and NMR experiments.

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## ANTIOXIDANT ACTIVITY OF SOME TURKISH MARINE DERIVED FUNGI

**Hajar Heydari and Belma Konuklugil**

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The marine habitat is a rich source of bioactive natural compounds with pharmaceutical potential. Some of them are novel compound with novel mechanisms of action (1). Marine derived Fungi which isolated from marine species such as sponges, anemone, tunicate and etc. have become a focus of interest. In recent years marine fungi have arised as the new sources of antioxidants in the form of their wide variety of secondary metabolites such as alkaloids, benzoquinones, flavanoids, phenols, steroids, terpenoids, tetralones, and xanthenes(2,3). Numerous studies about diverse and unique compounds of marine fungi and their biological activities including antimicrobial, antioxidant, anticancer, anti-inflammatory and antiviral properties have been reported (4). In this study seven marine-derived fungi were isolated and identified from marine invertebrates and investigated with regard to their antioxidant activity. Antioxidant activity of extracts was determined by DPPH, ABTS, NO and SO assay. According to the obtained results *A.chevalieri* and *A. terreus* showed high antioxidant activity in every four assays. On the other hand, *A.awamori*, *M.globose*, *M. tassiana\_1* and *T. harzianum* showed lowest activity. This is the first study about habitant of marine-derived seven fungi of Turkey's coasts and their antioxidant, activity. Besides, it is also the first report about antioxidant activity of *C. Funiculosum* and *A. awamori*

### Acknowledgement

This work was supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK), Project No: BMBF,114S916.

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## PRELIMINARY PHYTOCHEMICAL INVESTIGATION ON MAMA POWDER — AN APPROVED HERBAL ANTIMALARIAL IN NIGERIA

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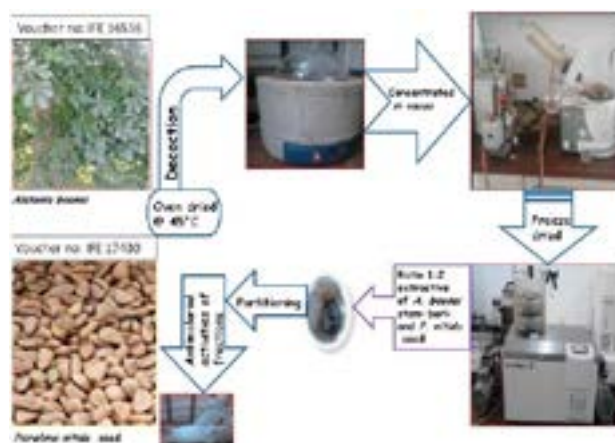
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**Statement of the Problem:** Malaria is an endemic disease in Africa where one out of four mortalities is reported regularly. Despite the discovery of artemisinin-combination therapy (ACT), high mortality rate persists due to chloroquine-resistant *Plasmodium falciparum*; hence, there is a need for new antimalarial drugs. A mixture of powdered *Alstonia boonei* De Wild (Apocynaceae) stem-bark (A) and powdered *Picralima nitida* (Stapf) T. Durand & H. Durand (Apocynaceae) seed (P), in the ratio 1:2 (Mama powder), is an FDA-approved herbal antimalarial in Nigeria. Hitherto, no phytochemical investigation on the mixture has been reported; hence, a preliminary phytochemical investigation is hereby communicated for the first time.

**Methodology:** The two plant materials were collected from their locations in Ile Ife, Nigeria, authenticated in IFE Herbarium, Obafemi Awolowo University, Nigeria, oven-dried at 45°C and separately pulverized. The two powdered samples were used to compose Mama powder as above, decocted in distilled water, concentrated in vacuo at 60°C and lyophilized. The lyophilized crude extractive was re-constituted with distilled water and partitioned with n-hexane, dichloromethane, ethyl acetate and n-butanol. The resulting fractions were tested for antimalarial activities orally on chloroquine-sensitive *Plasmodium berghei*-infected mice at 13, 26 and 52 mg/kg with chloroquine (5 mg/kg) as positive control.

**Findings:** At 52 mg/kg, the n-hexane, dichloromethane, ethyl acetate and n-butanol fractions gave chemosuppressive activities of 78.8, 47.2, 85.4 and 64.9%, respectively while chloroquine (5 mg/kg) gave 88.5%.

**Conclusion & Significance:** The highest activity was obtained in the ethyl acetate fraction which is being further purified in our laboratories in order to isolate and characterize the active constituent(s) of Mama powder.



**Figure 1:** Flow chart showing the preparation and antimalarial evaluation of solvent-partitioned fractions obtained from Mama powder decoction.

### Recent Publications

1. Ajayi C O, Elujoba A A and Adepiti A O (2015) Antiplasmodial properties of *Alstonia boonei* stem-bark and *Picralima nitida* seed in different combinations. *Nigerian Journal of Natural Products and Medicines* 19:71–77.
2. Adepiti A O, Elujoba A A and Bolaji O O (2014) *In vivo* antimalarial evaluation of mama decoction on *Plasmodium berghei* in mice. *Parasitol Res.* 113:505–511.
3. Pulcini S, Staines H M, Pittman J K, Slavic K, Doerig C, et al. (2013) Expression in yeast links field polymorphisms in PfATP6 to *in vitro* artemisinin resistance and identifies new inhibitor classes. *Journal of Infectious Diseases* 208(3):468–478.
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# Pharmacognosy and Medicinal Plants

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5. Iwu M M and Klayman D L (1992) Evaluation of the *in vitro* antimalarial activity of *Picralima nitida* extracts. *Journal of Ethnopharmacology* 36(2):133–135.
6. Asuzu I U and Anaga A O (1991) Pharmacological screening of the aqueous extract of *Alstonia boonei* stem-bark. *Fitoterapia* 63:411–417

## Biography

Clement Olusoji Ajayi is a PhD student in Pharmacognosy, being mentored in botanical, biological, physico-chemical, toxicity and chemical standardization of African medicinal plants for building-up monographs as necessary requirements for herbal pharmacopoeias.

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## EFFECTS OF HIGH DOSES OF LEMON BALM (MELISSA OFFICINALIS L.) ESSENTIAL OIL ON MICE BEHAVIOR AND SERUM BIOCHEMICAL PARAMETERS

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**Statement of the Problem:** The essential oil of *Melissa officinalis* leaves is held in high esteem for its use in aromatherapy; however, due to the low yield of the essential oil, its production cost is very high. A myriad of beneficial biological activities of this essential oil was reported and its composition has been extensively studied. Strangely, up to date, no studies exist on the acute toxicity of this essential oil. Prompted by this, in this work, the toxicity of orally administered *M. officinalis* essential oil was assessed.

**Methodology & Theoretical Orientation:** The hydrodistilled essential oil used in the current study was obtained from fresh plant material (leaves, yield 0.087%, w/w) and the detailed analyses (GC and GC/MS) showed that the tested essential oil contained high amounts of geranial (22.1%), neral (17.6%), citronellal (4.2%), nerol (1.3%) and geraniol (1.2%), as expected for *M. officinalis* (3). The acute toxicity was evaluated in female BALB/c mice that were orally treated with the essential oil (in the dose range 0.5-3 g/kg). During a 24-h period, the animals' behavior was monitored, and after that, the survived animals were sacrificed and, in their sera, liver damage-related parameters were evaluated.

**Findings:** Doses over 1 g/kg decreased animal movement, produced abdominal writhings, tumbling, atony, spastic movements and in some cases muscle rigidity. All these symptoms were dose dependent and could probably be brought in connection with the amount of citronellal in the applied doses. Serum levels of ALT and AST, as well as their ratio (AST/ALT), increased with the applied essential oil in doses >1 g/kg, indicating liver toxicity.

**Conclusion & Significance:** In conclusion, by causing a wide panel of both behavioral alterations and changes in serum biochemical parameters in mice, *M. officinalis* essential oil can be deemed as being moderately toxic.



*Melissa officinalis*



Female BALB/c mice

### Recent Publications

1. Ajayi C O, Elujoba A A and Adepiti A O (2015) Antiplasmodial properties of *Alstonia boonei* stem-bark and *Picralima nitida* seed in different combinations. *Nigerian Journal of Natural Products and Medicines* 19:71-77.
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3. Pulcini S, Staines H M, Pittman J K, Slavic K, Doerig C, et al. (2013) Expression in yeast links field polymorphisms in PfATP6 to *in vitro* artemisinin resistance and identifies new inhibitor classes. *Journal of Infectious Diseases* 208(3):468-478. Iwu M M and Klayman D L (1992) Evaluation of the *in vitro* antimalarial activity of *Picralima nitida* extracts. *Journal of Ethnopharmacology* 36(2):133-135.
4. Asuzu I U and Anaga A O (1991) Pharmacological screening of the aqueous extract of *Alstonia boonei* stem-bark. *Fitoterapia* 63:411-417

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### Biography

Nikola Stojanovic obtained his MD Degree from the Department of Medicine (Faculty of Medicine, University of Niš, Serbia) and was awarded as the Best Graduated Student for the graduation year 2014/2015. He began his research work during the second year of his studies and he is now doing a large number of specialized *in vivo* and *in vitro* experiments in the fields of Pharmacology, Toxicology, Biology, Immunology and Microbiology. His main focus currently involves the effects of essential oils on the levels of anxiety in both humans and animals. Up to now, he had participated and won several prizes, on national and international congresses in different fields of research. Besides that, he is an Author and Coauthor of a number of publications in highly esteemed peer-reviewed journals.

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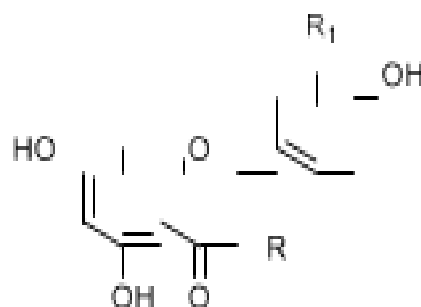
Lucía Bada et al., Am J Ethnomed 2018, Volume 5  
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## PHYTOCHEMICAL AND PHARMACOLOGICAL STUDY OF DIANTHUS HYSSOPIFOLIUS L., A MEDICINAL PLANT FROM THE “SIERRA DEL CAUREL”

Lucía Bada, Dolores Viña and Elías Quezada

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In recent years, natural resources have regained great importance in the development and research of new pharmacological agents. “Sierra del Caurel” is a natural spot located in the heart of Galicia (Spain). It is presented as a potent reservoir of bioactive secondary metabolites. *Dianthus hyssopifolius* L. is an herbaceous plant belonging to the Caryophyllaceae family. It has been used as a medicinal plant for decades by the inhabitants of the “Sierra del Caurel” because of its antirrhematic and anticatarrhal properties. However, to date, few phytochemical and pharmacological data have been reported on species of the genus *Dianthus*. Therefore, *Dianthus hyssopifolius* L. has been selected to study its composition and therapeutic activity. To obtain the secondary metabolites, extraction with organic solvents was first carried out. The whole plant was macerated fresh in methanol at room temperature. The crude extract was resolved in methanol:water (3:1) and extracted with hexane. The polar phase was evaporated under reduced pressure to methanol: water (1:5) after extraction with dichloromethane. After evaporation of the solvent, three oil extracts were obtained: hexane, dichloromethane and methanol. The methanol extract was solubilized in hot ethanol to yield a yellow precipitate. Sephadex LH-20 and silica gel column chromatography as well as HPLC were used in order to fractionate the precipitate and to purify the obtained compounds. Using one- and two-dimensional NMR experiments and mass spectrometry, two flavonoids have been identified: a glycoside derived from quercetin and another from kaempferol. According to the bibliography review, study of antibacterial, anticancer, antifungal, anti-inflammatory, and antioxidant properties for the different extracts are currently in progress.



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1. Aliyazicioglu R, Demir S, Badem M, Sener S O, Korkmaz N, et al. (2017) Antioxidant, antigenotoxic, antimicrobial activities and phytochemical analysis of *Dianthus carmelitarum*. Records of Natural Products 11:3.
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### Biography

Lucía Bada is a Graduate in Pharmacy from the Universidade de Santiago de Compostela (Spain), 2015. She has a Master's Degree in Research and Development of New Drugs. Her passion lies in natural products, especially medicinal plants. She is interested in medicinal and chemical properties of phyto-preparations and phytotherapy researches in order to assess their scientific value, promote their pharmacological valorization and stimulate sustainable drug development with a high socioeconomic potential that would enhance the welfare of communities.

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## ANTIPROLIFERATIVE ACTIVITY OF THREE SESQUITERPENES ON HUMAN BLADDER CANCER CELL LINES

Mila Radan, Franko Burcul and Vedrana Cikes-Culic

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Sesquiterpenes are C<sub>15</sub>-terpenoids built from three isoprene units. These compounds are commonly found in higher plants, marine organisms and fungi. Naturally, they occur as hydrocarbons or in oxygenated forms including lactones, alcohols, acids, aldehydes, and ketones. Over the last decade, sesquiterpenes have attracted significant attention because of the roles they play in biological systems and their diverse biological activities, such as anti-inflammatory, antibacterial, antioxidant and anti-carcinogenic. The antiproliferative activity of three sesquiterpenes (thuyopsene, nerolidol and farnesol) was evaluated on three lines of human bladder cancer (T24, UM-UC-3, TCCSUP) using MTT assay. Thuyopsene is one of the major sesquiterpene constituents of cedarwood, while farnesol and nerolidol can be found in the essential oils of many types

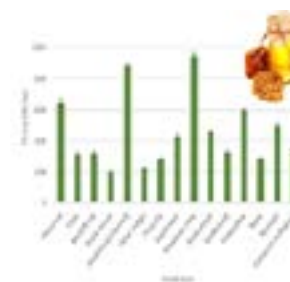
of plants and flowers. The tested compounds showed different antiproliferative activity on the cancer cell lines. Farnesol showed the highest impact on T24 cells growth (52% growth inhibition) at concentration of 90 μM, while thuyopsene showed the strongest growth inhibition of 95% and 27% on UM-UC-3 and TCCSUP cell line at concentration of 65 and 611 μM, respectively. Generally, nerolidol showed the lowest antiproliferative potential on the cancer cells. In order to determine type of cell death induced by treatment, Annexin-V-FITC assay was used for the detection of apoptosis by flow cytometry. The results obtained for these three sesquiterpenes open a perspective for their use as antiproliferative agents.

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DOI: 10.21767/2348-9502-C1-006**BOTANICAL ORIGIN AND ANTIOXIDANT ACTIVITY OF UNI-AND MULTIFLORAL HUNGARIAN HONEYS****Alexandra Bodó, Ágnes Farkas and Marianna Kocsis**

University of Pécs, Hungary

**H**oney is a supersaturated solution of sugars, which contains phenolic compounds, enzymes, free amino acids, minerals, vitamins and proteins acting as minor components. It is well established that honey has antioxidant effect, for which the phenolic acids and flavonoids are responsible. The aim of this study was to evaluate the antioxidant capacity of some floral and honeydew honeys available on the Hungarian market, with four different antioxidant techniques; and to classify the honeys according to melissopalynological analysis. We applied spectrophotometric methods: Folin-Ciocalteu (FC) assay and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay for antiradical activity, trolox equivalent antioxidant capacity (TEAC) assay for total antioxidant activity, ABS450 for color intensity and one fluorimetric method: oxygen reactive antioxidant capacity (ORAC) for the antilipoperoxidant activity. To classify the botanical origin of honeys, we carried out qualitative and quantitative melissopalynological analyses. The antioxidant capacity measured by the Folin reagent reactivity correlated with the color parameters, as well as with the antioxidant activities measured with the other methods. Black locust honey samples with pale color had the lowest (116 mAU), while dark honeydew honeys called "meadow clary (*Salvia pratensis*) honey" exhibited the highest (1617 mAU) radical scavenging activity. Values ranged from 197.93±4.64 to 937.64±28.43 mg GAE/kg, 89.14±26.9 to 240.15±47.3 µM TE/100g, 61.76±2.85 to 5.47±0.02, and 14.78±1.15 to 114.89±10.43 µM TE/g for the FC, TEAC, DPPH (IC<sub>50</sub>), and ORAC assays, respectively. However, the melissopalynological analysis revealed that the "meadow clary honey" was in fact of multifloral origin. Among unifloral honeys the chestnut (*Castanea sativa*) honeys (1037 mAU) had the highest antioxidant activity (PC: 636.48±31.9 mg GAE/kg, TEAC: 171.23±2.0 µM TE/100g, DPPH (IC<sub>50</sub>): 17.37±0.57, ORAC: 75.2±4.71 µM TE/g). The results of this study demonstrated that the botanical origin and the color intensity of honeys have the greatest influence on their antioxidant activity.



**Figure 1:** Antioxidant activity of some Hungarian honey samples, measured by Folin-Ciocalteu assay.

**Recent Publications**

1. Gorjanović S Z, Alvaraz-Suarez J M, Novaković M M, Pastor F T, Pezo L, et al. (2013) Comparative analysis of antioxidant activity of honey of different floral sources using recently developed polarographic and various spectrophotometric assays. *Journal of Food Composition and Analysis* 30:13–18.
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**Biography**

Alexandra Bodó is a PhD student at the University of Pécs, Hungary. She has been researching on honey for a couple of years, first as a beekeeper, later as a researcher. She finds it important to investigate the quality and bioactivity of honeys, in order to avoid adulterations and provide customers with honeys of certified origin and beneficial health effects. Besides the research work, she takes part in teaching plant anatomy and plant physiology courses.

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## DEVELOPMENT OF A HERBAL FORMULATION CONTAINING EXTRACTS OF CAMELLIA SINENSIS AND LIMONIUM ALGARVENSE AND ASSESSMENT OF ITS IN VITRO BIOACTIVITIES

Maria J Rodrigues<sup>1</sup>, Marta M Oliveira<sup>1</sup>, Vanessa F Neves<sup>1</sup>, Andreia Ovelheiro<sup>1</sup>, Catarina A Pereira<sup>1</sup>, Nuno R Neng<sup>1</sup>, José M F Nogueira<sup>2</sup>, Luísa A Barreira<sup>1</sup> and Luísa M Custódio<sup>1</sup>

<sup>1</sup>Centre of Marine Sciences, Portugal

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The growing interest of stakeholders and consumers in herbal products urges the need for research aiming to unravel innovative products with health improvement potential. Green tea (*Camellia sinensis* (L.) Kuntze, CS) is commonly sold in mixtures combining different herbs, fruits or spices in order to improve or add beneficial properties to such beverages. Having this in mind, and following previous promising results obtained with the halophyte sea lavender (*Limonium algarvense* Erben, LA) this work prepared and evaluated herbal beverages containing mixtures of CS and LA flowers in terms of antioxidant capacity and inhibition of enzymes related with Alzheimer's (acetyl- and butyrylcholinesterase) and Type 2 diabetes *mellitus* ( $\alpha$ -amylase and  $\alpha$ -glucosidase). The phenolic profile was determined by HPLC. Both synergistic and antagonistic interactions were observed. LA and CS samples had strong antioxidant activity, whereas LA and CS mixtures exhibited higher OH radical-scavenging and anti-lipid peroxidation capacity. LA samples had higher cholinesterase inhibition than CS and mixtures resulted in stronger enzymatic inhibition. CS had the highest  $\alpha$ -glucosidase inhibition, which decreased when combined with LA. CS had higher phenolic contents, and its combination with LA increased the phenolic diversity of the mixtures. Results showed that LA and CS infusions and decoctions and their combinations have relevant *in vitro* antioxidant, neuroprotective and antidiabetic properties. Infusions and decoctions of LA and CS mixtures should thus be further explored as potential innovative functional beverages able to prevent oxidative stress and lipid oxidation related diseases, and to reduce the progression of neurodegenerative diseases and diabetic complications.



Figure 1: *Limonium algarvense* flowers.

### Recent Publications

1. Rodrigues M J, Custódio L, Lopes A, Oliveira M, Neng N R, et al. (2017) Unlocking the *in vitro* anti-inflammatory and antidiabetic potential of *Polygonum maritimum*. *Pharmaceutical Biology* 55:1348–1357.
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3. Rodrigues M J, Neves V, Martins A, Rauter A P, Neng N R, et al. (2016) *In vitro* antioxidant and anti-inflammatory properties of *Limonium algarvense* flowers' infusions and decoctions: a comparison with green tea (*Camellia sinensis*). *Food Chemistry* 200:322–329.
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## Biography

Maria J Rodrigues has a Degree in Biology (Major in Biomedicine) and a Master's in Biomedical Sciences. She has interest in exploring new biotechnological applications and products from marine organisms (halophytes, microalgae, macroalgae and marine invertebrates) found in the Algarve coast. She has been working as a Research Fellow in the Marine Biotechnology group under the scope of different projects: SEABIOMED (marine photosynthetic organisms of the Algarve coast with biomedical applications, XtremeBio (halophytes: a precious resource nutritional elements and bioactive compounds, MaNaCruzi (searching for marine origin molecules against *Trypanosoma cruzi*, and recently Xtreme Gourmet (extremophile plants in the gourmet cuisine). Since 2014, she has been working on her PhD project: "Unravelling the biotechnological potential of halophytes species of the Algarve coast". During this time, she participated in the preparation of 27 scientific articles published in international peer-reviewed journals and 17 communications in national and/or international conferences (oral and poster).

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## PERSPECTIVES ON DERMATOLOGICAL AND COSMECEUTICAL PROPERTIES OF COMPOUND K

**En Hyung Kim**

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**G**insenosides are representative pharmaceutical compounds found in various forms in *Panax ginseng*, a traditional medicinal plant. There have been many reports describing the biological activities, including anti-inflammatory, anti-tumor, and anti-dementia effects, of several ginsenosides. The biological actions of these ginsenosides have been closely related to their biotransformations by intestinal microbiome. They are converted to their metabolites Rg2, Rg3, compound K, and others by human intestinal microflora following ingestion. The main functional component detected in mammalian blood or organs after oral administration of ginseng or ginsenosides is compound K. Compound K has been reported to exhibit diverse biological functions, including antitumor, antidiabetic, antiallergic, and anti-inflammatory effects *in vitro* and *in vivo*. Recently, antiaging effects of ginsenosides in human skin have been reported from clinical trial and *in vitro* model data. Ginsenosides have hence been proposed as promising natural cosmeceutical agents. We reviewed the biotransformation and delivery of compound K. Also biological effects of ginsenosides, especially compound K, on skin health and its potential use as cosmeceutical agents was studied.

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2. Kim E H (2017) A case of facial partial unilateral lentiginosis treated with low-fluence 1,064 nm Q-switched neodymium-doped yttrium aluminum garnet laser. *Case Rep Dermatol.* 9(2):30–34.
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### Biography

En Hyung Kim finished her residency in Dermatology at Ajou University Hospital and is currently the Director of Department of Dermatology at Cheil General Hospital and Women's Healthcare Center. As a Scientist, she is interested in Skin Physiology and Inflammatory Mechanisms. As a Clinician, she makes effort to find new innovative ways to treat her patients effectively and safely.

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## INVESTIGATIONS ON SOME MEDICAL PLANTS FROM NORTHERN CYPRUS

**Ali Hikmet Meriçli** and **Filiz Meriçli**

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Cyprus is the third largest island of the Mediterranean Sea and the point of the encounter of several different eco-systems. Flora of Cyprus is represented with 1800 plants and 22 of them are endemic plants in Northern Cyprus. Besides a rich plant diversity, Cyprus is also very rich in cultural variation depend on historical situations. The plant and cultural diversities in Cyprus provide very rich traditional treatments and folk medicines. The active principles of some medicinal plants growing wildly in Northern Cyprus and their uses as sources of herbal medicinal products have been investigated by our research group. *Ceratonia siliqua*, *Matricaria recutita*, *Prunus dulcis*, and *Thymus capitatus* are the widespread medicinal plants used traditionally in Northern Cyprus. On the other hand, Mediterranean fever disease is a serious health problem in the island and colchicine containing medicinal plants are valued for treatment. *Colchicum pusillum* is widespread in Northern Cyprus, the active principles of the bulbs and aerial parts of *C. pusillum* are investigated. The anticancer activity of the bulb extract is also determined. Our researches on some medicinal plants from Northern Cyprus and their possibility of herbal medicines production will be summarized.



**Figure 1:** *Colchicum pusillum* Sieber (photos: by AH Meriçli)  
*Matricaria recutita* L.

### Biography

Ali Hikmet Meriçli graduated from Istanbul University, Faculty of Pharmacy. He entered as an Assistant at the Faculty of Pharmacognosy at Istanbul University. He has contributed to the education and development of New Century University Faculty of Pharmacy between February 2012 and August 2013 and served as the Senator of New Century University Faculty of Pharmacy. By 2015, he has published 141 original scientific articles, 3 books, 3 books in chapter books, 40 compilations and current writings. He is working on isolation and structure elucidation of natural compounds and biological activities of medicinal plants and also herbal medicines.

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## EXAMINATION OF THE ANTIOXIDANT POTENTIALS OF PHENOLIC EXTRACTS OF IRANIAN HONEY BY DIFFERENT METHODS

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**Statement of the Problem:** Honey is a natural substance produced by bees and from ancient times, this compound has been consumed by people. In oldest civilizations, honey was consumed for both nutritional and medical purposes. Honey, prevents lipid oxidation in meat and retardations oxidation reactions in food, caused by light, heat and metals.

**Objective:** In the present study, four phenolic extracts of Iranian honey were examined for antioxidant potentials by DPPH and NO radicals scavenging, reducing power and determination of phenolic and flavonoid contents.

**Methodology & Theoretical Orientation:** For preparation of phenolic extracts of honey, Amberlite XAD-2 resin was used. For DPPH radical scavenging, honey samples at different concentration levels were mixed with DPPH. For evaluation of NO radical scavenging, nitroprusside was used. For evaluation antioxidants potential by FRAP method, FeCl<sub>3</sub>, acetate buffer, and TPTZ solution was used. For determination phenolics, Folin-Ciocalteu was used as a reagent. Flavonoid content of the samples was determined using NaNO<sub>2</sub>.

**Findings:** With respect to antioxidant properties, Gavan sample presented the highest phenolic (3817±1.52 mg GAE/100 g) and flavonoid contents (3.1±0.005 mg QE/100 g) and DPPH radical scavenging (IC<sub>50</sub>= 2± 0.003 mg/ml). Bahareh honey possessed the maximum NO radical scavenging (IC<sub>50</sub>=0.0403 ± 0.0009 mg/ml) and Meymand honey presented the highest reducing power by FRAP method (IC<sub>50</sub>= 0.0018± 0.000003 mg/ml).

**Conclusion & Significance:** Honey samples presented antioxidant potentials especially by NO radical scavenging.

**Table 1:** Antioxidant potentials of 4 honey phenolic extracts by different methods in comparison with antioxidant standards.

Samples	DPPH radical scavenging (IC <sub>50</sub> , mg/ mL)	Nitric oxide scavenging ability% (200 mg/mL)	Antioxidant potential by FRAP method (IC <sub>50</sub> , mg/ mL)
Gavan, bee	2± 3.09	0.054 ± 0.002	0.652± 0.002
Zataria	>3.200	0.045± 0.0017	0.294± 0.0014
Bahare	>3.200	0.0403 ± 0.0009	>3.200
Meymand	>3.200	0.05± 0.0014	0.0018± ± 0.000003
Quercetin	0.0265± 0.00006	0.07± 0.0016	0.009 ± 0.00003

\*Results are given as mean± SD values.

**Table 2:** Total phenolic and flavonoid contents of four honeys phenolic extracts.

Sample	Phenolic content (mg GAE/100g honey) <sup>b</sup>	Total flavonoids(mg QE/100g honey) <sup>c</sup>
Gavan	3817± 1.52	3.1± 0.005
Zataria	102± 1	2.3± 0.015
Bahare	58± 1.06	1± 0.0015
Meymand	866± 1.15	2.7± 0.005

aValues are expressed as mean± SD of three parallel measurements (p<0.05); bGAE: Gallic acid equivalent; cQE: Quercetin equivalent.

6<sup>th</sup> Edition of International Conference on  
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Medicinal Plants****Recent Publications**

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## IN SILICO EVALUATION OF NEW POTENTIAL TARGET PROTEINS OF FLAVONOLIGNANS FROM SILYBUM MARIANUM

Antonia Diukendjieva<sup>1</sup>, Mattia Mori<sup>2</sup>, Petko Alov<sup>1</sup>, Ivanka Tsakovska<sup>1</sup>, Maurizio Botta<sup>2</sup> and Ilza Pajeva<sup>1</sup>

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<sup>2</sup>University of Siena, Italy

The medicinal plant *Silybum marianum* (milk thistle) has been used from antiquity for treatment of liver and gallbladder disorders of different etiologies. Its main active component, silybin, occurs in two diastereoisomeric forms, silybin A and B. Silybin has been shown to exert a broad spectrum of bioactivities including cardioprotective, neuroprotective, antidiabetic and anticancer activities; however, the mechanisms of these actions have not been elucidated yet. In the current study, we assessed the chemical similarity of the silybin diastereoisomers to all approved drugs in the DrugBank database using ROCS software. Tanimoto Combo index, taking into account features obtained by shape and chemistry alignment of the compounds, was used as similarity estimator. The drugs scored with Tanimoto Combo indices  $\geq 0.9$  (9 drugs for silybin A and 9 drugs for silybin B) were filtered and analyzed in terms of target pathology and mechanisms of action. Among them three drugs exert antidiabetic (canaglifozin, dapaglifozin, empaglifozin) and two other drugs possess antitumor activities (vemurafenib and vismodegib). Since silybins have been reported to possess antitumor activities, the similarity with these drugs is of a particular interest when studying their mechanism of action. Since the X-ray structures of the antitumor drug targets, Smoothened homolog (vismodegib) and BRAF kinase (vemurafenib) are available, further docking studies of silybins in these receptors were performed and the possibility of silybin interactions with them was estimated. The results suggest that silybins can be accommodated in the binding sites of BRAF kinase and Smoothened homolog performing specific interactions with particular residues, including also those vemurafenib and vismodegib interact with. Experimental studies are necessary to prove the hypothesis that silybins can act as inhibitors of these proteins.

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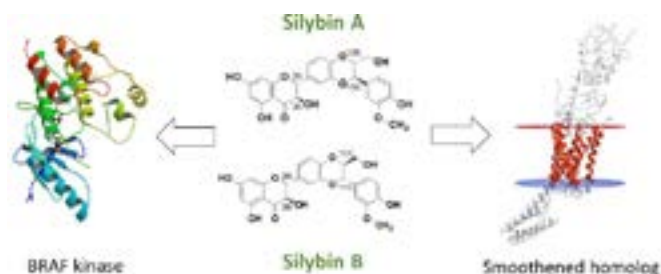


Figure 1. *In silico* evaluation of silybin interactions with new potential target proteins based on similarity with their ligands

### Biography

Antonia Diukendjieva got her BS Degree in Biotechnology and MS Degree in Biochemistry from Sofia University "St. Kliment Ohridski", Faculty of Biology. Currently she is a PhD student at the Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences. Her main scientific interests are in the field of Predictive Toxicology and *In Silico* Drug Design. Her most recent investigations relate to pharmacokinetic and pharmacodynamic evaluation of naturally-derived flavonoids.

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## IN SILICO STUDIES OF PLANT TRITERPENOID: PHARMACOPHORE-BASED VIRTUAL SCREENING AND TOXICITY EVALUATION

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**Statement of the Problem:** The metabolic syndrome (MS) represents a complex pathological condition featured by hypertension, type 2 diabetes (T2D) and obesity. It is among the risk factors for development of cardiovascular diseases (CVD) and is characterized by increasing global prevalence. Modulation of the nuclear peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) by natural compounds is considered a promising pharmacological strategy for targeting the MS. Within this study, we aimed at applying a virtual screening protocol for investigating the potential of triterpenoid saponin of natural origin to act as PPAR $\gamma$  partial agonists. Furthermore, an *in silico* estimation of their safety profile has been performed.

**Methodology & Theoretical Orientation:** An in-house virtual library with more than 70 triterpenoids had been screened by a pharmacophore-based docking of the unique aglycons in the PPAR $\gamma$  binding pocket using MOE software (v.2016.0802). The toxicity of these aglycons was evaluated using Derek Nexus knowledge-based system (v.5.0.1.).

**Findings:** Potential partial agonist-like binding modes were evaluated based on the binding energy scores of the protein-ligand (PL) complexes resulting from the docking simulation. The PL interactions and poses relevant to the PPAR $\gamma$  partial agonists' binding pattern were predicted. Potential toxicity effects, including chromosome damage and developmental toxicity, were outlined for particular triterpenoid aglycons.

**Conclusion & Significance:** The results of this study contribute to the mechanistic explanation at molecular level of the effects of triterpenoid saponins/sapogenins by a potential PPAR $\gamma$ -mediated mode of action. This research can direct further studies of naturally-derived triterpenoids as potential MS modulators.

### Recent Publications

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Figure 1. Virtual screening and toxicity prediction of naturally derived triterpenoids supporting the discovery of potential type 2 diabetes and metabolic syndrome modulator and the prevention of cardiovascular diseases therapeutics.

6<sup>th</sup> Edition of International Conference on  
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Medicinal Plants**

**Biography**

Merilin Al Sharif got her BS Degree in Biotechnology and MS degree in Biochemistry from Sofia University "St. Kliment Ohridski", Faculty of Biology, and PhD in Biological Sciences from the Bulgarian Academy of Sciences (BAS). Currently she holds the position of a Senior Assistant Professor at the Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences. Her main scientific interests relate to predictive toxicology and in silico drug design. Her most recent investigations in the field of naturally-derived triterpenoids could aid in the discovery of promising leads for design of PPAR $\gamma$ -targeting modulators of the metabolic syndrome.

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## ETHNOPHARMACOLOGICAL SURVEY OF MEDICINAL PLANTS WITH HALLUCINOGENIC EFFECT AND USED AGAINST PAIN, INFLAMMATORY DISEASES, DIABETES AND URINARY LITHIASIS IN ZAGORA “MOROCCO”

Hicham Boufous<sup>1</sup>, Fatimazahra Marhoume<sup>1</sup>, Abderrahman Chait<sup>2</sup> and Abdellah Bagri<sup>1</sup>

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**Aim:** The aim of this study was to identify different plants used in folk medicine for treating pain, inflammatory diseases, diabetes and kidney stones by the population of Zagora province, in southeast Morocco. This investigation was undertaken during more than two years started in 2013 and ended in 2015.

**Materials and Methods:** A total of 1400 persons with different ages between 20 and 80 years, in 12 areas were included in this survey; 348 were diabetics, 292 were suffering from kidney stones and 760 were healthy. Data collected were separated into two parts. The first part concerned interviewee information (age, sex, and level of education) and the second part was designed for plants uses (vernacular names, uses, parts used, and mode of preparation). Use value (UV), fidelity level and family UV (FUV) were calculated.

**Results:** We inventoried 83 plants species belonging to 40 families that were used. Ranunculaceae family showed the highest significance (FUV=0.36). Six species with the highest UV were *Zygophyllum gaetulum* L. (0.44), *Nigella sativa* (0.36), *Rosmarinus officinalis* L. (0.36), *Trigonella foenum-graecum* L. (0.35), and *Thymus satureioides* L. (0.35). We identified 50 species used for treating or managing pain, 45 for diabetes, 19 for kidney stone, seven for treating inflammatory diseases, and only three species that were recognized with hallucinogenic effects.

**Conclusions:** This study shows that folk medicine in Zagora still occupies a high level in primary health care. Data collected may help to preserve knowledge about different plants used and their mode of preparation.

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## ANTI-INFLAMMATORY EFFECTS OF PHENOLIC COMPOUNDS FROM QUERCUS MONGOLICA ON UVB-IRRADIATED HUMAN SKIN CELLS

Min Won Lee and Han Hyuk Kim

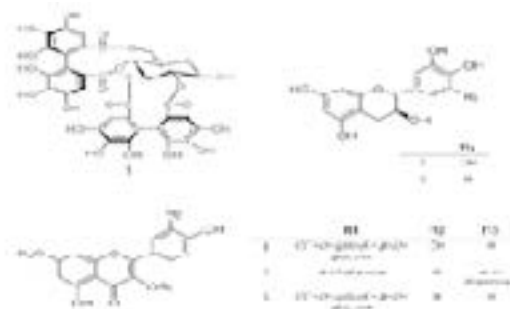
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**Q**uercus mongolica (QM) is a species of Quercus native to Eastern Mongolia, Siberia, China, Japan, and Korea. Species of Quercus have been used as an oriental traditional medicine in north-east Asia for the treatment of inflammation of the oral, genital, or anal mucosa, and externally for inflammation of the skin. Previous studies on the chemical composition of Quercus species have led to the isolation of various triterpenoids, flavonoids, and phenol glucoside gallates exhibiting a variety of bioactivities including gastrointestinal disorders, anti-bacterial and anti-oxidative activities. Previously, we conducted isolation and elucidation of the structures of the known compounds from QM including one ellagitannin [pedunculagin (PC)], five flavanoids [(+)-gallocatechin, (+)-catechin, quercetin-3-O-(6"-O-galloyl)- $\beta$ -D-glucopyranoside (QGG), kaempferol-3-O- $\beta$ -D-glucopyranoside-7-O- $\alpha$ -L-rhamnopyranoside, kaempferol-3-O-(6"-galloyl)- $\beta$ -D-glucopyranoside]. In this work, we measured inhibitory activities on chemokine and cytokine production of the extracts and compounds isolated from QM. The activities of QM and its compounds against MCP-1, TARC, IL-6, IL-8, IL-10 and IL-13 in keratinocytes irradiated with UVB showed that EtOAc fraction and PC and QGG showed the best activities. Based on the inhibitory activities of cytokines and chemokines, PC and QGG were selected as candidate the treatment of chronic skin diseases and evaluated their protein and mRNA levels of inflammatory mediators including COX-2, PGE2, cytokines and chemokines in UVB irradiated HaCaT cells and also quantified by western blotting and RT-PCR. PC and QGG diminished UVB-irradiated protein level expression of COX-2 and PGE2, downstream products in dose-dependent manners. These results suggest that PC and QGG are potential anti-inflammatory for treating inflammation of skin.

### Recent Publications

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2. Sung Hye Youn, Min Won Lee, et al. (2017) Anti-Inflammatory and Anti-urolithiasis effects of polyphenolic compounds from *Quercus gilva* Blume. *Molecules* 22(7):1121.
3. Manh Heun Kim, Min Won Lee, et al. (2016) Two new phenolic compounds from the leaves of *Alnus sibirica* Fisch. ex Turcz. *Natural Product Research* 30(2):206–213.
4. Jun Yin, Min Won Lee (2016) Inhibitory activities of phenolic compounds isolated from *Adina rubella* leaves against 5 $\alpha$ -reductase associated with benign prostatic hypertrophy. *Molecules* 21(7):887.
5. Han Hyuk Kim, Min Won Lee, et al. (2015) Inhibition of matrix metalloproteinase-1 and type-I procollagen expression by phenolic compounds isolated from the leaves of *Quercus mongolica* in ultraviolet-irradiated human fibroblast cells. *Archives of Pharmacal Research* 38(1):11–7.



6<sup>th</sup> Edition of International Conference on  
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**Biography**

Min Won Lee has his expertise in Pharmacognosy and Natural Product Derived Medicine. He is a Professor at the College of Pharmacy, Chung-Ang University and served as a President in the Korea Society of Pharmacognosy in 2017. He has extensive experience in working on phytochemical constituents from natural herbal medicine and finding effective compounds to treat various kinds of chronic inflammatory diseases including atopic dermatitis and benign prostatic hyperplasia. Because of these successful biological activities, the isolated compounds and extract of natural herbal medicine were registered on several patents in Korea and in US and China. Many papers using these results including effective compounds isolated from natural plants have been published in famous international journals.

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## HISTOPATHOLOGICAL EFFECTS OF HYDROALCOHOLIC EXTRACT OF BERBERIS VULGARIS ON EXPERIMENTAL STOMACH PEPTIC ULCER IN RATS

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<sup>2</sup>Islamic Azad University, Iran

**B***erberis vulgaris* L. (Berberidaceae) is an evergreen plant. The fruits of the plant are used to enhance the food taste. *Berberis* L. species are effective as anti-insomnia, liver protective, antibacterial, antifungal and anti-inflammatory, and have different constituents such as alkaloids, flavonoids, mineral compounds and phyto-acids. Peptic ulcer disease is one of the most common diseases, it means disintegration of gastric or duodenal mucosa integrity and one of the symptoms is burning epigastric pain that is selected by food intake and exacerbated by starvation. In this study, 24 male Wistar rats were fasted for 48 hours. Peptic ulcer was induced by Indomethacin (50 mg/kg). The rats randomly were divided in to four groups of six. *Berberis vulgaris* hydroalcoholic fruits extract (BVHFE) (50, 100 mg/kg), omeprazole (40 mg/kg) and normal saline (5 mL/kg) were administered orally to the rats for 14 days. After five hours of the last dose, all animals were anesthetized by ether and sacrificed. Their stomachs were prepared to J. Score by loop and the histopatologic examination. Both doses of BVHFE had significant effects on improvement of ulcers in the stomach mucosa, reduced inflammation, accelerated wound healing and BVHFE (100 mg/kg) was found to be the best.

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## CHEMICAL STUDY, ANTIOXIDANT ANALYSIS AND EVALUATION OF THE LARVICIDAL POTENTIAL AGAINST AEDES AEGYPTI LARVAE OF ESSENTIAL OIL OF OCIMUM BASILICUM LINN.

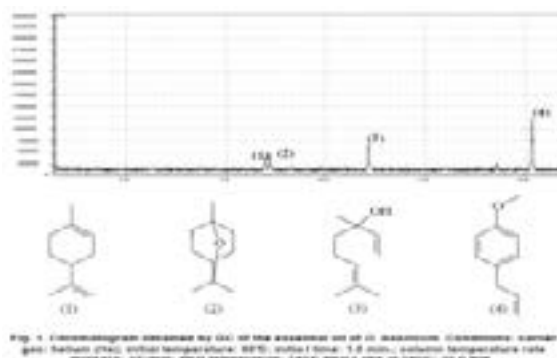
Sheylla S M S Almeida, Rosany L Martins, Ana L F Farias, Alex B L Rodrigues and Érica M Rabelo

Universidade Federal do Amapá, Brazil

The purpose of this research was to accomplish chemical study, antioxidant analysis and evaluation of the larvicidal potential against *Aedes aegypti* larvae of essential oil from the leaves of *O. basilicum* Linn. The research was carried out in the Pharmacognosy and Phytochemistry Laboratory, Department of Biological and Health Sciences, Federal University of Amapá (UNIFAP), between July 2013 and March 2014. Arthropoda Laboratory, Department of Biological and Health Sciences, Federal University of Amapá (UNIFAP) between September 2013 and March 2014. The essential oil was obtained by hydrodistillation; the identification and quantification of components was achieved with the use of GC-MS analysis. The antioxidant activity was evaluated by the method of sequestration of DPPH. The essential oil was tested in the third larval state of the development of the mosquito *Aedes aegypti*. The third larval instar were exposed to different concentrations of the oil (500, 400, 300, 200 and 130 ppm) in triplicates. Chromatographic analysis identified that the major constituents found in essential oil of *O. basilicum* were limonene (13%), 1,8-cineole (15%), linalool (20%) and methyl chavicol (45%). In trials of free radicals sequestration, the essential oil showed (AA%) 67.35±1.11 in the highest concentration and inhibitory concentration, IC<sub>50</sub> value of 61.517 mg/mL. The essential oil of *O. basilicum* showed larvicidal potential with CL<sub>50</sub> of 67.22 ppm. A more detailed study should be done to verify the larvicidal potential and biological mechanism of action, as several authors claimed that the constituent of essential oils affect the nervous system of the mosquito *Aedes aegypti* and the action mechanism is not yet fully elucidated. New studies demand the development of tests using samples of lower concentrations to verify the degree of toxicity in other animal species, including man, and preparation of formulations that may function as a natural alternative to combat mosquito larvae.

### Recent Publications

1. Almeida S S M S (2017) Chemical composition and *in vitro* antioxidant, cytotoxic, antimicrobial, and larvicidal activities of the essential oil of *Mentha piperita* L. (Lamiaceae). *The Scientific World Journal* 2017:1-8.
2. Almeida S S M S (2017) Mineral composition of leaves, ethanolic leaf extract and infusions of *A. occidentale* L. from Amazon in Northern Brazil. *Mintage Journal of Pharmaceutical E Pharmaceutical Research* 6:8-11.
3. Almeida S S M S (2016) Chemical composition: an antioxidant, cytotoxic and microbiological activity of the essential oil from the leaves of *Aeollanthus suaveolens* Mart. ex Spreng. *Plos One* 11:1-10.
4. Almeida S S M S (2016) Antioxidant effect of plant extracts of the leaves of *Tithonia diversifolia* (Hemsl.) A. Gray on the free radical DPPH. *Journal of Chemical and Pharmaceutical Research* 8:1182-1189.
5. Almeida S S M S (2016) Larvicide and antioxidant activity of the ethanol crude extract from the stem bark of *Pseudoxandra cuspidata* (Annoaceae). *Journal of Chemical and Pharmaceutical Research* 8:841-846.



6<sup>th</sup> Edition of International Conference on  
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**Biography**

Sheylla S M S Almeida is a Graduate in Pharmacy and is a qualified Clinical Analyst from the Federal University of Pará. She holds a Master's in Chemistry with a specialization in Organic Chemistry from the Federal University of Pará. She has a PhD in Chemistry in the area of Chemistry of Natural Products (Organic Chemistry) from the Federal University of São Carlos. She is Professor of Pharmacognosy, Organic Chemistry and Mechanisms of Organic Reactions at the Federal University of Amapá. Also, she is a Permanent Teacher of the Master's Course in Pharmaceutical Sciences, at the same university. She is the State Coordinator Amapá and Permanent Professor of the Doctoral Program of the Postgraduate Program in Biodiversity and Biotechnology of the Bionorte Network. She works in several lines of research in the areas of Biotechnology, Biodiversity, Essential Oils and Natural Product Chemistry.

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# E-POSTERS

Abstracts



6<sup>th</sup> Edition of International Conference on  
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Howyda H Mohamed Salih et al., Am J Ethnomed 2018, Volume 5  
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## EVALUATION OF THE NEPHROPROTECTIVE ACTIVITY OF VICIA FABA LEAVES EXTRACTS AGAINST GENTAMICIN INDUCED NEPHROTOXICITY IN RATS

Howyda H Mohamed Salih and Ikram Mohamed Eltayeb

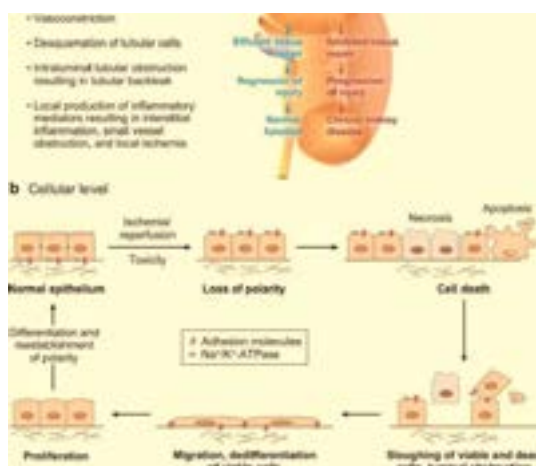
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The marine habitat is a rich source of bioactive natural compounds with pharmaceutical potential. Some of them are novel compound with novel mechanisms of action. Marine derived fungi isolated from marine species such as sponges, anemone, tunicate and etc. have become a focus of interest. Numerous studies about diverse and unique compounds of marine fungi and their biological activities including antimicrobial, anticancer, anti-inflammatory and antiviral properties have been reported. As part of our ongoing chemical investigation of biologically active metabolites from marine fungi, two known compounds, echinuline and preechinulin have been isolated from the marine-derived fungus *A. chevalieri*. The structures of the metabolites were determined on the basis of mass spectroscopy and NMR experiments.

### Recent Publications

1. Singh A and Mamibhushan N (2013) An assessment of faba bean (*Vicia faba* L.) current status and future prospect. African journal of Agriculture Research 8(50):6635–6641.
2. Abdel-Raheem I T, Abdel-Ghany A A and Mohamed G A (2009) Protective effect of quercetin against gentamicin-induced nephrotoxicity in rats. Biological and Pharmaceutical Bulletin 32(1):61–67.
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4. Ali B H (1995) Gentamicin nephrotoxicity in humans and animals: some recent research. General Pharmacology 26(7):1477–1487

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**Figure 1:** Schematic representation of (a) Pathophysiological and (b) Cellular surviving cells that remain adherent to contribute to repair.

## EVALUATION OF PHYTOCHEMICAL AND ANTIDIABETIC COMPONENTS OF CHROMOLAENA ODORATA L. KING AND ROBINSON (ASTERACEAE) CULTIVATED IN NIGERIA

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**M**edicinal plants provide the best option as a safer and more effective medication due to the presence of various compounds. Presently, available therapeutic agents for non-insulin-dependent diabetes mellitus such as insulin, oral hypoglycaemics, and dietary modification all have limitations. *Chromolaena odorata* has been reported to be traditionally used in Nigeria for the treatment of diabetes without scientific justification. This study was carried out to investigate the presence of antidiabetic bioactive constituents present in the leaves of *C. odorata* and also to determine the best solvent suitable for extracting its various phytochemical compounds. *C. odorata* leaves extract obtained by soxhlet extraction using seven solvents of varying polarity, were quantitatively analyzed using standard laboratory methods for the presence of phytochemical constituents with diabetic activity. Results obtained revealed the

presence of tannins, saponins, flavonoids, anthraquinones, cardiac glycosides, triterpenoids and sterols in all the solvent extracts of the leaf sample of *C. odorata*. There was however, absence of alkaloids. The aqueous solvent showed higher dissolution and extraction of the bioactive compounds followed by ethanol, methanol, ethyl acetate, chloroform, acetone and petroleum ether in descending order. The results from the study could be useful in the selection of appropriate solvents for the extraction of pharmacologically active constituents in the formulation and development of antidiabetic herbal drug from *C. odorata* leaves. The presence of the various compounds could be responsible for the antidiabetic activity of *C. odorata*, thus justifying the folkloric use of the plant for treating diabetes.

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ACCEPTED

Abstracts



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## IMPACT OF GEOGRAPHICAL VARIATION ON CHEMOTYPIC VARIABILITY AND BIOLOGICAL POTENTIAL OF GLORIOSA SUPERBA L. COLLECTED FROM GANGETIC PLAINS AND CENTRAL INDIA

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The present study reports the chemotypic variability of bioactive alkaloids (colchicine and gloriosine) and phenolics (quercetin and kaempferol) through calibrated HPTLC method in *Gloriosa superba* L. (tuber), collected from 17 locations of Central India and Gangetic plains. The effect of phytogeography on their antioxidant and anti-inflammatory potential was also established. Quantification data reveals that the content of colchicine (COL) and gloriosine (GLO) varies from 0.02–0.513% and 0.028–0.165%, respectively. Maximum content of colchicine and gloriosine was reported in NBG-10 (Kanth, U.P) and NBG-11 (Mohanlalganj, U.P) having 0.513% and 0.165%. Quercetin and kaempferol content varies from 0.0007% to 0.122% and 0.005% to 0.075%, with is maximum report in NBG-13 (Bheragha, M.P) germplasm. The investigated test extract showed promising antioxidant activity which was found in significant correlation

to total phenolic and flavonoid contents. Although varied results were observed against *in vitro* anti-inflammatory activity, the best results were observed in NBG-01 (0.0038%), whereas, lowest activity was observed in NBG-78 (0.0117%). Based on statistical evaluation on quantitative analysis of bioactive metabolites and bioactivity, five germplasm were identified as elite chemotypes of *G. superba* (NBG-1, NBG-10, NBG-11 and NBG-13) in the targeted phyto-geography. Furthermore, our study proves significant variability in biological potential of *G. superba* extract with the change in phytogeographical content. Thus, it will aid in site specific exploration of high metabolite yielding chemotype(s) with validated pharmacological action to meet out the medicinal and commercial demands.

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## MEDICINAL PLANTS SOLD AS ANTI-HAEMORRHAGIC IN THE COTONOU AND ABOMEY-CALAVI MARKETS (BENIN)

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<sup>2</sup>University of Lomé, Togo

**M**arket herbalists are one of the primary uses of primary health care for people in developing countries. They contribute to the conservation of endogenous plants and knowledge. In order to identify plants with antihemorrhagic properties sold in markets in Southern Benin, an ethnopharmacological survey was carried out among 34 herbalists in 17 markets in Cotonou and Abomey-Calavi. The method used is triplet purchase of medicinal recipes (ATRM). A total of 38 plant species in 24 families were identified. The most represented family is the *Rubiaceae* (13.16%). The most cited species are *Cissampelos mucronata* (12.96%), *Hybanthus*

*enneaspermus* (9.26%) and *Cassytha filiformis* (8.02%). Considering the plants mentioned in single use, *C. mucronata* (37.5%), *C. filiformis* (12.5%) and *N. laevis* (10%) were the most cited species. The leafy stem (71%) is the most used part. Two methods of preparation are mainly used, maceration (45%) and decoction (55%). The extracts of these plants could be a source of improved traditional medication (AHT) for the treatment of hemorrhages.

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## ANTIMICROBIAL ACTIVITIES OF TOTAL CRUDE SAPONINS FROM PARINARI CURATELLIFOLIA PLANCH EX. BENTH ROOT BARK

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**Statement of the Problem:** Parinari curatellifolia (Planch ex. Benth Chrysobalanaceae) is used in traditional medicine in Nigeria for the treatment of various diseases including microbial infections. The aim of the present study was to determine the potentials of the total crude saponins (TCS), extracted from the root bark of the plant against some selected pathogenic fungi and bacteria.

**Methodology & Theoretical Orientation:** The plant was collected from Zaria and authenticated at the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. The TCS was extracted using standard procedures and was subjected to qualitative phytochemical tests. The antibacterial activities of TCS were evaluated against clinical isolates of Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa using the cup-plate method. The TCS demonstrated strong antibacterial activities with zones of inhibition (in diameter) ranging between 8.0 and 21.0 mm, showing concentration-dependent responses on each of the test microorganisms. Staphylococcus aureus was the most susceptible producing the longest zone of inhibition (21.0 mm) at 30 mg/mL with MIC and MBC of 3.75 mg/mL, indicating that the TCS was bactericidal

on *S. aureus*. Similarly, the antifungal activities of the TCS were also evaluated using the agar diffusion cup-plate method against clinical isolates of Trichophyton mentagrophytes, Trichophyton rubrum and Aspergillus niger with typed isolates of Candida albican (ATCC 102311). TCS showed zones of inhibition ranging from 12–18 mm at 140 mg/mL on Trichophyton mentagrophytes, Trichophyton rubrum and Aspergillus niger. An MIC<sub>90</sub> and MFC<sub>90</sub> of 195 µg/mL were obtained for Aspergillus niger using the micro-broth dilution. This investigation revealed that the total crude saponins possessed fungicidal effect on Aspergillus niger. There was no activity observed on Candida albican.

**Conclusion & Significance:** This study has revealed both antifungal and antibacterial properties for the total crude saponins, extracted from Parinari curatellifolia root bark.

**Recommendations:** Further work should be conducted on the separation, isolation, characterization and evaluation of antimicrobial activity of the pure saponin(s) from this plant.

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## ANTIMICROBIAL ACTIVITIES OF TOTAL CRUDE SAPONINS FROM PARINARI CURATELLIFOLIA PLANCH EX. BENTH ROOT BARK

**Halilu M E, Ifeanyi G I and Ungokore H Y**  
Usmanu Danfodiyo University, Nigeria

## HEPATOPROTECTIVE ACTIVITY OF RHUS MYSORENSIS ROOT EXTRACT ON CARBON TETRACHLORIDE INDUCED HEPATO-TOXICITY IN WISTAR RATS

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The roots of *Rhus mysorensis* of *Anacardiaceae* family is used by the tribes of Andhra Pradesh, India, for the treatment of various hepatic disorders. In this study, the root of *Rhus mysorensis* was selected for the investigation of its hepatoprotective activity against CCl<sub>4</sub> induced hepatotoxicity. The rats were given a daily pre-treatment with ethyl acetate *Rhus mysorensis* root 200, 400 and 800 mg/kg and silymarin (25 mg/kg) followed by CCl<sub>4</sub> at a dose of 1.25 ml/kg orally for seven days. On the eighth day, blood was collected from the retro orbital plexus of all the rats. Serum was separated by centrifugation and analyzed for serum glutamic

pyruvate transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (ALKP), cholesterol (CHL), total protein (TPTN) and albumin (ALB). The result of the present study suggests that ethyl acetate extract of *Rhus mysorensis* root showed better hepatoprotective activity due to the presence of phytoconstituents such as steroids, triterpenoids and flavonoids. Thus, the present study provides a scientific and rational for the traditional use of this plant on the management of liver diseases.

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## ACUTE TOXICITY AND ANTI-NOCICEPTIVE ACTIVITY OF METHANOLIC EXTRACT OF HYOSCYAMUS MUTICUS IN MICE

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The objective of this study was to investigate the acute toxicity and potential activity of methanolic extract of *Hyoscyamus muticus* (Me-HM) to assess nociception in mice. The acute toxicity was studied in both oral and intraperitoneal route. LD50 was determined using Probit method and the effect of extract against nociceptive was studied by thermal stimulus (hot plate) and injection of chemical substances such as formalin (formalin test) and acetic acid (writhing test). Morphine was used as positive drug in hotplate test and acetylsalicylic acid was used in formalin and writhing test. The antinociceptive activity was determined by observed increase of latency time in hotplate test, decrease of abdominal constriction in writhing test and decrease of stretching in formalin test. The LD50 of intraperitoneal administration of Me-HM was  $1000 \pm 42, 89 \text{ mg/kg-1}$ . Our extract produced a significant ( $P \leq 0.001$ ) and dose dependent increase of

latency time in hotplate test. The optimal effects were observed after 90 mins of oral administration of both doses. In the formalin test, the both doses reduce significantly ( $P \leq 0.001$ ) the effect produced by intraplantar injection of formalin with maximum inhibition recorded in neurogenic phase with 49.36% and 42.67% successively for 100 and 50 mg/kg of Me-HM. Morphine and acetylsalicylic acid produced desired anti-nociceptive activity in tests used in this study. The antinociceptive effect of *H. muticus* extract can be explained probably by binding of scopolamine and hyoscyamine from extract to muscarinic receptors and 5-HT<sub>3</sub> involved in pain pathways. It was concluded that Me-HM shows a remarkable antinociceptive activity in thermal and chemical model of nociception in mice.

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## NORMALIZATION OF INSULIN RESISTANCE, GLUCOSE INTOLERANCE AND LIPID PROFILE BY SWIETENIA MAHAGONI LEAF EXTRACT IN FRUCTOSE INDUCED DIABETIC RATS

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**S***wietenia mahagoni* is a medicinal plant used in various medicinal practices for the treatment of various human ailments. Present study evaluates the anti-diabetic potential of *Swietenia mahagoni* aqueous extract (MAE) in fructose induced diabetic rats with insulin resistance. Diabetes was induced in the rats by treating the rats with 20% fructose (W/V) in drinking water for 40 days. Diabetes and hyperinsulinemic condition was confirmed by assessing the glucose and insulin levels in the blood of the experimental rats. After the confirmation of diabetic condition, rats were treated with the MAE and the standard drug metformin and one group was left without any treatment and labelled as positive control. MAE treatment improved glucose levels, reduced insulin levels, improved insulin sensitivity,

improved glucose tolerance, improved pancreatic  $\beta$ -cell health, decreased glycated hemoglobin content, reduced lipid peroxides, improved glycogen content and activity of enzymes involved in the synthesis of glycogen in the liver. There were also overall improvements in anti-oxidant status (improved levels of vitamin C, SOD and catalase and decreased amount of lipid peroxides) of the treated groups when compared with the diabetic control. The treatment also improved the lipid profile (improved HDL and lowered LDL and triglycerides) of diabetic rats. Results confirmed the anti-diabetic potential of MAE in animal models indicating its scope for use as diabetic adjuvant in T-2 diabetic subjects.

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## CURATIVE EFFECT OF ARJUNOLIC ACID FROM TERMINALIA ARJUNA W. & ARN. ON CELLULAR AND RODENT MODELS OF NON-ALCOHOLIC FATTY LIVER DISEASE

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**Statement of the Problem:** The prevalence of NAFLD is estimated as 25.24% and it is increasing globally. NAFLD is a progressive disease leading to fibrosis, cirrhosis and carcinoma. Terminalia arjuna W. & Arn. (Combretaceae) is an endemic tree found in India and Sri Lanka and used traditionally for its cardioprotective and hepatoprotective effects. Arjunolic acid (AA) is a triterpenoid found majorly in the heart wood of *T. arjuna*.

**Methodology & Theoretical Orientation:** AA was isolated from the heart wood of *T. arjuna* by the method of King et al. The structure of AA was confirmed using spectroscopic studies. Steatosis was induced to Hep G2 cells using FFA mixture and the effect of AA on triglyceride accumulation and lipotoxicity were assessed. *In vivo* effect of AA on NAFLD was assessed using high fat diet fed rats.

**Findings:** The treatment with AA showed GI50 value of 746.34  $\mu$ M to Hep G2 cells. The treatment with AA significantly lowered the oil red O concentration by 26.44% and triglyceride accumulation by

58.96% at 100  $\mu$ M concentration. Fenofibrate treatment showed 29.15 and 57.56% reductions, respectively. The treatment with AA and fenofibrate showed 74.90 and 73.28% reduction in the leakage of LDH, compared to the vehicle treated group. The *in vivo* findings clearly demonstrated that the animals treated with AA at 50 and 100 mg/kg concentrations showed a significant decrease in the levels of transaminases, phosphatases and LDH levels. Further, the treatment increased the albumin to globulin ratio. The liver histology of the treated animals was normal (Figure 1). The treatment with AA, significantly upregulated the expression of genes viz., PPAR- $\alpha$ , FXR- $\alpha$  and CPT-1.

**Conclusion:** These evidences suggested that AA might be a promising lead to treat NAFLD. Future robust scientific studies on AA will shed more light on its usefulness for the treatment of NAFLD.

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## CUCURBITACIN D INHIBITED PRO-INFLAMMATORY CYTOKINE PRODUCTION AND PROLIFERATION IN KERATINOCYTES

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**P**soriasis is a chronic, relapsing inflammatory skin disease, characterized by erythematous plaques covered by silvery scales. While the pathogenesis of psoriasis is still unclear, most findings indicated that intensifying T helper 1(Th1) and Th17 are required for the development of psoriasis. Cucurbitacin D is chemically classified as steroid with anticancer activity on endometrial and ovarian cancer cells. First, we evaluated the effects of cucurbitacin D on inflammatory mediator and chemokine production in IL-1 $\alpha$ , IL-17A, IL-22, oncostatin M, and TNF- $\alpha$  (named M5 combination)-stimulated 3D reconstituted human epidermis. Cucurbitacin D significantly inhibited the

production of IL-1 $\beta$ , IL-20 and GM-CSF. Second, we examined whether cucurbitacin D influences the expression of proliferation marker in IL-22 stimulated HaCaT keratinocytes. Cucurbitacin D significantly suppressed the expression of K16, K17 and Ki67 in IL-22 stimulated HaCaT cells at a concentration of 30 $\mu$ M. Cucurbitacin D also suppressed M5 induced phosphorylation of ERK 1/2 in HaCaT cells. These results suggest that cucurbitacin D may be useful as an anti-inflammatory agent, especially for psoriasis.

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## ISOLATION AND CHARACTERIZATION OF ANTICANCER BIOACTIVE COMPOUNDS FROM LEAVES OF CONYZA SUMATRENSIS, A PLANT REPUTED FOR ANTICANCER ACTIVITIES

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The plant *Conyza sumatrensis* is used in traditional medicine in parts of Africa for the treatment of numerous health ailments like inflammation, skin diseases as well as cancers. Bioassay guided fractionation of the methanol extract of the leaves of *Conyza sumatrensis* against breast cancer (MCF-7), lung cancer (NCI-H460) and NIH 3T3 (mouse embryonic fibroblast normal cell line) at 1-250 µg/mL was carried out. Fractions and isolated compounds were as well tested at 1-100 µg/mL and 1-100 µM against the cell lines. Extracts of *C. sumatrensis* was partitioned into aqueous and chloroform fractions and both fractions were tested for their effects on MCF-7 and NCI-H460. Further chromatographic and biological studies of the active chloroform fraction yielded two compounds whose identities

were revealed as Stigmasterol 3-O-beta-D-glucoside (A) and 2, 3-dihydroxylpropyl hexacosanoate (B) through NMR and MS studies. These compounds were observed to give  $-16.50 \pm 0.14$  and  $-21.71 \pm 0.23\%$  cytotoxicities against MCF-7 at 100 µM with GI<sub>50</sub> and TGI of  $40 \pm 0.10$ ,  $50 \pm 6.0$  µM and  $22.67 \pm 1.33$  and  $69.33 \pm 1.33$  µM respectively. These compounds were also cytotoxic against NCI-H460 cell lines but less than doxorubicin, the anticancer drug used. The overall results showed that the plant can be used to prevent the proliferation of breast and lung cancer cells and hence justify the ethnomedicinal uses of the plants in treating tumour related ailments.

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## ISOLATION AND CHARACTERIZATION OF ANTICANCER BIOACTIVE COMPOUNDS FROM LEAVES OF CONYZA SUMATRENSIS, A PLANT REPUTED FOR ANTICANCER ACTIVITIES

## PHYTOCHEMICAL ANALYSIS OF EMBELIA RIBES SEEDS FOR ANTIOXIDANT ACTIVITY

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Many plants contain antioxidant compounds where it protects cells against the damaging effects of reactive oxygen species (ROS) such as singlet oxygen, superoxide, peroxy radicals, hydroxyl radicals and peroxynitrite which results in oxidative stress leading to cellular damage. Cell damage caused by ROS appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction. The study was carried out to demonstrate the antioxidant property in *Embelia ribes* seeds. *Embelia ribes*, commonly known as *Vayu vilanga*, is a medicinally valuable, woody climber, a well-known drug in *Ayurvedic system*. *E. ribes* is identified and recognized by Medicinal Board, Government of India. *E. ribes* is one of the red list plant mainly found in semi evergreen and deciduous forests at

an altitude of 400 to 1500 m of Northern Western Ghats of India, Sri Lanka, China. The dried powdered seeds were subjected to sequential extraction by solvents like acetone, petroleum ether, water, methanol, and chloroform in increasing order of their polarity. The phytochemical screening of seed extracts revealed that the seeds were rich source of secondary metabolites. The results of DPPH and ABTS assays for antioxidant activity showed great free radical scavenging activity under low concentrations for methanolic extracts. Upon quantification of methanol and acetone extract, they showed the highest amount of flavonoid and phenolic compounds, respectively. The presence of flavonoids and phenolics are responsible for the antioxidant activity as they are free radical scavengers *in vivo*.

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## USE OF SOUTHERN BENIN PLANTS IN THE TREATMENT OF TYPHOID FEVER: ROLE OF HERBALISTS

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**S***almonella* spp. is pathogenic bacteria responsible for typhoid and paratyphoid fevers. Herbalists are one of the main users of primary health care for people in developing countries. They contribute to the conservation of plants and endogenous knowledge. The aim of this study was to establish the potential of the Benin flora for the treatment of salmonellosis. It was carried out thanks to an ethnopharmacological study of medicinal plants sold. It was conducted with 90 herbalists located in 30 markets in Southern Benin. The method used is Triplet Purchase of Medicinal Recipes. This technique consisted in visiting the same herbalist three times in a weekly interval. These visits allowed us to buy plants suspected of treating a patient suffering from typhoid fever. At each visit, the herbalist was told that his treatment was

effective but that the treatment was very difficult for the patient. The technique then allowed to check the concordance in the recipes proposed by these herbalists during the three visits and to identify the most cited plants in its recipes. In this study, 57 plant species sold by herbalists were identified. *Senna siamea* (8.32%), *Phyllanthus amarus* (4.16%), *Uvaria chamae* (3.56%), *Acacia siberiana* (2.97%), *Heterotis rotundifolia* (2.97%), *Crateva adansonii* (2.77%), *Citrus aurantiifolia* (2.77%), *Acanthospermum hispidum* (2.57%), *Corchorus olitorius* (2.57%) were the most sold. Waiting to go through further biological explorations of their activities, extracts from these plants could be a source of Improved Traditional Medicines for the treatment of typhoid fever.

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## INDUSTRIAL CHEMOTAXONOMY - AN APPROACH FOR EXPLORATION OF POTENTIAL BIOMOLECULES FROM VARIED PHYTO-GEOGRAPHY OF INDIA

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**A**ccurate plant identification and right quality plant material from best location of different phyto-geographical zones is the foundation of effective usage of plant based natural health products in pharmaceutical industry. Herbal drug technology is used for converting botanical materials into medicines, where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge is important. The use of chromatographic techniques and marker compounds to standardize botanical preparations has proven industrial usage for commercial exploitation of medicinal

diversity, their variable sources and chemical complexity. This has huge opportunity in the area of drug development and discovery, where variation in metabolite content plays an important role. A chemotypic fingerprinting and related technique provides an optimal characterization of botanical materials. This present contribution provides an overview and a brief account of various such studies conducted that are useful in identifying best location of right material from different phyto-geographical zones of India.

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## ANALGESIC, ANTI-INFLAMMATORY AND ANTICANCER ACTIVITIES OF COMBRETIN A AND COMBRETIN B ISOLATED FROM COMBRETUM FRAGRANS F. HOFFM (COMBRETACEAE) LEAVES

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**P**harmacological and phytochemical research shows that *Combretum fragrans* F. Hoffm (Combretaceae) is a plant possessing numerous therapeutic virtues and rich in active secondary compounds. In this study, we investigate the *in vivo* (antinociceptive, anti-inflammatory) and *in vitro* (anti-cancer, anti-TNF $\alpha$ , ROS and NO inhibitor) capacity of Combretin A and Combretin B, the triterpenes (cycloartane-type) isolated from *Combretum fragrans*. ROS production from phagocytes, TNF- $\alpha$  production, NO production, anticancer and cytotoxicity assay were done by using chemiluminescence technique, ELISA kit, colorimetric method, MCF-7 Cells and MTT assay, respectively. Antinociceptive and anti-inflammatory activity was estimated using a model of acetic acid, formalin and carrageenan. Combretin

A and Combretin B significantly ( $p < 0.001$ ) inhibited extracellular ROS production. These compounds also possess significant ( $p < 0.001$ ) reduced TNF- $\alpha$  and NO production. The compound decreased cell viability in MCF-7 cells. Concerning the pain induced by acetic acid and formalin as acute inflammation in rat induced by carrageenan, Combretin A and Combretin B exhibited significantly ( $p < 0.001$ ) antinociceptive and anti-inflammatory activity. Antinociceptive, anti-inflammatory and anticancer potential associated with inhibitors effect on ROS, TNF $\alpha$  and NO production revealed in this study approve that, Combretin A and Combretin B are considered as a promising chemotherapeutic agent in breast cancer treatment and inflammatory disease.

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## ANTIHYPERTENSIVE EFFECT OF CELERY SEED ON RAT BLOOD PRESSURE IN CHRONIC ADMINISTRATION

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**T**his study investigated the effects of different celery (*Apium graveolens*) seed extracts on blood pressure (BP) in normotensive and deoxycorticosterone acetate-induced hypertensive rats. The hexanic, methanolic, and aqueous-ethanolic extracts were administered intraperitoneally and their effects on BP and heart rate (HR) were evaluated in comparison with spironolactone as a diuretic and positive control. Also, the amount of n-butylphthalide (NBP), as an antihypertensive constituent, in each extract was determined by HPLC. The results indicated that all extracts decreased BP and increased the HR in hypertensive rats, but had no effect on normotensive rats. The data showed that administration of 300 mg/kg of hexanic,

methanolic, and aqueous-ethanolic (20/80, v/v) extracts of the celery seed caused 38, 24, and 23 mmHg reduction in BP and 60, 25, and 27 beats per minute increase in the HR, respectively. Also, the HPLC analysis data revealed that the content of NBP in the hexanic extract was 3.7 and 4 times greater than methanolic and aqueous-ethanolic extracts. It can be concluded that celery seed extracts have antihypertensive properties, which appears to be attributable to the actions of its active hydrophobic constituents such as NBP and can be considered as an antihypertensive agent in chronic treatment of elevated BP.

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# Pharmacognosy and Medicinal Plants

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## QUALITY CONTROL AND STANDARDIZATION OF MEDICINAL AND AROMATIC PLANTS — FROM CULTIVATION OF MEDICINAL PLANTS TO ITS CLINICAL APPLICATION

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In spite of recent developments of antibiotics and newer synthetic drugs, a vast majority of people depend on traditional medicines for their primary health care needs and it can safely be presumed that a major part of traditional therapy involves the use of plant extracts or their active principles. In the recent years with ever growing commercialization in the field of herbal medicines, there has been an instant demand for quality control of the drugs used in this system. The studies on the identity, purity and quality of the genuine drug will enhance information in checking the adulteration. A set of standards would not doubt be deterrent on substitution and adulteration and also an aid for 'drug law enforcement'. The present talk incorporates study from birth of the plant to its clinical application which is a dire need for all concerned to have knowledge of GAP, GFCP, GLP, CGMP and the possible adulterations. All modern instrumentation used for quality control will be discussed with special reference to GC/MS for aromatic plants. Besides above protocols, this study deals with approaches towards establishing the safety and quality

starting from preliminary examination of a medicinal plant, its morpho-anatomical, pharmacognostic, physicochemical and analytical parameters, foreign organic matter, pesticide residue, radioactive and microbial contamination, chemical assay, finger printing of different extractives using modern extractors, chromatographic and spectroscopic techniques, phytochemical screening, quantitative analysis of inorganic constituents and standardization with special reference to marker compounds in plant species and their fingerprinting along with its modern perspectives. Different stages, i.e. quality control studies of raw medicinal plant, controlled studies on method of processing, quality control studies of finished phytomedicines, standardization procedures at each stage from birth of the medicinal plant up to clinical application of herbal medicine have been described. Emphases have been given on the protocols which are required for registration of phytomedicines.

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## SOIL PARAMETERS FROM DIFFERENT GEOGRAPHICAL AREAS IN SOUTH AFRICA: A HINT FOR A PREEMINENT ANTIOXIDANT COLLECTION SITES OF HYPOXIS HEMEROCALLIDEA

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**Statement of the Problem:** Hypoxis hemerocallidea is a commonly used African herbal medicine that is wild harvested, due to its strong antioxidant activity. Its anti-oxidant activity is linked to plant stressors like soil, heavy metals concentrations, organic matter content and pH. Although, minerals are known to increase antioxidant capacity, the use of this plant from a wild source may pose a safety concern to the person consuming the said plant or its naturally harvested corm may not be completely safe.

**Objective:** The purpose of this study is to determine the relationship between the concentration of selected soil metal concentrations, the H. hemerocallidea corm metal concentrations and corm antioxidant activity.

**Methodology & Theoretical Orientation:** H. hemerocallidea corms were collected from five different geographical regions of South Africa. Qualitative and quantitative evaluation of anti-oxidant activity of the corms was done. Heavy metal, organic matter content and pH analysis were also evaluated.

**Findings:** The soil and corm metal concentrations varied by site. In general, the highest corm and soil metal concentrations for any sites were Fe, Mn, and Cr. Among the trace metals investigated, Fe was the highest, particularly for the corm collected from Ga-Rankuwa  $83.7 \pm 0.03 \mu\text{g g}^{-1}$ . The soil and corm crude samples from Ga-Rankuwa with high levels of metals (e.g. Fe, Cr, Ni, Pb) yielded greater antioxidant activity (EC<sub>50</sub> of  $1.68 \pm 0.49 \mu\text{g/ml}$ ). We were, however, unable to link the corm's antioxidant activity to environmental conditions.

**Conclusion & Significance:** The results from this study however highlight the dangers of using naturally harvested bulbs in undefined soils as the dangers of naturally harvested medicines and indicate that the plant has the ability to bio accumulate heavy metals.

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## ANTI-TRYPANOSOMAL ACTIVITY OF HPLC PURIFIED PRECOCENE I FROM *AGERATUM HOUSTONIANUM* LEAVES AGAINST *TRYPANOSOMA EVANSI*

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Freshly harvested leaves of *Ageratum houstonianum* were dried under shade and powdered. Leaf sample of *A. houstonianum* was extracted by process of hydrodistillation using a Clevenger-type apparatus for the preparation of essential oil. Extract from *A. houstonianum* was prepared by dissolving 5 µL of the essential oil in 10 mL methanol. All the sample was filtered through a Whatman (Maidstone, England) stainless steel syringe assembly using a 0.22 µm Durapore (Millipore: Milford, USA) membrane filter. HPLC analysis was carried out via a Waters HPLC system consisting of model 510 and 515 pumps, a Rheodyne injector, a Novapak C18 column (250 x 4.6 mm i.d.; 4 µm), a model 490E multi-channel detector and Millennium 2010 sata manager. The mobile phase constituents were filtered using a Durapore 0.22 µm membrane filter. The elution was carried out with a linear gradient of acetonitrile: water (40:60) to pure acetonitrile in 60 min at a flow rate of 1 mL/min. detection was at 210, 240, 280 and 320 nm. The precocene was eluted within 25 min, the peak areas showed good reproducibility (average relative standard deviation were 0.78%), and the calibration curves (i.e. mass of precocene standard injected vs. peak area detected at 210 nm) were linear over the range of 0.05-10 µg (for precocene I,  $y = 6654454 x + 176626$ ,  $r^2 = 0.99$  and for precocene II,  $y = 4618457 x + 133472$ ,  $r^2=0.99$ ). Standard sample containing precocene I (1 mg/mL) and

precocene II (1 mg/mL) obtained from Sigma (St Louis, MO, USA) were prepared in methanol. Identified precocene I was screened against *Trypanosoma evansi* for trypanocidal activity on Vero cells grown in Dulbecco's Modified Eagle Medium (DMEM) and supplemented with foetal calf serum (FCS) 20-40% at appropriate conditions. *In vitro* cytotoxicity test of precocene I at concentrations (1.56–100 µg ml<sup>-1</sup>) was done on Vero cells but without FCS. *In vitro* trypanocidal activity varied from immobilization, reduction and to the killing of trypanosomes in corresponding ELISA plate wells. At 250 µg ml<sup>-1</sup> of purified precocene I, there was drastic reduction of average mean trypanosomes count to complete killing of trypanosomes (40.±0.0 to 0.00±0.00) at 9 h of incubation, which was statistically the same as diminazine acetate (50 µg ml<sup>-1</sup>) at 4 h. Trypanosomes counts decreased in concentration and time –dependent manner with significant difference ( $P \leq 0.05$  to 0.01)). During *in vitro* cytotoxicity test, Purified precocene I and diminazine acetate standard drug, were cytotoxic to Vero cells at all concentrations except at concentrations of 6.25–1.56 µg ml<sup>-1</sup> and 1.56 µg ml<sup>-1</sup>, respectively. Precocene I was responsible for higher anti-trypanosomal activity. Precocene I could be the near future trypanocidal compound for a new trypanocide.

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## ANTI-THROMBOCYTOPENIC AND ANTI-LEUKOPENIC PROPERTIES OF SYZYGIUM CUMINI (L.) SKEELS (MYRTACEAE) LEAVES IN A MURINE MODEL

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Dengue has remained to be a national epidemic in the Philippines and has been one of the leading causes of mortality in children. Although there is no quick dengue infection confirmation test, a complete blood count might show thrombocytopenia and leukopenia. *Syzygium cumini* (L.) Skeels (Myrtaceae) is widely used for various diseases and is particularly abundant with quercetin. The air-dried *S. cumini* leaves underwent sequential extraction using hexane, ethyl acetate, and methanol followed by phytochemical screening, where the methanolic extract was found to contain the highest amount of flavonoid (87.5 mg QE/gram), followed by the ethyl acetate extract (56.1 mg QE/gram), and the hexane extract (32.5 mg QE/gram), respectively. The methanolic leaf extract, given its numerous toxicity studies to be safe from 5 to 2,000 mg/kg as per OECD 423 guidelines, was then subjected to *in vivo* bioassay utilizing 24 male Sprague-Dawley rats. The rats were divided into four groups (n=6) namely: distilled

water 10mL/kg p.o., hydroxyurea 15 mg/kg p.o., methanolic extract 400 mg/kg p.o., and methanolic extract 800 mg/kg p.o., where hydroxyurea was used to induce thrombocytopenia and leukopenia in all groups. The thrombocyte and leukocyte counts were measured before induction to get the baseline, after induction, and at the 1st, 3rd, and 6th day of treatment. Applying one-way ANOVA and Duncan Test as post-hoc, results revealed that the methanolic leaf extract of *S. cumini* exerted an anti-thrombocytopenic property at both doses of 400 and 800 mg/kg and an anti-leukopenic property at the dose of 800 mg/kg. This study significantly claimed for the first time that the leaves of *S. cumini* can be orally active and effective in increasing platelets and WBCs in hydroxyurea-induced thrombocytopenia and leukopenia. Hence, it is a potential candidate for further research leading to the development of an herbal therapeutic agent for dengue.

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## CARDIOPROTECTIVE EFFECT OF TWO CAMEROONIAN SPICES AGAINST DOXORUBICIN INDUCED CARDIOTOXICITY ON H9C2 CELLS

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**Background:** Doxorubicin (Dox) is a highly active chemotherapeutic drug used to treat several solid and hematologic tumors. However, its clinical use is limited by its severe cardiotoxic side effects including, reactive oxygen species (ROS) production. The aim of this study is to investigate the protective effects of plants extracts used as spices against the Dox-induced toxicity on H9c2 cells and elucidates their mechanism of action.

**Methodology:** The cytoprotective effects of the extracts of *Afrostyrax lepidophyllus* (GEH) and *Monodora myristica* (AEH) were tested by assessing the growth and viability of H9c2 cells treated with doxorubicin in presence of the extracts using sulforodamine B (SRB) and resazurine assays. The effects of extracts on morphological and biochemical changes of the cells were explored through the mitochondrial membrane potential ( $\Delta\Psi_m$ ), the production of ROS, and the activity of caspases 3 and 9.

**Results:** The SRB assay showed that the samples AEH (60.56±9.83%) and GEH (65.26±9.29%) at the concentration of

25 µg/mL have revealed a significant ( $P<0.05$ ) protective effect of cell growth and the resazurine assay confirmed their effect on cell viability with the respective values of  $68.94 \pm 6.00\%$  and  $74.68 \pm 5.84\%$  that were significantly higher compared to the control treated with Dox. We found an increase of the  $\Delta\Psi_m$  in presence of the samples: GEH ( $68.75 \pm 7.99\%$ ) and AEH ( $68.41 \pm 3.21\%$ ) compared to the group receiving the Dox ( $56.91 \pm 4.18\%$ ). Also we noted a decrease production of ROS in living H9C2 cells and reduction of the activity of the caspases 3 and 9. The morphological assessment showed a reduction of apoptotic cells, nucleus fragmentation and the TMRE showed an increase of the  $\Delta\Psi_m$  in the presence of AEH and GEH.

**Conclusion & Significance:** AEH and GEH extracts demonstrated protective effect against the deleterious effects of Dox on cardiomyocytes. Their mechanism implied the reduction of apoptosis in cardiac cells and oxidative stress. Therefore, they could be used as inhibitor of Dox cardiotoxicity to improve cancer treatment.

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