

CONFERENCE ABSTRACTS

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New medicines

In vitro activity and antioxidant investigation of fruit wines

Uroš Čakar^{1*}, Nikolina Lisov², Ivana Plavšić², Aleksandar Petrović², Brižita Đorđević¹

¹Faculty Of Pharmacy, University of Belgrade, Serbia

²Faculty of Agriculture, University of Belgrade, Serbia

* uroslion@gmail.com

Background: Fruit is a rich source of biologically active compounds which are essential for the human organism. It is also important to highlight the various fruit products which can be considered important constituents of nutrition; among these products, the authors would like to highlight fruit wines.

Objectives: The aim of this study was to determine the phenolic profile, antioxidant and in vitro activity of analysed fruit wine.

Methods: Fruit wines were produced from black chokeberry in different controlled conditions of micro vinification. Wines were produced with the addition of sugar and enzymatic preparation and without addition. Two different yeasts were used in separate fermentations. Identification and quantification of phenolic compounds were conducted by UPLC TQ-MS/MS, while antioxidant activity was detected by the FRAP method. Alpha-glucosidase inhibitory activity was measured by using alpha-glucosidase and substrate solution, p-nitrophenyl alpha-D-glucopyranoside.

Results: In the black chokeberry wine were quantified phenolic acids and flavonoids. From phenolic acids, it is possible to highlight the content of chlorogenic, caffeic, and p-coumaric acids. From flavonoids, a considerable amount of

quercetin and rutin was observed. Antioxidant activity measured by the FRAP method showed that values for chokeberry wine were in the range of 70.77 to 88.57 mmol/L Fe²⁺. The alpha-glucosidase inhibitory activity showed that black chokeberry wine has the ability to inhibit the enzymatic activity of the above-mentioned enzyme. The values were in the range of 25.75 to 52.37 µg/ml. The control was acarbose, whose inhibitory activity was 73.7 µg/ml. Higher content of selected phenolic compounds and better antioxidant activity was observed in wines produced with sugar and enzymatic preparation addition. Also, better inhibitory activity against alpha-glucosidase was observed for the same wines.

Conclusions: Besides quantified phenolic compounds, the synergistic and antagonistic effect of many other biologically active compounds in analysed black chokeberry wines are responsible for antioxidant and in vitro inhibitory activity against alpha-glucosidase.

Influence of different treatments of Vožd cultivar on total phenolic content and antioxidant capacity of wine

Uroš Čakar^{1*}, Nikolina Lisov², Ivana Plavšić², Aleksandar Petrović², Ljiljana Gojković-Bukarica¹

¹Faculty Of Pharmacy, University of Belgrade, Serbia

²Faculty Of Agriculture, University of Belgrade, Serbia

* uroslion@gmail.com

Background: The red grape skins and stalk are particularly rich in extractable polyphenols responsible for the major sensorial characteristics of wine, such as colour, astringency and ageing ability. During vinification, colourless phenolic increase during alcoholic fermentation, reaching maximum

values at pressing, and remain stable during malolactic fermentation and subsequent storage. The profile of wine polyphenols depends on the grape variety and other factors affecting berry development, such as climatic conditions or geographical location.

Objectives: The aim of this paper was to investigate the influence of treatment preparations (adding grape stalk) on the total phenol content and antioxidant capacity of Vožd wine.

Methods: The grape variety Vožd (vintage 2020) was harvested in optimal enological maturity and originated from vineyards around the town of Vršac (Serbia). Grapes were prepared with crashing and destemming and with treatment preparations by adding a grape stalk in 30% and 50%. For all of the samples, the maceration time was the same (14 days). Total phenolic content in wine samples was determined by the Folin–Ciocalteu's (FC) method using gallic acid as a standard. The antioxidant capacity of wine was analysed with anti-DPPH radical activity.

Results: The minimal amount of total phenolic content was detected in wine obtained without adding a grape stalk (2380 mg GAE/l), while wine with treatment preparations (50% grape stalk) gave the maximum amount of total phenolic content in wine (2640 mg GAE/l). While wine prepared with the addition of 30% grape stalk showed the most potent DPPH free radical-scavenging activity.

Conclusions: The total phenol content was higher when the crashing grapes were added to the grape stalk, but the antioxidant capacity of the wine was not accompanied by an increase in the TPI value.

Cannabidiol: knowledge and perceptions of society

Abigail Calleja*, Janis Vella Szijj, Anthony Serracino-Inglott

University of Malta, Malta

* abicalleja14@gmail.com

Background: Cannabidiol (CBD) and tetrahydrocannabinol (THC) are cannabinoids found in the cannabis plant. Research and marketing efforts about the use of CBD increased the public's interest and knowledge about its use.

Objectives: To assess the knowledge and perception of the general public about CBD.

Methods: A questionnaire targeting the knowledge and perception of the general public about CBD was developed, validated and disseminated. Ethics approval was granted. The

questionnaire consisted of four sections: Demographics, Knowledge, Perception and Barriers related to CBD use.

Results: 400 individuals (62% female, 41% aged 26 - 40 years, 42% having a tertiary level of education) answered the questionnaire. 75% of respondents from the general public (n = 257) learned about CBD from social media/news, and 88% (n = 247) of the participants discussed the use and effects of CBD with friends/family. 96% of participants (n = 384) agree that CBD has a therapeutic effect, with 79% (n = 314) believing that CBD has an analgesic effect and 76% (n = 305) believing that CBD has an anxiolytic effect. 45% (n = 180) believe that CBD causes sedation and drowsiness, 3.8% (n = 15) do not know if CBD has any side effects, and 8% (n = 32) believe that CBD has no side effects. 50% (n = 202) of the participants are of the opinion that CBD products should be prescription-only-medicine, and 69% (n = 277) disagree that CBD is a gateway drug. 85% (n = 342) of the participants believe that CBD products should be accessible in Malta for medicinal use, and 77% (n = 306) agree that CBD products recommended or prescribed by a healthcare professional are more likely to be used by patients. 53% (n = 210) of the participants agree with the statement that use of CBD might lead to judgemental issues or conflicts between healthcare professionals. Participants who were knowledgeable about CBD (67.9%) believe that 'Social stigma associated with the use of CBD for medicinal use would be a potential barrier related to CBD use'. Participants who are not knowledgeable about CBD (53.8%) perceive the 'Risk of impaired driving' and 'Misuse of CBD products as potential barriers related to CBD use'.

Conclusions: The findings demonstrate that participants were aware and knowledgeable about CBD. The majority of the participants (n = 314) believe that CBD has an analgesic effect, yet there is no approved CBD medicinal product indicated for painful conditions on the market. Findings demonstrate that there are participants who do not know whether CBD has any side effects or believe that it has none, indicating a lack of knowledge among the public with regard to the side effects caused by CBD. Results indicate that CBD products recommended or prescribed by a healthcare professional are more likely to be used by patients.

Reflections on the use of cannabidiol for medicinal purposes and the views of healthcare professionals

Abigail Calleja*, Janis Vella Szijj, Anthony Serracino-Inglott

University of Malta, Malta

* abicalleja14@gmail.com

Background: The cannabis plant has more than 100 cannabinoids. The two most researched cannabinoids are tetrahydrocannabinol (THC) and cannabidiol (CBD). Demand

for CBD products grew, with healthcare professionals recommending CBD for potential therapeutic effects.

Objectives: To assess the knowledge and perception of healthcare professionals (HCPs) about CBD.

Methods: A questionnaire targeting the knowledge and perceptions of HCPs about CBD was developed, validated and disseminated to medical doctors, pharmacists, nurses, podiatrists and other HCPs. Ethics approval was granted. The questionnaire consisted of four sections: Demographics, Knowledge, Perception and Barriers related to CBD use.

Results: 150 HCPs (58% female, 53% aged 26-40 years, 49% pharmacists, 23% medical doctors, 8% nurses, 7% podiatrists, and 5% psychologists) answered the questionnaire. 70% (n = 97) of HCPs obtained information about CBD from social media/news, 61% (n = 91) had patients asking them about CBD and its use, and 69% (n = 104) felt comfortable in prescribing or recommending CBD for pain. HCPs (n = 105) believe that sedation/somnolence are from common to very common side effects caused by CBD, while fever (n = 90), infections (n = 80) and drooling (n = 59) were believed to be rare to very rare side effects of CBD. 15% (n = 22) were aware of Epidiolex/Epidyolex, 60% (n = 90) believed that Epidyolex should be available in Malta, and 53% (n = 79) believed that CBD products intended for medicinal use without a marketing authorization should not be legally available in Malta. 67% (n = 101) of HCPs believe that CBD products should be prescription-only medicines, whereas 39% (n = 58) are of the opinion that CBD products should be prescribed following the same procedure for controlled and dangerous substances. 61% (n = 91) disagree that CBD is a gateway drug, 45% (n = 67) agree that CBD products used for recreational purposes should be decriminalised, and 65% (n = 97) of HCPs deemed their personal beliefs as a barrier to CBD use.

Conclusions: The majority (n = 145) of the HCPs claim that CBD produces an analgesic effect, yet there is no approved CBD product for the management of pain. HCPs (n=58) believe that CBD products should be considered narcotics. Sedation and somnolence were believed to be common very common side effects caused by CBD. Drooling, fever and infections were believed to be rare to very rare side effects of CBD. These results indicate different levels of knowledge among HCPs. The side effects mentioned in the questionnaire were listed as common to very common side effects in the SmPC of Epidiolex / Epidyolex. Awareness and education about the potential side effects caused by CBD products are proposed.

Therapeutic potential of cannabidiol

Abigail Calleja*, Janis Vella Szijj, Anthony Serracino-Inglott

University of Malta, Malta

* abicalleja14@gmail.com

Background: Cannabidiol (CBD) is one of the most prevalent phytocannabinoids found in the cannabis plant. CBD and tetrahydrocannabinol (THC) have a similar chemical structure though they differ in spatial configuration, which leads to differences in their pharmacological profiles. There is growing interest in the promising pharmacological properties of CBD.

Objectives: To investigate the potential therapeutic benefits of CBD in different medical conditions.

Methods: A systematic literature review of studies on the potential therapeutic benefits of CBD was carried out. PubMed was used to retrieve peer-reviewed open-access and full-text articles from January 2010 until December 2020. Publications that were neither experimental studies nor observational studies and studies with ongoing results or no results were excluded. Publications describing the approved or potential therapeutic effects of CBD used alone in different medical conditions were included. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses style method was employed. The impact factor of the journals containing the studies was identified.

Results: A total of 2,637 articles were identified, of which 126 articles met the inclusion criteria for review. CBD was reported to have beneficial effects on mental health disorders (33), inflammatory conditions (27), neurological disorders (21), tumours (15), cardiovascular disease (11) and neuropathic pain (6). CBD demonstrated neuroprotective effects (5) and other therapeutic effects (8). 55 of the 126 studies were 'in vivo' studies, 33 were human studies, 23 were 'in vitro', and 15 were 'in vitro and in vivo' studies. The journals, including the studies which demonstrated that CBD has an effect on neurological conditions, had the highest average impact factor: 14.39. The 21 studies reporting the effect of CBD on neurological conditions demonstrated that CBD reduced the frequency and severity of seizures and improved cognitive and motor abilities and behaviour.

Conclusions: Results gathered from the systematic literature search support that CBD has recognised therapeutic effects and has a promising pharmacological purpose. CBD is a naturally occurring compound derived from cannabis that has beneficial and therapeutic effects and also adverse effects. Further human studies investigating the therapeutic effects need to be carried out on a wider scale with carefully structured clinical trials.

Methanol extracts and volatiles of telekia speciosa (Schreb.) Baumg

Ermina Cilovic Kozarevic^{1*}, Jelena Arsentijevic², Zoran Maksimovic², Esmeralda Dautovic¹, Martin Kondza³, Esmeralda Dautovic, Vera Kerleta - Tu zovic^{1,4}

¹University of Tuzla, Faculty of Pharmacy, Bosnia and Herzegovina

²University of Belgrade, Faculty of Pharmacy, Serbia

³University of Mostar, Faculty of Pharmacy, Bosnia and Herzegovina

⁴Agency for Healthcare Quality and Accreditation in FB&H, Bosnia and Herzegovina

* ermina.cilovic@unitz.ba

Background: Telekia speciosa (Schreb.) Baumg is widespread in Eastern and Central Europe and the Balkan Peninsula. In Bosnia and Herzegovina, the root smoke of this plant is used in inhalations for bronchial asthma. Studies on the secondary metabolites and volatiles of this plant have been done sporadically.

Objectives: The objective of the present study was to analyse the presence of phenolic acids in methanol extracts from the aerial and underground parts of Telekia speciosa. The aim of the study was also to determine their antioxidant capacity. Volatiles from both parts of the plant were analysed.

Methods: RP-HPLC method was used for qualitative and quantitative analysis of the extracts. Component identification was performed by comparing their retention times and UV spectra with those obtained from standards. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assay and the ferric reducing antioxidant potential (FRAP) assay were used for the evaluation of the *in vitro* antioxidant capacity. The volatile of aerial and underground parts of T. speciosa were determined with GC-FID/MS. The identification of the individual compounds was based on the comparison of their retention times (tR), retention indices (RIs), and mass spectra with those obtained from authentic samples and/or listed in the NIST, Wiley mass spectral libraries, and the literature. For the quantification, the relative area percentages obtained by FID were used.

Results: The chlorogenic acid (CGA) and caffeic acid derivatives were present in the extracts. The CGA content was 977.8 ± 85 mg per 100g extract for underground and 272.0 ± 18 mg per 100g extract for aerial parts of T. speciosa. The extract of the underground plant parts (IC₅₀ = 129.57 ± 3.93 µg/mL-1, FRAP = 1337.48 ± 48.03 µmol Fe²⁺g-1) had three and a half times higher antioxidant capacity in comparison with the extract of the aerial plant parts (IC₅₀ = 497.79 ± 31.86 µg/mL-1, FRAP = 384.32 ± 9.54 µmol Fe²⁺g-1). The volatile constituents were characterised by the presence of a high concentration of oxygenated sesquiterpenes (dominantly isoeucalyptol).

Conclusions: Obtained results contribute to a better knowledge of the phytochemical properties of T. speciosa. The data about the antioxidant capacity of this plant have not been found by now. There were no available literature data about phenol acids in T. speciosa except some derivatives of phenol acids from a T. speciosa flower.

An alternative approach to circumvent low aqueous solubility for a BCS Class II compound using lipid-based suspensions

Gabriel Correia*, Dipanwita De

Thermo Fisher Scientific, Tilburg, The Netherlands

* gabriel.correia@thermofisher.com

Background: Most new chemical entities intended for oral use require non-traditional formulation approaches to circumvent low solubility in the GI fluids and insufficient intestinal permeation.

One of the strategies used to improve the bioavailability for BSC class II and IV makes use of lipid-based drug delivery systems, where the drug can be either dissolved or suspended in a mixture of excipients (oil, surfactant, or cosolvents)

Objectives: This study aimed to assess the efficacy of two suspensions with a shear thinning behaviour in enhancing the bioavailability of a BSC II molecule. The first suspension (F1), classified as type I according to Lipid Formulation Classification System (LFCS), consists of a combination of oils and a wetting agent. The second suspension (F2), classified as type II, is composed of a mixture of oils, water-insoluble surfactant (HLB=9), and a wetting agent.

Methods: Rheology was evaluated using a Modular Compact Rheometer, and flow curves were obtained at 25°C in rotational mode with a controlled shear rate from 0.1 to 100 s⁻¹. *In vitro* gastric dispersion was performed using a USP dissolution apparatus type 2 filled with 300 mL of simulated gastric fluid (SGF). *In vitro* lipolysis was performed according to a protocol established by the LFCS consortium.

Results: The release of the API from lipophilic matrices in simulated gastric fluid (SGF) was moderate, primarily due to limited fat digestion and low solubility in this medium. About 7-8% (vs claim) of the API was recovered for F2, while F1 yielded only 4-5% recovery. However, during lipolysis, a substantial increase in API recovery was observed in the aqueous phase, particularly for F2 with 60% recovery, whereas F1 exhibited a mere 1% recovery.

These results indicate that the process of digestion significantly affects the drug's solubility in the aqueous phase. This can be attributed to the ability of lipid-based

formulations to form various colloidal structures, thereby enhancing the API's solubility.

Conclusions: Lipid-based suspensions offer a promising approach for formulating above the drug's saturation solubility point and promoting its solubilization in the intestine.

Identification of novel compounds targeting the mu-, kappa- and delta-opioid receptors

Roxana Damiescu^{1*}, Thomas Efferth²

¹*Institute of Pharmaceutical and Biomedical Sciences, Johannes Gutenberg University, Germany*

²*Department of Pharmaceutical Biology, Institute of Pharmaceutical and Biomedical Sciences, Johannes Gutenberg University, Germany*

* r.damiescu@uni-mainz.de

Background: Opioid overdoses have been one of the main causes of death in the United States for the past several decades, and it has been declared a public health emergency. Although the number of prescriptions has been decreasing over the last few years, there is still an increased number of people misusing pain relievers. Additionally, the COVID-19 pandemic has only made things worse and has settled a new negative record of opioid overdoses, many with deadly outcomes. The biggest problem is that currently, opioids are the strongest available analgesics on the market, and there are no other alternatives that work as strong against pain without causing addiction and tolerance in time.

Objectives: This study aims to search for new possible compounds isolated from natural products with potential analgesic effects but without the addictive effect by using different computational methods and in vitro testing.

Methods: Virtual screening was used to select from our chemical library, containing over 40000 natural compounds, the ones with the highest affinity toward the mu-, kappa- and delta-opioid receptors. The top hundred ligands were then docked with their receptors, respectively. Various parameters, including Lipinski's rule of five, were applied to select the compounds with the best molecular properties, which were then further tested in vitro.

HEK cells expressing the mu-, kappa- and delta-opioid receptors, respectively, were then incubated for 24 hours with the compound of interest, and the changes in cAMP accumulation were analysed using HTRF technology. In parallel, through microscale thermophoresis, the binding affinity of these selected compounds towards the protein was then assessed.

Results: Data regarding the binding affinity as well as cAMP accumulation of these compounds are still being collected.

For the mu-opioid receptor, the microscale thermophoresis analysis revealed various promising candidates. However, for some compounds, high concentrations were required. Nonetheless, preliminary results reveal one possible candidate that selectively interacts with the mu-opioid receptor and shows both a strong binding affinity as well as relevant dose-dependent changes in cAMP accumulation. The measurements of the cAMP accumulation for the KOR- and DOR-HEK cells displayed a common candidate, yet different from the one interacting with the MOR-HEK cells.

Conclusions: This study identified new possible candidates isolated from natural products that can be further used in the development of new possible candidates with analgesic effects.

A comparative study on four Egyptian allium species: In vitro antioxidant, total phenolic, and flavonoid content.

Yasmin A. Elkhawas*, Noha Khalil

Future University In Egypt, Egypt

* yasmien.alaa@fue.edu.eg

Background: The genus *Allium* has many vital crops for human health. They include a variety of health-promoting chemicals, including polyphenols (particularly flavonoids), sulphur compounds, vitamins, mineral elements, and antioxidants.

Objectives: This study was designated to investigate the total phenolic and flavonoid contents as well as evaluate the in vitro antioxidant activity of the methanolic extracts of four *Allium* species growing in Egypt. Two of them are subspecies of *Allium cepa* L. (green and red onion), and the other two species are *Allium sativum* L. (garlic), *Allium porrum* L. (leek).

Methods: The Folin-Ciocalteu assay was used to quantify total phenolic content, and three assays were used to determine antioxidant activity, including 2,2-diphenyl-picrylhydrazyl (DPPH) assay, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABST) assay and ferric reducing antioxidant power (FRAP) assay.

Results: Results showed that total phenolic content (TPC) ranged between 9.81 ± 0.17 to 24.75 ± 2.06 mg gallic acid equivalent (GAE)/100mg dry weight (DW), while total flavonoid content (TFC) ranged between 8.88 ± 0.65 to 19.80 ± 0.64 mg (rutin equivalent) RE/100 gm DW. *Allium porrum* L showed the highest absorbance in the FRAP assay, 118.8 ± 7.21 μ M/mg. The results showed that *A. cepa* (green onion) and *Allium sativum* have the highest phenolic contents. On the other hand, in vitro antioxidant activity revealed that *A.*

cepa (green onion) and *A. porrum* have high antioxidant activities.

Conclusions: In this study, the assessment of antioxidant activity indicates that edible wild leafy plants with higher phenolic and flavonoid contents could be a significant source of natural antioxidants. There is a positive correlation between the antioxidant activity and total phenolic contents of the tested *Allium* species.

Maqui [*Aristotelia chilensis* (Molina) Stuntz] anthocyanin concentrates as a potential nutraceutical

Alexander Gamboa*, Amador Alburquenque, Cristian Tapia, Dra Lilian Abugoch

Universidad de Chile, Chile

* agamboa@ciq.uchile.cl

Background: Natural polyphenols are bioactive compounds widely distributed in berries, where maqui can be highlighted as a rich source of anthocyanins. These molecules are responsible for the colour of these fruits and whose extract is marketed mainly as a solid dispersion in maltodextrin.

Objectives: The objective of this work was to prepare high-purity concentrates of maqui anthocyanins as a potential nutraceutical.

Methods: Three extraction solvent systems consisting of ethanol, methanol, and acetone in a 1:1 ratio with water were studied. All the products obtained were characterized (after lyophilization) in terms of antioxidant capacity (ABTS), percentage of anthocyanins, weight yield, and moisture. The best extract was reconstituted and purified again by preparative chromatography in the new Pure C-850 system and characterized by HPLC PDA and UHPLC MS.

Results: The weight yield of the extracts was 43.0%, 45.4%, and 46.5% for the 1:1 water cosolvents of ethanol, methanol, and acetone at a moisture content of 21.5%, 22.8%, and 19.2%, respectively. In these, the antioxidant capacity was 45.1, 53.3, and 72.9 mmol/L Trolox equivalent, respectively, and the maximum content of anthocyanins was 93.3% of the total chromatographic area. From the acetone: water cosolvent extract, two fractions were obtained by preparative chromatography, where the first was rich in delphinidin glycoside and the second in cyanidin glycoside, according to the analytical data.

Conclusions: In conclusion, this study indicates two highly purified fractions of maqui anthocyanins with potential use as a nutraceutical were obtained.

Correlation between phenolic composition of three varieties of Egyptian date seeds (*Phoenix dactylifera* L.) and their antidiabetic activity

Noha Khalil^{1*}, Mokhtar Bishr²

¹Department of Pharmacognosy and Medicinal Plants - Faculty of Pharmacy - Future University In Egypt, Egypt

²Arab Company for Pharmaceuticals and Medicinal Plants (Mepaco Medifood), Egypt

* noha.hassan@fue.edu.eg

Background: Date seeds are produced in huge amounts as a waste by-product from the date fruit industry. However, they are medicinally important as they are rich in valuable secondary metabolites.

Objectives: This study aimed at profiling the phenolic constituents present in the aqueous extracts of three date varieties cultivated in Egypt: Ajwa, Sukkari and Zaghlool (Khalal stage), together with determining their antidiabetic effect.

Methods: Phenolic constituents were identified using ultrahigh performance liquid chromatography–electrospray ionization mass spectrometry (UPLC-ESI-MS/MS). The antidiabetic activity was evaluated through in-vitro inhibition of dipeptidyl peptidase IV (DPPIV), α -amylase and α -glucosidase enzymes, and in-vivo in streptozotocin-induced diabetic rat models.

Results: Results showed that total phenolic content (TPC) ranged between 65.66 \pm 3.25 - 169.15 \pm 5.22mg gallic acid equivalent (GAE)/100mg dry weight (DW), while total flavonoid content (TFC) ranged between 28.45 \pm 1.88 - 112.69 \pm 6.35 mg (rutin equivalent) RE/100 mg DW. The seed extracts also exhibited good radical scavenging activity on DPPH with IC₅₀ ranging between 42.02 \pm 3.11 - 78.41 \pm 8.25 μ g/ml. The highest TPC, TFC and, thus, the antioxidant effect were recorded for Zaghlool date seeds. UPLC-ESI-MS/MS profiles of the extracts were assessed. Among the identified polyphenolics, hydroxycinnamic and p-coumaric acids prevailed in the three seed extracts. All tested extracts displayed potent DPPIV inhibitory activities with IC₅₀ values of 3.66 \pm 0.89, 9.83 \pm 1.09 and 11.59 \pm 1.33 μ g/ml for Sukkari, Ajwa and Zaghlool seeds, respectively. The highest α -glucosidase inhibition was recorded for Zaghlool seed extract, with IC₅₀ 139.58 \pm 4.22 μ g/ml relative to the standard acarbose with IC₅₀ 130.68 \pm 2.64 μ g/ml. The highest α -amylase inhibitory effect was for Sukkari seed extract with IC₅₀ 3.64.58 \pm 0.78 relative to the standard acarbose with IC₅₀ 1.22 \pm 0.04. Moreover, the three seed extracts showed a significant ($p < 0.05$) reduction in levels of glucose, triglycerides and cholesterol in diabetic rats, almost like standard gliclazide, with the highest activity recorded for Zaghlool seed extract.

Conclusions: In conclusion, date seeds, as a potential nutraceutical rich in polyphenolics, represent a promising source for antioxidant and antidiabetic drug therapy.

Direct N^1 -selective alkylation of hydantoins using potassium bases

Takuya Kumamoto^{1*}, Yumi Shintani¹, Koichi Kato², Masashi Kawami¹, Mikihiisa Takano¹

¹Hiroshima University, Japan

²National Center of Neurology and Psychiatry, Japan

* tkum632@hiroshima-u.ac.jp

Background: Hydantoins, including the antiepileptic drug phenytoin, contain an amide nitrogen and an imide nitrogen, both of which can be alkylated. Due to the higher acidity of its proton, N^3 can be more easily alkylated than N^1 under basic conditions.

Methods: In this study, the authors explored methods for direct N^1 -selective methylation of phenytoin.

Results: When phenytoin was subjected to the conventional methylation reaction (NaH, CH_3I , DMF), a mixture of N^3 -monomethylated and N^1, N^3 -dimethylated phenytoins were obtained. After the trials using various bases and solvent, it was found that conditions using potassium bases [potassium tert-butoxide (tBuOK) and potassium hexamethyldisilazide (KHMDS)] in tetrahydrofuran (THF) gave N^1 -monomethylated phenytoin in good yield (69-79% yield). The reaction completed within 5 min. The applicable scope of this reaction system was found to include various hydantoins and alkyl halides. To explore the function of methylated hydantoins, the effects of a series of methylated phenytoins on P-glycoprotein were examined, but none of the methylated products showed inhibitory activity toward rhodamine 123 efflux by P-glycoprotein.

Conclusion: The authors optimised the reaction conditions for N^1 -selective alkylation on hydantoins such as phenytoin and found that reaction systems using potassium bases such as tBuOK and KHMDS in THF gave N^1 -methylated products in good yield and regioselectivity within short reaction period (5 min). Even though no inhibitory activity of methylated hydantoin toward P-gp efflux of rhodamine 123 was not observed in this experiment, it is under discussion whether phenytoin and its derivatives can be a substrate for P-gp.

Pre-clinical study of a dietary supplement prototype of pentacyclic triterpenes obtained from *Cecropia angustifolia* roots as a modulator of type 2 diabetes

Johanna Valentina Lopez Cortes*

Universidad Icesi, Colombia

* jvlopez1@icesi.edu.co

Background: Pentacyclic triterpenes are a large group of secondary metabolites that show biological activity. This has been evidenced through different studies carried out on various plants, such as *Eribotrya japonica*, *Cecropia telenitida*, and *Eucalyptus tereticornis*, among many others. Its biological activity stands out as anti-inflammatory, anticancer, antiviral, and mainly antidiabetic. Much research has been done on their potential use in regulating metabolic disturbances such as dyslipidemia, glucose tolerance, insulin resistance, inflammation, obesity, and hypertension. In addition, these factors are related to a higher risk of type 2 diabetes mellitus and other cardiovascular diseases, which is why they are of great interest in the investigation since DM2 has a high incidence in Colombia.

According to figures from the Colombian Ministry of Health and Social Protection, DM2 is one of the main causes of death in people between 30 and 70 years of age, and it is estimated that three in every ten Colombians suffer from DM2.

Objectives: Considering the above and what we have shown in previous studies carried out by the research group on the therapeutic potential of pentacyclic triterpenes, the objective of this research was to evaluate the safety and efficacy of a prototype dietary supplement made from a fraction of chemically controlled formulation of pentacyclic triterpenes obtained from the roots of *Cecropia angustifolia*, transported in a nutritional oil of Sacha Inchi.

Methods: Pharmacokinetic parameters and bioavailability of the prototype were initially determined in healthy male New Zealand rabbits after a single dose was administered, either orally or intravenously. In this section, it was possible to increase the bioavailability five times concerning the fraction of non-formulated pentacyclic triterpenes, showing that Sacha Inchi oil is a suitable vehicle to increase the intestinal absorption of these metabolites. Subsequently, in vitro tests were performed on cell lines to explain the obesity model. From this it was obtained that the pentacyclic triterpenes present in our prototype do not affect lipid metabolism or glucose uptake; however, they increase insulin production and resistance to it and inhibit the secretion of certain proinflammatory cytokines. Finally, in vivo tests were performed on male C57BL/6J mice in a prediabetic state, where a biochemical and toxicological analysis was performed during and after continuous administration of the dietary supplement prototype and the respective controls.

Results: From this, it was obtained that after continuous administration of the prototype, animals treated with a high-calorie diet showed a decrease in glucose levels during fasting and better glucose and insulin tolerance, as well as higher insulin levels in these.

Conclusions: These results demonstrate the great potential of these metabolites to modulate important factors to control DM2. And in addition to generating pre-clinical evidence, they give way to subsequent studies where the safety and efficacy of healthy and sick humans are evaluated at a clinical level. All the results are part of my current master's thesis and project 67730 – Colciencias 2020-2023.

Remdesivir loaded nanoparticles as a potential treatment for COVID-19

Salam Massadeh^{1,2,3,4,5*}, Manal Alaamery^{1,2,3,4,5}

¹King Abdullah International Medical Research Center, Saudi Arabia

²King Saud University for Health Sciences, Saudi Arabia

³Ministry of National Guard Health Affairs, Saudi Arabia

⁴KACST-BWH Centre of Excellence for Biomedicine, Joint Centers of Excellence Program, King Abdulaziz City for Science and Technology (KACST), Saudi Arabia

⁵Saudi Human Genome Project, King Abdulaziz City for Science and Technology, Saudi Arabia

* massadehsa@ngha.med.sa

Background: Remdesivir (GS-5734) is a broad-spectrum antiviral prodrug with activity against RNA viruses from several families. It specifically targets viral RNA-dependent RNA polymerase (RdRp) and demonstrates no activities against human RNA pol II or mitochondrial RNA polymerase.

Objectives: In this study, the authors attempted to load this drug into Tri-block Poly (lactide) poly (ethylene glycol) poly(lactide) copolymers (PLA-PEG-PLA) based nanoparticles.

Methods: The use of PLA-PEG-PLA copolymers as Drug carriers have been approved by the Food and Drug Administration (FDA) and the European Medicine Agency (EMA). Preparation of PLA-PEG-PLA nanoparticles was achieved by applying the double emulsion method.

Results: This study's synthesis protocol achieved a polydispersity index of 0.29. This indicates uniformity in size and lack of aggregation. The drug-to-polymer ratio used here was 1:20 with a theoretical drug load of 5%. A total of 87.23 µM was encapsulated within approx. 4mg of Remdesivir loaded NPs. The result shows that this synthesis protocol achieved a 21.75% encapsulation efficiency.

Microglial NMDA receptors and mitochondria-derived superoxide mediate Aβ1-42 oligomer-induced neurotoxicity in primary rat brain cultures

Ramune Morkuniene^{1,2*}, Aiste Jekabsone², Silvija Jankeviciute¹, Katryna Pampuscenko¹, Vilmante Borutaite¹

¹Institute of Neurosciences, Lithuanian University of Health Sciences, Lithuania

²Faculty of Pharmacy, Lithuanian University of Health Sciences, Lithuania

* ramune.morkuniene@ismuni.lt

Background: Ageing is the main risk factor for most neurodegenerative diseases, including Alzheimer's disease (AD). More than 55 million people suffer from dementia, and there is currently no effective treatment available to cure ageing-related neurodegenerative diseases. The pathology of AD involves abnormal accumulation of amyloid beta (Aβ), and the link between Aβ1-42 structure and toxicity is of major interest, in particular, the neurotoxic potential of oligomeric species. The proposed mechanisms of neurotoxicity of Aβ1-42 oligomers include disruption of Ca²⁺ homeostasis, promotion of inflammatory reactions or mitochondrial dysfunction. NMDA receptors (NMDA-R) are thought to be essential in Aβ-affected neurons, but the role of this receptor in the abnormality of glia is unclear. In addition, Aβ effects on the redox state of neurons and glial cells, which may have an impact on the activation of inflammation and neuronal viability, are not entirely understood.

Objectives: In the present study, the authors investigated whether various Aβ1-42 species, small oligomers (z-high < 5 nm), large oligomers (> 5 nm), insoluble fibrils and monomers are capable of producing neurotoxic effects via microglial NMDA-R activation and changes in mitochondrial redox state in primary rat brain cultures.

Results: This study found that small Aβ1-42 oligomers induced rapid and significant increase in intracellular Ca²⁺ in primary microglial cell culture. Moreover, small Aβ1-42 oligomers induced concentration- and time-dependent microglial necrosis, whereas bigger Aβ1-42 aggregates or monomers did nothing for intracellular Ca²⁺ or microglial death. Importantly, NMDA-R inhibitors MK801, memantine, or D-2-Amino-5-phosphopentanoic acid (DAP5) completely prevented a small Aβ oligomer-induced increase in microglial intracellular Ca²⁺ concentration. However, the inhibitors were not as effective in preventing microglial death caused by Aβ. Next, small Aβ1-42 oligomers, but not larger Aβ1-42 species, induced neuronal and microglial mitochondrial superoxide generation and mitochondrial depolarization in mixed primary neuronal-glia cultures. These effects were prevented in the presence of selective mitochondria-targeted antioxidant MitoTempo, which also suppressed glutamate release to the culture medium and neuronal death.

Conclusions: In summary, small A β 1-42 oligomers, but not monomers, large oligomers or fibrils, elevate intracellular Ca²⁺ concentration in microglia by triggering NMDA receptors. In addition, only the small A β 1-42 oligomers induce mitochondrial ROS-mediated mitochondrial depolarisation and glutamate release to the extracellular medium, contributing to neuronal cell death. Hence, pharmacological inhibition of microglial NMDA-R and mitochondrial ROS can protect neurons from small A β oligomer-induced damage in Alzheimer's disease.

Molecular docking and molecular dynamic study of some flavonoid and terpenoid compounds for Alzheimer therapy

Fauzan Zein Muttaqin^{1,2}, Dewi Setyani², Sophi Damayanti³

¹Indonesian Pharmacist Association, Indonesia

²Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Bhakti Kencana University, Indonesia

³School of Pharmacy, Bandung Institute of Technology, Indonesia

* fauzanzein@iai.id

Background: Alzheimer's disease is the most common cause of dementia. AChE inhibitors are one of the most potent drug molecules against Alzheimer's disease. Flavonoid and terpenoid are lead compounds which expected to act as AChE inhibitors. Molecular Docking and Molecular dynamic are important computational for identifying the structural modelling and predicting the drug activity.

Objectives: This study aims to observe and compare the ability of flavonoid and terpenoid compounds to inhibit the AChE receptor.

Methods: Molecular Docking using Autodock 1.5.6 software and Molecular dynamic simulation using Gromacs with simulation time during one ns were carried out to predict the ligand affinity to the receptor. The molecular dynamic simulation was carried out for three flavonoids and three terpenoids.

Results: Sudachitin (Flavonoid) and Siderol (Terpenoid) showed better activity than other compounds of ligand. The free binding energy and inhibition constant of sudachitin were -5.18 kcal/mol and Ki 160.89 μ M, respectively, while Siderol were -8.30 kcal/mol; Ki 821.71 nM, respectively. Molecular Dynamic simulation results showed that the RMSD value of the ligand-receptor complex was lower than the RMSD value of the receptor. It indicated that the ligand stabilized the complex.

Conclusions: This study revealed that Sudachitin and Siderol had better potential than the other compounds as AChE inhibitors for anti-Alzheimer candidates.

Medicinal cannabis use in rare diseases

Jekaterina Parovincaka*, Janis Vella-Szjij, Anthony Serracino-Ingloott

University of Malta, Malta

* livshina@inbox.lv

Background: Rare diseases (RDs) affect over 300 million people worldwide. There are approximately 7,000 recognised RDs. RDs are usually genetic, with childhood or adulthood onset and are associated with severely debilitating symptoms which persist for a patient's lifetime. RD patients often face multiple issues, ranging from difficulty in establishing an accurate diagnosis to a lack of accessible treatment options. Medicinal Cannabis (MC) is used to relieve symptoms, such as pain, anxiety and muscle spasticity, which may be commonly experienced by patients with RDs.

Objectives: To identify RDs for which MC is of interest.

Methods: A systematic literature review was carried out. Open access peer review journal articles published in English in PubMed Central or MEDLINE databases between January 2011 – September 2021 were included.

Results: Thirty-eight identified articles describe the use of MC as a possible therapeutic option in 23 RDs: epileptic conditions (n = 7), neurodegenerative diseases (n = 6) and skin disorders (n = 4), a number of them of early childhood onset (n = 12). Literature suggests that MC can be used in RDs which are associated with pharmaco-resistant seizures, such as Dravet Syndrome (n = 14), Lennox-Gastaut Syndrome (n = 13), Tuberous sclerosis complex (n = 4); in neuropathic pain and spasticity (Neurofibromatosis type 1 (n = 1), Multiple Sclerosis (n = 4)); in skin disorders (Epidermolysis bullosa (n = 1), Scleroderma (n = 1)); also obesity in Prader-Willi syndrome (n = 1), gastrointestinal symptoms in chronic intestinal pseudo-obstruction (n = 1). Studies show improvement in patients' Quality of Life (QOL) and low incidence of severe adverse events associated with MC use. Studies reported the use of CBD (Cannabidiol, n = 16), Cannabinoid-based medicines (CBMs, n=7), synthetic derivatives of MC (n = 4) or 'Sativex' (THC: CBD in 1:1 ratio, n = 2) in patients with RDs. The number of publications in 2020 and 2021 on the MC use in RDs has increased, demonstrating that research on the use of MC as a therapeutic option in RDs is emerging.

Conclusions: Literature suggests that MC can be used in certain RDs. In lack of efficacious treatment options, MC can be an alternative therapy for symptom relief. There is need

for further studies involving academia and industry investigating the use of MC therapies in RDs.

A computational approach of structure-based virtual screening towards the discovery of selective 5 α -reductase type II inhibitors for BPH treatment

Hanan Refaat*, Nasser S.M. Ismail, Asmaa A. Mandour

Future University In Egypt, Egypt

* Hanan.Refaat@fue.edu.eg

Background: 5 α -Reductase type II (5 α R2) inhibition has been considered one of the most promising strategies for Benign Prostatic Hyperplasia (BPH) treatment. This enzyme catalyses androgen dihydrotestosterone (DHT) formation from testosterone leading to elevated levels of DHT responsible for the abnormal growth of the prostate in male humans.

Objectives: In this research, a computational approach based on virtual screening, including ligand-based 3D pharmacophore modelling, 2D QSAR, and molecular docking simulations, was adopted to develop novel, potent and selective 5 α R2 inhibitors.

Methods: Virtual screening of Maybridge and National Cancer Institute databases was performed to discover potential hits. The Hits were filtered via the validated best pharmacophore model (Hypo4) and 2D QSAR model regarding the fit value and estimated log IC₅₀, then subjected to a molecular docking experiment. Hereby, docking was performed on the recently determined co-crystallized structure of 5 α R2, not the 5 β R-related structure as in previously published research. Visual inspection was used to discover the mechanism of action and the binding interactions with the essential amino acids compared to finasteride ligand and DHT, as reference.

Results: Three hits named Hit 1, 2 and 3 were identified and explained the role of binding to Glu57 and Tyr91 for 5 α R2 selective inhibition based on dihydro finasteride and NADP (NADP-DHF) adduct formation. Alignment between Hit 2 and Finasteride in the binding pocket showed a similar binding mode of sulphonamide moiety with the key amino acids of 5 α R2.

Conclusions: Biological activity prediction of the three hits using Pass Online-Way2Drug showed potential antitumor and androgen targeting activity that encourages their use as BPH potential therapy.

Characterisation and purification of antimicrobial biosurfactants towards their individual usage in pharmaceutical applications

Maissa Dardouri¹, Rita Mendes¹, Johannes Frenzel², Isabel Ribeiro^{1*}

¹Research Institute for Medicines, iMed.Ulisboa, Faculty of Pharmacy, Universidade de Lisboa, Portugal

²Pharmaceutical Institute, Rheinische Friedrich-Wilhelms-Universität Bonn, Germany

* iribeiro@ff.ulisboa.pt

Background: Sophorolipids (SLs) and rhamnolipids (RLs) are glycolipid biosurfactants that have been at the centre of scientific and industrial research. Recently, these biosurfactants have gathered attention not only for their physicochemical properties, low toxicity, and biodegradability but also for their antibiofilm and antimicrobial activity, making them very promising molecules with diverse applications. These special properties arise from the different structures of biosurfactants present when produced. Nevertheless, occurring in nature as mixtures represents a drawback towards specific applications in pharmaceutical, cosmetics and biomedical fields.

Objectives: This work aimed at the separation and purification of SLs and RLs by developing and improving different chromatographic methodologies.

Methods: Thus, Thin Layer Chromatography (TLC), Flash Chromatography (FC), Solid Phase Extraction (SPE) and Ultra High-Performance Liquid Chromatography-Tandem Mass Spectrometry (UHPLC-MS/MS) methods were used.

Results: With this work, a novel dichloromethane based-TLC mobile phase was proposed and proven to be suitable to replace the mobile phases usually used for SLs and RLs separation that includes the chloroform. Besides achieving the separation of most active compounds through FC, a new and alternative SPE method was achieved. A reverse phase C18 solid phase extraction (SPE) showed a selective isolation and purification of the biosurfactants C18:0 and C18:1 lactonic diacetylated SLs, with a purity of 85.7% and 94% respectively. Moreover, RhaC8:0C8:0, RhaRhaC8:0C12:0, RhaRhaC10:0C10:0 and RhaC10C10:1 RLs congeners were obtained with a purity of 99.56%, 89.9%, 86.6% and 81.2% respectively. Additionally, eight SLs and 13 RLs congeners were identified by an alternative UHPLC-MS/MS fast method.

Conclusions: To conclude, these optimised methods revealed to be a good alternative for the laborious, time consuming and the toxic conventional ones used for biosurfactants separation and purification and will certainly reduce the hard work of researcher teams focused on the biosurfactants production and purification.

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Evaluation of the neuroprotective potential of ibogaine in an in vitro neurodegeneration model for Alzheimer's disease

Manuela Bentura¹, Florencia Arredondo¹, Ignacio Carrera², Cecilia Maldonado³, Eduardo Savio^{1*}

¹Centro Uruguayo de Imagenología Molecular, Uruguay

²Departamento de Química Orgánica, Facultad de Química (UdelaR), Uruguay

³Departamento de Ciencias Farmacéuticas, Facultad de Química (UdelaR), Uruguay

* eduardo.savioq@gmail.com

Background: Ibogaine, found in the root bark of the African shrub *Tabernanthe iboga* is the most studied of the iboga alkaloids, a group of naturally occurring and synthetic indole alkaloids that possess unique biological activities. Particularly, ibogaine displays an interesting anti-addictive property that has been found in pre-clinical models and in observational and open-label clinical trials in human beings.

This anti-addictive effect is long-lasting, transcending the pharmacokinetic elimination of ibogaine and its active metabolite (noribogaine) in the body. Although the mechanisms of action of ibogaine and noribogaine have not been elucidated, their long-lasting effects suggest the induction of plasticity processes in the nervous tissue. Previous studies carried out in Uruguay show that the administration of ibogaine in rats promotes the expression of GDNF and BDNF (neurotrophic factors derived from glial cells and from the brain, respectively), proteins linked to the survival, repair and maintenance of nervous tissue. Taking into account that the decrease in GDNF expression has been linked to the development of neurodegenerative diseases such as Alzheimer's and Parkinson's disease, ibogaine and structurally related alkaloids could represent interesting neuroprotective activities in this regard.

Objectives: Characterisation and evaluation of the neuroprotective potential of ibogaine in an in vitro neurodegeneration model for Alzheimer's disease.

Methods: Different concentrations of ibogaine were placed on cultures of 3xTg-AD transgenic astrocytes, grown in 96-well plates and maintained using DMEM + FBS 10%, to determine its toxicity (IC₅₀). Subsequently, primary cultures of cortical/hippocampal neurons of C57B6J mouse embryos (E15-17), cultured for 12 days in vitro (DIV), were subjected

to neurotoxic damage through exposure to a conditioned medium of astrocytic cultures derived from 3xTg-AD treated with different concentrations of ibogaine and vehicle. In this manner, the capacity of ibogaine to reduce the toxicity of astrocytes, and thus prevent or attenuate neuronal death in this paradigm was evaluated. A 96-well plate format was used to assess neuronal viability as a screening method for the neuroprotective potential of ibogaine using the MTT assay.

Results: An IC₅₀ value of ibogaine on astrocytes 3xTg-AD of 169 microM was obtained. The MTT assay is used to measure cellular metabolic activity as an indicator of cell viability, proliferation and cytotoxicity. In the treatment of cortico-hippocampal neuronal cultures with astrocyte-conditioned media, a great reversal of the toxicity of these cells was observed when increasing the concentration of ibogaine in the treatments. In the case of the treatments with higher concentrations of ibogaine (150 and 200 microM), greater metabolic activity is observed in the neurons than in the positive controls.

Conclusions: Ibogaine is toxic to transgenic astrocytes at concentrations close to 170 microM. A reversal of the toxicity of astrocytes on neurons is observed when treated with concentrations of ibogaine close to its IC₅₀, encouraging further assays to assess and characterize the neuroprotective effect of this alkaloid.

In vitro plant cell culture as a source for production of pentacyclic triterpenes acids from the roots of a Colombian native plant for management of prediabetic state

Juan Sebastian Vasquez Delgado*

Universidad Icesi, Colombia

* jvasquez1@icesi.edu.co

Background: Plants are a powerful source of molecules for treating many diseases. In Colombia, due to the high biodiversity, the authors are able to research many native plants that have the capacity to produce secondary metabolites of high interest in the pharmaceutical industry. In this study, the genus *Cecropia* spp. was taken as the focus of the research due to the presence of pentacyclic triterpenes acids in their roots. These molecules have been extensively researched due to their promising results in different investigations and their broad spectrum of action in different diseases.

Objectives: With regard to this, the authors decided to investigate pentacyclic triterpenes acids' properties against metabolic diseases and alternative sources for obtaining them. A very important part of this project was the standardization of the metabolic profile and the expression of

these metabolites, standardizing the in vitro growing conditions with the aim of securing a chemically controlled fraction.

Methods: The first step was the collection, drying and extraction of vegetal material obtained in Pance, Valle del Cauca. Then, the raw extract was purified using normal phase flash chromatography and ethyl acetate/dichloromethane as the mobile phase. Once obtained a group of cleaner extracts, the authors proceeded to characterise them, obtaining their metabolic profile using mass spectrometry. The results of this allow us to construct a pentacyclic triterpene metabolic library for this plant.

Results: Due to the high environmental impact generated by obtaining roots from wild trees and the variability associated with the metabolic behaviour of the wild plants, the authors opted for searching the best conditions to produce these roots with in-vitro plant cell culture techniques. In this order, the authors determined the best conditions for growing biomass in-vitro, together with the optimisation of the production of secondary metabolites. This study achieved important results, concluding that the best way to obtain a larger quantity of biomass together with the highest concentration of pentacyclic triterpenes is the use of a temporal immersion system together with Methyl Jasmonate as the best abiotic elicitor for this class of compounds.

Conclusions: To determine metabolic differences between wild plant extracts and the obtained from in vitro sources, we used mass spectrometry techniques including UPLC – SQD2 and MALDI – TOF, obtaining significantly different results concerning to production and distribution of the secondary metabolites and the very clear differences between the metabolism of in vivo and in vitro plants.

Small extracellular vesicles derived from adipose tissue mesenchymal stem cells: Possible new therapy against osteoarthritis

Alvaro Compañ-Bertomeu^{1*}, María Luisa Ferrandiz¹, María Isabel Guillen², María José Alcaraz¹

¹Universitat De Valencia, Spain

²Universidad Cardenal Herrera-CEU, Spain

* alcomber@alumni.uv.es

Background: Osteoarthritis is the most common joint problem in older people due to age overload due to obesity, oxidative stress and inflammation. Mesenchymal stem cells of adipose tissue (AD-MSC) represent an attractive option in regenerative medicine due to their repairing and immunosuppressive potential. The chondroprotective effect of the conditioned medium (CM) from AD-MSC has been

demonstrated. These effects are largely mediated by small extracellular vesicles (sEV) present in CM. sEV is actively secreted by cells and represents a mechanism for cell-to-cell signalling in physiological and pathophysiological responses.

Objectives: Update advances in the therapeutic use of sEV in the treatment and diagnosis of osteoarthritis.

Methods: To carry out this study, the Pubmed search engine and the Clinical Trials database have been used using the keywords: extracellular vesicles, adipose tissue mesenchymal stem cell, and osteoarthritis in the last five years in high-impact specialised journals.

Results: The authors have collected evidence that human AD-MSC CM sEV, as well as from other sources, exert immunomodulatory and protective effects on osteoarthritis chondrocytes. However, we have found a small number of clinical trials.

This review tests a new form of immunotherapy, enhancing the physiology of cartilage and maintaining the viability and functionality of the joint, which has an impact on a higher quality of life for the patient.

Conclusions: Despite the promising results of AD-MSC sEV, it is necessary to deepen both their knowledge and the molecular mechanisms that govern the process of osteoarthritis pathophysiology.

Rational design of heat shock protein (HSP-70) inhibitors

Laurent Grech, Claire Shoemake*

University of Malta, Malta

* czer1@um.edu.mt

Background: The heat shock protein 70 (HSP-70) has a cytoprotective role against tumour necrosis factor alpha apoptosis, enhancing cancer cell survival. HSP-70 is often overexpressed in triple-negative breast cancer (TNBC) cells. Various small molecule inhibitors targeting different TNBC survival pathways are in clinical trial phases.

This study was the first step in the identification and design of small molecules with the potential to challenge the tumour-protective role of HSP-70. A small molecule HSP-70 inhibitor VER-155008 scaffold was designated as a lead molecule for this study.

Methods: This study adopted a dual approach- virtual screening and fragment-based de novo design. In the virtual screening approach, two high affinity HSP-70 modulators, VER-155008 and ADP468, were identified, superimposed in

LigandScout, and a unique pharmacophore incorporating the critical features of both molecules was modelled and read into the ZincPharmer molecular database. Filters compliant with the rule of 3 for lead-likeness were imposed during the search process. The retrieved molecular cohort was then docked into a protomol. A protomol is a virtual ligand binding pocket that describes the area at the core of the receptor, which can be further stabilised through ligand binding. The affinity of each hit molecule was quantified in Sybyl-X as a total score, and the structures were ranked in order of affinity.

Results: A 2D topology map, showing the critical interactions between VER-155008 and the HSP-70 ligand binding pocket, as described in pdb crystallographic deposition 4IO83, was modelled in Discovery Studio. This guided the de novo design process. The bioactive HSP-70 ligand binding pocket was modelled in LigBuilder. Fragments capable of sustaining novel growth were docked into the modelled ligand binding pocket and tethered together in such a way as to ensure Lipinski Rule compliance and synthetic feasibility.

Conclusions: The molecular cohorts obtained through each approach were assessed in terms of affinity and physicochemical parameters, and the optimal structures were selected and compared. In vitro assays are being carried out for a primary assessment of bioactivity prior to further founts of optimisation.

Methods: Two-fold serial oil dilutions were prepared in a concentration range 10-0.005%. Amoxicillin was the reference antibiotic, prepared in two-fold serial dilution in a concentration range 1-0.0005 mg/ml. The inoculation was carried out according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines using the *Staphylococcus aureus* strain (ATCC25923) in saline solution and the 1:10 dilution in a nutrient broth. During the experiment, the Miler-Hinton agar temperature of 55±5°C was maintained, so it could remain liquid. The individual and the combined effects of individual oil and amoxicillin were examined.

Results: Minimum inhibitory concentrations (MIC) for amoxicillin and EOL were: 0.015 mg/ml and 10.43 mg/ml (1.2%) respectively. Synergism (fractional inhibitory concentration index, FICI=0.375) was observed when EOL and amoxicillin were combined.

Conclusions: The agar microdilution method has proven to be suitable for performance in a minimally equipped laboratory, and it distinguishes itself from others in terms of inexpensiveness, simplicity and reduction in the occurrence of separation of hydrophobic essential oils and hydrophilic medium.

Investigation of antimicrobial potential of essential oil of *Lavandulae officinalis* using agar microdilution method, *in vitro*

Jelena Antic Stankovic¹, Dubravka Bigovic²

¹University of Belgrade, Belgrade, Serbia

²Institute for Medicinal Plant Research Dr Josif Pancic, Belgrade, Serbia

*jelena.stankovic@pharmacy.bg.ac.rs

Background: Natural products, either pure compounds or standardised plant extracts, provide unlimited opportunities for novel drug treatments because of their unmatched range of chemical diversity. Due to the increasing incidence of antibiotic resistance, the study of ingredients of herbal extracts (essential oils) and the discovery and optimisation of new methods for their examination are being sought.

Objectives: The aim of this study was to determine the antimicrobial potential of essential oil of lavender (*Lavandulae officinalis aetheroleum*, *Lamiaceae*; EOL), as the effect of the simultaneous use of essential oils with amoxicillin *in vitro*, performing the agar microdilution method.