Antibiofilm Potential of Essential Oils: a New Approach for Fungal Infections

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ORIGINAL ARTICLE

ABSTRACT

Introduction: Fungal infections or mycosis represent a huge health and economic burden affecting over 1 billion individuals worldwide and accounting for around 13 million infections per year[1]. Moreover, patients with underlying health issues or a weakened immune system, including chronic lung disease, prior tuberculosis, HIV, cancer, or diabetes mellitus are at higher risk[2]. In this context, both dermatophytosis and candidiasis constitute a very serious health concern that requires effective therapeutic strategies. Despite the recent developments in antifungals, invasive fungal infections continue to impact patients' quality of life and mortality rates remain high. These poor outcomes are usually associated with antifungals´ high toxicity, unpleasant side effects and drug interactions and, most importantly, the emergence of resistant strains. To mitigate these negative impacts, the development of effective antifungals is imperative with aromatic plants standing out as promising sources of bioactive compounds that have shown antifungal properties[3]. Herein, we intend to deepen our knowledge on the antifungal potential of essential oils by exploring their effect on dermatophytes and Candida albicans biofilms, as these virulence factors are very difficult to eradicate due to their high resistance to both therapy and host defenses.

Methods: The effect of the essential oils was assessed on biofilm mass, extracellular matrix, and biofilm viability using crystal violet, safranin, and XTT assays, respectively. In addition, morphological alterations were confirmed by optical and scanning electron microscopy.

Results: Our studies point out promising essential oils being that of *Lavandula multifida*, rich in carvacrol, one of most promising. This essential oil significantly decreased biofilm mass and viability in all the tested strains and reduced dermatophytes extracellular matrix, particularly during biofilm formation. Moreover, fungi morphological alterations were evident in mature biofilms with a clear decrease in hypha diameter.

Discussion: These results are quite relevant as fluconazole, the most widely used antifungal in the clinic, fails to inhibit biofilms even at doses 200x higher than its minimal inhibitory concentration. Overall, the essential oils´ antibiofilm effects and inhibitory properties on fungal growth, justify the development of effective essential oil based-antifungals.

Keywords: antifungal, candidiasis, dermatophytosis, lavender, virulence.

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Coccidiostats, Chicken Eggs and Human Health

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ORIGINAL ARTICLE

ABSTRACT

Introduction: Aside from providing animal protein, eggs also contribute to our daily mineral, vitamin, and fatty acid needs offering a moderate calorie source and assuming a very important component of a healthy diet[1]. Chicken eggs have always been part of human diets throughout history. Food and Agriculture Organization (FAO) reported a sharp increase, of about 50%, in egg consumption since 2000. In order to prevent and control coccidiosis caused by Eimeria spp., poultry industry widely uses coccidiostats as feed additives. This can be harmful to consumers[2], affecting physiological functions, like drug resistance, hypersensivity, poisoning, carcinogenicity and teratogenicity[3].

Methodology: The main objective of this study was to evaluate the presence of nine synthetic/ionophore coccidiostats (lasalocid, narasin, salinomycin, monensin, maduramicin, halofuginone, robenidine, diclazuril, and nicarbazin) in 62 egg samples by UHPLC-MS/MS.

Results and Discussion: Overall, detection frequency and average concentration were 90.3% (56/62) and 106.3 μg kg^{-1}, respectively. Only diclazuril and nicarbazin were detected. Diclazuril, only found in home raised eggs, showed an overall detection frequency of 8.1% (5/62), with an average and a maximum concentrations of 0.46±1.90 μg kg^{-1} and 13.6 μg kg^{-1}, respectively. Nicarbazin presented an overall higher frequency, 88.7% (55/62), with levels up to 744.8 μg kg^{-1}. Additionally, 4 samples (6.5%) presented both nicarbazin and diclazuril. Home raised egg samples (n=28) showed a detection frequency of 89.3% being nicarbazin found in more samples (85.7% vs. 17.9%) and at higher levels (266.3±169.4 μg kg^{-1} vs. 0.91±2.78 μg kg^{-1}), when compared to diclazuril. In supermarket samples (n=34) only nicarbazin was detected, in 31 samples (91.1%), with an average of 167.6±62.2 μg kg^{-1}.

Considering the average contamination scenario, and according to the risk assessment performed[4], consumers' health should not be adversely affected by egg consumption. However, in every scenario considered, children were the most vulnerable population group. Given the scarcity of published studies, it is imperative to pay greater attention to this topic.

Keywords: coccidiostats in eggs, UHLC-MS/MS, risk assessment.

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Subprodutos Industriais de Frutos da Bacia do Mediterrâneo: Potencial para Reintrodução na Cadeia de Alimentar

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ORIGINAL ARTICLE

RESUMO

Introdução: Os frutos são alimentos essenciais na dieta mediterrânea devido ao seu grande conteúdo nutricional e benefícios para a saúde. Todos os anos são geradas toneladas de subprodutos tais como cascas e sementes de frutos como consequência do seu processamento industrial. Além disso, podem encontrar-se nestes subprodutos um maior teor de compostos bioativos, como compostos fenólicos, do que na parte edível. No entanto, a sua segurança pode ser comprometida por riscos químicos como micotoxinas ou resíduos de pesticidas. Este estudo avaliou a possibilidade de reintrodução destes subprodutos de frutos na cadeia alimentar.

Metodologia: No âmbito do projeto ValICET foram selecionados vários subprodutos de frutos tais como citrinos, cerejas, tâmaras e uvas para serem avaliados quanto ao valor biológico de forma a aferir o seu potencial de reutilização pela indústria alimentar. Estes subprodutos de frutos foram recolhidos de várias indústrias dos países parceiros do projeto, nomeadamente França, Itália, Portugal e Tunísia. Para a caracterização da capacidade antioxidante, os extratos etanólicos concentrados foram testados utilizando o ensaio de branqueamento do β–caroteno e o ensaio de inibição do radical DPPH. O perfil de fenólicos dos subprodutos foi determinado por Cromatografia Líquida de Ultra Resolução combinada com Espectrometria de Massa (UHPLC-ToF-MS). Este estudo também avaliou a presença de micotoxinas e resíduos de pesticidas através de duas metodologias (baseadas em extração sólido-líquido e QuEChERS) seguidas de cromatografia líquida acoplada a espectrometria de massa.

Resultados: Em geral, todos os subprodutos de frutos estudados obtiveram uma elevada capacidade antioxidante. Os extratos de caroços de tâmara e grainha e uva apresentaram a maior capacidade antioxidante e níveis mais elevados de fenólicos e flavonoides totais. A catequina e a epicatequina foram os principais compostos

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fenólicos quantificados no subproduto de tâmara (63,15 mg/g e 57,91 mg/g, respetivamente), reconhecidos pelas suas propriedades antioxidantes. Relativamente aos contaminantes químicos, nenhum dos 155 resíduos de pesticidas ou das oito micotoxinas foram encontrados nos subprodutos de frutos acima dos limites máximos para cada contaminante, comparativamente à matriz edível.

Discussão: Estes resultados mostram que os subprodutos de frutos são ricos em compostos bioativos com alta atividade antioxidante e, por isso, são uma potencial fonte de antioxidantes naturais de baixo custo. A reintrodução destes subprodutos de frutos, que têm reduzida utilização comercial, na cadeia de abastecimento alimentar, é uma solução para reduzir o desperdício alimentar e contribuir para a economia circular.

Palavras-chave: subprodutos industriais, frutos, compostos fenólicos, capacidade antioxidante, economia Circular.

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Heavy Metals and Metalloids in Children's Food: The Nickel Example

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ORIGINAL ARTICLE

ABSTRACT

Introduction: Children are the most vulnerable population group to food contaminants due to their relationship between food ingestion and body weight. In the case of heavy metals and some metalloids, there is an increased concern due to their toxicity and bioaccumulation properties[1]. Several of these compounds pose a threat to humans, namely through the consumption of contaminated food, like mercury, chromium, lead and nickel[2]. Although some of these compounds have been evaluated for a long time, nickel is a growing problem. Nickel is used in electroplating, batteries, electronic equipment, alloys like stainless steel, pesticides, fertilizers and herbicides. This led to increased use in recent years and a growing burden on the environment which can represent a serious hazard to human health[3].

Methodology: Therefore, two studies were conducted to evaluate the risk posed by the exposure of children to nickel through commercial premade baby foods and homemade baby foods in Portugal, observing the Commission Recommendation (EU) 2016/1111. The analytical methodology used for the determination of nickel was dry ashing followed by graphite furnace atomic absorption spectrometry which allowed for a method detection limit of 17.4 $\mu g.kg^{-1}$.

Results and discussion: For the commercial premade baby foods 26 ready meals, 31 fruits, 8 deserts and 20 paps were analyzed. The results showed that 78 (91.8 %) samples contained nickel, with values up to 225.7 $\mu g.kg^{-1}$. The group with the highest average concentration was the fruits (50.1 $\mu g.kg^{-1}$) followed by the ready meals (40.2 $\mu g.kg^{-1}$). Samples from organic farming presented higher detection frequency and average concentration (54.7 $\mu g.kg^{-1}$) than the others. As for homemade foods, 146 samples were evaluated including soups, main courses, fruits and non-commercial paps. From these, 121 (82.9%) were contaminated, with the non-commercial paps presenting the highest average (134 $\mu g.kg^{-1}$) and maximum (350 $\mu g.kg^{-1}$) nickel concentrations. These results were similar to those obtained for Portuguese commercial premade baby foods, discarding the possibility of nickel migration from the containers and lids alloys. Moreover, the estimated daily intake for the different age groups, when using average results (1.12-3.55 $\mu g.kg^{-1}$ b.w.), were significantly lower than the actual tolerable daily intake (13 $\mu g.kg^{-1}$ b.w.) (Table 1).

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Table 1. Nickel daily intake in commercial premade and homemade baby food.

Age		**Total commercial premade ($\mu g.kg^{-1}$ b.w.)**	**Total homemade ($\mu g.kg^{-1}$ b.w.)**
6 months	Average	1.12	1.16
	Worst-case scenario	4.88	2.28
1 year	Average	2.76	2.70
	Worst-case scenario	12.22	5.95
2 years	Average	3.13	3.55
	Worst-case scenario	13.90	8.33

Keywords: human nickel exposure, food analysis, baby food.

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Study of the Anti-inflammatory and Antioxidant Activity of Dragon Fruit (Hylocereus costaricensis) Extract

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ORIGINAL ARTICLE

ABSTRACT

Introdution: Inflammation is a defense mechanism of the body as long as it is a self-limiting process. However, when acute inflammation becomes persistent and exaggerated, together with oxidative stress, it is usually related to the development of several noncommunicable chronic diseases. There are foods that, because they are rich in some vitamins and phenolic compounds, have antioxidant and anti-inflammatory properties and can help to prevent diseases, such as diabetes, cardiovascular diseases, and some types of cancer. This study aims to evaluate the antioxidant and anti-inflammatory activity of a dragon fruit phenolic extract.

Methodology: The antioxidant activity of the phenolic extract was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and by the assay of intracellular production of reactive oxygen species in HaCaT cells, using the probe 2', 7'–dichlorodihydrofluorescein diacetate (DCFH-DA). The anti-inflammatory activity was studied using the model of acute local inflammation, the paw edema induced by carrageenan, with pitaya extract (5mg/kg and 10mg/kg) daily, orally administered for 14 days in male Wistar rats.

Results: The dragon fruit extract showed a scavenging activity of 33.4% indicating it to be a potential antioxidant and demonstrated ability to reduce ROS under conditions of oxidative stress of H2O2 and ultraviolet light. Regarding the anti-inflammatory capacity, the group of animals treated with a dose of 5mg/kg of pitaya phenolic extract, after administration of carrageenan, showed an increase in paw volume (paw edema) of 46.1% and 35.4% (at 3h and 6h, respectively) which was similar to the control group (46.7% and 34.4%). However, the 10 mg/kg dose caused a statistically significant decrease in edema, 34.9% ($p<0.02$) and 24.3% ($p<0.06$) at 3h and 6h, respectively, when compared to the control group.

Discussion: Thus, the results obtained by the DPPH method and the assay of intracellular production of ROS in HaCaT cells demonstrated that pitaya extract has antioxidant activity. The administration of the dragon fruit extract in animal model of acute local inflammation showed that the extract has anti-inflammatory activity.

Keywords: hylocereus costaricensis, antioxidant activity, anti-inflammatory activity.

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The Immunomodulatory Effect of the Roots of Withania Somnifera Encapsulated Into β - Glucan Particles

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ORIGINAL ARTICLE

ABSTRACT

Introduction: *Withania somnifera* (WS) is used in the traditional Indian medicine system, being responsible for having anti-inflammatory properties. Nevertheless, the molecular mechanism of action underlying its effect is poorly understood[1]. To enhance drug delivery systems and to allow a controlled release, has been explored the use of β-glucan particles (GPs) as delivery vesicles such as β-glucan, normally found in the cell walls of yeast[2], having strong immunostimulants and immunomodulatory effects. This is possible since GPs are phagocytosed and processed by cells with a myeloid origin, being responsible to engage different receptors of antigen-presenting cells[3]. The study aims to evaluate the *W. somnifera* immunomodulatory effect in vitro studies, to understand if the use of GPs can be responsible for boosting the effects of WS components.

Methodology: WS components were obtained using ethanol extractions being, posteriorly encapsulated with β-glucan particles (WS-loaded GPs) prepared from alkaline/acid treatment of *Saccharomyces cerevisiae*. ROS, NO production, and cell viability were evaluated on the murine macrophage cell line, RAW 264.7. Proliferation assays were made on PBMCs after 96 hours of incubation. ROS was also evaluated on human neutrophils. Cytokine quantification was performed by ELISA in CD-1 mice spleen cells, in RAW 264.7 supernatant and in human neutrophils.

Results: WS-loaded GPs showed an increase in ROS production, contrary to the WS extracts that showed to inhibit in a concentration-dependent manner. Besides, it was observed an increase of TNF-α and IL-6 levels on RAW 264.7. with the use of GPs. The production of TNF-α was also observed in spleen cells, with no production of any cytokine with the use of WS extracts. It´s possible to observe a proliferation effect with the use of GPs on PBMCs. There´s no production of IL-10, Il-12, or TNF-α on human neutrophils. All the tested concentrations showed a cell viability percentage above 70%, compared with unstimulated cells.

Discussion: Moreover, WS-loaded GPs results demonstrated a contrary effect compared with the WS since the active components when encapsulated are responsible for altering their properties. This way, the studies demonstrated that WS has an anti-inflammatory effect and GPs a pro-inflammatory effect, where GPs showed to have an adjuvant effect on the cytotoxicity of active components of WS.

Keywords: *Withania somnifera*, β-glucan particles, immunomodulatory.

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Avaliação *in vitro* do Potencial Anticancerígeno de Decocções de Ayahuasca: Proliferação Celular, Apoptose e Stresse Oxidativo

Gonçalves J.[1,2*], Feijó M.[1], Socorro S.[1], Luís A.[1,2], Gallardo E.[1,2], Duarte A.P.[1,2]

ORIGINAL ARTICLE

RESUMO

Introdução: A Ayahuasca é uma bebida psicoativa, amazónica, preparada originalmente a partir de folhas de Psychotria viridis (P. viridis) e do caule de Banisteriopsis caapi (B. caapi). Possui o alucinogénico N,N-dimetiltriptamina, que, por ação das β-carbolinas sobre a MAO-A, acede à corrente sanguínea atuando no sistema nervoso central. Contudo, ao longo dos anos foram surgindo algumas variações na preparação desta bebida, sendo usados análogos sintéticos ou naturais, como a Mimosa hostilis (M. hostilis) e a Peganum harmala (P. harmala), para substituírem a P. viridis e a B. caapi, respetivamente. O seu consumo mundial tem aumentando, levando ao aumento do número de estudos sobre os seus potenciais efeitos terapêuticos, porém até ao momento não existem referências sobre o seu potencial anticancerígeno.

Metodologia: Este estudo visa a avaliação da atividade anticancerígena da Ayahuasca em células humanas de adenocarcinoma colorretal (Caco-2). Assim, foram escolhidas duas decocções de plantas usadas na preparação de Ayahuasca (M. hostilis e P. harmala) e de uma mistura das mesmas plantas, com base no IC 50 determinado através do ensaio de viabilidade celular MTT. Em seguida, foi avaliada a atividade da Caspase-3 e determinado o índice de proliferação celular através da análise imunocitoquímica do marcador de proliferação celular Ki-67. A indução de stresse oxidativo foi avaliada através de duas sondas fluorescentes (diacetato de 2',7'-diclorodihidrofluoresceina e dihidroetidio), e avaliou-se também a atividade das enzimas antioxidantes: Glutationa Peroxidase (GPx) e Superóxido Dismutase (SOD).

Resultados: Os resultados demonstraram que as amostras induziram significativamente a apoptose nas células Caco-2, enquanto a proliferação celular diminuiu muito significativamente após exposição aos extratos. Foi também verificado que as amostras de M. hostilis e a mistura de M. hostilis e P. harmala reduziram stresse oxidativo de forma significativa, contrariamente à amostra de P. harmala. Aquando da avaliação da atividade da GPx verificou-se um aumento significativo após exposição às amostras de M. hostilis e à mistura de M. hostilis e P. harmala. Contudo na avaliação da atividade da SOD não foram verificadas diferenças significativas.

Discussão: Considerando os resultados, é possível verificar que as três decocções foram capazes de reduzir a viabilidade e a proliferação celular de células Caco-2, tendo também efeito pró-apoptótico. Os extratos também demonstraram a capacidade de reduzir o stresse oxidativo, e embora não tenham sido verificadas diferenças significativas na atividade da SOD, os resultados da atividade da GPx sugerem que os extratos levam ao desencadeamento de uma resposta de defesa contra o stresse oxidativo.

Palavras-chave: ayahuasca, propriedades anticancerígenas, Caco-2.

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Explorando o uso da *Salicórnia* como Substituto do Sal – Um Caso Prático de Aplicação no Fiambre

Lopes M.[1,2*], Akyüz G.[1], Komora N.[3], Azevedo M.A.[4], Salazar M.[5], Cavaleiro C.[6], Ramos F.[1,2]

ORIGINAL ARTICLE

RESUMO

Introdução: Nos últimos anos, a preocupação das entidades de saúde e dos consumidores em relação aos níveis altos de ingestão de cloreto de sódio (NaCl) tem sido crescente, em particular, devido ao risco cardiovascular associado. Tal tem levado as indústrias alimentares, nomeadamente a dos produtos de charcutaria, a procurar estratégias de redução do uso de sódio nos seus produtos. Contudo, é sabido que a adição de NaCl aos produtos cárneos melhora a sua qualidade, particularmente ao nível da capacidade de retenção de água, capacidade de emulsificação, textura, sabor e inibição do crescimento microbiano. Assim, a remoção completa do sal em produtos cárneos não é viável, no entanto, urge encontrar soluções que permitam a redução das quantidades usadas. As halófitas são espécies capazes de prosperar em condições muito adversas, em particular sob níveis de elevada salinidade, representando um recurso relativamente abundante, mas subaproveitado do ponto de vista tecnológico. Estas espécies destacam-se por exibirem respostas metabólicas diferentes das plantas convencionais, observando-se, por exemplo, uma síntese aumentada de fibra e antioxidantes. Por isso, as halófitas são fontes interessantes destes compostos que, atualmente, estão tão em voga pelos seus efeitos benéficos. Além disso, de entre as diferentes espécies, as do tipo acumulador, como a *Salicórnia*, têm a capacidade de reter níveis importantes de minerais de interesse como o potássio, cálcio e magnésio nas suas partes aéreas. Por conseguinte, o seu uso enquanto ingrediente funcional merece ser devidamente explorado, sem esquecer os aspetos de segurança alimentar.

Metodologia: Desta forma, o presente estudo, visa investigar o efeito da substituição moderada ou grande de NaCl em fiambre de porco da perna pelo uso de *Salicornia ramosissima* (do agregado de espécies da *S. europae*[1]). Os fiambres desenvolvidos, controlo e teste, foram elaborados usando os ingredientes e a metodologia de confeção da empresa Primor Charcutaria - Prima, S.A. A *Salicórnia* foi adicionada à matriz na forma de pó, tendo sido fornecida pela empresa RiaFresh e produzida num sistema de hidroponia para assegurar uma qualidade controlada. De modo a caracterizar os protótipos de fiambre desenvolvidos, efetuou-se o estudo dos seus parâmetros físico-químicos e microbiológicos utilizando metodologias convencionais.

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Resultados: No geral, a substituição moderada, i.e., 80% de NaCl + 20% de *S. ramosissima*, revelou-se adequada e promissora, tendo permitido uma redução do teor de NaCl de 8,2%.

Discussão: No seu conjunto, os dados reforçam o enorme potencial do uso das espécies halófitas na indústria alimentar.

Palavras-chave: *salicórnia*, teor de sal reduzido, fiambre.

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Exploiting the Antioxidant, Anti-Inflammatory, Wound Healing, and Senescence Properties of *Thymbra capitata* (L.) Cav.

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ORIGINAL ARTICLE

ABSTRACT

Introdution: *Thymbra capitata* (L.) Cav. (T.capitata) belongs to the Lamiaceae family and can be found in the Mediterranean region. In the traditional medicine, the aerial parts of this aromatic plant are used in inflammation disorders, skin diseases, and wounds[1]. In this work, the essential oil (EO) and the hydrodistillation residual water (HRW) were obtained, and their phytochemical profiles assessed. In accordance with the traditional uses the antioxidant, anti-inflammatory, wound healing, and senescence activities were evaluated.

Methodology: Phytochemical characterization of EO and HRW was performed by GC-MS and HPLC-PDA-ESI-MSn, respectively. The DPPH, ABTS, and FRAP methods were used to assess the antioxidant activity. Anti-inflammatory activity was evaluated through NO production, iNOS, and pro-IL-1β protein levels in lipopolysaccharide-stimulated macrophages RAW 264.7. The wound healing activity was disclosed by the scratch wound assay. The cellular senescence was performed by the etoposide-induced senescence assay.

Results: The major phytoconstituents in EO and HRW were carvacrol and rosmarinic acid, respectively. HRW showed higher activity in DPPH and FRAP assays compared to EO. In the anti-inflammatory assay, both extracts reduced the levels of NO, iNOS and pro-IL-1β. In the wound healing assay, the EO did not have any effect, whereas the HRW reduced the migration of the cells. Regarding senescence, EO showed anti-senescence properties while HRW induced senescence.

Discussion: This study confirms some of the medicinal uses of T.capitata related to anti-inflammatory and wound-healing effects. Prolonged inflammation delays wound healing[2], so its anti-inflammatory potential makes it an excellent candidate for treating chronic wounds. Inflammation and senescence are two of the hallmarks of aging. Herein, we report for the first time the anti-senescence potential of EO. Together with its anti-inflammatory activity, EO showed potential as an anti-ageing agent of cosmetic interest. HRW demonstrated anti-inflammatory activity, cell migration inhibition and cellular senescence induction. Combined, these activities are of interest in the cancer field. However, to prove its anti-tumour activity, further studies are required. Overall, this work highlights the industrial potential of *T.capitata* in the cosmetic/pharmaceutical industry through the valorisation of the by-product (HRW) of EO hydrodistillation and the promotion of a sustainable green bioeconomy.

Keywords: Thymbra capitata, inflammation, rosmarinic acid.

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The Role of the European Pharmacist in Food Supplement Advice - Regulatory Aspects and Beyond

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ORIGINAL ARTICLE

ABSTRACT

Introduction: Food Supplements (FS) are concentrated sources of nutrients or other substances with a nutritional or physiological effect, which are marketed in small "dose" form (tablets, liquids in measured doses). A wide range of nutrients and other ingredients might be present in FS, including micronutrients, amino acids, fiber, plants and herbal extracts, and others. They are intended to correct nutritional deficiencies, maintain an adequate intake of certain nutrients, or support specific physiological functions. They are not medicines and, as such, cannot exert a pharmacological, immunological, or metabolic action. Therefore, their use is neither intended to treat or prevent diseases in humans nor to modify physiological functions.

Methodology: Research was carried out on the state of the art of dietary supplements in Europe and especially in Portugal. Relevant references from grey literature, including legislation, were included.

Results: In the European Union, FS are regulated as food. Harmonized legislation regulates the vitamins and minerals, and their sources, which can be used in the manufacturing of FS. For other ingredients, the European Commission has established harmonized rules to protect consumers against potential health risks. FS cannot replace a regular and healthy diet but has the aim of increasing human health. European legislation about FS is only partially harmonized and the tools to support health professionals when advising their patients on these products are practically nonexistent. In Portugal, the transposition of Directive 2002/46/EC into law 118/2005 does not guarantee that pharmacists will provide duly informed advice on these products. Although there are countries such as France, Italy, and Belgium, that have implemented procedures that aim to ensure safer advice on these products.

Discussion: There is a growing concern about the misuse of FS, their potential for interaction with medications, and their confusion with drugs. Additionally, FS can be sold not only in pharmacies, but also in dietetic stores, supermarkets, or on the internet. This range of points-of-sale raises the problem of a lack of pharmacists' advice. In addition, the few market studies performed until now, disclose that FS are consumed by at least 20% of Portuguese. All these data reinforce the importance of the intervention of pharmacists in improving the regulation of FS by regulatory affairs specialists; of industry pharmacists in the development and production of safer FS and of community pharmacists in counselling and dispensing them.

Keywords: food supplements, regulation, pharmacists.

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Cantanhede (Bairrada): Vinho Monocasta Baga Amigo do Ambiente e da Saúde?

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ORIGINAL ARTICLE

RESUMO

Figura 1. Casta Baga

Introdução: O presente estudo preliminar teve como objetivo avaliar o potencial da casta Baga, cultivada no Concelho de Cantanhede (Região da Bairrada), na produção de vinho com características organoléticas e bioatividade similares ou superiores a vinhos de outras castas. Nesse sentido o vinho de casta Baga foi comparado com os vinhos monocasta Touriga Nacional, casta nacional transversal a todas as regiões vitivinícolas e com Merlot, casta internacional.

Metodologia: As amostras foram recolhidas em dois diferentes momentos do processo de vinificação, resultantes de 2 modos de produção diferentes: Proteção Integrada e Biodinâmica. A avaliação das amostras foi realizada por meio de testes físico-químicos e sensoriais (visão, olfato e paladar). A quantificação dos fenóis totais foi realizada pelo método de Folin Ciocalteu, enquanto o método da Farmacopeia Portuguesa foi utilizado para determinar o teor total de taninos. A avaliação do perfil fenólico foi realizada por cromatografia líquida de alta eficiência com detetor de fotodíodo (HPLC-PDA). A atividade antioxidante foi avaliada pelo método difenilpicrilhidrazil (DPPH), antes e após a remoção dos taninos (amostras destanizadas).

Resultados e Discussão: Os compostos encontrados, com a exceção dos estilbenos e do derivado do ácido benzoico, são comuns a todas as amostras. A presença dos derivados dos ácidos benzóicos é comum tanto nas uvas como nos vinhos das amostras Baga e Merlot. Apesar das amostras serem muito semelhantes no nível de compostos encontrados, diferem, no entanto, na sua quantidade. Esta diferença foi notória aquando da realização do doseamento de fenóis, taninos e também da atividade antioxidante. A casta Baga apresenta maior quantidade de taninos, conferindo dessa forma adstringência e estrutura (corpo) aos vinhos feitos a partir desta casta. A casta

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Baga é a que produz vinhos menos ácidos e com mais baixo teor alcoólico. Quando se compararam as duas amostras de vinhos Baga, verificou-se que é na amostra de Vinho Baga Biodinâmico que a acidez total e o título alcoométrico total apresentam valores mais baixos. Assim, os vinhos resultantes de uvas de produção biodinâmica para além de apresentarem valores mais elevados em taninos, de serem menos ácidos e menos alcoólicos não apresentam características organoléticas diferentes dos demais, pelo que a mudança de modo de produção permite transformar a produção agrícola tradicional numa produção mais amiga do ambiente, contribuindo para a sustentabilidade do planeta, bem como um importante complemento na alimentação mediterrânica saudável.

Palavras-chave: atividade antioxidante, polifenóis, taninos.

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The need of the One Health Approach to Address the Mycotoxin Challenge: Case Study of Aflatoxins in Portugal

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ORIGINAL ARTICLE

ABSTRACT

Introduction: Mycotoxins are secondary metabolites of fungi, considered natural and inevitable contaminants of food and feed that can adversely affect animal and human health. It is acknowledged that the environment plays a determining role in the production and occurrence of these contaminants, that directly (non-animal derived foods) and indirectly (animal derived foods) can enter human food chain. Just as humans are trying to adapt and respond to climate change, mycotoxin-producing fungi are also adapting. Climate change, along with economic drivers, globalization and agriculture practices, contribute to variation of the geographical pattern of mycotoxin contamination and hinder the aimed control and reduction of the risk of exposure to these contaminants.

Methods: The complex nature of this public health issue calls for a comprehensive and multidisciplinary approach. As a case study, the surveillance of carcinogenic (IARC 1) aflatoxins (AFs), in different food and biological samples previously analyzed by our research group, is used to highlight the importance of the One Health approach.

Results and Discussion: Commercialized UHT milk features higher AFM1 levels and frequency of contamination when cows are reared on an extensive, semi-extensive, and organic production mode. Goat and sheep milk can also present AFM1 contamination, with the first species presenting lower levels. Despite the lower AFM1 levels, comparing with the ones reported in bovine, it is of concern that almost all of the small ruminant milk is destined to cheese production. AFM1 concentration in cheese is estimated to be 2 times higher than in milk given that AFM1 associates with milk proteins. In human breastmilk, one third of the analysed samples were AFM1 contaminated, with the risk assessment of the breastfed babies showing an EDI higher that the proposed TDI. In the exposure pattern, contamination of breastmilk was associated with summer collection, lower mother's educational level, early lactation phase and the maternal consumption of rice and chocolate. The survey of AFB1 in tea and medicinal plants destined to prepare infusions demonstrated that over two thirds of the samples were contaminated, especially tea samples.

The AFs case study clearly shows that engaging multisectorial experts and sharing data is crucial to assess and manage mycotoxins in a changing global environment.

Keywords: one health, mycotoxin, public health, aflatoxins, food, feed.

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Validation of a Chemiluminescence Immunoassay for Nine Mycotoxins in Cereals Grains

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ORIGINAL ARTICLE

ABSTRACT

Introduction: Cereal grains are widely cultivated agricultural commodities, and they constitute a key source of energy and high nutritive qualities[1]. However, many of them are affected by fungal contamination, and consequently, by the presence of mycotoxins (secondary metabolites). They can colonize a huge variety of food and feed commodities and generate mycotoxins during the pre-harvest or post-harvest stages of the food processing chain. In this line, food safety concerns have grown worldwide[2]. The development of monitoring techniques to evaluate exposure, is therefore of interest. Immunoassays have recently emerged as potential alternatives for the study of mycotoxins. This study focuses on a validation of a biochip array technology (BAT) for multi-mycotoxins screening in oat, barley, rye, and wheat grains.

Methodology: The Evidence Investigator Myco 7 (RANDOX Food Diagnostic), based in a competitive chemiluminescent immunoassay, was used for the simultaneous semi-quantitative detection of the mycotoxins immunoassays: aflatoxin B1 (AFB1) and aflatoxin G1 (AFG1), ochratoxin A (OTA), zearalenone (ZEA), toxin T2 and HT2 (sum of T2 and HT2), fumonisins (sum of FB1 and FB2) and deoxynivalenol (DON)[3]. A single extraction step with acetonitrile:methanol:water (50:40:10, v/v/v) was used.

Results: According to validation results, spiked cereals grain samples have not shown any false positives, and a maximum of 5% of false negatives were found for FB1+ FB2, OTA, AFB1, and T2+HT2, in agreement with European Union performance criteria for mycotoxins determination[4]. For the validation of the method, it was taken into account the parameters such as those indicated in Commission Regulation (EC) No. 401/2006, of 23 February 2006, which establishes sampling and analysis methods for the official control of levels of mycotoxins in foodstuffs[5].

Discussion: This BAT immunoassay provides important benefits for the rapid and efficient screening of several mycotoxins in cereal grain samples at various levels. The screening procedure is further made easier by the multi-analytical approach because only samples suspected of contamination need confirmation testing using a more precise technique, like LC-MS/MS.

Keywords: mycotoxins, chemiluminescence immunoassay, rice.

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Beyond the Capsule: Exploring the Oxidative State of Fish Oil-Based Supplements

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ORIGINAL ARTICLE

ABSTRACT

Introduction: Fish oil-based supplements have gained immense popularity due to their potential health benefits attributed to long-chain omega-3 fatty acids. However, the oxidative stability of these supplements is a critical concern, as lipid oxidation can compromise their efficacy and safety. This study aims to evaluate the oxidative status of fish oil supplements through a comprehensive analysis, encompassing various parameters related to lipid oxidation.

Methodology: In 2022, a total of 25 fish oil supplements, abundant in EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid), were bought in the Portuguese market, including pharmacies, health shops, and supermarkets. The samples were subjected to rigorous testing using standardized analytical methods1. The analysis included fatty acid composition, glyceride forms and vitamin E profiling, measurement of peroxide index, p-anisidine index, absorbances, and total acidity.

Results and Discussion: Preliminary findings show significant oxidative variations in tested fish oil supplements. Vitamin E levels vary, with some below optimal levels. Additionally, p-anisidine and peroxide indices reveal differing lipid oxidation levels, some exceeding legal limits. Fatty acid profiles differ in EPA + DHA content, aligning with label specifications. No significant correlation was observed between the composition of the supplements and their oxidation status, as well as their price. The observed variations in vitamin E levels, fatty acid composition, and oxidative markers emphasize the importance of quality control measures in the production of these supplements. This comprehensive analysis sheds light on the oxidative status of fish oil supplements, providing valuable insights for both consumers and manufacturers. Further optimization of formulations and manufacturing processes are necessary to ensure the delivery of safe and efficacious fish oil supplements to consumers.

Keywords: fish oils, Long chain omega-3, lipid oxidation.

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Exploring Biotechnology to Improve the Bioactivity and Bioavailability of Dietary Polyphenols in Tropical Juices

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ORIGINAL ARTICLE

ABSTRACT

Introduction: Polyphenols (PP), including bioflavonoids, play multiple roles including antioxidant, antimicrobial, anti-inflammatory, antiangiogenic, antitumor, exhibit improved pharmacokinetic properties; moreover, PP display various mechanisms of action in the reduction of inflammatory response in the human body, namely relating to gut microbiome, and reduce the risk of disease. Alterations in the structures of bioflavonoids or their (de)glycosylation can change biological activity. Bioavailability determines polyphenol bioactivity and is affected by food matrix, enzymatic and microbial degradation, and gastrointestinal absorption.

Methodology: This work entails enzymatic deglycosylation systems to produce polyphenols to be used as active substances in functional food, tropical juices, and evaluate their role in bioavailability. Fruit samples were collected in Maputo, province of Mozambique, and juices of *Strychnos spinosa, Landolphia kirkii, Vangueria , Garcinia livingstonei* were prepared at a 35%±2% (fruit/water) ratio. Fruit extracts were characterized, and compounds identified by HPLC(MS/MS). Physicochemical and nutritional characterizations were carried out. 3D hydrogels of pectinase were developed and thermal, operational, and storage stabilities of bioimmobilizates assessed. Assays were carried out with pectinase free and immobilized in polyvinyl alcohol (PVA) beads. The bioimmobilizates were tested in bioconversion trials using the different juices in stirred and pneumatic bioreactors, as well as the biologic activity. Antioxidant and anti-inflammatory activities were evaluated.

Results: The results of key parameters on the characterization of the fruits are given in Table 1.

Table 1. Characterization of the different tropical fruits juices.

Characterization	Fruits			
	Strychnos spinosa	*Lndolphia kirkii*	*Vangueria infausta*	*Garcinia livingstonei*
Reducing sugar (mg/mL)	1.30	0.98	3.51	1.08
Reducing sugar (mg/mL)**	2.25	2.67	0.61	1.30
Phenolic content (mg/mL)	2.4	1.22	0.31	0.30
Protein (mg/mL)	0.1774	0.18	0.14	0.17
Antioxidant activity IC50	102.37	84.39	144.72	42.87

** after enzymatic action

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Discussion: The assessed juices showed significant content of reducing sugars, total phenolic compounds and antioxidant activity. *V. infausta* depicted the lowest phenolic titre, albeit the highest antioxidant activity, suggesting the presence of a highly active compound. Pectinase action on the juices resulted in increase of reducing sugars, safe for *V. infausta*.

Keywords: polyphenols, pectinase, bioavailability.

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Impact of Oral Administration and Vascular Microenvironment on Brewing Bioactive Peptides' Efficacy as Angiotensin-Converting Enzyme Inhibitors

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ORIGINAL ARTICLE

ABSTRACT

Introduction: Food-derived bioactive peptides may act as inhibitors of angiotensin-converting enzyme (ACE), which converts angiotensin (Ang) I into the vasopressor Ang II, thus having the potential of managing hypertension. Indeed, peptides derived from brewing by-products (PDBB), namely brewer's spent grain (BSG) and yeast (BSY) and a 50:50 mixture of both (MIX), have proven to be good ACE inhibitors by in vitro assays[1]. However, the impact of oral administration and/or vascular microenvironment on their potential efficacy in inhibiting endogenous ACE remains to be verified. Accordingly, we aimed to evaluate ex vivo the ACE-inhibitory activity of BSG and MIX peptides before (BSG_I, MIX_I) and after (BSG_F, MIX_F) *in vitro* simulated oral administration (gastrointestinal digestion, intestinal absorption, and liver metabolism) and compare these results with those obtained by in vitro assays.

Methodology: *In vitro*, a fluorometric ACE activity assay kit (CS0002, Sigma) was used to assess the bioactivity of PDBB (0.87 mg of protein/mL); *ex vivo*, iliac arteries from adult spontaneously hypertensive rats were studied with isometric tension recording at an organ bath[2] to estimate the effects of PDBB (0.87 mg of protein/mL) or captopril, an ACE-inhibitory drug, on vascular ACE by performing cumulative curves to ACE substrate (Ang I, 10-9-10-6M).

Results: Either *ex vivo* or *in vitro* the peptides' bioactivity was retained or even increased after simulated oral administration (Tabela 1). Although *in vitro* all PDBB demonstrated ACE-inhibitory capacity, the ex vivo assays revealed that only BSGF promoted a marked decrease in vasocontraction evoked by the generated Ang II, an effect comparable to captopril, thus, revealing an equivalent efficacy/potency as the antihypertensive drug. *Ex vivo* assays also revealed that BSGI lacked vascular effect, whereas MIXI enhanced Ang I-induced vasoconstriction, opposing the in vitro results. A Michaelis-Menten and Lineweaver-Burk plots were obtained indicating a mixed inhibitory mechanism by BSGF.

Discussion: The present study reveals an evident impact of the oral route and vascular microenvironment on the efficacy of BSG and MIX as ACE inhibitors, increasing or decreasing their bioactivity, respectively. Moreover, these results indicate BSG as the best PDBB, supporting its use as ACE-inhibitors with promising impact in the management of hypertension.

Keywords: brewing by-products cardiovascular diseases, renin-angiotensin system.

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Table 1. Impact of natural peptides derived from brewing by-products on ACE activity *in vitro*

Compound	in vitro (% control)	ex vivo (% control)
$\mathbf{BSY_{initial}}$	72,9 ± 3,5***a##	
$\mathbf{BSY_{final}}$	45,5 ± 1,8***a	
$\mathbf{BSG_{initial}}$	46,8 ± 1,4***b	129,7 ± 25,5d###
$\mathbf{BSG_{final}}$	36,9 ± 1,5***b#	53,4 ± 12,7*d
$\mathbf{MIX_{initial}}$	65,2 ± 2***c##	152,2 ± 35,7*###
$\mathbf{MIX_{final}}$	32,9 ± 2***c##	89,4 ± 16,5#
$\mathbf{Captopril_{1\mu M}}$	47.4 ± 0.8***	28,2 ± 8,3***

Effect of 0.87 mg/mL of initial (before) and final (after) simulation of oral administration) brewer's spent grain (BSG), brewer's spent yeast (BSY), and 50:50 mixture of BSY:BSG (MIX) protein hydrolysates as well as 1 μM of captopril drug on angiotensin-converting enzyme (ACE). Results expressed as percentage of respective controls.
In vitro: ACE without treatment was used a control. Data presents means ± SEM from 3 independent experiments in each group. Ex vivo: Constriction elicited by angiotensin-converting enzyme substrate angiotensin I (10-7 M) on iliac segments from adult SHR. Angiotensin I alone used a control. Data presents means ± SEM from ≥6 rats in each group. Significant differences (One-way ANOVA followed by post hoc Tukey t-test): * $p<0.05$ and *** $p<0.001$ against the control; # $p<0.05$, ## $p<0.01$ and ### $p<0.001$ against Captopril; between respective initial and fina groups presented in different letters.

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Level of 4-Methylimidazole, a Carcinogenic Compound, in Beverages

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ORIGINAL ARTICLE

ABSTRACT

Introduction: Food presentation is an important factor for market acceptance. So, to make it more attractive, color additives are widely used. Among the oldest food color additives are caramel colors, which are used nowadays by food industry in a wide range of foods and beverages due to their color, flavor, and other properties, such as stabilizing colloidal systems and preventing haze formation in beers. 4-methylimidazole (4-MeI) is one of the degradation products of caramel IV color (E150d) which has been proven to be a neurotoxic and carcinogenic agent. European legislation establishes maximum limits for the presence of 4-MeI in caramels, although it does not establish limits for its presence in food. The main objective of this study was to quantify the 4-MeI content in 37 samples of carbonated drinks (energy drinks, soft drinks, dark beers), acquired in several countries, containing caramel IV in their composition in order to compare the levels of 4-MeI found with the situation observed in the market in 2010.

Methodology: The samples were prepared according to the scheme represented at figure 1. To determine the presence of 4-MeI in caramel, a GC-MS method based on ion pair-extraction with bis-2-ethylhexylphosphate (BEHPA) and isobutylchloroformate derivatization have been successfully applied (Figure 1).

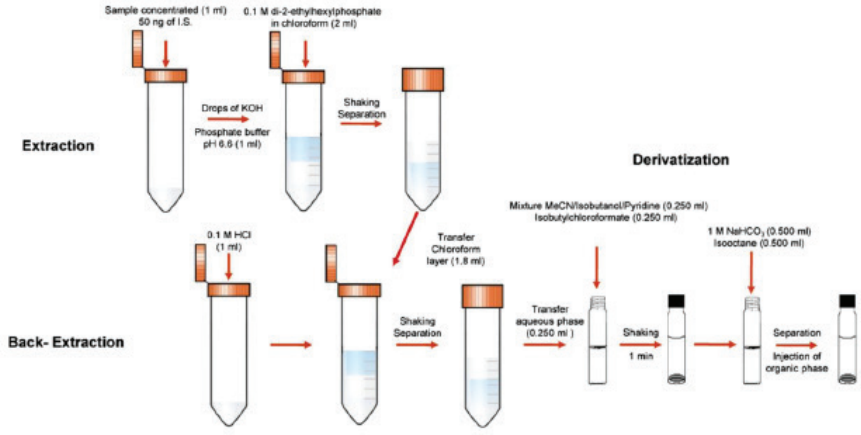

Figure 1. Sample preparation scheme used in this study.

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Results: Overall, soft drinks showed higher amounts of 4-MeI (ranging from 431 to 1381 μg/L) than those found in the dark beers (ranging from 90 to 437 μg/L), with colas presenting the highest levels.

Discussion: 4-MeI was also found in two of the three energy drinks studied. In general, the levels found in 2023 are higher than those reported in 2010, with soft drinks from India presenting the highest levels.

Keywords: 4-MeI, beverage, beers.

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New Dry Powder Aerosolization Device Designed for In Vitro Applications - Validation Tests

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ORIGINAL ARTICLE

RESUMO

Introdução: O cancro do cólon é o segundo tipo de cancro com maior número de mortes na Europa[1]. A sua incidência tem vindo a aumentar assim como a busca por novas alternativas terapêuticas de origem natural que permitam ultrapassar as limitações associadas ao tratamento anticancerígeno convencional. O ambiente marinho tem mostrado ser uma excelente fonte de produtos com potencial terapêutico por apresentar uma elevada biodiversidade e, consequentemente, uma grande variedade de compostos bioativos[2]. As microalgas marinhas têm sido alvo recente de estudos por produzirem compostos com propriedades benéficas para a saúde[3]. Este estudo teve como objetivo avaliar a citotoxicidade de extratos produzidos a partir da biomassa de uma microalga marinha do grupo das diatomáceas e família das Staurosiraceae, numa linha celular do carcinoma colorretal humano - HCT116.

Metodologia: Foram selecionados três solventes com polaridades distintas (etanol, acetato de etilo e n-hexano) para obtenção de extratos constituídos por diferentes compostos bioativos. A citotoxicidade destes extratos foi avaliada *in vitro* através de dois ensaios, um de determinação da atividade metabólica (3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazólico - MTT), e outro de exclusão de corantes, com recurso ao trypan blue. A gama de concentrações testada variou entre 100 e 0,1 μg/mL e os tempos de exposição foram de 3 e 24h.

Resultados: Os resultados mostraram que a concentração mais elevada (100 μg/mL) de todos os extratos em análise exibiu citotoxicidade no ensaio de MTT às 3 e 24h de exposição, registando-se valores de viabilidade celular abaixo dos 70%. Quando aplicado o ensaio de trypan blue para a mesma concentração, os extratos também se mostraram citotóxicos, pois o número de células vivas contabilizado após tratamento foi sempre menor que após exposição ao meio de cultura celular (controlo). O extrato que evidenciou maior citotoxicidade foi o de acetato de etilo ao induzir uma viabilidade celular de 38% e 22% após 3 e 24h de incubação, respetivamente, bem como o menor número de células vivas após 24h de exposição (2,5 cel/μL).

Discussão: Este estudo demonstra o potencial das microalgas marinhas no desenvolvimento de terapias oncológicas alternativas, sendo necessária a realização de estudos adicionais que permitam a sua caracterização química e farmacológica.

Palavras-chave: diatomáceas, testes de toxicidade, células HCT116.

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Plant Vesicles from Food Waste: a Sustainable Innovative Approach for Drug Delivery

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ORIGINAL ARTICLE

ABSTRACT

Introduction: Plant vesicles are currently at the center of attention, particularly in the health sector as potential drug delivery platforms. This interest is due to their high stability, lack of toxicity and low immunogenicity, compared to animal vesicles, already explored in this context[1]. In fact, many vesicles of animal origin are involved in the development of tumors, being their potential biological risk unknown[1], which further reinforces the interest in vesicles of plant origin. It addition, plant vesicles can be obtained on a large scale and at low cost[2], from undervalued or wasted plant parts, thus contributing to a circular economy. As only a very small number of plants have been used to obtain vesicles for drug delivery purpose, it is necessary to broaden current knowledge to other vegetables/fruits. It should also be noted that some vesicles seem to have greater affinity for certain target cells, as several features such as size, surface charge and type of membrane proteins can influence their distribution and interaction with target cells[3].

Methods: The isolation of vesicles from distinct vegetable waste parts, obtained from the Canteens of the University of Coimbra, was optimized and vesicles yield, size and morphology characterized by Nanoparticle Track Analysis and Transmission Electron Microscopy. In addition, samples purity was confirmed by Western Blot analysis.

Results: The infiltration-centrifugation technique enabled the extraction of clean apoplastic washing fluid, being the most effective infiltration obtained with a vacuum flask. Overall, the vesicles obtained from vegetable wastes presented a similar size and morphology as that reported for animal extracellular vesicles. Interestingly, a very high yield of vesicles was obtained using roots. Western blot and metabolic analysis are being carried out to better characterize the population of vesicles obtained from different plant parts and identify relevant metabolites.

Discussion: These preliminary studies are the basis of a broader project that aims to develop a breakthrough approach using plant vesicles to deliver active molecules to specific organs. This strategy will greatly improve compounds bioavailability by guarantying their efficient delivery, reducing therapeutic dose and protecting off-target organs. Moreover, by adding value to less used plant parts and/waste products, a more sustainable production is attained, thus contributing to circular economy.

Keywords: circular economy, extracellular vesicles, organ targeting.

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New Contributions to the Study of an Intercontinental Food Plant: Lemongrass

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ORIGINAL ARTICLE

ABSTRACT

Introduction: Lemongrass, *Cymbopogon citratus (*DC.) Stapf, is a medicinal plant, widely distributed around the world, which is part of the traditional medicine of several countries. It is an aromatic plant from the Poaceae family also used in several countries in the pharmaceutical, food, cosmetics and perfumery industries, in traditional medicine in communities in Asia and South America. It is used daily, with meals, in Angola, S. Tomé and Príncipe and Cape Verde. In Cape Verde, *C. citratus* is commonly known as Xáliz (or chali) on the islands of Sotavanto and Belgata in the Barlavento islands and is an allochthonous species that contributes to the biodiversity of aromatic plants in the archipelago[1].

Methodology: The essential oil (EO) was extracted from the aerial parts of *C. citratus* grown at four locations: Lisbon and Setúbal (Portugal), São Vicente Island (Cape Verde) and São Tomé and Príncipe. Essential oils were extracted by hydrodistillation and analyzed by Gas Chromatography and Gas Chromatography coupled with Mass Spectrometry. Subsequently, an exploratory study was carried out on the possibility of clarifying the genetic diversity among accessions from different regions using ISSR markers-Inter Simple Sequence Repeats. At the same time, a questionnaire was carried out on habits and motivations for consumption.

Figure 1. Lemongrass from Cape Verde

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Results: The yield of *C. citratus* EOs varied from 0.75% (v/d.w.), 0.26%, 0.86% and 0.52%, respectively from plant material collected at the four locations above. The EOs main component was geranial (37-43%), reaching the maximum in the EO of Cape Verde plants (43%)[2]. The second major compound, neral, attained the highest level in the sample from São Tomé and Príncipe, and varied between 28% and 29%. β–Myrcene (16-19%) reached the highest percentage in the EO from Setúbal sample. The most important reasons described by respondents (48) were the effect of digestive well-being (46.5%), relaxation and relief from insomnia (18.6%), as medicinal tea for mild infectious conditions (11.6%) and by taste (9.3%)[3]. PCR amplification with ISSR markers has allowed to see differences among accessions.

Discussion: The EOs dominant components are within the percentage range reported in the literature for these EOs[2]. The ISSR technique will allow the detection of DNA polymorphisms and offers great potential for determining intra-and interspecific levels of genetic variation.

Keywords: *cymbopogon citratus*, chemotypes, genetic diversity.

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Essential Oils Potential as Anti-Fungal Agents: *in vitro* Studies and Proof-of-Concept

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ORIGINAL ARTICLE

ABSTRACT

Introduction: When perspiration occurs, germs like fungus can grow on our skin, shoes, and socks, developing a disease known as plantar bromhidrosis. Dermatophytes are a group of pathogenic fungi that are highly relevant due to their role in the morbidity-associated with superficial mycoses. Furthermore, they can cause invasive diseases in immunocompromised hosts. Some essential oils (EO) may have anti-fungal activity that might help treat this condition[1,2]. This study accessed the antifungal activity, viability, and permeability of an EO blend in dermatophytes and immortalized normal human dermal fibroblasts (NHDF), respectively, while also evaluating its antioxidant (AI) activity.

Methodology: To determine the cells viability, an MTT colorimetric test was employed, incubating the cells with 1%, 0.5%, 0.25%, and 0.1% of the EO blend in culture media for 12 hours. To determine cell permeability a TEER test was performed with an incubation of 0.1% EO blend in culture media for 12 hours. The antioxidant activity was evaluated by DPPH method. The antifungal activity of the EO blend was evaluated by disk diffusion susceptibility testing and by determining the values of minimum inhibitory concentration (MIC) against several strains of dermatophytes (*Epidermophyton floccosum FF9, Trichophyton verrucosum* CECT 2992, *Trichophyton mentagrophytes* var. *interdigitale* CECT 2958, and *Trichophyton rubrum* CECT 2794).

Results: The cell viability obtained was 26.92%, 26.53%, 56.77% and 85.25% for the 1%, 0.5%, 0.25% and 0.1% EO blend, respectively. The TEER test showed that for the selected concentration, the cell permeability was not significantly affected, and DPPH demonstrated that the AI of the EO blend was weak. The EO blend was effective against all the tested dermatophyte strains, being *E. floccosum* FF9 and T. *mentagrophytes* var. *interdigitale* CECT 2958 the most susceptible, presenting diameters of inhibition zones >90 mm and MIC values of 0.08 μL/mL.

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Discussion: The 0.1% was the only concentration that did not significantly affect the cells growth and survivability. Once the permeability did not change, it can be considered this a safe concentration for human skin contact. The DPPH results indicated that the EO blend does not present antioxidant activity. However, the EO blend presented interesting antifungal activity.

Keywords: essential oils, anti-fungal, *in vitro* assay.

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Evaluation of Olive oil Shelf Life for Domestic use: Exploring the Potencial Benefits of Vacuum Storage

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ORIGINAL ARTICLE

ABSTRACT

Introduction: After opening an EVOO bottle, oxidative stress rises, compromising its quality and the anticipated benefits like antioxidant capacity, phenolic compounds, vitamin E, and unsaturated fatty acids. This study assesses how different closure systems impact EVOO quality in common household conditions. Specifically, it explores whether vacuum storage, by reducing the headspace air, can help preserve EVOO characteristics.

Methodology: For the purpose, two distinct batches of commercial EVOO were acquired and distributed into dark glass bottles. One set was closed with conventional stopers (N=3), while the other one involved manual vacuum sealing caps (N=3), like the ones used for wine preservation. To mirror household use, approximately 20 mL was withdrawn from each bottle every 2 days throughout the study (84 days). At 8-day intervals, the EVOO were analysed for acidity, oxidation (peroxide value and UV absorbance), fatty acid composition, total content of phenols derived from tyrosol and hydroxytyrosol, and vitamin E.

Results and Discussion: Results indicate stable acidity and fatty acid composition in all sample groups over 84 days. There's a slight increase in oxidation (higher peroxide value and UV absorbance, $p < 0.05$), along with reduced antioxidant compounds (phenols and vitamin E). Vacuum closure shows a slightly slower degradation rate, but without statistical significance. In summary, throughout this test period, vacuum and conventional closures exhibit no statistical differences in preserving against oxidation or key nutritional components in EVOO. The vacuum system, despite reducing headspace air, doesn't offer additional advantages in preserving EVOO's characteristics. More research is needed to assess long-term effectiveness and oxidation reactions under vacuum storage, supporting these findings and exploring innovative closure systems for home use.

Keywords: olive oil, oxidation, vacuum, quality control.

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Validation of a HPLC-Based Hydrolysis Method for Accurate Quantification of Total Hydroxytyrosol and Tyrosol-Derived Compounds in Olive Oils

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ORIGINAL ARTICLE

ABSTRACT

Introduction: Olive oil phenolics, known for their cardiovascular benefits, have authorized health claims for specific compounds like tyrosol (Tyr) and hydroxytyrosol (Htyr). Analyzing these compounds by international guidelines is complex, costly, and demands advanced chromatography. To simplify quantification, we aimed to optimize and validate a high-throughput, reliable method for estimating total Htyr and Tyr derivatives, with a focus on hydrolysis conditions.

Methodology: An all-in-one acid hydrolysis method followed by HPLC-DAD separation was selected. Tests involved hourly monitoring of Htyr and Tyr released up to 7h of hydrolysis, side-by-side with hydrolysis of oleuropein standard, the main bioactive substance from this class. The stability of both phenolic alcohols and internal standard (IS,syringic acid) was evaluated during the hydrolysis tests and after refrigerated storage (24h). Method validation included linearity, LOD, LOQ, precision, and accuracy.

Results and Discussion: The study highlights the importance of a 6-hour hydrolysis for completeness, corroborated with oleuropein total conversion to Htyr. The IS maintained over 95% integrity after 6-hour hydrolysis but the 24-hour storage induced subtle concentration shifts for both compounds. The chromatographic conditions were optimized under isocratic elution, allowing for 6 min runs. Linearity was verified (0.1 - 19.0 μg/mL and r2=0.9999 for both target analytes) and low limits of detection and quantification were obtained for Htyr (6.5/21.8ng per injection; 8.2/27.3mg/kg of olive oil) and Tyr (4.6/15.2 ng/injection; 5.7/19.0mg/kg of olive oil). Precision was assessed and all results showed a relative standard deviation below 1%. The method also showed excellent accuracy with recoveries, i.e., above 99%. The method was applied to 103 olive oil samples from centenarian olive trees from the Coa Valley region (Portugal), with variable total contents of Htyr and Tyr derivatives, ranging from 7.6 to 36.3 mg/20g of olive oil, all above the limit established (minimum of 5 mg/20g), which demonstrates the richness of these the olive oils.

Keywords: phenolic compounds, olive oil, oxidation.

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Effect of Processing on Reduction of Pesticides Residues in Long Grain Rice

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ORIGINAL ARTICLE

ABSTRACT

Introduction: Pesticides play a key role in preventing and controlling pests and illnesses, allowing to enhance the quality of agricultural products during cultivation and storage. Good practices in pesticides management are crucial because these compounds are associated with adverse health side effects mainly due to chronic toxicity[1]. Rice is a primary staple food for a significant portion of the global human population[2]. For this reason, there is an increasing interest in the development of methodologies to detect pesticides residues in rice-based food and feed samples with low detection limits.

Methodology: In the present study, different processing methods were tested in order to evaluate the effects of washing, washing with vinegar, cooking (traditional method) and steam cooking on reduction of 121 pesticides residues in long grain rice. The traditional cooking method consists of placing the rice submerged in water, where the water is twice the amount of rice. QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) was chosen as the pesticide extraction method and the extract was analyzed by high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). A rice sample (200 g) was contaminated at a level of 50 μg/kg and afterwards sub-samples were subjected to different processing methods.

Results: The washing method with vinegar presented the greatest efficacy, with a reduction of pesticides residues in the range of 26.8-80.3%. The rice samples submitted to both processing methods (wash with vinegar and cooking) presented higher reduction factors, where more than half of the pesticides are below their LOQ, in a total of 62 pesticides. In this line, it is recommended to use, at domestic and industrial levels, both methods in order to better guarantee food safety.

Discussion: The removal of pesticide residues due to processing is affected by the degree of adsorption of pesticides by the cereals' grains, pesticide residues' solubility in water and heat-induced breakdown.

Keywords: rice, pesticide residues, retention factors.

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Estudio Comparativo de Extractos Enriquecudos en Fucoidanos de Undaria pinnatifida (ALARIACEAE) del Golfo San Jorge, Argentina

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ORIGINAL ARTICLE

RESUMEN

Introducción: Las algas pardas biosintetizan carbohidratos de importancia farmacéutica. Los fucoidanos, polisacáridos constituidos principalmente de L-fucosa y D-galactosa, varían su composición entre especies y espaciotemporalmente[1]. A fin de analizarlos en Undaria pinnatifida, se prepararon extractos por distintos protocolos.

Metodología: Se colectaron esporofitos en Comodoro Rivadavia, en otoño, conservando ejemplares en el Herbario Regional Patagónico (7163, 7652, 7861). El resto de material se secó y molió. Una parte se extrajo secuencialmente con n-hexano, cloroformo y metano[2]; otras 2 partes, con etanol a temperatura ambiente y a 70 °C. Los marcos de etanol y de metanol se extrajeron con agua a temperatura ambiente (UpOW1, EUpOW1, UpOW1-3E) y a 70 °C (UpOW2, EUpOW2, UpOW2-3E). Otra porción de un marco etanólico y el marco de la obtención de UpOW2, se trataron con HCl 0,1 M (MUpOHA, UpOHA). Se efectuaron cuantificaciones y análisis por CG-MS[3].

Resultados y Discusión: El contenido de carbohidratos expresados como L-fucosa fue de 24,6; 13,5; 28,0; 18,2; 29,6; 20,6; 2,4 y 1,8 %, respectivamente. Los extractos acuosos fueron semejantes, siendo mayoritaria la fucosa, seguida de galactosa y restos de otros monosacáridos; el grado de sulfatación fue variable. Los extractos a temperatura ambiente contenían restos de alginatos; los de 70 °C, proteínas, y los ácidos baja proporción de fucoidanos y alta de alginatos.

Conclusión: Los resultados demuestran una composición polisacarídica compleja, siendo posible obtener productos enriquecidos en fucoidanos mediante protocolos sencillos y ambientalmente compatibles. Agradecimientos. Al FONCYT, UNPSJB, CONICET.

Palabras claves: *Undaria pinnatifida*, Patagonia Argentina, fucoidanos.

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Fabricacion de Cerveza sin Alcohol a partir de una Levadura no Saccharomyces Cyberlindnera Saturnus

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ORIGINAL ARTICLE

RESUMEN

Introducción: El consumo de cerveza sin alcohol está creciendo a nivel mundial por lo que se está investigando sobre métodos alternativos a la forma de fabricación tradicional (desalcoholización). Uno de los desafíos más destacados en el campo de la investigación y desarrollo de fermentaciones, es el empleo de levaduras no Saccharomyces, las cuales presentan una selectividad única al consumir los azúcares. En este caso, se ha puesto la atención en la levadura no Saccharomyces *Cyberlindnera saturnus,* con el objetivo de conseguir los anhelados aromas afrutados que se asemejan a las características de una cerveza fermentada con levadura Saccharomyces.

Metodología: Para alcanzar este objetivo, se han llevado a cabo diversos experimentos en laboratorio, estableciendo las mejores condiciones para que la levadura desarrolle de manera óptima los aromas deseados en nuestra cerveza. Los resultados obtenidos en las investigaciones de este proyecto dan a conocer las condiciones óptimas para el desarrollo de la levadura no Saccharomyces *C. saturnus* en un medio cervecero. Además de los análisis físico-químicos, se han llevado a cabo evaluaciones sensoriales para obtener una comprensión más profunda de cómo esta levadura trabaja bajo las condiciones establecidas.

Resultados: Los resultados muestran que la levadura estudiada presenta su mejor desempeño a una temperatura de 20 °C y con un grado plato promedio de 12°P. Se cumplió el objetivo específico de fabricar una cerveza sin alcohol con una levadura no saccharomyces *(Cyberlindnera saturnus)* que dio como resultado un perfil organoléptico sobresaliente para el consumidor.

Palabras clave: cerveza sin alcohol, Cyberlindnera saturnus, elaboración.

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