

Proceedings | *Resumos*

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P1 - DEVELOPMENT OF AN IN VITRO TEST FOR THE EVALUATION OF THE SWEAT-RESISTANCE EFFICACY OF DECORATIVE COSMETICS – AN EXPLORATORY STUDY

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Palavras-chave: Decorative cosmetics, Anti-sweat, Sweat-resistance, Method development, In vitro

The decorative cosmetics market is becoming more and more demanding regarding product safety, quality and performance. In 2015, the most popular demand of consumers in the United States was “long-wearing”. The claim “anti-sweat” is starting to be used by the cosmetic industry, associated with the trend of people living more active lives and practicing sports. The aim of this project was to develop and optimize an in vitro test capable to assess sweat-resistance efficacy of decorative cosmetics.

Two test foundations were produced, A: 15% of Granresin MQI-2450 (Isododecane (and) C24-28 Alkyldimethylsiloxy Trimethylsiloxy silicate) and B: 5% of Unimer U-15 (VP/Eicosene Copolymer). The positive and negative controls used were a commercial sweat-resistant foundation and an aqueous paste, respectively. Two different methods were developed and optimized in an attempt to evaluate the sweat resistance of the foundations. Both tests were performed using polymethylmethacrylate (PMMA) plates as substrates. One of the tests involved the immersion of the plates in warmed water (Immersion 1, Immersion 2), while the other involved spraying warmed water on the plates (Spray 1, Spray 2). The amount of foundation lost was calculated considering the weight of the plates before and after the assays. Following test optimization, the colour of the samples and controls remaining on the plates was further measured using a colorimeter.

Sample B seemed to provide better sweat-resistance than sample A with respect to both the percentage of foundation loss and colour variation. Test Spray 2 showed a better performance when compared with the test Immersion 2. Not only it mimics more closely the real use of foundations, but it also clearly differentiated foundations A and B. With this work, it was implemented for the first time a method to evaluate sweat resistance efficacy. Although presenting some limitations, the optimized method had the ability to discriminate the two developed foundations.

Future studies should address the improvement of the method sensibility, either by modifying the test's parameters, such as the volume of water and drying method, or the use of different positive and/or negative controls.

P2 - PORTAL INFOCOSMÉTICOS: AN INITIATIVE OF FFUP FOR HEALTH PROMOTION AND EDUCATION IN THE COSMETOLOGY FIELD

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Palavras-chave: Portal infoCosméticos

Despite of the vast range of available information, there is an evident lack of scientifically supported content in the Cosmetology field, accessible to both consumers and professionals. Considering this, the Laboratory of Pharmaceutical Technology of the Faculty of Pharmacy from Oporto's University launched Portal infoCosméticos (PIC), using in-house academic knowledge and expertise to build an easy-access website projected to promote literacy regarding cosmetic ingredients and products.

Portal infoCosméticos' mission is to provide information to empower consumers while making safer and better-informed choices regarding cosmetic products. Likewise, professionals involved in cosmetic advice can obtain validated information, supported by up-to-date scientific evidence.

This educational project uses clear and lay language, short contents and infographics, and hyperlinks to other information sources that help with the understanding of the comprised contents. The content is developed by recent graduates and students of the Master Degree in Pharmaceutical Sciences in the format of an elucidation of a pre-defined question. After this stage an infographic is created and a process of validation and harmonization takes place before web publication. The scientific validation is ensured by national and international researchers and lecturers besides the national regulatory authority, INFARMED. Besides students, researchers and pharmacists, designers, web designers, communication specialists and website developers were also enrolled in the project team.

Cosmetics ingredients, formulation, safety and regulation of cosmetic products are some of the addressed topics, with focus on the main concerns of nowadays consumers. Legislation is a crucial issue in this field and thus PIC dedicated several questions to this topic such as the question “What were the most important changes introduced by the cosmetics european regulation that entered into force in 2013?”. PIC revealed that the main purposes of those amendments were to clarify cosmetics legislation, mainly regarding safety reports, notification rules, labelling, allowed claims and experiments in animals.

Portal infoCosméticos is an ongoing digital project, being the first of its kind in Portugal, which will be increasingly dedicated to the improvement of literacy and to regular updating of relevant information in the Cosmetology field.

P3 - TAKING TOTAL PROTECTION INTO THE BLUE

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The negative impacts of visible light, particularly blue light (wavelength 380-500nm), to skin are still not fully understood. A few studies have shown an increase of blue light-induced oxidative stress and hyperpigmentation suggesting a contribution to photoaging (1-3). Only two UV-filters were found to partially block wavelength >380nm, hence additional means of protection are needed. We investigated blue light induced cutaneous damages and screened for compounds to suppress these damages.

Vitamins B3, B6 and E could prevent beta-carotene from blue light-dependent degradation in-vitro on PMMA plates. To investigate the potential of such compounds to suppress blue light induced damages in skin, we developed an ex-vivo model of blue light irradiation. Using fluencies of up to 100 Jcm⁻² we could show a dose-dependent increase of reactive oxygen species, and we established protein carbonylation as a novel marker of blue light induced skin damage. We found that distinct compounds like vitamin B3 as well as an extract of the freshwater microalga *Scenedesmus rubescens* could suppress blue light induced damage significantly (p<0.05) ex-vivo.

P4 - CYTOTOXICITY EVALUATION OF SULFATED ANTIOXIDANT COMPOUNDS IN A HUMAN KERATINOCYTE CELL LINE

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Palavras-chave: citotoxicidade, antioxidante

Chlorogenic acid and ascorbic acid are well recognized antioxidant compounds used in cosmetic products. In our group, we have experience working with polysulfated derivatives and one aim of our research is obtaining new topical antioxidants; for that, chlorogenic acid persulfate (CAP) and ascorbic acid persulfate (AAP) were synthesized. The antioxidant potential, copper ion-chelating ability and high water solubility of chlorogenic acid persulfate were previously established leading to the conclusion that the use in skin care products, namely as anti-pollution active could be favored (1). In this work, cytotoxicity of these new compounds was evaluated in human keratinocytes (HaCaT cell line) with MTT reduction assay. HaCaT cells (104 cells/well, 96 wells microplates) were incubated for 24 h in DMEM supplemented with 10% fetal bovine serum and containing CAP or AAP (100-5000 µM). Cells were washed with phosphate-buffered saline 1x and incubated for 2 h with MTT solution (0.5 mg/mL). Formazan crystals were solubilized by adding DMSO and absorbance was measured at 570 nm. The AAP compound promoted a slight decrease in cell viability (78.88±0.67) at the lowest concentration studied (100 µM). However this effect was not concentration-dependent and up to 1000 µM, cell viability was around 75%, and may result from other effects such as an antiproliferative activity. For CAP, a significant decrease in cell viability has been detected only from the concentration of 500 µM. in comparison with the negative control. For this compound, a concentration dependent decrease of cell viability was observed. These results support the interest of application of these derivatives for skin care formulations, in particular chlorogenic acid persulfate. (1) M Correia da Silva, H Cidade, E Sousa, M Pinto. Searching for Small Molecules with Antioxidant and Anticoagulant Properties to Fight Thrombosis, Journal of Pharmaceutical Sciences and Nanotechnology, 2014, 1(1), 43-50.

P5 - DEVELOPMENT OF AN IN VITRO TEST FOR THE EVALUATION VERSUS TRADITIONAL: PHYSICAL-CHEMICAL AND SENSORIAL EVALUATION OF WETTABILITY AND SURFACE TENSION OF SHAMPOOS

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Palavras-chave: shampoo, Sulfate free, sugar-based non-ionic surfactants, contact angles, surface tension, sensory evaluation

A shampoo formulation comprises water, surfactants, thickening agents and other components. With the growing demand for environmentally-friendly products, such as sulfate-free, there has been an increasing request for naturally-based surfactants and thickening agents.

Shampoos with traditional surfactant composition (sodium lauryl ether sulfate based - SLES) and "green shampoos" composed, in this case, by sugar-based non-ionic surfactants such as coco glycoside, decyl glucosides, decyl glucosides with cocamidopropyl betaine and lauryl glucosides, were compared. Traditional and sugar-based thickening agents were also compared, in 14 different shampoo formulations.

An in vivo sensory evaluation was performed with two final formulations, one traditional (sodium laureth sulfate (SLES) 8.8%, Cocamidopropyl Betaine 5.0%, PEG-18 Glyceryl Oleate/Cocote 3.0%, PEG-7 Glyceryl Cocoate 2.5%, Phenoxethanol 0.5%, water 80.2%) and one "green" formulation with Coco-Glucoside 8.8% instead of SLES with subsequent analysis of the hair strands by scanning electron microscopy (SEM). For a traditional shampoo, although leaving the hair clean, it is possible to check the presence of some shampoo residue, and the hair cuticle was more open causing more scales on the hair strands resulting in a more damaged appearance hair. For the "green" shampoo, there was no evidence for shampoo residue and the hair cuticle was left uniform and closed, not causing its damage.

A physical-chemical evaluation of each formulation included the measurement of both wettability by contact angles on a α -keratine surface (simulating the surface of a human hair) and surface tension using du Noüy ring method. The "green" shampoos showed larger wettabilities with smaller contact angles on the α -keratine surface comparing with traditional ones. As expected, the critical micelle concentration (CMC) of anionic surfactants (SLES) showed larger than sugar-based non-ionic "green" surfactants, due to charge repulsion in micelle formation, leading to a more economic formulation with lower surfactant concentration and less environmental impact.

Although with better wetting properties for the hair, more environmentally economical due to lower CMC, and causing less damage to hair, "green" shampoos are more difficult to formulate than traditional shampoos and have less consumer acceptance because they have lower viscosity and produce less foam.

P6 - SUGAR-BASED SURFACTANTS SHOWER GELS DEVELOPMENT: IN VITRO AND IN VIVO CHARACTERIZATION

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Palavras-chave: sugar-based surfactants, rheology, Efficacy, Cytotoxicity

Introduction: Sodium laureth sulfate (SLES) has known ecological disadvantages and ocular and dermal irritation when it is used in cleansing cosmetic products (shower gels and shampoos).

Aim: The aim of this study was the development and characterization of sugar-based surfactants shower gels.

Methods: Several formulations containing lauryl and decyl glucosides with the same amount of active substance (10%) were developed and compared with a formulation with sodium laureth sulfate (SLES).

To guarantee the consumer required viscosity, different thickeners were used: cocamide DEA, PEG-200 hydrogenated glyceryl palmate (and) PEG-7 glyceryl cocoate and PEG-200 Glyceryl Stearate. All shower gels formulations were characterized concerning the pH (pH meter InoLab 730 WTW), rheology profile (Brookfield Rotation Viscosimeter), foam building (vortex method) and foam stability and in vitro cytotoxicity using immortalized human keratinocyte cell line, HaCaT, (CLS, Germany) and ARPE-19 cell line (ATCC, CRL-2302™). The biological effects (Epidermal capacitance and sebumetry) were evaluated in 12 volunteers after one single application. A sensorial analysis (questionnaire to the assessment of sensory attribute) was performed to evaluate the acceptability of each formulation.

Results: The pH (acid) and viscosity values are within the expected limits (pH 5.5 and 3000-4000mPa.s, respectively) when 5% of PEG-200 Glyceryl Stearate was added, as well as, the height of the foam formed (0.4 cm). Cytotoxicity studies demonstrated that decyl glucoside showed 87.7% and 90.0% of cell viability for HaCaT and ARPE-19, respectively, while the SLES showed 39.9% and 79.9% for HaCaT and ARPE-19, respectively. The in vivo results showed that there were no significant differences on both formulations concerning the hydration and sebum. The sensorial analysis met consumer appeal and acceptance requirements.

Discussion/Conclusion: This work supports the idea that, despite the difficulties in formulating sulfate free cleansing cosmetic products, the incorporation of sugar-based surfactants in shower gels can meet the consumers demands and match the already market leader.

P7 - RHEOLOGICAL METHODS TO EVALUATE THE MUCOADHESIVE PROPERTIES OF VAGINAL HYALURONIC ACID-BASED HYDROGELS

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Palavras-chave: mucoadhesivity, Hydrogels, sodium hyaluronate, Rheology
Hyaluronic acid (HA) is naturally present in the vagina and is responsible for maintaining hydration in moisture-sensitive environments. Its unique properties allow it to adhere to the vaginal mucosa, enhancing the residence time, thus hydrating and protecting the tissue. For the successful development of these systems the evaluation of mucoadhesiveness, that is the interactions between the polymer and mucins present at the vaginal mucous, is of utmost importance.

The aim of the present work was to investigate the mucoadhesivity of hyaluronic-acid based hydrogels intended to attenuate vaginal dryness. Gel formulations comprise sodium hyaluronate (SH) 0.3% (w/w) in an isotonic solution.

Two hydrogels containing 0.3% and 0.15% (w/w) of sodium hyaluronate were developed. Rheology (Malvern Kinexus Lab+ rotational rheometer, UK), oscillatory, tackiness, and zeta potential (ZP) measurements were conducted to assess the mucin interaction with the HA-based hydrogels. The adhesive strength was measured using the same equipment with a plate - plate geometry. The mucoadhesion interaction was determined by measuring the ZP of the mucin and hydrogel (Zetasizer Nanoseries Nano Z, Malvern, UK).

Oscillatory measurements demonstrated that with the mucin the G' and G'' increased in both products. At lower frequencies both products exhibited fluid-like properties (G'' > G'). The results of the tack testing show that the Mucin + HA 0.3% appears to be the tackiest with a peak normal force of -0.287 N, followed by the HA 0.30% (-0.287 N), Mucin (-0.228N), Mucin + HA 0.15% (-0.216 N) and HA 0.15% (-0.178 N). With the addition of mucin both HA 0.15% and HA 30% ZP values increased in absolute being the Mucin + HA 0.30% a more negatively charged system. Mucin can be described as a double-globular protein regions connected by highly glycosylated linkers containing carboxylic and sialic acid, which confers the negative charge at physiologic pH. All these results indicated that some kind of interaction occurred between the mucin and the HA being stronger with HA 0.30%. Physical entanglements and hydrogen bonding are possible forms of interaction.

The results highlighted the mucoadhesivity of the vaginal formulation and its ability to interact with the vaginal mucosa thus increasing the residence time in the vagina.

Hyaluronic acid-base hydrogels gels are suitable hydrogels for vaginal application with enhanced mucoadhesive properties as demonstrated by viscosity measurements.

P8 - FORMULATION STUDIES OF INNOVATIVE TOPIC SYSTEMS WITH N-ACETYL-D-GLUCOSAMINE AND QUERCETIN

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Palavras-chave: Emulsions, Processing, topical delivery systems, droplet size

Skin is the largest organ of the human body and one of its main functions is to protect the organism and to maintain temperature. With ageing, skin loses its structural and morphologic characteristics, and therefore its functions. Both quercetin and GlcNAc have been extensively studied regarding their potential to prevent skin ageing.

The aim of this work was to prepare and characterize two topical emulsions, one containing quercetin (lipophilic substance) and other one with GlcNAc (hydrophilic substance).

Quercetin's required solubility studies to incorporate it in topical delivery systems containing an EO free concentrate (Ethylhexyl Stearate, Sorbitan Laurate, Phenoxethanol, Cotton Seed Oil, Polyglyceryl-4 Laurate and Dilauryl Citrate) and a medium polarity emollient (Ethylhexyl Palmitate) was determined. Factors such as method of preparation, the use of different equipment (EUROSTAR 60 digital, T25 digital ULTRA-TURRAX® and Turbotest evo) and different concentrations of excipients were studied to understand their influence on the stability of the formulations. Quercetin and GlcNAc were then added to the optimised formulations and were characterised regarding their rheology, pH, conductivity, droplet size and microscopic structure.

The droplet size distribution was not affected by the addition of the oil phase to the aqueous phase or vice-versa and the homogenisation by ULTRA-TURRAX® was the most effective to obtain smaller droplets; the average diameter value corresponding to percentile 90 was 6.954 μ m, 9.396 μ m and 26.793 μ m for the ULTRA-TURRAX®, TURBOTEST evo and EUROSTAR 60 digital. The oil/water ratio was also studied and the results showed that smaller particles were obtained with highest volume fraction of water, as well as the highest conductivity and lowest viscosity. The rheological profiles of both placebos and final formulations suggested a shear-thinning behaviour and the addition of quercetin and GlcNAc did not influence these profiles, as well as the particle size distribution, pH and conductivity.

The emulsions so far obtained, suggest promising results concerning their efficacy as antiaging quercetin and GlcNAc innovative topic systems.

P9 - PREBIOTICS: THE NEW WAY TO PROTECT THE SKIN

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¹QUIMBIBIOS

Palavras-chave: PREBIOTICS

INTRODUCTION: The skin is a complete ecosystem in which countless microorganisms coexist. This ecosystem is called microbiota

•Each person has a different microbiota determined from our birth and that varies depending on age, lifestyle and environment

•Microbiota should be in perfect balance and communication with our stratum corneum but external and internal factors (pollution, sun, temperature and lifestyle, diet, treatments) can affect the balance and favor inflammatory processes, irritations, redness or bad odors

The studies confirms that stress causes the loss of variety and diversity of our microbiota and to solve this problem, Quimbibios proposes the use of preBIULIN FOSTM, a new nutrient and rebalancing asset of the microbiota that offers good results in the skin health

Aplicaciones

•Day and Night care

•Sensitive skin

•Elderly cosmetics

•Skin identical cosmetics

•Cosmeceuticals

•Oral care

•Hygiene gels

•Nutri cosmetics

•Skin identical

Technical data

INCI: Inulin, Fructose

DOSAGE: 0,5-5%

ASPECT: Powder

Results: Demonstration of activity Fermentation: The foreign microorganism don't show a decrease in the pH and this it means that there isn't fermentation.

Inhibition test P. acnes: P. acnes was incubated in anaerobic conditions for 24h with different concentrations of active. The presence of the bacteria decrease with preBIULIN FOSTM

Growth inhibition of St. salivarius: Streptococcus salivarius was grown containing 1% preBIULIN FOSTM during 8h. PreBIULIN FOSTM improves the growth with 26%. Streptococcus salivarius protects our teeth

Inhibition test Staphylococcus Hominis: Skin microbiota were disturbed with S. Hominis, preBIULIN FOSTM and Ethanol and compared with Placebo. preBIULIN FOSTM boosts the effect of hygienic ingredients such as ethanol

The skin was disturbed with ethanol without preBIULIN FOSTM. After 4h still 33% was not repaired. The skin was disturbed with ethanol and stabilized with preBIULIN FOSTM. After 4h more than 92% was repaired

CONCLUSIONS

•Balance the flora

•Strengthens the microbiotic stratum

•Combat bad smell (deo, foot care, geriatric care)

•Protects the skin from external aggressions (pollution, UV, ...)

•Protect the teeth.

P10 - OPTIMIZATION OF EXTRACTION OF PASSIFLORA EDULIS SEED OIL FROM MADEIRA ISLAND FOR COSMETIC APPLICATION

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Palavras-chave: Passion fruit, extraction, cosmetic

Purple passion fruit is the fruit of *Passiflora edulis* Sims var. *edulis*, a tropical climbing plant. The passion fruit is one of the most popular fruits of Madeira Island, Portugal, both for its exceptional flavour and for the various uses that it has. Currently, only the pulp is used for producing fruit juice and foods. The skin and the seeds aren't used in the food industry, being considered as waste. However, the seeds of passion fruit have been widely used as a multifunctional ingredient in certain cosmetic industries because of their antioxidant properties and UV protection efficacy, which are important in the prevention of photoaging. The aim of this study was to compare the effectiveness of Soxhlet and ultrasound extraction methods for obtaining the oil of *Passiflora edulis* seeds from Madeira Island. The Soxhlet method was selected as a conventional extraction technique. For each extraction, 10 g of passion fruit seeds from Madeira Island were packed in porous cellulose filter thimble and inserted in the Soxhlet extractor. Then, were added 250 ml of the solvent (Ethanol, Acetone, Isopropanol or n-Hexane) and the system was heated until boiling. Reflux was kept for 8 h, then the extraction solvent was eliminated in a rotary vacuum evaporator. The ultrasound extraction was performed in an ultrasonic bath (35kHz/80W) at room temperature. A preliminary study was carried out to choose the best extraction time (5, 10, 15, 30, 60 and 120 minutes) using a 1:4 (m/v) solid (seeds) to solvent ratio. The best extraction time was 60 minutes. The extraction yield using the Soxhlet method with acetone (34.54%) was higher than with ethanol (33.84%), isopropanol (28.52%) and n-hexane (24.39%). As shown in the first method, using ultrasound, ethanol was the solvent that showed the best results (22.95%), followed by acetone (11.46%). The extraction with isopropanol (9.72%) and n-hexane (10.47%) was lower than with the other solvents. The results of this work demonstrated that the extraction by Soxhlet, using acetone or ethanol as solvent, could be the better choice and may be useful for obtaining passion fruit oil used in the production of dermocosmetic formulations.

P11 - EVALUATION OF DIFFERENT SHAMPOOS CONTAINING BEER

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Palavras-chave: Beer, Shampoo, Rheology, Foam

Nowadays, among hair care cosmetics with a temporary effect, shampoos have gained an important role in people's health concerns, as well as in physical appearance. Besides, cosmetics containing beer also included shampoos, complementing these products with antioxidant and antiseptic properties or as a support in hair growth, volume and shine. In this study was performed the evaluation and comparison between five shampoos (A, B, C, D and E), with some commonly used tests. The most important characteristics of shampoos, from the consumer's perspective, such as rheology and foam properties, cleanliness, shine, scent, softness and overall aspect of the hair at the end of the treatment, were evaluated. Rheological properties were evaluated using a viscometer, coupled to a thermostatic bath at 20 °C. The foam index evaluation was performed with a high shear homogenizer and the foam stability was evaluated after the first and fifth minutes. The other mentioned properties were measured using natural hair strands. None of the tests mentioned above were performed in animals, in accordance with UE cosmetic regulations. Each of the tested shampoos presented a pseudoplastic behavior and shampoos D and E also showed thixotropy. Regarding foam index and stability, shampoos B and C showed the best performance and shampoo A was the one that presented the worst results. Again, the shampoo C was classified as the best of the five, giving better overall aspect to the natural hair strands at the end of the treatment. We can conclude that, among the five tested shampoos, the shampoo C presented the best characteristics. Other tests can be done in order to obtain more information. For example, it would be important to make a sensorial analysis with the different shampoos.

P12 - INFLUENCE OF SUSTAINABLE INGREDIENTS ON THE STRUCTURAL BEHAVIOR AND SPREADABILITY PROPERTIES OF ECO-FRIENDLY EMULSIONS

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Palavras-chave: Sustainability, Green emollients

Introduction: The interest in sustainable cosmetic products has increased along the years, since the choice of products, packaging and production processes have a great impact on the environment. One of the main challenges is to replace the synthetic cosmetic ingredients for "green" alternatives. Select the right ingredients could be a great challenge, because is essential to ensure the safety and the function of each ingredient, the stability of formulation and the consumers preference.

Aim: Evaluate the influence of the replacement of synthetic ingredients, namely hydrocarbons and silicones, by sustainable ingredients on its rheological and spreadability properties.

Methods: An o/w emulsion containing Petrolatum and Dimethicone was used as a control. Four o/w emulsions were prepared differing on the emollient, as follows: F1-Ricinus Communis Seed Oil; Hydrogenated Castor Oil; Copernicia Cerifera Cera; F2-Butyrospermum Parkii; F3-Butyrospermum Parkii and PEG-8 Beeswax 1:1; F4-PEG-8 Beeswax. The silicone alternative used in F1-F4 was Hydrogenated Ethylhexyl Olivat (and) Hydrogenated Olive Oil Unsaponifiables. Structural experiments were performed with a controlled stress Kinesus Rheometer (Malvern). Rotational viscosity was determined using a cone and plate geometry, with an angle of 4°. Dynamic viscosity measurements were carried out between 1 and 1000 Pa on a logarithmic increment. Oscillation frequency sweep tests were performed at frequencies ranging between 0.01 and 1 Hz. The adhesive strength was measured using the same equipment with a plate - plate geometry and a gap 0.2mm. Droplet size distribution was determined using a Malvern Mastersizer (Hydro 2000).

Results: F1 show similar rheological profile to the control; F1 and F2 show similar adhesion and tackiness properties to the control. Regarding the oscillatory test, in all formulations, the $G' > G''$, meaning these formulations have the elastic module superior to the viscous module and presenting strong network that allows good spreadability, adhesion and tackiness of emulsions. Concerning droplet size distribution, all formulations show a monomodal population with a similar profile.

Discussion/Conclusion: The replacement of synthetic ingredients for "green" alternatives has effectively an impact on structural properties of emulsions. The viscosity has a great impact on emulsions' spreadability and the chemical structure of low viscosity esters has an impact on their spreading behavior.

P13 - SCALING-UP THE PRODUCTION OF ECO-FRIENDLY O/W EMULSIONS

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Palavras-chave: Scale-up, manufacturing process, w/o emulsions, defloculator
Introduction: Scaling up of emulsions is considered a challenge as cosmetic emulsions are thermodynamically unstable. During the scale-up process, the effect of operational variables on the emulsification process and physical properties affecting overall attributes need systematic evaluation. Most of the work in the field of emulsions manufacturing still depends on empirical studies and the principles of geometric similarities. Thus, the scale-up of a cosmetic product could have a largely influence on the final product characteristics, due to the need to use different conditions and appropriate equipment, which is why choosing the right production-scale equipment is fundamental.

Aim: The aim of the present research work was to assess the influence of different manufacturing processes on the final product characteristics, in terms of stability, structure and rheological features.

Methods: Three eco-friendly o/w emulsions were developed, exactly with the same qualitative and quantitative composition, at room temperature. Different equipments were used in the emulsification phase: F1 - High shear homogenizer (1 min. at 400 rpm/min) + stirrer at 400 rpm until total cooling; F2 - Stirrer at 400 rpm until total cooling; F3 - Defloculator (1 min. at 1000 rpm/min) + stirrer at 400 rpm until total cooling. Structural experiments were performed with a controlled stress Kinesis Rheometer (Malvern). Rotational viscosity was determined using a cone and plate geometry, with an angle of 4°. Dynamic viscosity measurements were carried out between 1 and 1000 Pa on a logarithmic increment. Oscillation frequency sweep tests were performed at frequencies ranging between 0.01 and 1 Hz. Droplet size distribution was determined using a Malvern Mastersizer (Hydro 2000).

Results: F1, F2 and F3 show similar rheological profile when compared to the control. Regarding the oscillatory test, in all formulations, $G' > G''$, meaning these formulations have the elastic module superior to the viscous module (i.e. structured fluids) and presenting strong network allowing good spreadability. In terms of droplet size distribution, all formulations show a monomodal population with a similar profile.

Discussion/Conclusion: The results showed that the use of different manufacturing process does not influence the stability, structure and rheological properties of this type of emulsions.

P14 - DEVELOPMENT OF A QUESTIONNAIRE FOR THE CHARACTERIZATION OF POSOLOGIC INSTRUCTIONS IN DERMATOLOGY

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Palavras-chave: Questionnaire, Posologic instructions, Dermatology, Topical treatment
Introduction: Dermatoses have high prevalence worldwide. The posology instructions patterns associated with prescription in Dermatology are insufficiently characterized, and there are no data regarding the situation in Portugal. The omission of treatment duration and the site of application were some of the reported shortcomings

Aim: Development of a questionnaire to characterize the posology instruction patterns in dermatology

Methods: The development and validation of the questionnaire included the following stages: 1) State of the art on dermatoses topical regimens and existing measures of posologic instructions, 2) Construction of a pool of items and definition of scales; 3) Review by a panel of experts comprising dermatologists, pharmacists, expert in instrument development and validation expert in dermatopharmacy physician and health public researcher, with respect to the clinical relevancy and clarity of each of the items produced. Two rounds of discussion were conducted to obtain a pre-test version; 4) Implementation of a pre-test with a group of 3 physicians (pediatrician, family's physician and, allergist and clinical immunologist) following a discussion between pre-test participants and the authors; 5) Implementation of an electronic final version; 6) Approval of the study by the IUCS Ethics Committee

Results: The questionnaire is anonymous and contains 30 items. Initially, a short description of the questionnaire and the aims of the study are stated. An informed consent is a mandatory item before initiating. The following 5 dimensions are contemplated: Socio-demographic characteristics, instructions on topical prescription, methods of transmitting instructions, instructions according to the therapeutic group, dosage forms most prescribed for a given dermatosis. Likert-like and nominal scales were used. The instrument was deemed easy to understand and fill by physicians. About 7 min are needed to fill this instrument

Discussion/Conclusion: This questionnaire is a new instrument in Portuguese language that will allow a deeper understanding of posology instruction patterns in dermatology in Portugal. It could be also valuable to other Portuguese-speaking countries after cultural adaptation. The data obtained will allow to establish guidelines for an adequate use of topical medicines and to promote adherence to this type of treatment

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P15 - INFLUENCE OF SOCIODEMOGRAPHIC FACTORS ON PATIENT SATISFACTION WITH TOPICAL TREATMENT OF PSORIASIS

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Palavras-chave: patients preferences, topical treatment, psoriasis, sociodemographic characteristics

Introduction: Adherence to topical therapy in psoriasis is low. One of the factors that influence adherence is the characteristics of the topical medicines

Aim: Investigate the influence of sociodemographic characteristics of patients on their preferences for topical medicines for the treatment of psoriasis

Methods: A questionnaire for the assessment of satisfaction with topical medicines used for psoriasis treatment was developed. The attributes (21) classification was made using a 7-point Likert-like scale. The instrument was applied to a total of 79 patients that were members of the PSOPortugal or outpatients of a dermatology unit of a central hospital. Due to inclusion criteria only adults with diagnosis of psoriasis, treated with a topical medicine for the past 6 months were studied. Both electronic (LimeSurvey® program) and paper versions were available. Approval by the Ethics Committee of the IUCS was obtained. Statistical analysis was conducted with IBM SPSS® for Windows® version 25 (Armonk, NY: IBM Corp, USA). For the evaluation of the results age was categorized in elderly (age>65 years) and not elderly (age≤65 years) and level of education in higher education or not higher education. The level of significance was fixed at p=0.001 for high significant, p=0.05 for significant and p=0.1 for low significant

Results: Results indicate that women had greater dissatisfaction with the fact that the topical formulations stained clothes (p=0.018) and with the adhesive/sticky feeling on the skin (p=0.047). Regarding the influence of the age, elderly patients were not so satisfied with the medicines concerning the sensation of the adhesive/sticky feeling on the skin (p=0.096), skin gloss (p=0.071) and staining of clothes (p=0.075) as younger patients. Participants with higher education had a higher degree of satisfaction with consistency (p=0.043), easiness of application only on the lesions without achieving healthy skin (p=0.089), time taken for application (p=0.049), degree of hydration (p=0.057) and residue in the lesions (p=0.039)

Discussion/Conclusion: The influence of the sociodemographic characteristics on the degree of satisfaction with the treatment could be helpful in supporting the selection of the dosage form in clinical practice and promotion of the adherence to topical treatment
Acknowledgements: This Work is supported by CESPU under the Grants POSOL_DERM_CESPU_2016, PHARM4ADHER_CESPU_2017

P16 - TOPICAL DELIVERY OF N-ACETYL-D-GLUCOSAMINE: AFFORDABLE OPTIMISATION STRATEGIES

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Palavras-chave: N-acetyl-D-glucosamine, Topical delivery, In vitro release, In vitro permeation

Advanced delivery systems have proven their efficacy in improving skin delivery, but often lack customisable, cost-effective methods. Through the Formulation for Efficacy (FFE) concept it is possible to optimise skin delivery using affordable raw materials and equipment. FFE has been used to improve the delivery of hydrophobic compounds, but there is limited information on hydrophilic compounds. This study explored the use of FFE in optimising the topical delivery of N-acetyl-D-glucosamine (GlcNAc), a monomer of hyaluronan, an important skin component both structurally and physiologically. GlcNAc is hydrophilic (log P = -2.1) and has shown to be a versatile and efficacious cosmetic ingredient.

Solubility studies were performed with GlcNAc in water, glycerol and propylene glycol (PG). Carbomer (0.5%) gels neutralised to pH 6.0 with NaOH were formulated with the following ratios of solvents: water (F1); water : glycerol at 1 : 2, 1 : 1 and 2 : 1 (F2, F3 and F4, respectively); water : PG at 1 : 2, 1 : 1 and 2 : 1 (F5, F6 and F7, respectively); and water : glycerol : PG at 1 : 1 : 1 (F8). In vitro release studies were performed with vertical Franz diffusion cells (VDC Test System Model HDT 1000, Copley Scientific™, UK) and HT Tuffryn® membrane (Pall Corporation®, USA) (pore size = 0.2 µm); the receptor phase was a phosphate-buffered solution (pH 7.4) kept at 32 °C and stirred at 500 rpm. Samples were collected after 0.5, 1, 2, 3 and 4 h. Under the same conditions, in vitro permeation studies were performed with Strat-M® membrane (EMD Millipore, Germany). GlcNAc was quantified by UV spectroscopy at 202 nm with a NanoDrop™ 2000 (Thermo Fisher Scientific, UK).

The solubility of GlcNAc was: 206.333 g.L⁻¹ in water, 40.375 g.L⁻¹ in glycerol and 7.390 g.L⁻¹ in PG. The hydrogels that presented the best in vitro release profiles were: F1, F2, F3, F7 and F8. In the in vitro permeation studies, the best profiles were obtained with the hydrogels F6 and F8. GlcNAc was more soluble in glycerol than PG, which corresponded to better release profiles obtained for higher concentration of glycerol and lower concentration of PG. This suggests that if the solubility of the active is reduced below an optimal level, its release may be hindered. In the permeation studies, PG, less hydrophilic than glycerol, showed a better capacity to promote permeation through a more hydrophobic membrane. A synergistic effect was apparent when both solvents were used.

P17 - INNOVATIVE STRATEGIES FOR EFFICACY ASSESSMENT OF PROPOLIS FORMULATIONS

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Palavras-chave: efficacy assessment, propolis, antioxidant activity, skin regeneration
Propolis is produced by bees from plant resins, and has been found to have antioxidant, anti-inflammatory and skin regenerator properties. However, the majority of the studies conducted on propolis have been done in vitro or using animal models. The main purpose of this work is, thus, to assess in vivo and in human volunteers the wound healing and antioxidant capacity of propolis formulations.

20 volunteers participated in this study after informed consent. In the study of regeneration properties, four sites were selected in the ventral forearm: one untreated (control), one for the blank formulation, and in the other two different propolis formulations were applied. Baseline values for transepidermal water loss (TEWL), SC hydration and erythema were obtained. Using tape-stripping, a controlled lesion of the epidermis was induced in each of the sites. The formulations were applied for 7 days, during which daily measurements of all the cutaneous variables were made.

In the study of the assessment of the antioxidant capacity three sites (2 for propolis formulations and 1 untreated for control) were outlined on the volar forearm. A 2 h pre-treatment with the formulations was made using non-occlusive patches. After this, an erythema was induced in each area, by the application of ethyl nicotinate. Measurements of the individual test sites were then recorded continuously for 20 minutes with Laser Doppler flowmetry equipment. The onset time and the slope of the tangent line in the hyperaemia area were then determined in all sites.

Results show slight differences in the recovery of the sites treated with one of the propolis formulations, where a return to basal values of erythema occurred sooner. In the TEWL results no significant differences were found between the control and the treated sites. A decrease in SC hydration was observed in all sites, and this was the only variable where, after 7 days, a return to basal values had not been achieved.

The results of the antioxidant ability study indicated that the propolis formulations decreased the intensity of the erythema caused by ethyl nicotinate when compared to the control site, as noted in the slope of the tangent line in the hyperaemia parameter. Longer onset times were observed in the treated sites.

Our findings confirm the bioactivity of topical formulations containing propolis, corroborating that it is an active ingredient with wound healing and antioxidant properties.

P18 - PORTUGUESE SMELL TESTS FOR THE DIAGNOSIS OF OLFACTIVE DYSFUNCTION

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Palavras-chave: Olfactive function, Smell test, Portuguese population, Rheology, Structure

The assessment of olfactory dysfunction (partial or total anosmia) is very important in the early diagnosis of neurodegenerative diseases. Currently, there is no diagnostic test for partial or total anosmia, validated for the Portuguese population.

The aim of this study was the development, structure characterization (rheological, DSC and microscopy) and sensorial characterization of a smell test adapted to the Portuguese population containing orange fragrance to evaluate the dysfunction in patients with partial or total anosmia (frequent pathology in patients with Alzheimer's and Parkinson's). Different PEG - based formulations were developed. Viscoelasticity studies were performed in the Rheometer, Kinesus (Malvern) using the oscillatory method. DSC (calorimeter DSC Q200, TA Instruments, USA) and Microscopy (Olympus BX40, Japan) were assessed as well as the volatile components analysis by GC-MS of the orange fragrance. The sensory evaluation was carried out by a panel of volunteers and based on the responses obtained, a formulation was selected.

The orange odor (fragrance) was chosen by 55.43% of the population that answered, to questionnaires. The type and amount of PEG influences the morphology and crystal structure of the formulations. The sensorial evaluation of the products under study allowed to identify the formulation that best adapted to the use on smell tests. Results pointed out there was a preference (199/359 of answers).

For formulations containing different percentages of PEG-400 and PEG-1500 higher G' values than G'' were obtained. Both elastic and viscous module increased as the percentage of polyethylene glycol 1500 increases.

Smell tests for the diagnosis of olfactive dysfunction with a structured and sensorial characteristic adapted to the Portuguese population were developed allowing the physician to use them as a diagnostic method for total or partial anosmia.

P19 - IONIC LIQUIDS-POLYMER NANOPARTICLE SYSTEMS FOR DELIVERY OF POORLY SOLUBLE DRUGS

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Palavras-chave: Ionic Liquids-Nanoparticles Hybrid Systems, Encapsulation Efficiency, Rutin, Polymer Nanoparticles, PLGA, Choline-based Ionic Liquids

Introduction: Poor drug solubility and low skin permeation are challenges to the Pharmaceutical and Cosmetic Industries. Nanotechnology may avoid them, by allowing drug protection, controlled and/or targeted delivery and improving transdermal uptake(1). Ionic liquids (ILs) are salts, used to increase drug solubility and/or permeation(2).

Aim: Development of IL-nanoparticle hybrid systems for topical delivery of a poorly soluble drug, rutin.

Materials and Methods: Poly(lactic-co-glycolic acid) (PLGA) 50:50 or 75:25 were used to produce nanoparticles by modified solvent-evaporation double emulsion technique(3). The inner phase was an aqueous solution of 0.2%(v/v) of IL, (2-hydroxyethyl)-trimethylammonium-L-phenylalaninate[Cho][Phe] or (2-hydroxyethyl)-trimethylammonium-L-glutamate[Cho][Glu](2,4), dissolving rutin at its maximum solubility. They were also prepared at the isoelectric point of the drug(pH 6.7)(5). The diameter, polydispersity index(PdI) and zeta potential(ZP) of nanoparticles and the association efficiency(AE) and loading capacity(LC) of rutin were evaluated. In vitro antioxidant activity was assessed by DPPH assay.

Result: Nanoparticles had a diameter of 250-300nm with PdI between 0.2-0.4 and good colloidal stability. The AE was above 50%, with no relevant differences found between PLGA ratios. At pH 6.7, the PdI and colloidal stability were similar and the diameters ranged from 300-450nm. LC ranged from 0.4-1.2%.

Discussion/Conclusion: When using [Cho][Phe], a superior AE (75%) was obtained when compared with [Cho][Glu] (50%). This is relevant given the low drug solubility. At pH 6.7, a significant increase in diameter was observed while maintaining good PdI and ZP results. For the AE, an enhancement of about 8% was only observed with [Cho][Glu] for both PLGA ratios. The nanoparticles were able to protect the antioxidant activity of rutin. Results show the potential of IL-nanoparticle hybrid systems to deliver poorly soluble drugs, since they allow higher drug loading, compared to systems without ILs. The formulations were stable and robust, regardless of pH adjustment.

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P20 - EXPLORING THE CUTANEOUS VASCULAR RESPONSE TO MASSAGE BY COMPONENT ANALYSIS OF LDF AND PPG

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Palavras-chave: Massage; LDF; PPG; skin microcirculation

Massage is an ancient technique, commonly used for health and well-being, namely to improve skin aesthetics and relieve disease-related symptoms. Skin is regarded as an appropriate organ to address a wide range of functional aspects of microcirculation vascular effects, so that several non-invasive technologies, such as LDF (laser Doppler flowmetry), and PPG (photoplethysmography) use it to evaluate microcirculation changes.

The aim of this study is to clarify the consequences of in vivo massage on skin's microcirculation response, through the analysis of LDF, PPG and its oscillatory components.

16 young healthy subjects (28.0 ± 7.5 y.o.) both gender, were subjected to a massage protocol, applied in the upward direction (upM), consisting of three phases - 10 min baseline (phase I), 5 min massage (phase II) and 10 min recovery (phase III). One randomly chosen limb was massaged (test limb), while the contralateral was used as control. Skin perfusion was measured on the second and first toes of both feet with LDF and PPG, respectively. The wavelet transform (WT) analysis was then applied to registered signals, and their main activity components (cardiac, respiratory, myogenic, sympathetic, endothelial NO-dependent (NOD) and endothelial NO-independent (NOI)) identified. Descriptive and comparative statistics (nonparametric) were applied for phase comparisons (p<0.05 adopted).

Massage increased perfusion in both feet, but only significantly in the intervention foot for the LDF, probably due to a direct mechanical mobilization of the blood. Significant decreases in pulse rate and in systolic and diastolic blood pressures, and a significant increase in the respiratory rate were also observed. Both techniques recorded a significant increase in the respiratory activity in the intervened limb. LDF recorded from the intervened limb has shown a significant increase in the cardiac activity and a significant decrease in the NOD activity. PPG recorded in both feet, have shown a significant increase in the myogenic component, together with a significant decrease in the NOI activity.

In the present experimental conditions, the massage manoeuvre modified the microvascular reactivity, and increased skin perfusion both in the test and control limbs, with LDF and PPG recording different oscillatory features. These results confirm the usefulness of this model to study the cutaneous skin physiology, and the effect of topically applied products including cosmetics.

P21 - NEW PARAMETERS TO EXPLORE SKIN VISCOELASTICITY BEHAVIOR FROM 3D REPRESENTATIONS

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Palavras-chave: Skin biomechanics, 3D representation variables, CutiScan®, Age differences

Introduction: Several devices have been used to assess human skin's viscoelastic behavior, using different methods, but none was able to perform a successful analysis along a wide range of angles. A recently introduced device, the CutiScan®, allows the assessment of skin biomechanics in a 360 degree angle perspective from a single measurement. This device seems to offer a wide range of possibilities and a remarkable potential for the quantitative assessment of human skin biomechanics. However, it is still a novelty to explore.

Aim: Our aim is to propose new quantification parameters obtained from CutiScan® data and test their usefulness in comparing the viscoelasticity profiles from young and older subjects.

Methods: Data was collected from the forehead of 13 female subjects with healthy skin (7 younger - 20.0 ± 1.2 y.o., 6 older - 50.2 ± 2.4 y.o.). The CutiScan® (Courage-Khazaka, Germany) probe applied a negative pressure of 400 mbar for 2 seconds on skin surface, and a camera quantified the height of displacement in pixels for each of the 360 degree angles. We first created a 3D polar representation of the elasticity profile (time vs height vs angle), which served as the basis for the construction of several 2D plots. From these plots different functional parameters were calculated: stretchability, defined as the mean height of displacement, obtained from the height vs angle plot; stretching speed, defined as the mean highest slope, obtained from the height max slope vs angle plot; stretching time, defined as the mean time to max displacement; rise time, defined as the mean time-to-max-slope, obtained from the time-to-max-slope vs angle plot.

Results: From our proposed plots, results suggest that older subjects display larger displacement heights, as well as larger height max slopes, and higher stretching and rise times, irrespective of the angle under consideration.

Discussion / Conclusion: These results seem to confirm that these new proposed quantification parameters are sensible and useful for the characterization of skin viscoelasticity, and in this particular case, of skin aging.

P22 - CREAM PRODUCT DEVELOPMENT BASED ON A QUALITY BY DESIGN APPROACH

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Palavras-chave: Cosmetic, Cream, Quality by Design

Introduction: Over the last years, academic and industrial have experienced a breakthrough in cosmetics formulations research. Besides the protective function, skin is recognized as an important administration route of cosmetics ingredients and topical formulations have offered a targeted approach to enhance or modify skin appearance. The effectiveness of this route is hampered by the skin barrier nature and different strategies are investigated to improve the permeation of cosmetics substances through the stratum corneum. Pharmaceutical companies are currently undergoing a significant transformation to provide production flexibility and controls, and to reduce regulatory burden. In order to achieve these purposes, Quality by Design (QbD) reveals to be an excellent initiative. Although the application of QbD concept is recommended for different pharmaceutical dosage forms, topical formulations are still in the early stage. Employing this systematic methodology on cosmetic development will ensure a meaningful improvement in product design.

Aim: This work aims to define all stages of process design and development of a conventional cream for cosmetically purpose, applying QbD approach. Methods: The implementation of such methodology includes quality target product profile (QTPP) and critical quality attributes (CQAs) product definition, the achievement of risk assessment through an Ishikawa diagram construction and a Failure Mode Effects Analysis (FMEA), the accomplishment of design of experiments (DoEs), the establishment of a control strategy and the continual improvement and innovation throughout the product lifecycle.

Results: Identification of critical variables is the preliminary step in optimization methodology and it is established over a screening process. Screening designs are used to identify the most influencing factors: critical material attributes (CMAs) and critical process parameters (CPPs). After performing screening experiments, significant variables are then further explored in the optimization phase to define their optimal settings and consequently to define the design space.

Conclusion: The robust manufacturing of a conventional cream, with their complex formulation components, requires a thorough understanding of CMAs and CPPs. Therefore, implementing QbD in cream development, as planning system, is useful to optimize its formulation and processes parameters, providing fundamental data to understand how to develop an optimal cosmetic cream.

P23 - ASSESSING SKIN MICROCIRCULATION REACTIVITY DURING DORSIFLEXION IN THE STANDING POSITION

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Palavras-chave: muscle pump, lower limb, LDF, PPG

The assessment of microvascular dynamics is critical to understand the impact of muscle activity on lower limb perfusion. The calf muscle pump is an important determinant of lower limb perfusion regulation and its activation during concentric plantar flexion effects have shown to alter peripheral microcirculation. However, the effect of dorsiflexion activity has not yet been addressed. In this study we explore the influence of dorsiflexion in standing in skin peripheral microcirculation. Six young (31 ± 9 years) healthy (nonsmokers, with no known cardiovascular disease) subjects enrolled in this study after giving informed written consent. Subjects performed a protocol consisting of three phases – 5 min standing with parallel feet, 1 min of dorsiflexor muscle group contraction, and 5 min recovery in the initial position. Skin foot perfusion was evaluated with photoplethysmography (PPG) and laser Doppler flowmetry (LDF) sensors. Pulse rate (PR) was obtained from PPG and skin temperature obtained from LDF. Nonparametric statistics were used for phase comparisons. Foot dorsiflexion changed blood flow significantly, having PPG recorded a decrease (p=0.028) while LDF recorded an increase (p=0.028). PR and skin temperature did not change during the protocol. Foot dorsiflexion seems to cause different responses in the skin microvascular networks, which might be attributed to the different measuring depths of the techniques employed, justifying further studies.

P24 - HAIR COLOR USING CLAY NANOTUBES-BASED COSMETICS

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Palavras-chave: halloysite, nanotube, microtube, cosmetic, hair, color, dye, nanoformulation, formulation, pharmaceuticals, pharmaceutical technology, biomaterial

Introduction: Halloysite clay microtubes are naturally and abundantly formed in mines. Being safe and biocompatible, this material is used with minor processing, becoming inexpensive and viable for large-scale industrial utilization. Direct application of the coating to the hair surface makes hair coloring a natural choice for application. We present a paradigm in hair cosmetic application using self-assembly of halloysite clay microtubes onto the hair surface², after a few minutes-washing. The second part of the invention involved the sustained delivery of molecules stored in the hollow lumen of microtubes directly to hair. The lumen is engineered to load and release color molecules that are difficult to formulate regarding hair care.

Aim: Formulate hair care halloysite clay microtube-based cosmetics for the release of natural hair colors without alkalines.

Methods: Combinations of aqueous formulations-containing water-insoluble, hydrophobic colors inside the microtube lumen are incorporated into the lumen after hydrophobization. The clay microtubes self-assembly on hair is achieved with simple hair washing. The chemicals inside the lumen can be delivered in between the cuticle where maximum absorption takes place.

Results: The lumen is naturally hydrophilic and hence has to be modified to make it hydrophobic to load a compound like Lawsone. However, the microtubes retain their dispersibility in water and can be used as an aqueous formulation.

Discussion/Conclusion: A broad spectrum of cosmetic applications of halloysite microtubes includes hair thickening loss and keratin treatments. In this work, clay microtubes loaded with lawsone efficiently colored hair without using current alkaline damaging compounds. Present formulations employ alkaline compounds which are highly damaging. Not only does the microtube formulation bypass the alkaline compounds, its slightly acidic nature restores the natural pH of hair. The above coloring formulation is extendable to include both hydrophilic and phobic dye compounds. Microtubes loaded with dye molecules can be incorporated into shampoos or currently marketed formulations by simple addition and admixture.

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P25 - SKIN SENSORIAL ANALYSIS, A NEW METHOD FOR DETECTING MEISSNER CORPUSCLES BY LASER SCANNING CONFOCAL MICROSCOPY

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Palavras-chave: Meissner

Introduction: Sensorial evaluation has been a key aspect in the analysis of cosmetic products and is one of the most important characteristics to be pursued by any formulation.

Meissner corpuscles (MC) are skin superficial sensorial nerve endings responsible for touch-pressure sensations. His density and superficial accessibility influences the touch sensation and can be detected by skin biopsies which are a highly invasive and non-practical methodology. Laser Scanning Confocal Microscopy (LSCM), as a practical non-invasive skin biopsy substitute is a candidate to evaluate and detect these sensorial receptors.

Aim: The aim of this study was to define a new quantification method of Meissner corpuscles on the 1st and 4th finger of the dominant hand.

Methods: Following an informed written consent and an authorization from the Ethical Committee, we select 45 women with ages between 18-65 years old. Meissner corpuscles images were obtained in each subject by sampling a 2,5x2 mm area over the midpoint of the volar aspect of the distal phalanx of Digit I, and Digit IV, on the dominant hand. An in vivo LSCM (Vivascope 1500, Lucid Inc., NY) was used to obtain all the images at a specific depth (where dermal papillae can be observed). The sampling images were examined using an image analysis routine to detect the number of Meissner corpuscles inside the dermal papillae, and to calculate their density. A comparison between the densities obtained at Digit I and IV in order to define eventual differences and select the best analysis method. All the statistical analysis was performed using MS Excel 2016 and SPSS 22. A 95% confidence interval was defined.

Results: The used method was capable of detect MC in both Digit I and IV images. The densities were similar, although the detection algorithm was easier to apply in Digit IV. No statistical differences were obtained between both fingers.

Discussion/Conclusion: Detection of Meissner corpuscles on Digit I was more difficult than on Digit IV mainly because the skin thickness. This higher skin thickness leaves the MC at also a higher depth and therefore inaccessible to detection. On the contrary it is easier to detect Meissner corpuscles on Digit IV and also observe and calculate some shape descriptors that characterize this nerve terminals. These findings suggest that is more reliable to detect Meissner Corpuscles on Digit IV than on Digit I, and that the calculations obtained are more reliable and specific.

P26 - CHEMICAL MAPPING OF ACTIVE COMPOUNDS FOLLOWING SKIN PERMEATION

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Palavras-chave: Skin permeation, stratum corneum, hair follicle, ToF-SIMS

Introduction: Current understanding of the skin permeation of topically applied active compounds is limited, particularly regarding the route or mechanism of stratum corneum penetration, the rate-limiting barrier to skin permeation. Knowledge of the mechanism of stratum corneum penetration of an active ingredient is important for the development of topical products with targeted delivery.

Aims: To investigate transepidermal and follicular permeation of a variety of active ingredients, with differing LogP values, through ex vivo porcine ear skin tissue.

Methods: An oil-in-water emulsion was used as permeation vehicle for all active ingredients. An infinite dose was applied, and after 24 hours, skin sections were snap frozen and cross-sectioned via cryo-ultramicrotome. The use of tissue fixation/embedding agents was avoided. Vertical cross-sections were analysed using time-of-flight secondary ion mass spectrometry (ToF-SIMS). ToF-SIMS is a mass spectrometry imaging technique useful for mapping various small molecules at the surface of biological samples. The stratum corneum and hair follicles were analysed for presence of the active compounds.

Results: The permeation of the active ingredients was monitored with the molecular secondary ion peak intensity. Ascorbic acid and imiquimod were detected to permeate transepidermally through the stratum corneum by a 7.2 fold and 6.2 fold increase in the molecular secondary ion peak intensity in treated skin vs negative control respectively. α -tocopherol was observed to permeate via the hair follicle by a 3.7 fold increase in the molecular secondary ion peak intensity in treated skin vs negative control.

Conclusion: ToF-SIMS, with its high sensitivity and chemical specificity, allows for label-free mapping of active ingredients in skin tissue. Initial data collected, from one biological repeat, has suggested the follicular permeation of α -tocopherol, following topical application of an oil-in-water emulsion.

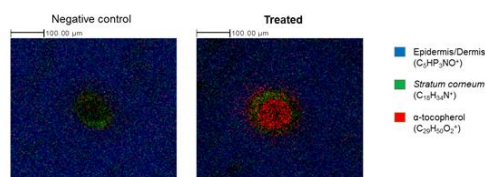


Figure 1. ToF-SIMS ion map of a hair follicle showing the distribution of α -tocopherol

P27 - DEVELOPMENT AND VALIDATION OF IN VIVO AND EX VIVO TECHNIQUES FOR NAILS ANALYSIS

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The nails are important, not only aesthetically, but also structurally and functionally to the fingernails. They have an important role in motor tasks, in touch sensations and protecting the distal phalanx.1 Regular nail care is very important both for esthetical appeal and to keep the nails in good condition.2 Therefore it is important to evaluate the quality of nail lacquers and nail plates, assuring the health and good functioning of the nails and the consumer acceptability of the products. Although there are a great variety of techniques, the majority of them are performed in vitro, lacking in vivo methods that study the product by actual application on the live nails.3 There is a need to develop new techniques that allow analysing various characteristics of nail plates and nail lacquers. The aim of this study was to develop and validate new techniques that can help to understand and analyse some of the features of nail plates.

In this communication it is presented the development and validation of a customized protocol for the analysis of the nail surface roughness and the hardness of the nail plate. One of the innovations developed is the analysis of the roughness of the nail surface with PRIMOSpremium, usually used to analyse wrinkles, scars and other aspects on the skin. Another development made was the use of a Texturometer, a device often used in the cosmetics, pharmaceuticals and food industries, among others, to analyse mechanical textural parameters, in order to evaluate the hardness of nail clippings' samples, with the help of a proper needle probe.

A special thanks to Pedro Castro and Joana Pereira from Escola Superior de Biotecnologia - Universidade Católica do Porto for their help with the texturometer.

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P28-EVALUATION OF CHEMICAL AND PHYSICAL ALTERATIONS IN HAIR FIBRES WITH DIFFERENT TECHNIQUES

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The aim of these project was to look at the chemical composition and physical alterations on the surface of healthy hair and damaged hair fibres using well known techniques, but not yet as explored for hair analysis, validating these techniques for the ex vivo analysis of hair fibres. This can help us to understand and analyse different features of the hair, as well as to properly explore the efficacy of different cosmetic hair products.

In the first place we expected to see a significant difference between normal hair fibres and damaged hair fibres and perhaps to verify chemical changes that did not result in mechanical changes. After these observations, we analysed treated hair fibre samples to see if a certain treatment/product has protected or changed the hair chemical composition and possible physical alterations on the surface of the hair fibres.

Mainly two techniques were used: Optical Profilometry (a light microscope technique which can take 3D measurements from the sample) and ToF-SIMS (which allows chemical surface analysis of the first few nanometres depth of the sample and helps to understand lipid differences on the hair surface), being developed and validated protocols and methodologies for the analysis of hair fibres through these different procedures.

Regarding the Optical Profilometry, it is possible to see and calculate the differences between virgin hair and damaged hair roughness (Ra). However, the differences are not statistically significant and the methodology needs to be polished in order to have more significant results.

The protocol designed for the ToF-SIMS analysis helped us to understand and calculate the damage degree of the hair fibres through the 18-MEA (methyl eicosanoic acid) level of degradation. These protocol was validated, as differences between normal hair and damaged hair were statistically significant.

A special thanks to Long Jiang of University of Nottingham for his help with the techniques

P29 - METHOD FOR ASSESSING THE ANTI-POLLUTION EFFICACY OF SKIN CARE COSMETIC PRODUCTS

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Palavras-chave: anti-pollution, skin, tabacco smoke, malondialdehyde

Nowadays pollution is a major concern of humanity especially on the bigger cities and industrial areas. So, the cosmetic industry has been betting on the development of products with anti-pollution properties.

The aim of this study was to develop a method for assessing the anti-pollution efficacy of skin care products.

Tobacco smoke is a good pollution representative as it contains the major classes of pollutants that we are daily exposed to, including heavy metals. Considering the main constituents of tobacco smoke it is expected to induce lipid oxidation, which leads to an increase of the concentration of the enzyme Malondialdehyde (MDA) that is a marker of lipid oxidation. Therefore on this study the effect of the tobacco smoke on the skin malondialdehyde (MDA) concentration of 5 subjects was evaluated. For that purpose a system for the skin exposure to tobacco smoke was developed. The smoke from the cigarettes combustion was sucked into a chamber that is in contact with the skin of the subjects. The total exposure time was 20 minutes. After that, samples of skin surface layer were collected and analyzed by Gas chromatography-mass spectrometry (GC-MS).

The results showed a mean 23.86 ± 12.46 % increase on the MDA concentration after the smoke exposure, validating the hypothesis. This method can be now used for evaluation of the efficacy of skin care cosmetic products in the protection of the damages caused by pollution, particularly products that might have anti-oxidant effects and, therefore reduce lipid oxidation induced by the cigarette smoke.

P30 - HAIR DAMAGE BY CIGARETTE SMOKE AND CARBON BLACK EXPOSURE

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Palavras-chave: pollution, hair, cigarette smoke, Carbon Black

Pollution is an increasing concern of the humanity, especially on the bigger cities and industrial areas. Therefore, cosmetic products with protective properties of the damage triggered by pollution have been developed. Pollutants can exert negative effects on hair and skin mainly by two ways: deposition and adhesion on the surface; or triggering chemical and/or physical damage.

The aim of this study was to investigate a good pollutant representative to use on a method to evaluate the efficacy of hair products with anti-pollution proprieties.

We conducted a series of tests on how the exposition to cigarette smoke or carbon black (CB) particles affect the hair fibers. Also, the exposure to UV radiation was used. Seven different treatments of hair fibers were prepared for analysis: no damage; cigarette smoke only; CB particles only; bleaching only; bleaching and UV radiation; bleaching, UV radiation and cigarette smoke; bleaching, UV radiation and CB. To the cigarette smoke exposure, the smoke was sucked into a kitasato flask with the hair fibers inside. For the Carbon Black exposure to different methods were used: one where a CB dispersion was sprayed between exposures to UV radiation; and another where the hair fiber was covered by CB particles for one week. Samples of all the hair treatments were analyzed by Scanning Electron Microscopy (SEM) and differential scanning calorimetry (DSC). The samples of bleached hair were also analyzed in a texturometer, recording the rupture force.

The texturometer results show a significant decrease of rupture force only between before UV exposure and after UV exposure (-24.29 %) and between before UV exposure and after UV exposure and CB exposure (-24.76 %). These results indicate that only UV radiation has effects on rupture force of the hair fibers. So neither cigarette smoke nor CB particles had an influence on the rupture force. However, on SEM analysis we can see greater cuticle damage on the hair fiber expose to smoke or CB when compare with the control hair sample. Also, this cuticle damage is more pronounced in tobacco smoke exposure when compared to exposure to CB.

We conclude that cigarette smoke and CB particles exposure affect the cuticle of the hair fibers, being greater the effect of cigarette smoke. To evaluate the degree of damage, SEM is the most appropriate analysis, since the damage caused by the pollutants investigated occurs at the cuticle level.

P31 - EFFECT OF PORTUGUESE THERMAL WATERS ON CELL VIABILITY AND AGAINST SKIN COMMENSAL AND PATHOGENIC MICROORGANISMS

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Palavras-chave: Thermal Waters; cell viability; antimicrobial activity; skin

Introduction: Thermal waters (TW) are well recognized for their therapeutic application in different dermatological disorders. It is known that the bioactivity of TW is related to their chemical profile. However, opposing to what is observed in the major European thermal centers, there is no scientific evidence supporting the use of Portuguese TW as valuable assets for clinical applications.

Aim: The present work aims to evaluate the in vitro effect of different TW, grouped by similar chemical profiles, on skin cells viability as well as their potential to act as antimicrobial agents against specific bacteria involved in skin infections.

Methods: Cell viability of Skin fibroblasts (3T3) and keratinocytes (HaCat) treated with Portuguese TW (sulphurous, Bicarbonated-sulphurous, Bicarbonate-silicated and hyposaline-silicated) was determined by the MTT assay. Five bacterial and one yeast collection strains of *S. aureus* (ATCC 6538), *E. coli* (ATCC 8739), *S. epidermidis* (DSM 28764), *C. amycolatum* (ATCC 49368), *P. acnes* (DSM 1897), *C. albicans* (ATCC 10231) were included in this study. The antimicrobial activity was determined using the CLSI M07-A10, CLSI M45-A2, CLSI M11-A6 and CLSI M27-A3 microdilution methods.

Results: Fibroblasts cell viability increased about 16% after treatment with sulphurous TW while treatment with other tested TW resulted in a reduction on cell viability for both fibroblasts and keratinocytes.

Regarding bacterial growth, sulphurous and bicarbonated-sulphurous TW reduced *E. coli*, *S. epidermidis* and *C. amycolatum* growth by a maximum of 35%. For *S. aureus* only one of the tested waters was able to reduce bacterial growth by 10%. Bicarbonate-silicated TW led to a significant increase of bacterial growth for the mentioned bacteria. One sulphurous water was able to reduce *C. albicans* fungal growth by 20%. A significant reduction of *P. acnes* growth was observed for sulphurous and bicarbonate-sulphurous TW by a maximum of 65%.

Discussion/Conclusion: The reduced viability observed on skin cells after TW exposure may be of interest for the development of new therapeutic alternatives in hyperproliferative skin diseases.

Additionally, the sulphurous and bicarbonated sulphurous TW enrolled in the study significantly reduce overall bacterial growth, which favors the use of these waters for future clinic investigation. The general increase observed on microbial growth by some TW might indicate a protective cellular mechanism that needs to be clarified.

P32 - ASSESSMENT OF THE IMPACT OF FEMALE INTIMATE HYGIENE PRODUCTS ON SKIN PROPERTIES

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Palavras-chave: hydration, lipid content, skin pH, intimate hygiene products

Introduction: Several female intimate hygiene products (IHP) are available as cosmetics aiming to promote vulvar cleansing with minimal skin aggression. These products have evolved from traditional alkaline soap with the development of new detergents as main components of more balanced formulas that may be associated with different cleansing performances and impact on skin parameters.

Aim: To study the impact of different commercial IHP formulas on skin parameters (pH, hydration and lipid content).

Methods: 5 IHP (Gino-Canefresh®, Saforelle®, Germisidin®, composed by amphoteric detergents; Saugella Attiva®- anionic and Lactacyd Intim® - mixture of amphoteric and anionic detergents) were tested in both forearms of 10 adult women (water and alkaline soap as negative and positive controls). IHP and positive control were previously diluted in water. pH, hydration and lipid content measurements were performed before and after (t0, t30 and t60 minutes) product application, by means of Corneometer, pHmeter and Sebometer devices (Courage-Khasaka Electronic). Variations from basal values were calculated.

Results: The positive control promoted a pH variation of 2 at t0 that was maintained during the test. Gino-Canefresh and Saforelle (similar non-acidic pH) induced an initial variation of approximately 1. Lactacyd and Germisidin showed a slight variation (aprox 0,3) and Saugella (product pH 3.7) presented a negative variation when compared with water. Physiologic pH was achieved for all IHP at the end of the test. The lowest variation of lipid content was obtained with Lactacyd followed by Germisidin, Saforelle and Gino-Canefresh (similar values and the same type of detergents). All products were less detergent than the positive control.

Concerning hydration, Germisidin and Saugella presented the most positive variation over time.

Discussion/Conclusion: The tested IHP present different profiles concerning skin parameters. Lactacyd presented a good performance for pH and lipid content while Germisidin showed good pH, lipid and hydration profile. A specific acidic profile was observed for Saugella as stated in the product claims. The improved hydration properties of this IHP and Germisidin may be due to the humectant ingredients they contain. Although the skin of the vulva is more sensitive than the forearm, this work has allowed for the comparison of the impact of IHP on skin properties being helpful for the development of new products.

P33 - ECHINACEA PURPUREA EXTRACTS AS A COSMETIC INGREDIENT: AN ASSESSMENT OF THE AVAILABLE MARKETED PRODUCTS IN PORTUGAL

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Palavras-chave: Echinacea purpurea; cosmetic ingredient; bioactive ingredient

Introduction: The immunomodulatory, anti-inflammatory, antioxidant and healing properties of Echinacea purpurea are the basis for its usefulness in the treatment or prevention of various pathological conditions (immune, respiratory, urinary, dermatological, cardiovascular, gastrointestinal disorders). In addition, particularly regarding its role in the regeneration of the skin, extracts of this plant show an increased market potential as cosmetic ingredient. In Portugal, the formulations containing Echinacea purpurea, which are included in the group of herbal products, are mainly marketed as dietary supplements. These products are not fully listed in any updated database listing label and formulation information accessible for the public.

Aim: The present work aimed to identify and describe the marketed products containing Echinacea purpurea.

Methods: The identification of commercial products containing Echinacea purpurea was performed in Portuguese community pharmacies and pharmaceutical distributors. Label information was collected and analyzed concerning the items considered crucial to define quality, safety and efficacy of the product (composition, part of the plant used, dosage form, health and well-being promoting claims).

Results: A total of 60 products were identified, classified in the majority as dietary supplements; but also cosmetics were found (three products). We found different dosage forms: cream, lipstick, sprays, single-dose oral solution, oral solution in drops, syrup, alcohol-free liquid extract, liquid extract with alcohol, tablet, coated tablet, effervescent tablet, tablet to suck, gum drug and capsule. The cosmetics were represented as lipstick (one product) and creams (two products). For the part of the plant used in the studied formulations, both aerial parts and the root of *E. purpurea* were found. Labels often referred the immunostimulant action of *E. purpurea*.

Discussion/Conclusion: We concluded that although the legislation concerning herbal products and dietary supplements has been modified and supplemented in recent years, there are still many weaknesses in the control of marketed products such as the lack of information. It is urgent, therefore, to invest in greater enforcement of the legislation concerning herbal products, pre and post-marketing, and the definition of criteria of quality, safety and efficacy.

P34 - THE POTENTIAL OF TOPICAL PRODUCTS DEVELOPMENT TO TREAT AND PREVENT CHILBLAINS: AN ASSESSMENT OF THE PREFERENCES OF THE PORTUGUESE POPULATION

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Palavras-chave: chilblains, natural therapy, prevention, topical products, consumer preferences

Introduction: Chilblains (scientifically referred to as pernio or erythema pernio) are defined as injuries involving inflammatory acral areas such as hands and feet, these being the areas of most frequent occurrence. Chilblains affect mostly women and are characterized by symptoms of pain, burning and itching.

Aim: As no data is available on the prevalence and management practices for this disease in Portugal, the objective of this study was to collect data on the lifetime prevalence of chilblains and the type of product used for treatment while identifying the preferred characteristics for an ideal product.

Methods: A population of 336 individuals was studied, living in the north and center regions of Portugal (districts of Braga, Porto, Viana do Castelo, Aveiro and Guarda), aged between 18 and 92 years old. All individuals answered a questionnaire. Statistical analysis was performed with the data obtained.

Results: In this population, 44.6% of the inquired reported to have/have had chilblains, mostly not chronic and self-limited, being more frequent in the winter (93.3%). The most frequent location is the hands (79.3%) and feet (37.3%) and the most evident symptoms were itching (82.7%), pain (75.3%) mainly pulsating/throbbing and burning (74%). When it comes to treatment, the vast majority reported the use of topical products for protecting and regenerating skin, like Friax® (40.7%, containing cetrimide, eucalyptus oil and benzyl alcohol), Akihiver® (34.7%, containing vitamins A, E, B5 and Ginkgo Biloba) and Carena® (10.7%, containing zinc oxide and codfish liver oil). However, most individuals suffering from chilblains reported that these were uncomfortable (39.3%) or oily (24.7%). Only 16% stated that the products were rapidly absorbed and that do not interfere with manual tasks. Most users refer that an ideal product should be presented as a spray (32.6%), followed by a gel (22%), a solution (11.3%), a cream (10.7%) or an ointment (2.7%). Noteworthy, only about half of the inquired believe the products they take/apply improve their quality of life.

Discussion/Conclusions: Regarding the treatment of chilblains, most individuals use topical products, in order to relieve symptoms and treat their condition. However, about half of the enquired population reported that these strategies are not satisfactory, which is an indication that there is a need in the market of more effective products, particularly in the form of sprays or gels.

P35 - EVALUATION OF THE APLICABILITY OF CISTUS LADANIFER ESSENCIAL OIL AS A NATURAL PRESERVATIVE IN COSMETICS FORMULATIONS

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Palavras-chave: Cistus ladanifer, natural preservative, antimicrobial activity, cosmetics
Introduction: The essential oil extracted from the Cistus ladanifer medicinal plant has been shown to present antimicrobial activity, due to the presence of phenolic compounds and compounds belonging to the terpene family. Therefore it presents a potential interest as a preservative for cosmetic products

Aim: This study intends to analyze the activity of the essential oil of Cistus ladanifer, a medicinal plant, against Staphylococcus aureus ATCC 6538, Pseudomonas aeruginosa ATCC 9027, Escherichia coli ATCC 8739, Candida albicans ATCC 10231 and Aspergillus brasiliensis ATCC 16404, in view of its application as a preservative in pharmaceutical or cosmetic formulations, thus contributing to the development of 100% natural formulations.

Methods: The microorganisms were incubated with the essential oil, marketed by Aromas do Valado® company, with the objective of determining the minimum inhibitory concentration (MIC) and the minimum lethal concentration (MLC). For this purpose, microdilutions were performed in 96-well plates. *S. aureus*, *P. aeruginosa*, *E. coli* and *C. albicans* were incubated with a range of oil concentrations of 0.25 µl/ml - 16 µl/ml. *A. brasiliensis* was incubated with a range of oil concentrations of 1 µl/ml - 64 µl/ml.

Results: For *S. aureus* and *E. coli*, the MIC and MLC was 16 µl/ml. The MIC for *P. aeruginosa* was 8 µl/ml and the MLC was 16 µl/ml; the MIC for *C. albicans* was 4 µl/ml and the MLC was 8 µl/ml. For *A. brasiliensis* the MIC was 16 µl/ml and the MLC was > 64 µl/ml.

Discussion/Conclusion: Our results indicate that there is great potential of this oil to be used as a natural preservative in pharmaceutical and/or cosmetic formulations since it inhibits the growth of the standard microorganisms necessary for its approval as a preservative ingredient.

P36 - MICROALGAE IN THE NATURAL COSMETIC INDUSTRY: NEW AND DIFFERENTIATED INGREDIENTS

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Introduction and Aim: The potential of using microalgae in cosmetics is being explored by ALLMICROALGAE. Depending on the species and on the strain, microalgae are rich in functional antioxidants, polysaccharides, pigments and other phytochemicals that are reported as having important role in skin health and beauty.

Methods: Several microalgae are being characterized concerning their biochemical and functional properties, and their potential use as natural ingredients for cosmetic applications.

Results: Microalgae strains such as *Phaeodactylum tricornutum* and *Haematococcus pluvialis* are rich in pigments with added value, namely fucoxanthin and astaxanthin respectively. These species have been produced at Algafarm (a microalgae industrial scale production plant, located in Pataias, Portugal), further analyzed and a high quality and pure biomass has been obtained (significant levels of high value compounds, low microbiological counts and residual levels of heavy metals / PAHs).

Discussion/Conclusion: In general, microalgae biomass is reported as having several nutritional and functional properties. *Chlorella vulgaris* is already recognized as having an anti-collagenase and an anti-elastase effect, and a stimulation effect on total collagen synthesis. The species which are being cultivated at Algafarm, may certainly be used as a natural active ingredient for cosmetic industry, and that may be supplied by Allmicroalgae as a powder, as a frozen paste, as a fresh concentrated culture and/or as an extract. These microalgae products seem to be aligned with the demanding specifications of the cosmetic sector, especially what concerns the purity and quality of the biomass and extracts. Further functional essays may be undertaken, to support the obtained results.