"worsened" (Fig. 3A). The results show that the treatment was effective in 12 subjects, was somewhat effective in 9 subjects, and was unchanged in 6 subjects. No subject experienced worsened conditions. The number of subjects evaluated as "effective" and "somewhat effective" was 21 of 27 (77.8%). In terms of safety, no adverse reactions were observed in any of the subjects as a result of the treatment of base make-up testing products (Fig. 3B). These results indicate that a treatment of base make-up products was safe to use and effective for the prevention of photosensitivity in most of the porphyria patients.

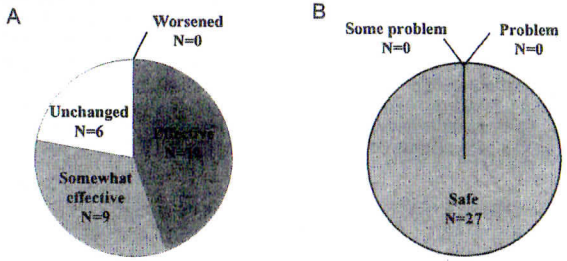

Fig. 3 Results of effectiveness (A) and safety (B) evaluations of base make-up cosmetics. The effectiveness was categorized as effective ¡ somewhat effective ¡ unchanged ¡ and worsened ¡ by dermatologists. The evaluation of safety was categorized as safe ¡ some problem, ¡ and problem ¡ by dermatologists. N indicates the number of subjects.

Conclusion

We have shown here that commercially available base make-up products can safely protect porphyria patients from photosensitivity by sun exposure in the visible light region. This suggests that the simple procedure of make-up application can markedly improve the quality of life of porphyria patients and possibly change their lifestyle. Our findings also suggest that the Porphyrin PF evaluation procedure reported here may be extended to identify effective cosmetics for other photosensitive diseases that induce adverse reactions in narrow regions of the visible spectrum. Finally, these findings demonstrate that cosmetic products can have a broader applicability than at present, serving important roles in the medical field by offering protection from visible light exposure.

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PHOTOTOXICITY EVALUATION OF SUCUPIRA EXTRACT: A POTENTIAL NEW COSMETIC INGREDIENT

TOCHIMI, M.U.[1]; GENESI, B. P.[3]; LOPES, P.S. [3]* (patricia.lopes@unifesp.br); **MATHOR, M.B.[1]; KANEKO, T.M.[4]; BARA, M.T.F.[5]; PAULA, J.R.[5]; OSTROSKY, E.A.[2]**

[1]IPEN/USP-Instituto de Pesquisas Energéticas e Nucleares – IPEN/CNEN- SP, Brazil, [2] FF - UFRN – Faculdade de Farmácia da Universidade Federal do Rio Grande do Norte – RN, Brazil, [3]UNIFESP - Universidade Federal de São Paulo – SP, Brazil, , [4]FCF-USP – Faculdade de Ciências Farmacêuticas da Universidade de São Paulo – SP, Brazil, [5]FF-UFG - Faculdade de Farmácia da Universidade Federal de Goiás, GO, Brazil

Sucupira (*Pterodon emarginatus* Vogel) is easily founded at Brazilian savanna, known as "Cerrado" and presents, as active substances, diterpenes, sesquiterpenes and flavonoids already identified and therapeutically proprieties as anti-inflammatory, analgesic and antimicrobial ones. In this context the goal of this study was evaluate the phototoxicity of sucupira hydroalcoolic extract in order to verify the potential use of this herbal product as cosmetic ingredient in antioxidant skin formulation. Phototoxicity is defined as a toxic response from a substance applied to the body which is enhanced after subsequent exposure to light. This study was conducted in fibroblast Balb/c 3T3 cells ($2x10^4$cell/well) and was performed using several sucupira extract different concentrations (31.7 to 2.1 $\mu g.mL^{-1}$) dissolved in methanol, as solvent, for 24h of contact at 37°C, 97% humidity and 5% CO_2 in cell culture flasks. The test requires that the sample and the solvent be exposed to UV light and plates with the same concentrations, but not irradiated, were used as controls. The viable cells were measured by MTS method, where the active component is a tetrazolium compound and the living cells reduce it to a colored formazan product that is quantified at 490 nm. The results showed that the cosmetic used concentration (10 $\mu g.100\ mL^{-1}$) is no phototoxic. The solvent also showed no phototoxicity at the same concentration. This results permits to determine the PIF (photo irritation factor) as 1, which means that sucupira extract does not have any phototoxic potential at the tested concentration. This test provides the ability to predict in vitro the phototoxic potency of new ingredients in the early stage of products development and so could be used for screening purposes preventing the use of animals in cosmetics products.

Key words: cosmetics, phototoxicity, natural antioxidant

Introduction

The interest of cosmetic industries for phytochemicals with antioxidant activity is increasing, as they present a promising strategy to minimize the damage caused by solar radiation on the skin (Rivelli et al., 2008). Exposure to sunlight, in addition to biological systems and cellular metabolism, is one of the main sources of reactive oxygen species (ROS), as superoxides, hydroxyl anion, nitric oxide, hydrogen peroxide and singlet oxygen. These are highly unstable and reactive molecules, which in its outer orbit, have unpaired electrons. Stability is achieved when these molecules capture electrons from other molecules and vital substances, such as DNA, proteins and lipids, causing oxidative damage and dysfunction of the endogenous antioxidant system. Such injuries can damage skin and regulatory pathways leading to photoaging, dermal inflammation and development of skin cancer (Steiner, 2011).

Antioxidants are substances that can prevent the formation of free radicals, neutralize the attack of these reactive molecules to cells and aid in removal of damage to the DNA molecule and cell membranes. These endogenous substances should be sufficient to keep the tissue homeostasis, but exposure to UV radiation alters the skin tissue, decreasing the amount of antioxidants in skin and contributing to the formation of exacerbated ROS (Steiner, 2011). The use of natural antioxidants is a very interesting strategy, because the plants are rich in antioxidants due to natural protection against the action of these unstable molecules, characteristic attributed to the evolutionary process of these species (Scotti et al., 2007). Antioxidant compounds widely found in plants and derivatives used in therapy include flavonoids, phenolic acids, nitrogen-containing compounds and monoterpenes, have presented powerful

antioxidant activity and are strong candidates for use in topical formulations to prevent and repair damage caused by the RL to the skin tissue (Chen et al., 2012; Dweck et al., 2002; Korać & Khambholja, 2011). Among the various classes of naturally occurring antioxidants, phenolic constituents have received much attention in recent years; especially by inhibit lipid peroxidation and lipoxygenase *in vitro*. The antioxidant activity of these substances is due mainly to its reducing properties and chemical structure. These features play an important role in neutralizing or adsorption of free radicals and transition metal chelation, acting both in the initiation step as in the propagation of oxidative process (DUTRA et al., 2008; Souza et al., 2007).

In some plant species, such as *Pterodon emarginatus* Vogel, family Fabaceae, also known popularly as "sucupira-branca" or "faveiro", a distinctive feature is the frequent presence of phenolic compounds in the integuments. Dutra et al. (2008) studied the total content of phenolic compounds and antioxidant activity of seeds of *P. emarginatus*.

The present work goal is the in vitro evaluation of the phototoxicity of sucupira hydroalcohoolic extract as a future candidate for antioxidant and photoprotector agent in cosmetic formulations.

Material and Methods

The tests were performed under sterile conditions, in a controlled environment, using materials and reagents, sterilized in the laboratory of radiation technology Center of Energy and nuclear research Institute (CTR-IPEN-CNEN/SP).

The samples of sucupira hydroalcohoolic extract were solubilized with methanol (7, 10, 15, 22, 32, 47, 69, 101µg .100 μL^{-1}) and placed at four 96 well plates seeded with Balb 3T3 ATCC CCL-163 (2.10^4 cells per well, cultured at DMEM supplied with 100 IU ml^{-1} penicillin, 100 $mg.ml^{-1}$ streptomycin, 0.025 mg ml^{-1} amphotericin 0,025 mg/mL, 4mM glutamine and 10 % (v/v) bovine fetal serum (ISO 10993-5, 2009) at 37°C and 5% CO_2.

The phototoxicity test was performed in a chamber specially built and qualified in accordance with OECD TG-432 and ©ECVAM DB-ALM: INVITTOX. Validation and qualification of the method and the chamber respectively were made with sodium lauryl sulfate, as recommended by OECD TG-432 (Sufi, 2013).

Two plates (sucupira and solvent) were exposed do UVA (+) and the other two plates were maintained at the same conditions but without UVA radiation (-) for one hour and forty minutes, to achieve 5 J/cm^2. The cells were washed and fresh medium were added and the plates were incubate for 24 hours. After this period the cells were submitted to MTS/PMS assay and the absorbance were evaluated using a plate spectrophotometer (Multiskan EX 355, Thermo Electron Corporation) at 490 nm.

The data were analyzed by Phototox® and Origin™ software.

Results and Discussion

Although this plant is widely studied, the phototoxicity of the hydroalcohoolic extract has not yet been reported. Following the global trend, the use of alternative methods for the assessment of cytotoxicity of herbal products is a reality (Esteves-Pedro et al, 2010) focusing on the attempt to prove the ability of in vitro methods to reduce the use of animals for toxicity screening plants and thus taking into account the principles of the 3Rs (RUSSEL & BURCH, 2009).

Some important factors such as solubility of plant extracts and the subsequent maintenance of their bioactive properties, as well as the use of solvents in non-cytotoxic concentrations (Esteves-Pedro et al., 2010) are fundamental adaptation of in vitro methods described (NIH, 2001; ICCVAM, 2006) for use in vegetable samples, with much more complex composition than synthetic chemicals. Another point that deserves mention is the concern to sterilize the samples without interfering in their biological activities.

In Figure 1it is possible to note that all tested concentrations of hydroalcoholic extract of sucupira were not phototoxic.

Also, at figure 2 the same results were observed for methanol solvent, as it did not show no phototoxicity at the evaluated concentrations.

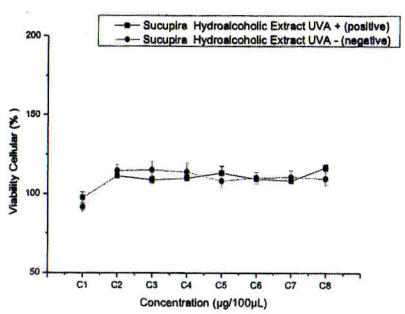

Figure 1. Graphical representation of cell viability at sucupira extract tested concentrations (7, 10, 15, 22, 32, 47, 69, 101 µg in 100 µl).

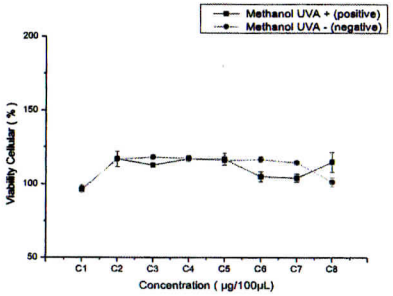

Figure 2. Graphical representation of cell viability at solvent methanol tested concentrations (7, 10, 15, 22, 32, 47, 69, 101 µg in 100 µl).

The OECD TG 432 describes the parameters of the PIF and MPE prediction values that indicate 1) Phototoxicity, 2) likely Phototoxicity, or 3) of Phototoxicity (Institute for In Vitro Sciences, 2013). Therefore, in accordance with the established with OECD the hydroalcoholic extract of sucupira due to MPE equal to 0.003 and PIF equal to 1 does not show Phototoxicity. As well as the solvent extract does not show Phototoxicity according to the value of the MPE -0.020 and PIF equal to 1.

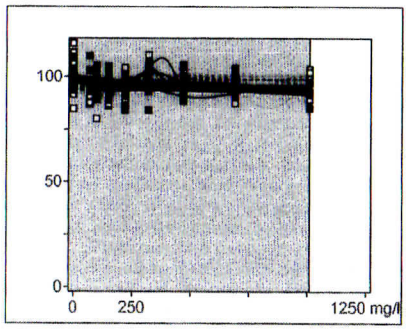

Figure 3: Chart created by Phototox ® program, with readings made of Sucupira Hydroalcoholic extract Phototoxicity.

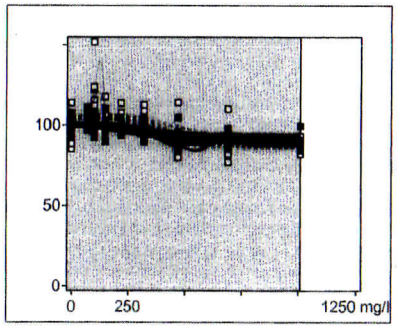

Figure 4: Chart created by Phototox ® program, with readings made of Methanol solvent Phototoxicity.

Conclusion

The hydroalcoholic extract of sucupira proved to be non-phototoxic under the tested conditions at 10 µg.100 μl^{-1} that is the usually concentration used as antioxidant action.

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EVALUATION OF THE PHOTOPROTECTIVE POTENTIAL OF MARINE NATURAL COMPOUNDS

FONTANEZI, BIANCA B.; Oliveira, Ana Lígia L.; Dias, Lara C.; Pereira, Karina C.; Debonsi, Hosana M., Gaspar, Lorena R.
Avenida do Café, s/nº, Faculdade de Ciências Farmacêuticas de Ribeirão Preto – Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil.

Key words: phototoxicity, photostability, marine compounds.

Due to the increasing concern about the UV-induced damage, many studies have been performed all over the world to find new photoprotective substances that present antioxidant and biological filter activities. However, some compounds present limited stability and there is almost no safety information regarding their use in cosmetic formulations, since they can have antioxidant or pro-oxidant behavior, leading to enhanced consumer exposure to unpredictable risks. Thus, the aim of the present study is to evaluate the photostability and phototoxicity of dicerandrol C and mycophenolic acid, which are compounds isolated from the red seaweed genus *Bostrychia* and associated fungi. For the photostability study, the samples were exposed or not to UVA radiation and they were analyzed by UV spectrophotometry to obtain the ratio of the mean UVA to the mean UVB absorbance. The phototoxicity was evaluated by using 3T3 monolayer fibroblast culture, which was submitted to UVA radiation for the determination of cell viability in the presence and absence of radiation, according to OECD TG 432 and to INVITTOX Protocol No. 78. The results showed that the substances dicerandrol C and mycophenolic acid were considered photostable and showed high absorption in the UVA and UVB band, respectively. They also showed similar profile to some mycosporine-like amino acids, which have been studied as candidates for UV-filters. In the phototoxicity studies, it was observed that only dicerandrol C presented cytotoxicity, and both substances did not present phototoxicity. The results are promising, because mycophenolic acid presented high UVB absorption and did not present cytotoxicity or phototoxicity, what can classify it as a good candidate for a new UV-filter.

1. INTRODUCTION

The use of natural substances that have antioxidant and biological filter activities, such as plant extracts, vitamins and products of marine origin is a worldwide tendency in photoprotective and anti aging formulations (F'GUYER et al., 2003; GASPAR; MAIA CAMPOS, 2007; CARDOZO et al., 2007). The red algae genus *Bostrychia* is part of the coastal flora of the State of São Paulo (Brazil) and are found different regions with peculiar characteristics, such as rocky shores and mangroves.

Those macroalgae are also subject to variations of salinity, sunlight incidence, temperature and level of nutrients, which induce the development of effective defenses to those aggressors, especially against prolonged UV radiation exposure damages (DUNLAP et al., 1998). Besides the compounds produced from algae that absorb UV radiation, some associated fungus species provide great protection to adverse natural conditions and also great potential in the production of a variety of new chemical structures (STROBEL, 2003; PONTIUS et al., 2008; KUMARESAN; SURYANARAYANAN, 2002). However, there are not many studies evaluating the photorreactivity of algae metabolites; thus these kinds of studies as well as the evaluation of UV absorption are extremely important for the evaluation of a new UV filter candidate, since this substance must be considered stable and safe, once it can also show pro-oxidant activity, leading to enhanced consumer exposure to unpredictable risks.

Thus, the aim of the present study was to evaluate the photostability and phototoxicity of dicerandrol C and mycophenolic acid, which are compounds isolated from the red algal genus *Bostrychia* and associated fungi.

2. METHODOLOGY

2.1. Marine Compounds