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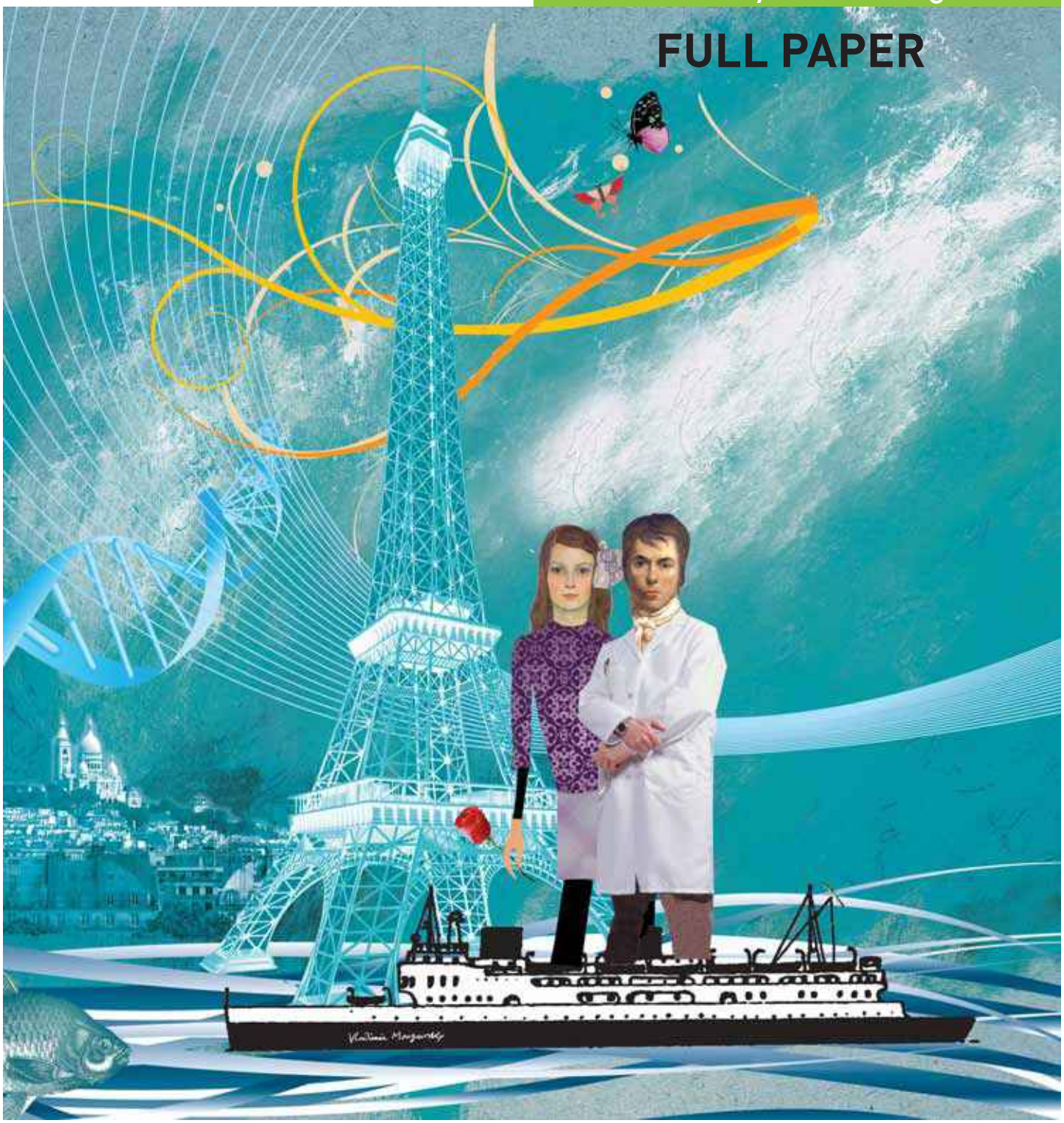


PACARI CITOTOXICITY – A POTENTIAL NEW ANTIOXIDANT COSMETIC

OSTROSKY, E.A.[1]; LOPES, P.S.[2]; UEMURA, M.T.[2,4]; MATHOR, M.B.[4]; KANEKO, T.M.[3]; FERRARI, M.[1], De PAULA J.R.[5], BARA, M.T.F.[5]

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

ABSTRACT

The concern about the sustainability of the production of biodiversity ingredients for the cosmetics industries, including respect for ethical trade-producing communities and the importance of secured processes for recognized certifications and verifications systems, was one of the world highlights. When observing more closely the industry, it is clear that this has increasingly potential to demand ingredients of biodiversity and, consequently, to influence positively or negatively the conservation of natural resources. *Lafoensia pacari* A. St.-Hil, known commonly as pacari, dedaleira and mangava-brava, belongs to Lythraceae family, and is commonly founded in the Brazilian cerrado. It is used as healing agent by the folks. The powder obtained from the dry leaf is also used during meals, as recommendation to assist in cases against gastritis and ulcer. Such properties have led researchers to evaluate their use as potential antioxidant and/or anti-aging cosmetic agent.

INTRODUCTION

The second largest biome in Brazil is the Cerrado, which corresponds to approximately 25% of the country area. It holds 33% and 5% of the national and global biodiversity respectively (Santos et al, 2010;. BRAZIL, 2013) and 44% of its flora is endemic (KLINK; MACHADO, 2005). Despite the richness of its flora and fauna, the Brazilian Cerrado is one of the most threatened biomes in the world, as a result of the expansion of agriculture, animal husbandry and extraction. It is now considered a global hotspot, or a priority area for conservation due to the high degree of threat of extinction (KLINK; MACHADO., 2005; FOGLIO et al, 2007; Santos et al, 2010;. BRAZIL, 2013).

Among the most representative families of the flora of the Cerrado are Fabaceae (Leguminosae), Asteraceae (Compositae), Orchidaceae, Poaceae (Gramineae), Rubiaceae, Melastomataceae, Myrtaceae, Euphorbiaceae, and Lythraceae Malpighiaceae (RIBEIRO, DIAS, 2007). Lythraceae generally comprises 22 genera distributed worldwide (JOLY, 2002), and in Brazil six species of the genus Lafoensia can be found: *Lafoensia pacari* A. St.-Hil, *Lafoensia densiflora* Pohl, *Lafoensia glyptocarpa* Koehne, *Lafoensia replicata* Pohl, *Lafoensia vandelliana* Cham. & Schltdl, *Lafoensia nummularifolia* A. St.-Hil. (MEIRA, 2000; SANO; ALMEIDA; RIBEIRO, 2008).

Lafoensia pacari A. St.-Hil. has a great tradition of popular use in Brazil due to its medicinal properties. It is popularly known as pacari, mangava-brava, dedal or dedaleiro (Lorenzi, 1992) (Figure 1).

Figure 1. General aspect the Lafoensia pacari in the city of Caldazinha / GO.

Scientific studies published in recent years have shown that the stem bark and leaves of pacari have diverse biological activities. Nascimento et al. (2011) showed that the stem bark has an antinociceptive effect regardless of the anti-inflammatory action involving mechanisms to reduce neurogenic pain. There is evidence that the extract from the bark of pacari has antidepressant effect (GALDINO et al., 2009). Rogério et al. (2003) demonstrated the potential of the ethanol extract of the stem bark of the *L. pacari* for use in eosinophilic inflammation, as those caused by nematodes such as Toxocara canis. It was also found that treatment with the ethanolic extract of *L. pacari* reduces cytokine production (IL-4, IL-5, IL-13) and that it may be an alternative treatment for asthma (ROGERIO et al ., 2008). Tamashiro et al. (2012) demonstrated the anti-ulcer activity of the methanol extract of the bark of pacari. Gastroprotection induction was detected in models of acute and chronic ulcers. This activity depends on the antioxidant activity and inhibition of pro-inflammatory cytokines, but was independent of gastrointestinal motility and secretion of mucus.

The phytochemical screening of the extract of the stem bark of *L. pacari* indicated the presence of tannins, saponins and steroids (SOLON et al, 2000; VIOLANTE et al, 2009). Solon et al. (2000) isolated and identified ellagic acid ether and ethyl acetate fraction of the stem bark and the ellagic acid was considered the main inhibitor of the formation of free radicals and xanthine oxidase. The leaf extract showed flavonoids as kaempferol-3-O-glucoside, 3-O-glucosyl-kaempferol glycosides and quercetin and 3-O-glycosides of quercetin (galactoside, glucoside and glycosyl xilosídeo) (SANTOS; SALATINO; SALATINO, 2000).

Sampaio et al. (2011) found a positive effect of temperature and micronutrients in the production of phenolic compounds (tannins, flavonoids and ellagic acid) in the leaves of pacari. The phenolic compound was found specially in the months of lower temperature, and the water soluble tannin were the compounds with the highest concentration (32 to 54%). According to Okuda et al. (2009), Lythraceae is known by the presence of hydrolysable tannins from the class of ellagitannins; these tannins were identified in several genres, including *Cuphea*, *Lagerstroemia*, *Lythrum and Woodfordia* (OKUDA; YOSHIDA; HATANO, 2000).

The tannins are phenolic compounds of high molecular weight (500-3000 Daltons), are water soluble and have the property of forming insoluble complexes with alkaloids and proteins (SANTOS, Mello, 2007). In plants, the tannins have an important role in the defense against herbivores and microorganisms (Monteiro et al., 2005). The tannins are responsible for some pharmacological activities as antiseptic, antidiarrheal, antimicrobial, as antidote against poisoning, wound healer, anti-inflammatory, antioxidant, antiviral and antitumor. The main properties responsible for the pharmacological activities were the reduction of metal ions, binding and precipitation of protein and antioxidant activity (OKUDA, 2005).

The interest of cosmetic industries in 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), which are highly unstable and reactive molecules, since they have unpaired electrons in its outer orbit. Stability is achieved when these molecules capture electrons from other molecules and vital substances, such as DNA, proteins and lipids, causing oxidative damage and disfunction 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).

Approximately 80% of the most significant signs of skin aging are caused by UV radiation and include wrinkles, roughness, appearance of fine lines, lack of elasticity and hyperpigmentation marks (BUCHLI, 2002; SARAF; KAUR, 2010).

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 (STEINER, 2011). 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 free radicals to the skin tissue (CHEN et al., 2012; DWECK et al., 2002; KORAC; KHAMBHOLJA, 2011).

According to what was said before about the potential of L. Pacari on antioxidante activity coupled with the fact an innovative cosmetic application justifies the preparation of this study, which main purpose is the assessment of the in vitro safety of pacari extract (obtained from plant leaves by reason of sustainability) as a promising candidate for antioxidante agent and sunscreen in cosmetic formulations.

EXPERIMENTAL

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 "pacari" extract were solubilized with methanol (3.9 to 1300 µg. mL^{-1}) and placed at 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 $mg.ml^{-1}$, 4mM glutamine and 10 % (v/v) bovine fetal serum at 37°C and 5% CO_2. The cytotoxicity test was performed three times, and also de methanol was assayed to evaluate the solvent cytotoxicity.

The cells were washed and the samples were added and the plates were incubate for 24 hours at 37°C, 97% humidity and 5% CO_2 in cell culture flasks. After this period the cells were submitted to MTS/PMS assay, 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 using a plate spectrophotometer (Multiskan EX 355, Thermo Electron Corporation). The data were analyzed by Origin™ software.

The data were also analysed using PHOTOTOX® (2008) software and the IC10 e IC50 values were determined (DOYLE; GRIFFITHS, 2000).

RESULTS AND DISCUSSION

There has been a continuous effort at World Wild, and particle EU level, to find alternative approaches which avoid testing on animals wherever possible. Whenever replacement is not possible, the development of methods which use fewer animals or cause least harm to the animals is supported. This 'Three Rs Principle' (replacement, reduction

and refinement of animal use) is present in all relevant world legislations (Adler et al, 2011).

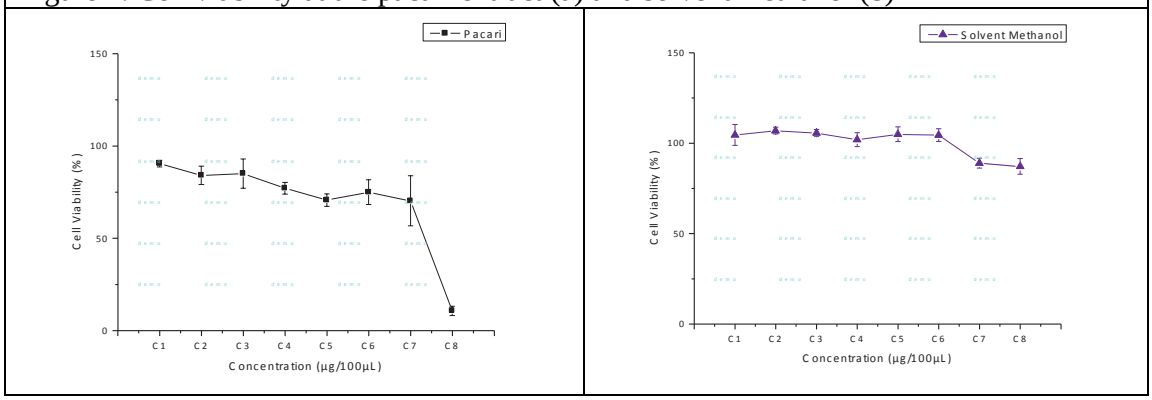
Although "pacari" is widely studied, the cytotoxicity of the extract has not yet been reported. Following the global trend, the use of alternative methods for the assessment of cytotoxicity of herbal products is a reality focusing on the attempt to prove the ability of *in vitro* methods to reduce the use of animals for toxicity screening plants and thus taking into account the principles of the 3Rs (Russel and Burch, 2009).

Recently in Brazil was approved a law to minimize the cosmetics tests in animals (not published yet), finally following the World efforts to attempt to 3Rs principles.

Some important points should be mentioned, such as the solubility of the plant extracts and subsequent maintenance of their bioactive properties. In addition, the use of solvents, at concentrations that do not have cytotoxic effects, is a major consideration when performing *in vitro* tests (ESTEVES-PEDRO et al., 2010). These points are of fundamental importance in already described *in vitro* tests (NIH, 2001; ICCVAM, 2006) to be adapted and used in herbal samples that are much more complex than synthetic chemistries. Another point of concern is the ability to sterilize the sample in a way that does not interfere with its biological activities.

In Figure 2 (a) it is possible to note that the lyophilized extract of "pacari" presents cytotoxicity just at the higher concentration. Also, at Figure 2 (b) the same results were observed for methanol solvent, as it did not show any toxicity at the evaluated concentrations.

Figure 2. Cell viability at the pacari extract (a) and solvent methanol (b)



Using the Phototox® software it is possible to calculate the IC50 and IC10 of "pacari" extract, 984.62 µg and 13.40 µg, respectively. The IC50 value corresponds to fifth percent of cell viability and the IC10 corresponds to tenth percent of cell viability being considerate as the non-cytotoxic value of the extract.

According to ICCVAM (2006), in order to determine a LD50 value based on the IC50, the following equation 1 is applied:

log LD50 (mg/kg) = 0.372log IC50 (µg/ml) + 2.024 (eq.1)

Therefore, the LD50 value for the *Lafoensia pacari* lyophilized extract corresponds to 1372.44 mg/kg. This value corroborate the studies performed by Matos et al (2008); Nascimento et al (2011) and Tamashir et al (2012) were the active pharmacological doses were between 0,5 and 1 g per kg weight.

The LD 50 determined value also could lead to an increase at the concentrations used by Violante at al. (2009) that studied the *in vitro* potential sunscreen activity of "pacari" and concluded that the assayed concentration (50 and 100 mg/mL) of ethanolic extract does not had any potential as plants with photo-protector potential.

CONCLUSION

The pacari extract proved to be non-cytotoxic under the tested conditions at 62.5 µg. mL^{-1} that is a usually concentration used as antioxidant action in cosmetic formulations. Also, it is possible to determine the IC10 and IC50 doses, which leads to the pre-determination of DL50 of "pacari" extract, preventing the use of animal tests and corroborating pharmacological already done studies.

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