EVALUATION OF THE TOXICITY PROFILE OF SHAMPOO PROTOTYPES FORMULATED WITH PLANT SURFACTANTS

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ABSTRACT

When developing new cosmetics, several analytical tests are carried out to prove the viability and safety of the product before production on a pilot scale. In the present work, three prototypes of innovative shampoo formulations containing plant extracts rich in biosurfactants from *Chenopodium quinoa* (quinoa), *Glycine max* (soy) and *Malpighia emarginata* (acerola), were analyzed regarding their environmental toxicity profile, genotoxicity and eye irritation due to means of cabbage seed phytotoxicity tests, analysis of the presence of micronuclei in onion root stem cells, and the HET-CAM assay, respectively. The results showed that the prototypes presented reduced or no toxic activity in the relative germination of the seeds, and the root growth rate was significant. Regarding the potential for eye irritation, the F1 and F3 prototypes were classified as little irritating and the F2 as non-irritating. In the genotoxicity assessment, the presence of micronuclei resulting from chromosomal changes was not verified, showing that the prototypes did not have a genotoxic effect. Such results indicate the biocompatibility and safety of the prototypes, thus presenting the potential for future commercialization and supply of new biotechnological products with high added value.

Keywords: Safe cosmetics. Quality control. Plant biosurfctant. Toxicological assessment.

1 INTRODUCTION

Shampoo is the main cosmetic used in hair care, being present in many people's daily routine. Currently, there is a wide range of shampoos for various applications, such as medicinal shampoos, shampoos for babies, people with sensitive skin and for each type of hair.[1] These shampoos contain a variety of key ingredients such as surfactants and foaming agents. Most of these ingredients present are capable of being absorbed by the skin and causing allergic reactions. Furthermore, prolonged exposure to some of these ingredients, of synthetic origin, is associated with carcinogenicity and mutagenicity.[2]

In addition to the negative effects on human health, residues of active ingredients and ingredients from cosmetic formulations are continually introduced into the environment, mainly through domestic sewage systems, due to the lack of effective removal of these residues by treatment plants, generating ecological impacts related to bioactivity, toxicity and bioaccumulation in aquatic systems.[3]

With this in mind, carrying out toxicity tests are extremely important, as cosmetics that appear harmless can cause serious damage to the health of users and the environment, therefore safety assessment must precede the placing of the product on the market. Several experimental models for evaluating toxicological effects are described, among these, alternative in vitro models, which replace the use of animals, have gained strength due to the prohibition of this practice in many countries, in addition to pressure from consumers to end the use of animals in tests.[4]

Based on this, the present work proposed to evaluate the environmental toxicity profile, genotoxicity and eye irritation potential of three prototype shampoo formulations, through in vitro tests. The prototypes use plant extracts rich in biosurfactants from *Chenopodium quinoa* (quinoa), *Glycine max* (soy) and *Malpighia emarginata* (acerola), as primary surfactants, com o objetivo de diminuir o uso de surfactates sintéticos derivados do petróleo, com potencial tóxico comprovado.

2 MATERIAL & METHODS

Obtaining extracts and producing prototypes

The seeds of *Chenopodium quinoa* and *Glycine max* and the dry fruit of *Malpighia emarginata* were used for the hydroalcoholic extraction of biosurfactants.[5] Three shampoo prototypes were produced according to previously developed formulation[6], using plant extracts of *C. quinoa*, *G. max* and *M. emarginata* as primary surfactants and disodium cocoyl glutamate (DCG) as a secondary surfactant. The combination of extracts in the prototypes is presented in Table 1. A formulation without the addition of surfactants, another containing only DCG, and a shampoo already commercialized were used as comparative standards.

Table 1 Surfactants used in prototypes.

Code	Combination of surfactants
F1	*C. quinoa* + *M. emarginata* + DCG
F2	*G. max* + *M. emarginata* + DCG
F3	*C. quinoa* + *G. max* + *M. emarginata* + DCG
F4	DCG
F5	No Surfactant

Environmental toxicity of prototypes

The phytotoxicity tests of the prototypes were evaluated at a concentration of 1%, through tests to estimate the germination rates and relative growth of the roots of *Brassica oleracea* var. capitata f. rubra (red cabbage), as described by Tiquia et al. (1996).[7]

Potential for eye irritation and genotoxicity of the prototypes

The hen's egg chorioallantoic membrane test (HET-CAM) was performed following the in vitro method described by Steiling et al. (1999)[8] for a semi-quantitative analysis of the irritant potential (IP) of the extracts at a concentration of 1% (w/v).

Genotoxicity of the prototypes

Genotoxicity was assessed using the Allium cepa test system according to Parvan et al. (2020).[9]

Statistical Analyzes

All tests were performed in triplicate and data are expressed as mean ± standard deviation. ANOVA analysis was used to determine significance. P values <0.05 were considered significant.

3 RESULTS & DISCUSSION

Environmental toxicity of prototypes

Cosmetics and personal hygiene products contain numerous ingredients and active ingredients that, when in contact with vegetable crops, can affect plant growth, therefore, the assessment of environmental toxicity is essential for the development of products that are also safe for the environment. In the case of the prototypes evaluated in this work, relative seed germination (RSG) was little affected when in contact with F2, and there was no interference in germination when in contact with F1 and F3. The relative root growth (RRG) was significant, with a rate above 65% for the three prototypes. The germination index (GI) of 77.8; 62.7 and 65.2% for F1, F2 and F3 respectively, were higher than those presented by the shampoo already sold. In formulations without the presence of plant extracts in the composition (F4 and F5), there was a significant drop in both the growth and germination rate of cabbage seeds (Table 2).

Table 2 Toxicity assays of prototypes with red cabbage seeds.

Samples	RSG (%)	RRG (%)	GI (%)
F1	100,00	77,84	77,84
F2	88,89	70,62	62,77
F3	100,00	65,29	65,29
F4	66,67	26,29	17,53
F5	77,78	24,40	18,98
SC	77,78	32,13	24,99

Barooah et al., (2022)[10] studied the effects of common brands of soap, shampoo and detergent on the germination and growth of chickpea (*Cicer arietinum*) and moth (*Vigna aconitifolia*) seeds. The results showed a more severe effect of detergents on seed growth and development compared to shampoo. The shampoo samples showed insignificant impacts on seed germination and growth, as the results obtained in this work.

Potential for eye irritation

The results of the ocular irritant potential (IP) of the prototypes, assessed by the chicken egg chorioallantoic membrane test (HET-CAM), are positive and are summarized in Table 3. Prototypes F1 and F3 were classified as little irritating (IP = 3.38 and 4.45 respectively), and F2 as non-irritating (IP = 0). Sodium lauryl sulfate 1%, used as standard in this test, and widely used as a surfactant in shampoos, proved to be severely irritating (IP = 18.38). Due to the low and no irritation potential of the prototypes, their use in cosmetic applications would not pose any risk.

Table 3 Results of the chicken egg chorioallantoic membrane test applied to prototypes.

Type of Irritation	Time Each Process Started (Seconds)						
	F1	F2	F3	F4	F5	SC	SLS
Lysis	158	-	139	-	112	-	55
Hemorrhage	-	-	-	-	-	-	14
Coagulation	-	-	-	-	-	-	39
Irritation potential	3,38	0	0	0	4,45	0	18,38

Genotoxicity of the prototypes

Cytogenetic research in plant species provides information about potential changes in plant chromosomes due to the presence of agents that cause mutations in their composition or as a result of their metabolism. Mutations can be caused by a variety of factors, including chemical substances, environmental conditions and radiation.[11] When evaluating the genotoxicity of the prototypes, the presence of micronuclei arising from chromosomal alterations was not verified, showing that the prototypes had no genotoxic effect on the meristematic cells of the root of *Allium cepa* L, that is, there is no presence of mutagenic agents in their composition (Figure 1).

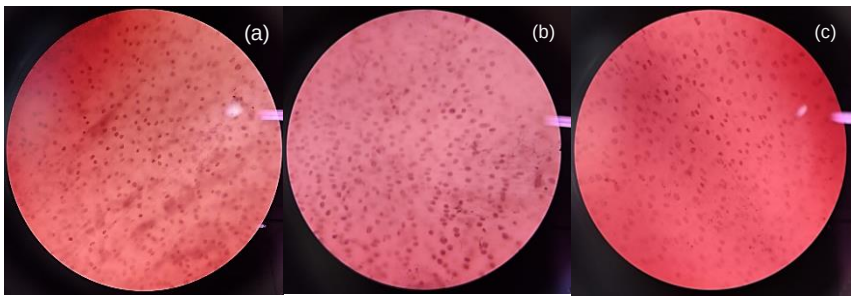

Figure 1 Meristematic cells of *A. cepa* treated with prototypes F1 (a), F2 (b) and F3 (c).

4 CONCLUSION

The prototypes evaluated here showed reduced or zero toxic activity for all tests carried out, validating the replacement of toxic synthetic surfactants with plant extracts with surfactant action in the formulation; indicating biocompatibility and safety for the environment and humans. The prototypes thus demonstrate potential for future commercialization, to supply new biotechnological products with high added value.

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