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Evaluation of the Environmental Toxicity Profile of Shampoos Formulated with Plant Biosurfactants

Káren G. O. Bezerra[a,c*], Keila I. da Costa[a], Júlio C. V. dos Santos[a], Ellanie S. C. da Silva[a], Juliana M. Luna[a], Fabíola C. G. de Almeida[c], Raquel D. Rufino[b,c], Leonie Asfora Sarubbo[a,c]

[a] Icam Tech School, Catholic University of Pernambuco, Rua do Príncipe, n. 526, Boa Vista, Zip Code: 50050-900, Recife, Pernambuco, Brazil;
[b]Health and Life Sciences School, Catholic University of Pernambuco, Rua do Príncipe, n. 526, Boa Vista, Zip Code: 50050-900, Recife, Pernambuco, Brazil;
[c]Advanced Institute of Technology and Innovation (IATI), Rua Potira, n. 31, Prado, Zip Code: 50751-31, Recife, Pernambuco, Brazil
karengercyane@gmail.com

In developing new cosmetics, a series of steps must be followed until the final product is reached. In general, the process goes through the initial idea and preliminary research before reaching the development of the prototype. After the development of the prototype, several analytical tests are carried out to prove the viability 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, of *Chenopodium quinoa* (quinoa), *Glycine max* (soy) and *Malpighia emarginata* (acerola) as cleaning agents were analyzed for their environmental toxicity profile. The plant extracts used aimed to reduce the use of toxic synthetic surfactants and to produce safe, renewable and biodegradable formulations, to help solve the challenges related to the damage caused to the environment due to the presence of synthetic surfactants in shampoo formulations and their toxic residues in soils and waters. The toxicity of the formulations was evaluated at a concentration of 1%, and the tests carried out were the phytotoxicity tests through the static test involving seed germination and root elongation of tomato (*Solanum lycopersicum*) and the *Allium cepa* L. root growth inhibition test. A toxicity test was also performed using the microcrustacean *Artemia salina* as a bioindicator. A formulation without the addition of surfactants, another containing only DCG, and a commercially available shampoo, whose acceptability is known, were used as comparative standards. The results showed that the formulations showed reduced or no toxic activity for the environmental bioindicator *Artemia salina*, for the seeds of *S. lycopersicum* and for the root growth of *Allium cepa* L., indicating the biocompatibility and safety of these formulations, thus presenting the potential for future commercialization, for the supply of new biotechnological products with high added value.

1. Introduction

Cosmetic and personal care products are used in large quantities all over the world, playing an essential role in people's daily lives. Various products are used daily, such as soaps, shampoos, toothpaste, deodorants, skin cream, perfumes, and makeup, and every day new varieties and products are emerging (VECINO et al., 2018). Along with the development of new products, there follows a growing number of chemical compounds that are added to the formulations of cosmetics and personal care products, such as fragrances, preservatives, stabilizers, dyes and surfactants, to enhance their quality, properties and shelf life. (BILAL et al., 2020).
However, the active residues of these ingredients and additives are continuously introduced into the environment, especially through domestic sewage systems, due to the lack of effective removal of these residues by sewage treatment plants (ANAND et al., 2022). Therefore, cosmetic products are continuously introduced into aquatic systems, generating ecological impacts related to bioactivity, toxicity and bioaccumulation.

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Furthermore, these compounds are accumulated in sewage sludge during wastewater processing, finding another path to the environment, due to the use of this sludge as a fertilizer crop (BILAL et al., 2020).
Among these ingredients are synthetic surfactants, especially sulfated ones, widely used in various cosmetic formulations. According to Johnson et al. (2021), the global use of surfactants exceeds 15 million tons per year, and about 60% ends up in the aquatic environment. The British Geological Survey, listed synthetic surfactants as organic pollutants, having been detected in surface waters, affecting plant life and microbial activity, interrupting the biological processes of these living organisms. Surfactants also increase the spread of other pollutants, such as heavy metals, which cause additional problems for the ecosystem (IVANKOVIĆ et al., 2010).
Given the above, bio-based ingredients are a good alternative and become a primary criterion for formulating sustainable cosmetic and personal care products. The bio-based content of an ingredient or formula is the percentage of carbon molecules in the formula derived from a renewable source, such as vegetables, for example, rather than a non-renewable and potentially toxic petroleum source (BONDI et al., 2015). Based on this, the present work proposed to evaluate the environmental toxicity profile of three prototypes of Shampoo formulations that use plant extracts rich in biosurfactants from *Chenopodium quinoa* (quinoa), *Glycine max* (soybean) and *Malpighia emarginata* (acerola), as primary surfactants.
Biosurfactants are produced from plants and microorganisms and have the same functional properties as synthetic surfactants. Plant biosurfactants, however, have received attention, especially due to the potential for better yields in extraction processes, when compared to the yield of biosurfactants from microorganisms, making them more viable for industrial applications. In addition, the possibility of using plant biosurfactants in the form of a crude extract is another positive point, since high expenses with purification processes are not required (DU et al., 2020). Therefore, plant extracts were used in formulations to reduce the use of toxic synthetic surfactants and to produce safe, renewable and biodegradable formulations, to help solve the challenges related to the damage caused to the environment due to the presence of synthetic surfactants in shampoo formulations and their products. toxic residues in soils and waters.
The prototypes underwent analytical tests to determine their toxic potential, intending to verify their environmental safety in a future commercial application. The toxicity of the formulations was evaluated through phytotoxicity tests, by seed germination and root elongation of tomato (*Solanum lycopersicum*), the Allium cepa L. root growth inhibition test and the toxicity test using the microcrustacean Artemia salina as a bioindicator.

2. Methods

2.1 Obtaining Extracts and Production of Formulations

The seeds of *Chenopodium quinoa* and *Glycine max* and the dry fruit of *Malpighia emarginata* were used for the hydroalcoholic extraction of biosurfactants (BEZERRA et al., 2021). Three shampoo prototypes were produced according to previously developed formulations, using plant extracts of *C. quinoa*, *G. max* and *M. emarginata* as primary surfactants and disodium cocoyl glutamate (DCG) as a secondary surfactant. The base formulation is presented in Table 1 and the combination of extracts in the formulations that were produced is presented in Table 2. A formulation without the addition of surfactants, another containing only DCG, and a shampoo already commercialized were used as comparative standards.

Table 1: Base formulation of prototypes

Component	Internacional Nomenclature of Cosmetic Ingredients	%	Function
Water	Aqua	q.s.*	Solvent
Extracts	*Chenopodium quinoa* seed extract and/or *Glycine max* seed extract and/or *Malphiguia emarginata* fruit extract	10	Surfactant
Amisoft CCS 22	Disodium Cocoyl Gluatamate	4	Surfactant
Glycerin	Glycerin	3	Wetting
Xanthan Gum	Xanthan Gum	0.85	Thickener
Coconut oil	Hydrogenated coconut oil	0.5	Moisturizer
Sodium benzoate	Sodium Benzoate	0.2	Preservative
Potassium Sorbate	Potassium Sorbate	0.2	Preservative
Lavender essential oil	*Lavandula Hybrida Grosso* Herb Oil	0.2	Fragrance
Sodium gluconate	Sodium Gluconate	0.1	Scavenger
Citric acid	Citric acid	q.s.*	pH corrector
Sodium hydroxide	Sodium Hydroxide	q.s.*	pH corrector

*Quantum sufficient

Table 2: Primary and secondary surfactants used

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

2.2 Phytotoxicity test

The phytotoxicity of the formulations was evaluated at a concentration of 1% in static assays to estimate germination rates and the relative root growth of *Solanum lycopersicum* (tomato), as described by Tiquia et al. (1996). The assays were performed in triplicate for 7 days in the absence of light. At the end of the experiments, the relative seed germination (RSG), relative root growth (RRG) (≥ 5 mm), and the germination index (GI) rates were calculated as in Equations (1)–(3), respectively.

$$\text{RSG (\%)} = \frac{\text{Germinated seeds in contact with the sample}}{\textit{Germinated seeds from control sample}}\ x\ 100 \quad (1)$$

$$\text{RRG(\%)} = \frac{\text{Average growth of roots in contact with the sample}}{\textit{Average growth of roots from control sample}}\ x\ 100 \quad (2)$$

$$\text{IG (\%)} = \frac{\text{RSG}}{\text{RRG}}\ x\ 100 \quad (3)$$

2.3 Toxicity test with onion (*Allium cepa* L.)

The phytotoxicity test was performed using onion (*Allium cepa* L.) as a bioindicator according to the method of Arambasic et al. (1995), with modifications. Onion bulbs (18 to 26 g) were purchased commercially in supermarkets and weighed individually (dry weight). The old roots were carefully removed and the base of the bulbs was placed in Falcon tubes (50 mL) containing samples of the solutions of the formulations at 1% concentrations and mineral water (control). Each sample consisted of 3 onion bulbs. Tests were performed at room temperature and in the dark. Every 48 h the samples were fed. After 7 days, the length of the roots was measured and each bulb was weighed (wet weight) to evaluate root growth and bulb mass to obtain the weight gain of each onion.

2.4 Toxicity Test with Artemia salina

The toxicity of the formulations was also evaluated using brine shrimp (*Artemia salina*) as the bioindicator. Brine shrimp eggs were obtained from the local market. The larvae were used within one day after hatching. After dilutions of the formulations in seawater, the assays were performed in a 50 mL beaker with 10 larvae in 20 mL of seawater + 20 mL of the formulations at 1% concentration. The mortality rate was calculated after 24h (SARUBBO et al. 2016).

2.5 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 and discussion

3.1 Phytotoxicity test

Cosmetics and personal care products contain numerous ingredients and actives that, when in contact with plant cultures, can affect plant growth. In the case of the formulations evaluated in this work, relative seed germination (RSG) was little affected when in contact with F1, and there was no interference in germination when in contact with F2 and F3. The relative seed growth (RRG) was significant, with an index above 50% for the three formulations. The germination index (GI) was estimated at 57.5, 58.5 and 64.6% for formulations F1, F2 and F3 respectively, higher rates than that presented by the shampoo already commercialized. Concerning formulations without the presence of plant extracts in the composition (F4 and F5), there was a significant drop in both growth and germination index of tomato seeds (Table 3).

Table 3. Toxicity assays of formulations with tomato seeds.

Sample	RSG %	RRG %	GI (%)
F1	85.7	67.1	57.5
F2	100	58.5	58.5
F3	100	64.6	64.6
F4	85.7	27.2	23.3
F5	85.7	24.5	21.0
CS	100	56.4	56.4

Barooah et al., (2022) studied the effects of common soap, shampoo and detergent brands on chickpea (Cicer arietinum) and moth (Vigna aconitifolia) seed germination and growth. 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, such as the results obtained in this work.

3.2 Toxicity test with onion (*Allium cepa* L.)

Plants, such as onions, have been widely exploited for the toxicological evaluation of various pollutants. The advantages of this plant organism are related to its low cost, easy growth, non-seasonal availability and viability for acute and chronic toxicity assays under laboratory or environmental conditions. Phytotoxicity, estimated by inhibition of root elongation in mature organisms or seed germination, is the most common indicator (PINTO et al., 2022). The experiments are quick and easy to perform and the toxic effect is considered taking into account weight gain and root elongation in contact with the product to be evaluated.

In the present work, the toxicity evaluation showed that the onions that grew in contact with the formulations obtained a small weight gain, however, there was no weight loss. Weight loss occurs when onions lose energy in self-defense against pollutants JARDIM (2004). Analyzing the length of the largest root, all samples provided the growth of small roots, except for the onion exposed to commercialized shampoo, which did not grow roots. Regarding the number of roots, among the formulations, F1 provided the highest number of roots, followed by formulation F4 (Figure 1).

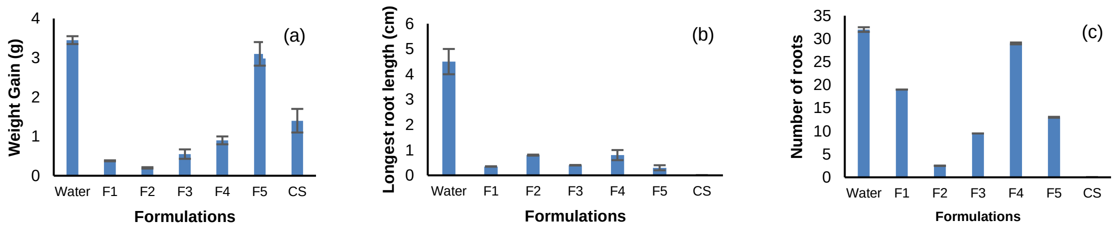

Figure 1. Weight gain of onions exposed to the formulations (a), Length of the largest root exposed to the formulations (b), and number of roots (c).

The toxicity test indicated that the formulations analyzed here do not have an inhibitory effect on the development of onions, unlike the commercialized shampoo. It is important to point out that by the time the ingredients and actives of cosmetics and personal care products reach the environment, they are mostly degraded and/or diluted (BONDI et al., 2015), therefore, it is necessary to offer toxic potential in low concentrations to cause negative effects on the environment, which is not the case of the formulations analyzed here, further confirming their safe and ecofriendly characteristics.

3.3 Toxicity Test with Artemia salina

Assessing toxicity against aquatic organisms is relevant in the case of any contact the product may have with aquatic ecosystems. Artemia salina is a standard marine living being commonly used in ecotoxicology due to the viability of maintenance on a laboratory scale, simple growth conditions and a short life cycle (PINTO et al., 2022). In addition, they have reduced tolerance to environmental changes and high specificity to external interference, ensuring the expression of clear results in the face of small variations in the quality of the environment (LIMA et al., 2019).

The test results showed that the F3 formulation had a high mortality rate (65%). Formulations F1 and F2, however, showed low or no mortality, respectively. About formulations F4 and F5, there was no mortality. It is interesting to note that the commercialized shampoo formulation had a mortality rate of 100% (Table 4).

Table 4. Toxicity of formulations using A. salina.

Formulations	Mortality Index (%)
F1	20 ± 5.0
F2	0 ± 0.0
F3	65 ± 5.0
F4	0 ± 0.0
F5	0 ± 0.0
CS	100 ± 0.0

According to Bondi et al. (2015), ingredients or formulations are considered toxic if they are lethal for half of the experimental population exposed at doses above 100 mg/L. Taking into account that the formulations were evaluated at a concentration of 1%, that is, 10000mg/L, it shows that the F1 and F2 formulations do not have toxic potential, even at a concentration 100 times higher. Concerning the F3 formulation, it will be necessary to evaluate the mortality rate of artemias exposed to this formulation at lower concentrations, to define its toxic potential.

4. Conclusions

The use and disposal of cleaning products release surfactants into the environment through domestic sewage systems. Therefore, the environmental toxicity profile is an important consideration when assessing the risks and benefits of using a particular surfactant in the formulation of products such as shampoos and cosmetics in general. The formulations evaluated here presented reduced or no toxic activity for *Artemia salina*, for the seeds of *S. lycopersicum* and the root growth of *Allium cepa* L., further validating the choice of plant extracts as surfactant agents in formulations, and indicating the biocompatibility and safety of these formulations, thus presenting the potential for future commercialization, for the supply of new biotechnological products with high added value.

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