

## Investigation of the antifungal action of natural products on seeds of *Euterpe oleraceae*: Bioproduct Potential



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### ABSTRACT

Açaí seeds (*Euterpe oleraceae*) are used in handicrafts by urban indigenous Sateré-Mawé present in Rio de Janeiro/Brazil, which brought the demand for the vast loss of seeds due to fungal contamination and the problems triggered by it. In search of simple, cheap and efficient solutions, total aqueous extracts of *Bixa orellana* (Attatto/Urucum), *Punica granatum* (Pomegranate) and *Tagetes erecta* (Carnation) and *Euphorbia tirucalli* (Aveloz/Firestick) were tested on *Euterpe oleraceae* (Açaí) seeds. Only *E. tirucalli* exerted antifungal activity (21.75%) at concentration tested, showing a promising action for the conservation of Açaí seeds. Based on the understandings generated by the experiments, new trials are being designed, inserted into the One Health perspective.

**Keywords:** Sateré-Mawé, *Euterpe oleraceae*, *Euphorbia tirucalli*, *Seeds*, *Antifungal effect*.

## 1 INTRODUCTION

Sateré-Mawé, people of the Waraná, are an indigenous Brazilian ethnic group from the Amazon region. In addition to the awareness of the preservation of their knowledge and "know-how", they deal with assertiveness regarding the use of the resource of biological diversity, such as guarana (*Paullinia cupana*), cultivated mainly in Maués and Bahia (ALVES, 2021).



A representative of this ethnic group, in an urban situation in Rio de Janeiro (ALVES, 2021), works as an artisan using açai seeds (*Euterpe oleraceae*) from the Amazon, in the making of handicrafts, aiming at financial autonomy. As a problematization, this indigenous artisan brought up the worrying issue of these seeds, stored in the bags in which they came packed, acquire fungi, release dust, which triggers severe respiratory allergy and, finally, rot. Therefore, in her worldview, seeds do not fulfill their purpose on Earth because they are not cultivated, nor ingested as food by any living being and not even employed for sustenance, since there is a great loss of seeds, evaluated by her as almost half of the packages she buys (approximately 47%).

In addition, these fungi-contaminated seeds attract ants into your home. It is known that any tender plant material, rich in carbohydrates, sucrose and fructose, feeds fungi already related in the literature to ant colonies. The smoke syrup is usually applied together with adhesive element, linseed or soybean oil, so that it is stabilized in the leaf and has an effect. Orange peel oil, rich in terpenoids, fungicide and bactericide, is usually sprayed on anthills of saúvas, cortadeiras (SANDRA ÁVILA GASPARGASPAR, oral communication 2022).

However, what is described for the treatment of açai seeds with natural products? Something simple to access and easy to apply for everyday use of these indigenous people who work with these (and other) seeds? Unfortunately, very little, given the potential resources existing in the rich Brazilian biodiversity.

However, it is known that some of the assisted crops of *Euphorbia tirucalli*, in pots with weight solutions of growth regulators, in addition to respective pharmacotechnical preparations for ultradiluted and energized solutions (SUD), exerted repellent effects on the voracious leaf-cutting ants, which selectively cut only plants from some pots, avoiding others (VARRICCHIO et al., 2006).

In addition, total extracts of *Euphorbia tirucalli*, *Bixa orellana* and *Punica granatum* exerted antifungal and dormancy activity in *Vigna unguiculata* seeds. Still, by the literature, the genus *Tagetes* sp., *Tagetes erecta* (Cravo) is described as antimicrobial (OLIVEIRA et al., 2019).

With antifungal actions already described for other models evaluated, can these plant species exert antifungal activity in açai seeds?

## 2 GOALS

To evaluate the antifungal effect of total aqueous extracts of *Euphorbia tirucalli* (Aveloz), *Bixa orellana* (Urucum), *Punica granatum* (Pomegranate) and *Tagetes erecta* (Clove) on seeds of *Euterpe oleraceae* (Açai). To evaluate the effects of the bioproduct obtained from the micropropagation of *E. tirucalli*, under different pharmacotechnical preparations applied on the cultivation of *Euterpe oleraceae*.



### 3 METHODOLOGY

#### 3.1 BOTANICAL CERTIFICATION

The exsicata of *E. tirucalli* was prepared with specimens of the garden plant from IPPN/UFRJ. The botanical identification was carried out by Dr. Luci de Senna Valle and the specimens of the testimonial material were deposited in the herbarium of the National Museum of Rio de Janeiro under Botanical Registration R – 204.406.

#### 3.2 ESTABLISHMENT OF IN VITRO CULTURE FOR THE EVALUATION OF THE EFFECT OF ULTRADILUTED AND STREAMLINED SOLUTION (ISOTHERAPEUTIC) ON MICROPROPAGATION

After botanical certification in the herbarium of UFRJ, disinfestation and culture of plant tissue were carried out. The cultures were kept in a growth room at a temperature of  $(25 \pm 1)^\circ\text{C}$ , illuminated with fluorescent lamps (Sylvania, Phillips/daylight), with a luminous intensity of  $30 \mu\text{moles/m}^{-2} \cdot \text{s}^{-1}$  and 16 hours of photoperiod. All in triplicate, with  $N = 40$ , for each test, in addition to the control with bideionized water.

After disinfestation, in a laminar flow chamber, the semisolid medium of basic composition according to Murashige & Skoog, 1962 (MS0 - with 30g/L of sucrose) was distributed in the Petri dishes, in triplicates. To each plate was applied 20 $\mu\text{L}$  of SUD 30CH of mother tincture (TM or 30% ethanolic extract submitted to succussions in the hahnemannian centesimal scale) and the respective control SUD 30CH of ethanol 30%, to be performed analysis of variance of the number of buds, shoots, oxidized stems and the presence of fungi at the thirtieth and sixtieth days. Commercial-branded seeds were purchased in the Amazon market, 3 seeds of açai (*Euterpe oleraceae*) were inserted in 10 cups, in each row, with a control solution (mineral water) and four test solutions: 100  $\mu\text{L}$  of 20% aqueous extracts of *Euphorbia tirucalli*, *Bixa orellana*, *Punica granatum* and *Tagetes erecta* were applied, acquired in pharmacy-school, making a total of 5 rows, with 150 seeds. All completed to 200mL of net volume of commercial mineral water. The pH of the mineral water used and the seeds in the cups were then measured.

The ambient temperature of the first day, the third day and the seventh day and the temperature of the cups of each of the rows were measured to establish an average temperature, seeking to investigate differences to evidence the presence of exothermic reactions.

To evaluate the effect of the test on seed germination morphology, the number of hypocotyls emitted, whether they were viable or not (detachable), their size and coloration were counted. The seeds were weighed on the first day before starting the experiment, and an average was established for the first day of the experiment and the seventh day.



As for abiotic factors, on the first, third and seventh day of cultivation, free chlorine (rupture), cyanuric acid (oxidative stress) and total alkalinity (recovery potential) were also measured by the Aquachek colorimetric method.

At the end, the experiment was repeated, in triplicate, and submitted to statistical analysis of variance by the ANOVA method.

## 4 FINDINGS

*Euphorbia tirucalli* (Aveloz), is a succulent considered exotic and attributed to the African continent, of traditional use by native peoples around the world including for cultivation, due to its allelopathic action, able to ward off invasive and competing species (MENDES et al., 2022).

### 4.1 CONSERVATION OF *EUTERPE OLERACEAE* SEEDS FOR SATERÉ-MAWÉ INDIGENOUS HANDICRAFTS WITH EXTRACTS OF *EUPHORBIA TIRUCALLI*, FROM BIOPRODUCTS OBTAINED FROM CROPS UNDER LIGHT CREEP ASSOCIATED WITH SUD (ISOPATHIC)

The results of the methodological approach *in vitro*, with isopathy, from the exposure of its ultradiluted and streamlined preparation, with 20 $\mu$ L SUD 30CH of mother tincture and its control LDS 30CH Ethanol, only once in the introduction *in vitro* with white light, were dramatically inhibiting. The evaluation initially scheduled for 30 and 60 days, needed to be brought forward to the end of the first week. There was extensive necrosis of the explants, with death ( $p \leq 0.05$ ). It was not possible to analyze the number of shoots, roots, nor was there time for the observation of fungal proliferation, so common to inoculation *in vitro* of this species from the first week of cultivation *in vitro*.

The extensive oxidation triggered was very negative, with blackening, drying and death ("sturricated", as if burned), a fact suggestive of energy overload in the mitochondrial chain, since previous micropropagation assay under different light fluencies, but in the absence of diluted and energized solution, had already been successful previously (VARRICCHIO et al., 2022).

Therefore, it is considered necessary to carry out the same experiment *in vitro* with other powers and respective controls, still on the same scale of succussion and dynamization (hahnemannian centesimal – CH). There are no data in the literature on micropropagation associated with SUD, in this case, with isopathy, for plants of the genus *Euphorbia* for discussion. Cultivation with isopathic in the same potency has already been performed in pots, having been extremely developmental inhibitor (VARRICCHIO et al., 2022).

Therefore, for this stage of the investigation, it was not possible to obtain extract in sufficient quantity from this micropropagation for testing. This study is original and promising for investigation of mechanism of action via mitochondrial chain and for the first time is being communicated.



#### 4.2 CONSERVATION OF *EUTERPE OLERACEAE* SEEDS FOR SATERÉ-MAWÉ INDIGENOUS HANDICRAFTS WITH EXTRACTS OF *EUPHORBIA TIRUCALLI*, *BIXA ORELLANA*, *PUNICA GRANATUM*, *TAGETES ERECTA*

The present assay was carried out with extracts collected and prepared in the summer, which had not induced the germination of *Vigna unguiculata* seeds. They maintained the desired seed dormancy. As an observation of the assay, there were no morphological modifications of the seeds with the test solutions, in relation to the respective controls. The extracts of *Bixa orellana*, *Punica granatum* and *Tagetes erecta*, at the tested concentration, were not able to reduce the fungal contamination of the seeds or the mineral water present in the cups, with massive loss.

The total aqueous extract of *Euphorbia tirucalli*, collected from the garden of the Ilha do Fundão Campus/UFRJ, was able to inhibit the fungal contamination of seeds and also of the mineral water where they developed (percentage rate of contamination of 21.75%), at the tested concentration.

Despite the promising results as an antifungal for acai seeds, *E. tirucalli* is a toxic plant species. The toxicity is given by the latex rich in esterified diterpenoids that cause lipid peroxidation, being known since they usually increase the fungal activity during the cultivation of cowpea exposed to these extracts (*Vigna unguiculata* - DELAUNAY DE SOUZA et al., 2023).

Previous micropropagations, with growth regulators, favored fungal contamination of explants (VARRICCHIO et al., 2022). Summer latex reduces the numerical amount of detectable terpenoids, while increasing the concentration of those that have not undergone volatilization process, due to excess heat and luminosity (DELAUNAY DE SOUZA et al., 2023).

Perhaps, this only substance detected at HPLC/UV in the summer may correspond to the diterpene ester of phorbol, considered a chemical marker of toxicity. And the process of dynamization of this matrix extract (TM) may have contributed to exacerbate the biological activity, overloading the thylakoids and photosystems I and II, resulting in oxidation, necrosis and death of the explants, and the second phase test cannot be performed.

Since the HPLC/UV of the oxidized explant yielded a different substance, which may be useful as a bioproduct, new tests will be carried out to finalize the detailing of this probable mechanism of action.

## 5 DISCUSSION

As postulated, the extract of *Euphorbia tirucalli* was able to inhibit the fungal contamination of the seed and also of the solvent medium, the mineral water, in the tested concentration, where they developed (percentage rate of contamination of 21.75%), evidencing a possible protective role, probably, through the Super Oxide Dismutase (SOD) pathway, to the seed film and/or due to membrane



lipid peroxidation with destruction or inactivation of the invasive fungus of the seed film of *Euterpe oleraceae* (Açaí).

Through the present essay shown here, it was reflected, then, on the potential alternative to seed conservation by inhibiting the growth of invasive fungi for informal work. There is also a promising potential to be a sustainable alternative through the use of light fluency associated with streamlined solutions, the effect of which can be observed from 7 days. Investigations continue to adjust this combination for final analysis of variance.

Mishaps during experiments should be taken seriously and thought through in depth. It is often nature itself showing us the best way forward in investigations. New assays will be carried out in order to evaluate the associated use of light and SUD in its action in water as a bioproduct, a natural antifungal, in already contaminated seeds.

It may also represent an affordable, low-cost and efficient strategic option for flooded areas, with people without immediate access to resources or the possibility of travel to purchase food. It is known that foods with fungal contaminations can trigger the presence of aflatoxins in the organisms, bringing, in the medium term, serious diseases. For all the reasons presented here, it is worth continuing this investigation.

Because it has existed since Pangaea, it has been recurrently published for many decades that indigenous peoples around the planet use Euphorbia, also *E. tirucalli*, in ethnomedicine and for economic uses. Specimens from the Arabian Plate and the Andes Mountains exhibit latex that are antitumoral, via mitochondrial uncoupling and programmed cell death. The popular use in Rio de Janeiro/Brazil cites its latex used for onychomycosis (VARRICCHIO & LAGE, 2020).

Brazilian shamans use it on their body for spiritual protection, evidencing its historical, cultural, medicinal and spiritual relevance (Cacique Carlos Machado/Doethyró-TUKANO oral communication *In* VARRICCHIO & LAGE, 2020; MACHADO/Doethyró-TUKANO et al., 2021).

The aveloz arrived in Brazil by the slave ships, because it was prepared as "axé soup" to increase their physical, mental and spiritual strength to these peoples. In fact, in the laboratory, its best plant chemical yield at 37°C confirmed the black historical account. There are records of the use of *E. virgata* for skin tumors, analysis of its resinous effect and plastic digester, as well as reports of the use of its latex as glue by calon gypsies from the northeast (VARRICCHIO & LAGE, 2020; VACITE et al., 2022).

Finally, actions of multicultural affirmative policies, respectful and peaceful intercultural coexistence, without impositions, were perpetrated. These cultural heritages permeate our society historically. Being considered distant cultures or second-class citizens are aspects that tell a lot about what Brazilian society has as value. The issue is historical-socio-economic (VARRICCHIO & LAGE, 2020).





Noteworthy, there is no intention to reduce the complexity of environmental sciences, however, given the current situation of climate emergencies, of several places with socio-environmental-climatic catastrophes simultaneously, we consider valid the attempts to use new methodologies, even if simple and focused, because they can bring well-being to small groups, as in family farming.

It aims at the promotion of Single Health, thinking about the best use of Brazilian natural resources and diversified pharmacotechnical preparations and this has been our trajectory (HANSEL-MARTINS et al., 2023). For control of zoonoses and their neglected diseases (GOMES et al., 2023); to remove environmental contaminants that chronically impregnate organisms and generate diseases (plastics in water – FREIRE SOUZA SILVA et al., 2019; Delaunay et al., 2023).

The discussion on the rational use of fresh drinking water in coastal areas where the sea has risen and invaded due to the potential participation of resistant halophyte plants, in this equation of use of the resource (GASPAR et al., 2023). Proposals for phytoremediation through phytoextraction of residual contaminants from water (medicines - KATHAR et al., 2023) with the potential to investigate other genera that could also be used in the filtration/removal of Leptospire and Hepatitis viruses present in flood waters.

## 6 CONCLUSION

The investigation of the biotechnological use of natural products, of plant origin, to avoid fungal contamination, was promising and of great relevance, since it aims to degrade or even prevent the fungal invasion in the film that belongs to the membrane of the açai fruit. New trials will be carried out for conclusion and continuation of the study of the mechanisms involved.

There was a healthy exchange of knowledge, contributing to the development of the ethical profile, engaged in socio-environmental issues and citizen integration, culminating in the protection of traditional knowledge, with effective actions to social innovation, of average potential for social transformation.

Intercultural coexistence, respectful and solidary, is the way to the sustainability of the planet.



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