International Journal of Phytocosmetics and Natural Ingredients 2020;7:10

doi 10.15171/ijpni.2020.10

Original Article

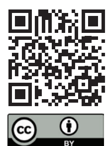
Photoprotective, antioxidant, antimicrobial and cytotoxic activities of guarana (*Paullinia cupana*) seed extracts

Edson Queiroz da Fonseca Junior[1,2*], Geverson Façanha da Silva[1,3], Luana Vieira Maquiné[1], Yasmin Matsdorff Monteiro[1], Raiana Silveira Gurgel[1,4], Elenn Suzany Pereira Aranha[5], Marne Carvalho de Vasconcellos[5], Patrícia Melchionna Albuquerque[1,2,4]

[1]Laboratório de Química Aplicada à Tecnologia, Escola Superior de Tecnologia, Universidade do Estado do Amazonas, Manaus-AM, Brazil
[2]Programa de Pós-Graduação em Biotecnologia e Recursos Naturais da Amazônia, Escola Superior de Ciências da Saúde, Universidade do Estado do Amazonas, Manaus-AM, Brazil
[3]Escola de Saúde, Centro Universitário Uninorte, Manaus-AM, Brazil
[4]Programa de Pós-Graduação em Biodiversidade e Biotecnologia - Rede Bionorte, Escola Superior de Ciências da Saúde, Universidade do Estado do Amazonas, Manaus-AM, Brazil
[5]Programa de Pós-Graduação em Biodiversidade e Biotecnologia - Rede Bionorte, Faculdade de Ciências Farmacêuticas, Universidade Federal do Amazonas, Manaus-AM, Brazil

***Correspondence to**
Edson Queiroz da Fonseca Junior, Email: edsonfonsecaqueiroz@gmail.com

Received 28 Mar. 2020
Revised 6 Nov. 2020
Accepted 16 Nov. 2020
ePublished 27 Dec. 2020



Abstract

Background: Guarana (*Paullinia cupana*) is a native Amazonian fruit and, due to its medicinal properties, stands out as one of the most promising species of Brazilian flora. Known for its invigorating and stimulating action, this species produces phenolic compounds responsible for its biological activities, which are of great interest to the phytocosmetic industry, in the preparation of anti-aging creams, for example. In this study, the antimicrobial, antioxidant, photoprotective and cytotoxic activities of extracts obtained from guarana seeds were evaluated.

Materials and Methods: Guarana seeds were obtained in Maués, Amazonas state, Brazil and, after drying, the seeds were ground in a knife mill. *P. cupana* seed extracts were obtained by the depletion method using hexane, dichloromethane and ethanol as solvents. Antioxidant activity assays were performed to determine the ability to scavenge the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•). To assess the sun protection factor (SPF), Mansur's spectrophotometric method was used. For the analysis of antimicrobial activity, the microplate dilution technique was used against strains of *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*. To evaluate the toxicity of the extracts, the lethal concentration of 50% of the population of the microcrustacean *Artemia salina* was determined. The cytotoxic effect of the extracts against human colorectal carcinoma cells (HCT 116) was also evaluated using the Alamar Blue test.

Results: The ethanolic extract from guarana seeds showed SPF value of 249.10 and a critical wavelength number (λc) of 383 nm, when evaluated at a concentration of 0.1% (m/m). The ethanolic extract also showed antioxidant activity, and a 50% efficient concentration (EC_{50}) of 647 µg mL^{-1}. The dichloromethane extract was active against *E. coli* and *P. aeruginosa*, with a minimum inhibitory concentration (MIC) of 20 mg mL^{-1} for both bacteria, and a MIC of 2.0 mg mL^{-1} for the yeast. This extract did not show toxicity against *A. salina* (LD_{50}> 1 mg mL^{-1}). The ethanolic and hexane extracts, however, presented low toxicity to the microcrustacean. On the other hand, none of the evaluated extracts inhibited the proliferation of HCT116 cells, showing no cytotoxicity.

Conclusions: *P. cupana* seeds have an important therapeutic value due to the presence of substances with antioxidant, antibiotic and photoprotective activities, though with low cytotoxicity, which allows their use as functional additives in phytocosmetics, as well as in the development of possible phytotherapeutic products.

Keywords: SPF, Free radical, Antibacterial, Antifungal, Toxicity, Caffeine

Background

The Amazon region, with its immense biodiversity, has vast potential for the discovery of new biologically active substances, which can be used as raw materials in many products, such as drugs, cosmetics and food. However, many Amazonian species have not been subjected to in-depth studies regarding the biological activities of their metabolites, thus, it is necessary to expand this knowledge.[1,2]

The addition of natural products, such as extracts and

Please cite this paper as: Fonseca Júnior EQD, Silva GFDS, Maquiné LV, et al. Photoprotective, antioxidant, antimicrobial and cytotoxic activities of guarana (*Paullinia cupana*) seed extracts . Int J Phytocos Nat Ingred. 2020;7:10. doi:10.15171/ijpni.2020.10.

oils, in cosmetic products provides, as a constituent of the formulations, a distinct value to the product. Plant metabolites, such as phenolic compounds, flavonoids, tannins, alkaloids, among others, provide a series of clinical activities desired by the cosmetic industry, such as antioxidant, anti-inflammatory, antimicrobial, anti-aging and photoprotective action.[3,4] Pharmacological properties of Amazonian species have been exploited in the development of nanosystems, such as microcapsules, liposomes and nanoemulsions, soaps and lotions, in order to improve the cosmetic performance of this type of product by using a natural active ingredient.[5-7]

Guarana (*Paullinia cupana* Kunth), of the Sapindaceae family, is a plant that is native to the Amazon region and is widely used in Brazil's beverage industry. The species has a wide range of pharmacological actions, which creates interest in chemical, therapeutic and agronomic studies of this species.[8,9] Thus, the preparation of extracts, the screening of biological activities, the characterization and isolation of bioactive substances are fundamental steps in adding value to guarana, which is known for its stimulating action.[10] Clinical and toxicological analyses, which are fundamental, are also necessary for such a study. Thus, this study aimed to evaluate the antimicrobial, antioxidant, photoprotective and cytotoxic activities of extracts obtained from guarana seeds, in order to verify the potential of this species in the preparation of phytocosmetics.

Materials and Methods

Plant material and extract preparation

The guarana seeds were provided by the company Luca D'Ambros ME, located in the municipality of Maués, Amazonas state, Brazil. The seeds were dried for 10 days at room temperature (25ºC), in the shade, and were ground in an electric knife mill, with a 3 mm screen.

The extraction of the dried and crushed seeds was carried out with the solvents hexane, dichloromethane and ethanol, in an increasing sequence of polarity. For cold maceration, 500 g of plant material and 1 L of solvent were used, the cycle being repeated for 3 consecutive days, by continuous solvent replacement. The extract was concentrated under reduced pressure at a temperature below 45°C in a rotary evaporator.

Determination of photoprotective activity

Guarana extracts were analyzed for sun protection factor (SPF) by spectrophotometric analysis. The absorption profiles of the extracts were determined from an alcoholic solution at a concentration of 1% (m/m) followed by successive dilutions to a concentration of 0.1% (m/m). The absorption of the samples was evaluated by scanning, in a UV-Vis spectrophotometer (Shimadzu UV-1800), between wavelengths of 280 to 400 nm in a quartz cuvette with 1.0 cm of optical path. Quercetin and benzophenone-3 (oxybenzone) were used as a standard. The SPF was determined from the values of UVB wavelength (290-320 nm), with increments of 5 nm, being applied to the equation of Mansur et al[11] (1).

$$\mathrm{FPS} = \mathrm{FC} \mathrm{x} \sum_{290\ \mathrm{nm}}^{320\ \mathrm{nm}} \mathrm{EE}(\lambda) \mathrm{x} \mathrm{I}(\lambda) \mathrm{x} \mathrm{Abs}(\lambda) \quad (1)$$

Here CF (correction factor) = 10; EE(λ) is the erythemal effect of radiation of the wavelength λ; I(λ) is the intensity of the Sun at the wavelength λ and Abs(λ) is the absorbance of the solution at the wavelength λ. The values of the constants EE(λ) x I(λ) were obtained from the correlation between EE and I at each wavelength.[11,12]

The ratio of the average UVA absorbance to the average UVB absorbance was obtained according to Boots the Chemist.[13]

$$\frac{\mathrm{UVA}}{\mathrm{UVB}} = \frac{\int_{320\ \mathrm{nm}}^{400\ \mathrm{nm}} \mathrm{A}\lambda, \mathrm{d}\lambda}{\int_{290\ \mathrm{nm}}^{320\ \mathrm{nm}} \mathrm{A}\lambda, \mathrm{d}\lambda} \quad (2)$$

where Aλ is the effective absorbance and related to the transmittance of the sunscreen Tλ by Aλ = -log [Tλ]. According to Donglikar and Deore,[14] the absorbance ratio is higher for products that exhibit more effective protection against UVA radiation, and are classified according to Table 1.

For the calculation of the critical wavelength (λc) of the guarana extracts, the area under the absorbance curve (AAC) was considered 100%. The λc is the wavelength where 90% of the AAC is found, in the range between 290 and 400 nm. According to Springsteen et al,[15] a product is considered to have a broad spectrum of photoprotection, when its critical wavelength is above 370 nm. The λc was determined using equation 3.[16]

$$\int_{290}^{\lambda c} A(\lambda) d\lambda = 0{,}9 \int_{290}^{400} A(\lambda) d\lambda \quad (3)$$

Determination of antioxidant activity

The extracts were evaluated using the indirect method of antioxidant activity by reducing the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•), which has maximum absorption at 517 nm. The methodology was described by Brand-Williams et al[17] and modified by Mensor et al.[18]

Quantification was performed by standardizing the antioxidant quercetin (800 μg mL^{-1}) with a calibration

Table 1. Star rating system, according to protection against UVA radiation[14]

Ratio UVA	Category (Stars)	Category Descriptor
0.0 to < 0.59	-	No UVA protection
0.6 to < 0.79	***	Moderate
0.8 to < 0.9	****	Good
over 0.9	*****	Maximum

curve (from 0 to 200 µg mL^{-1} of quercetin, $R^2 = 0.9082$). The stock solution of the guarana extracts was prepared at 1280 µg mL^{-1} in methanol, with successive dilutions being carried out to a concentration of 5.0 µg mL^{-1}. The test consisted of the reaction of 50 µL of the test solution, at different dilutions, with 1950 µL of DPPH• (0.06 M). The control was prepared by replacing the sample with methanol. After 30 minutes of reaction, the absorbance at a wavelength of 517 nm was verified. The percentage of inhibition (% PI) was determined using equation 4:

$$\%PI = \frac{(A_0 - A)}{A_0} x\,100 \quad (4)$$

where A_0 is the absorption of the control and A is the absorption of each sample added with DPPH•.

Through linear regression, the efficient concentration (EC_{50}) was determined; the sample concentration necessary for the scavenging of 50% of DPPH free radicals.

Determination of antimicrobial activity

The antimicrobial activity assay was evaluated against the strains of bacteria *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and the yeast *Candida albicans* ATCC 12031, which were obtained commercially. The bacteria were kept in Brain Heart Infusion broth (BHI) at 37ºC ± 2°C and the yeast in Sabouraud Dextrose broth (SD) at 30ºC ± 2°C. The inoculum was obtained in a 0.5 turbidity pattern on the MacFarland scale, equivalent to a final concentration of 2 to 5 × 10^6 UFC/mL.[19]

For each microorganism, the assays of antimicrobial activity were performed using the dilution method in a sterile microplate containing 96 wells. The test solution was prepared at a concentration of 40 mg mL^{-1} in 1% dimethyl sulfoxide (DMSO), which was used as a negative and sterility control, and then dilutions were performed to obtain the minimum inhibitory concentration (MIC). The volume of 100 µL, both of the test solution and of the inoculum, was used in the triplicate test. As a positive control, Rifampicin (20 mg mL^{-1}) was used for the antibacterial test and itraconazole (20 mg mL^{-1}) for the antifungal test. Subsequently, the microplates were incubated at 37°C for 24 hours. As a revelator, 30 µL of the indicator resazurin was used for the bacteria and TTC (2,3,5-triphenyltetrazolium chloride) for the yeast.

Toxicity test against Artemia salina

The toxicity of the guarana extracts was assessed using the *Artemia salina* Leach lethality test, as proposed by Meyer et al.[20] To obtain the culture medium, a synthetic sea salt solution (30 g L^{-1}) was prepared and 20 mg of *A. salina* eggs were incubated with exposure to artificial light for 48 hours in order to obtain the nauplii.

The test solution for the extracts was prepared at a concentration of 1.0 mg mL^{-1} with saline at the same concentration as the culture medium, initially being solubilized in 1% DMSO. Subsequently, serial dilutions were performed at a concentration of 0.125 mg/mL. In a microplate, 1.0 mL of the test solution and 10 larvae of *A. salina* were added. The tests were performed in triplicate. Counting of the number of dead larvae was performed after 24 hours and, from the dilutions of the solutions, the lethal dose for mortality of 50% of a population (LD_{50}), obtained through linear regression, was determined. Extracts with LD_{50} greater than 1.0 mg mL^{-1} were considered inactive.

Cytotoxicity test against the tumor cell HCT116

The Alamar Blue test was performed according to the methodology described by Ahmed et al.[21] Alamar Blue is a fluorescent/colorimetric indicator with redox properties. In proliferating cells it is reduced; the oxidized form is blue and non-fluorescent (indicating non-viable cell) and the reduced form is pink and fluorescent (indicating viable cell). The extracts were diluted to 50 µg mL^{-1} in 0.2% DMSO. The test was carried out in order to analyze the cell viability of the HCT 116 strain (human colorectal carcinoma).

The cells were grown in a culture bottle with DMEM medium (Dulbecco's Modified Eagle Medium) supplemented with 10% fetal bovine serum and 1% antibiotic (Penicillin-Streptomycin). They were transferred to 96-well microplates at a concentration of 0.5 × 10^4 cells/well. The plate was then incubated for 24 hours in an oven at 37ºC with an atmosphere of 5% CO_2. After this period, samples were added (50 µg mL^{-1}) and the plate was incubated for 72 hours under the same conditions. One control group received the same amount of DMSO in the well and the other Doxorubicin 10 µg mL^{-1}. After exposure to treatment, 10 µL of the Alamar Blue (resazurin) solution (0.4%) was added to each well of the plate. After 2 hours, fluorescence was measured using an Elisa plate reader (DTX 800 Beckman Coulter Multimode Detector) at excitation wavelength of 540 nm and emission of 585 nm. The samples were read before inserting the revelator at the same wavelength to obtain the blank values. The data were analyzed in relation to the control.

The percentage of cell viability was calculated according to equation 5:

$$\%viability = \frac{\Delta Ft}{\Delta Fc} x100 \quad (5)$$

where ΔFt = (cell fluorescence + medium + extract + resazurin) - (cell fluorescence + medium + extract) and ΔFc = (cell fluorescence + medium + DMSO + resazurin) - (cell fluorescence + medium + DMSO).

Extracts that showed the ability to reduce cell viability to 10% or less were considered cytotoxic.[22]

Statistical analysis

Results were expressed as the mean ± standard deviation (SD). Toxicity was expressed as LD_{50} and it was obtained

using linear regressions based on three replicates per concentration level. Differences between groups were determined by analysis of variance (ANOVA) followed by Tukey's test ($P<0.05$). All data obtained from three independent experiments were performed in triplicate.

Results

Production of guarana extracts

In the depletion of guarana seeds in solvents of different polarities, the dichloromethane extract showed a greenish color and was the one that showed the highest yield among the others, which was 1.35% (m/m). The hexanic extract was presented as a dark oil, with 0.83% (m/m) yield. The ethanolic extract was dark brown in color, with yield of 1.17% (m/m).

Sun protection factor

The results of the photoprotective activity showed that the guarana extracts at 0.1% (m/m) had SPF values above 6, which makes it possible, therefore, to use them in photoprotective formulations, as specified by the Food and Drug Administration (FDA).[23] Table 2 presents the SPF values and the UVA/UVB ratio obtained for the guarana extracts.

The ethanolic and dichloromethane extracts showed the highest SPF values, 249.1 and 88.0, respectively, both classified as good for UVA protection, and considered the most promising for this activity. The quercetin and benzophenone-3 patterns showed SPF equal to 272.2 and 289.8, respectively and a maximum classification against UVA radiation. The three extracts, as well as the standards, had a critical wavelength above 370 nm, indicating a wide range of protection in the UVA radiation region.

Antioxidant activity

From the results obtained for the guarana seed extracts (Table 3), it can be observed that the extract with the greatest antioxidant potential was ethanolic, with an EC_{50} of 647.4 μg mL^{-1}, which is capable of inhibiting 93.45% of the DPPH free radicals at the maximum concentration evaluated (1280 μg mL^{-1}). The dichloromethane and hexane extracts were not able to inhibit the free radicals, and had a maximum inhibition of 8.93 and 14.66, respectively. The standard antioxidant quercetin showed EC_{50} of 120.6 μg mL^{-1}.

Antimicrobial activity

The results of the antimicrobial activity of the guarana extracts presented in Table 4 showed that the dichloromethane extract possessed activity against all tested microorganisms, with a minimum inhibitory concentration of 20.0 mg mL^{-1} for *E. coli* and 10.0 mg mL^{-1} for *P. aeruginosa*. The hexanic and ethanolic extracts showed no activity against the evaluated bacteria. For the yeast *C. albicans*, the MIC of all extracts was 2.0 mg mL^{-1}.

Toxicity test against Artemia salina

From the test performed against the microcrustacean *A. salina*, with readings of average mortality performed within 24 hours of exposure to guarana extracts, toxicity was found in the hexanic and ethanolic extracts, with LD_{50} values of 0.667 mg mL^{-1} and 0.182 mg mL^{-1}, respectively

Table 2. SPF values, UVA/UVB ratio, classification against UVA radiation and critical wavelength (λc) for guarana extracts and commercial standards

Samples	SPF*	UVA/UVB	UVA Classification[14]	λ_c
Ethanolic extract	249.1^a	0.90	Good	383
Dichloromethane extract	88.0^c	0.88	Good	383
Hexane extract	8.21^b	1.45	Maximum	390
Quercetin	272.2^a	4.79	Maximum	385
Benzophenone-3	289.8^d	4.35	Maximum	378

*Identical letters indicate that there is no statistically significant difference, according to the Tukey test ($P < 0.05$).

Table 3. Antioxidant activity expressed as percentage of inhibition and efficient concentration (EC_{50}) of guarana seed extracts (*Paullinia cupana*)

Concentration	% Inhibition ± standard deviation		
(μg mL^{-1})	Ethanol extract	Dichloromethane extract	Hexane extract
80	3.44 ± 0.28	4.25 ± 0.44	5.17 ± 0.35
160	17.54 ± 6.14	4.56 ± 0.52	5.73 ± 0.44
320	29.01 ± 0.69	5.42 ± 0.17	8.87 ± 0.70
640	58.55 ± 7.27	5.36 ± 1.13	9.54 ± 0.78
1280	93.45 ± 1.47	8.93 ± 1.13	14.66 ± 0.17
EC_{50}	647.4 μg mL^{-1}	4.894 μg mL^{-1}	23.167 μg mL^{-1}

Table 4. Minimum inhibitory concentration (MIC) of guarana (*Paullinia cupana*) seed extracts against pathogenic microorganisms.

Extracts	MIC (mg mL^{-1})		
	Pseudomonas aeruginosa	*Escherichia coli*	*Candida albicans*
Ethanol	-	-	2.0
Dichloromethane	10.0	20.0	2.0
Hexane	-	-	2.0

(Table 5). The dichloromethane extract was not considered toxic, since the LD_{50} value obtained was greater than 1.0 mg mL^{-1}, according to the criterion adopted by Rebelo et al.[24]

Cytotoxicity test against HCT116 cells

The results of viability of the human colorectal carcinoma cell line (HCT116) when exposed to guarana extracts for 72 hours are shown in Table 6. It appears that the guarana extracts were not efficient in reducing the cell viability of the HCT116 line, since they are only considered promising as anticancer agents, when the samples are capable of reducing cell viability to 10% or less.[22] Therefore, the evaluated extracts did not show cytotoxicity. Contrary to what was observed in the assay against *A. salina*, the three guarana extracts showed similar results when used against the HCT116 strain.

Discussion

The extracts obtained from the guarana (*P. cupana*) seeds evaluated in the present study have properties of interest to the phytocosmetic industry, such as photoprotective, antioxidant and antimicrobial activities. Botanical extracts are complex mixtures of natural compounds with different chemical characteristics and biological properties, and their use in cosmetics has been well known since early times.[25] Despite the numerous scientific and technological advances of today, natural products,[26] like guarana, used on a large scale in beverage production,[27] are still crucial raw materials for the world economy.

Guarana is a plant originally from the Amazon, widely used in traditional Brazilian medicine, and is used in homemade and industrial drinks, as a tonic and stimulant. Among the main chemical constituents of guarana are methylxanthines (caffeine, theophylline and theobromine) and condensed tannins, the main ones being catechin and epicatechin.[28] These chemical classes have been described as having different pharmacological activities, such as those evaluated in this study and have wide application in the cosmetics industry.

Among the evaluated extracts, the ethanolic extract showed itself to be the most promising in regards to photoprotective activity, with an SPF value close to that of benzophenone-3. It has a 3-star rating for UVA protection and a wide range of photoprotection in the UV spectrum region, due to its λc of 383 nm (Table 2). Sunscreens are substances capable of absorbing, reflecting or refracting ultraviolet radiation and thus protect the skin from direct sunlight.[29] The biological activity of a sunscreen is evaluated by its ability to protect the skin from erythema and edema, and reduce the risk of burns and cell carcinoma of the basal and spinous layer.[30] Depending on their chemical structure, organic sunscreens can absorb UVB (280-315 nm) and UVA radiation (315-400 nm) and those capable of absorption in the two regions of the UV spectrum are called broad spectrum filters.[29] The ethanolic extract, rich in polar chromophore substances, therefore, proves to be interesting for this type of cosmetic application.

Since plant extracts are complex mixtures, composed of different molecules, some more and some less active, their maximum UV radiation absorption is not very well defined.[31] However, many natural extracts are being used as SPF enhancers in phytocosmetic formulations, and used as strategy for expanding the photoprotection and photostability spectrum of commercial sunscreens. Hubner et al,[32] for example, observed a synergism between the active molecules and an 81% increase in the SPF of photoprotectors plus grape extract (*Vitis vinifera* L). This result was associated with the presence of polyphenolic compounds in the extract and their ability to absorb UV radiation, as well as combat free radicals.[33,34]

Considering the benefit of reducing the damage caused by free radicals, antioxidants can be associated with sunscreens as a complement to photoprotection and stabilization of the formulation.[35,36] According to Basile et al,[37] due to the presence of polyphenols in its composition, *P. cupana* extract at 2.0 mg mL^{-1} is able to reduce lipid

Table 5. Toxicity of extracts obtained from guarana seeds (*Paullinia cupana*) against *Artemia salina*, expressed as the lethal dose (LD_{50})

Extracts	LD_{50} (μg mL^{-1})
Ethanol	182
Dichloromethane	> 1000
Hexane	667

Table 6. Effect of guarana extracts (50 μg mL^{-1}) on cell viability of the HCT116 tumor lineage, using the Alamar blue assay after 72 h of exposure

Extracts	Cell viability (%)
Ethanol	84.15
Dichloromethane	83.60
Hexane	77.61

peroxidation by 62.5%. The antioxidant activity of guarana was also reported by Mattei et al[38] who verified the inhibition of lipid peroxidation with low concentrations of guarana (1.2 μg mL^{-1}) in an *in vivo* assay. Peixoto et al[39] prepared an aqueous extract from toasted seeds, which showed significant antioxidant activity *in vivo*, and was able to increase resistance to cellular oxidative stress, thus exhibiting anti-aging properties.

The DPPH free radical scavenging method, although an indirect methodology for determining antioxidant activity, is commonly used in the screening of extracts and substances with this potential, as it is fast, reliable and reproducible.[40] In the present study, the ethanolic extract of guarana, in addition to being the most promising for photoprotective activity, was also the one that showed the highest antioxidant activity against the DPPH radical (EC_{50} of 120.6 μg mL^{-1}), and can therefore be considered as an enhancer of photoprotective activity for use in phytocosmetics.

In general, substances with a polar character have the ability to neutralize free radicals. Therefore, the extracts obtained using solvents of greater polarity are the most active. Dalonso and Oliveira[41] evaluated the antioxidant activity of the methanolic extract of the seeds of *P. cupana* at 10.0 mg mL^{-1} and found it had a high capacity to capture the DPPH radical (90.9%), a result that corroborates what was found in the present study, where the 1.28 mg mL^{-1} ethanolic extract was able to inhibit 93.45% of free radicals (Table 3).

Tannins are present in guarana seed extracts in concentrations ranging from 1.5 to 7.12%[27] and are responsible for the antioxidant activity of this species. Caffeine, in turn, present in extracts in concentrations ranging from 1.2 to 7.97%, depending on the type of extract and the genetic characteristics of guarana,[27] has no antioxidant activity, as shown in the study by Chu et al[42] The authors found that crude caffeine extracted from coffee had antioxidant activity and the ability to inhibit cyclooxygenase-2. Pure caffeine did not show these activities, leading the authors to conclude that those responsible for the antioxidant activity are other substances, extracted along with caffeine, in this case, the catechetical substances.

On the other hand, caffeine has, in its pure form as well as when combined with other substances, the ability to inhibit bacterial growth, and the proposed mechanism for this activity involves the inhibition of DNA repair routes in bacterial cells.[43] Lele et al[44] found in their study that pure caffeine inhibited the growth of multidrug-resistant isolates of *S. aureus* (MRSA), with MIC of 5.0 mg mL^{-1}. In addition, the authors verified the ability of caffeine to prevent the formation of biofilms, potentiating the action of antibiotics.

In the present study, the dichloromethane extract of the guarana was the one which revealed the greatest antimicrobial potential, with activity against *P. aeruginosa*, *E. coli* and *C. albicans*, and MIC values of 10.0 mg mL^{-1}, 20.0 mg mL^{-1} and 2.0 mg mL^{-1}, respectively (Table 4). Basile et al[37] observed antimicrobial activity of ethanolic extracts of guarana against *P. aeruginosa* and *E. coli*, however, the authors observed MIC values of 16 μg mL^{-1} and 32 μg mL^{-1}, more efficient in inhibiting the growth of these bacteria than the extract obtained in the present study. The crude guarana extract prepared in acetone and water 7: 3 (v/v) evaluated by Carvalho et al[45] presented MIC of 250 μg mL^{-1} against *E. coli*.

Majhenic et al[46] found that guarana extracts prepared at different temperatures, and with polar solvents methanol, or 35% acetone, or 60% ethanol, showed effective antibacterial activity against gram-negative and gram-positive strains. In addition, they observed antifungal activity of these extracts against *Aspergillus niger*, *Trichoderma viride* and *Penicillium cyclopium*. Antonelli-Ushirobira et al,[47] however, when analyzing the antimicrobial activity of *P. cupana* extracts in concentrations above 1 mg mL^{-1}, found that the acetone extract and the fractions obtained in ethyl acetate did not show activity against the bacteria *Staphylococcus aureus, Bacillus subtilis, E. coli* and *P. aeruginosa.* The ethanolic and hexane extracts evaluated in the present study were also not able to inhibit the growth of bacteria and were active only against *C. albicans* (Table 4), which indicates that the manner of obtaining the extracts, the type of solvent used and consequently, its chemical composition directly influences its biological activity.

The antimicrobial activity of natural products used in phytocosmetics is an interesting property, since the microbial contamination of cosmetic products can cause adverse effects, such as odors, changes in viscosity or color, in addition posing risks to the health of the skin. The addition of antimicrobial preservatives in cosmetic products is therefore necessary to prevent the growth of bacteria or fungi in the formulations.[48] The dichloromethane extract of the guarana evaluated in the present study has the potential to be utilized as a preservative in cosmetic formulations, due to its antibacterial and antifungal activity, replacing the currently used synthetic antimicrobials.

In order to use guarana extracts in cosmetics, it is necessary to assess their toxicity. In the present study, the toxicity of guarana extracts was initially evaluated against the microcrustacean *A. salina*. Bednarczuk et al[49] comment that this is a toxicological test that is easy to carry out, is cheap and materials are easy to obtain. The assay allows the assessment of general toxicity and is considered essential as a preliminary bioassay in the study of samples with potential biological activity.

The ethanolic and hexanic extracts of guarana showed LD_{50} values of 182 μg mL^{-1} and 667 μg mL^{-1}, respectively, with low toxicity against *A. salina*. The dichloromethane extract was non-toxic (Table 5). According to Nguta et al[50] samples with LD_{50} values below 100 μg mL^{-1} are

considered highly toxic, values between 100 and 500 µg mL^{-1} are moderately toxic, between 500 and 1000 µg mL^{-1} are mildly toxic, and above 1000 µg mL^{-1} are non-toxic.

In addition to the numerous health benefits and useful therapeutic indications for humans, guarana demonstrates low toxicological potential, as evidenced by several *in vitro* and *in vivo* studies.[27] In the present study, guarana extracts were not able to decrease the viability of cells of the tumor line HCT116, indicating, therefore, the absence of cytotoxicity of these extracts.

However, several studies report the existence of the cytotoxic activity of guarana. Fonseca et al.[51] evaluated the cytotoxic activity of the aqueous extract of guarana powder in prokaryotic cells and found that the complex formed between a flavonoid and caffeine was responsible for such activity. Carvalho et al[45] found antineoplastic activity in a guarana extract prepared with acetone and water, as well as from its fraction in ethyl acetate against HL-60 acute leukemia tumor cell strain. The authors suggest that the tannins present in guarana extracts are responsible for this effect. And Fukumasua et al[52] verified in an *in vivo* study that guarana showed inhibitory effects of hepatocarcinogenesis induced by N-nitrosodiethylamine in mice.

According to Marques et al,[27] in tests to assess toxicity, it is important to determine the sample dose, and take into account the manner of the pharmaceutical preparation of its extract, as well as specifying the amount administered, since the lack of standardization of these factors can raise doubts about the definition of a safe dose. Therefore, other tests should be performed with the extracts evaluated here, in order to confirm the safe dose for use in cosmetics.

Conclusions

The results obtained in this study demonstrated the potential of *P. cupana* seed extracts for use in phytocosmetics, since they present a number of pharmacological activities, such as antioxidant, photoprotective, antimicrobial and low toxicity. In addition, the use of extracts evaluated in the present study as additives in phytocosmetics can be considered promising in the development of different products, such as anti-aging emulsion, due to the photoprotective and antioxidant effect of the ethanolic extract, while the dichloromethane extract is a potential candidate to be applied as a preservative. It is also necessary to identify the substances present in each extract, in order to guarantee its standardization for use in different formulations.

Competing Interests

None.

Acknowledgements

The authors would like to thank FAPEAM (Universal Amazonas, Process 062.01337/2018) for the financial support for this study. We also would like to thank Mr. Matthew Miller for English revision.

References

1. Souza DP, Pimentel RBQ, Santos AS, et al. Fungicidal properties and insights on the mechanisms of the action of volatile oils from Amazonian Aniba trees. Ind Crops Prod. 2020;143:111914. doi:10.1016/j.indcrop.2019.111914
2. Costa RC, Nunez CV. Phytotherapy and phytocosmetic bioproducts market: management, technologies and innovation. Rev Fitos. 2017;10(3):295-306. doi:10.5935/2446-4775.20160023
3. Gurib-Fakim A. Medicinal plants: traditions of yesterday and drugs of tomorrow. Mol Aspects Med. 2006;27(1):1-93. doi:10.1016/j.mam.2005.07.008
4. Martins FJ, Caneschi CA, Vieira JL, Barbosa W, Raposo NR. Antioxidant activity and potential photoprotective from amazon native flora extracts. J Photochem Photobiol B. 2016;161:34-39. doi:10.1016/j.jphotobiol.2016.05.012
5. Liang Y, Li Y, Sun A, Liu X. Chemical compound identification and antibacterial activity evaluation of cinnamon extracts obtained by subcritical n-butane and ethanol extraction. Food Sci Nutr. 2019;7(6):2186-2193. doi:10.1002/fsn3.1065
6. Lucca LG, de Matos SP, Kreutz T, et al. Anti-inflammatory effect from a hydrogel containing nanoemulsified copaiba oil (*Copaifera multijuga* Hayne). AAPS PharmSciTech. 2018;19(2):522-530. doi:10.1208/s12249-017-0862-6
7. Aguilar MCA, Coronel PCR, Alvarado DAL, Sanchez FEV, Alcivar RT. Oil-in-water (o/w) emulsionable concentrate of Ishpink (*Ocotea quixos*) with thermodynamic stability. Rev Caatinga. 2019;32(3):590-598. doi:10.1590/1983-21252019v32n303rc
8. Bittencourt LS, Machado DC, Machado MM, et al. The protective effects of guaraná extract (*Paullinia cupana*) on fibroblast NIH-3T3 cells exposed to sodium nitroprusside. Food Chem Toxicol. 2013;53:119-125. doi:10.1016/j.fct.2012.11.041
9. Kober H, Tatsch E, Torbitz VD, et al. Genoprotective and hepatoprotective effects of Guarana (*Paullinia cupana* Mart. var. *sorbilis*) on CCl4-induced liver damage in rats. Drug Chem Toxicol. 2016;39(1):48-52. doi:10.3109/01480545.2015.1020546
10. Agência Nacional de Vigilância Sanitária (Anvisa). Memento Fitoterápico da Farmacopeia Brasileira. Brasília: Anvisa; 2016.
11. Mansur JS, Breder MNR, Mansur MCD, Azulay RD. Determination of sun protecting factor in human beings and by spectrophotometry: comparison between of the two methods. An Bras Dermatol. 1986;61(3):167-172.
12. Sayre RM, Agin PP, LeVee GJ, Marlowe E. A comparison of in vivo and in vitro testing of sunscreening formulas. Photochem Photobiol. 1979;29(3):559-566. doi:10.1111/j.1751-1097.1979.tb07090.x
13. Boots the Chemist Ltd. The Revised Guidelines to the Practical Measurement of UVA/UVB Ratios According to the Boots Star Rating System. Nottingham, England: The Boots Company PLC; 2004.
14. Donglikar MM, Deore SL. Sunscreens: a review. Pharmacogn J. 2016;8(3):171-179. doi:10.5530/pj.2016.3.1
15. Springsteen A, Yurek R, Frazier M, Carr KF. In vitro

measurement of sun protection factor of sunscreens by diffuse transmittance. Anal Chim Acta. 1999;380(2-3):155-164. doi:10.1016/s0003-2670(98)00577-7
16. Diffey BL, Tanner PR, Matts PJ, Nash JF. In vitro assessment of the broad-spectrum ultraviolet protection of sunscreen products. J Am Acad Dermatol. 2000;43(6):1024-1035. doi:10.1067/mjd.2000.109291
17. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT Food Sci Technol. 1995;28(1):25-30. doi:10.1016/s0023-6438(95)80008-5
18. Mensor LL, Menezes FS, Leitão GG, et al. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res. 2001;15(2):127-130. doi:10.1002/ptr.687
19. Clinical and Laboratory Standards Institute (CLSI). M27-A3: Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. 3rd ed. Wayne, PA: CLSI; 2009.
20. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 1982;45(5):31-34. doi:10.1055/s-2007-971236
21. Ahmed SA, Gogal RM, Jr., Walsh JE. A new rapid and simple non-radioactive assay to monitor and determine the proliferation of lymphocytes: an alternative to [3H] thymidine incorporation assay. J Immunol Methods. 1994;170(2):211-224. doi:10.1016/0022-1759(94)90396-4
22. Aranha ESP. Avaliação do potencial anticâncer in vitro de óleos essenciais de plantas do gênero Eugenia [thesis]. Manaus, AM: Universidade Federal do Amazonas; 2014.
23. FDA - Food and Drug Administration, Department of Health and Human Services. Labeling and effectiveness testing; sunscreen drug products for over-the-counter human use. FDA; 2011.
24. Rebelo MM, da Silva JKR, Andrade EHA, Maia JGS. Antioxidant capacity and biological activity of essential oil and methanol extract of *Hyptis crenata* Pohl ex Benth. Rev Bras Farmacogn. 2009;19(1b):230-235. doi:10.1590/s0102-695x2009000200009
25. Bouzabata A. Contemporary use of phytocosmetics in three districts from North-Eastern Algeria. Pharmacogn J. 2017;9(6):762-766. doi:10.5530/pj.2017.6.120
26. Costa IM. Phytocosmetics–where nature meets well-being. Int J Phytocos Nat Ingred. 2015;2:1-3. doi:10.15171/ijpni.2015.01
27. Marques LLM, Ferreira EDF, Paula MNd, Klein T, de Mello JCP. *Paullinia cupana*: a multipurpose plant-a review. Rev Bras Farmacogn. 2019;29(1):77-110. doi:10.1016/j.bjp.2018.08.007
28. Antonelli-Ushirobira TM, Yamaguti E, Uemura LM, Nakamura CV, Dias Filho BP, de Mello JCP. Chemical and microbiological study of extract from seeds of Guaraná (*Paullinia cupana* var. *sorbilis*). Lat Am J Pharm. 2007;26(1):5-9.
29. Schalka S, Steiner D, Ravelli FN, et al. Brazilian consensus on photoprotection. An Bras Dermatol. 2014;89(6 Suppl 1):1-74. doi:10.1590/abd1806-4841.20143971
30. Toyoshima M, Hosoda K, Hanamura M, Okamoto K, Kobayashi H, Negishi T. Alternative methods to evaluate the protective ability of sunscreen against photo-genotoxicity. J Photochem Photobiol B. 2004;73(1-2):59-66. doi:10.1016/j.jphotobiol.2003.09.005
31. Ferrari M, Oliveira MSC, Nakano AK, Rocha-Filho PA. [In vitro and in vivo determinations of sun protection factor (SPF) of emulsions with andiroba oil (*Carapa guianensis*)]. Rev Bras Farmacogn. 2007;17(4):626-630. doi:10.1590/s0102-695x2007000400023
32. Hubner A, Sobreira F, Vetore Neto A, et al. The synergistic behavior of antioxidant phenolic compounds obtained from winemaking waste's valorization, increased the efficacy of a sunscreen system. Antioxidants (Basel). 2019;8(11). doi:10.3390/antiox8110530
33. Cefali LC, Ataide JA, Moriel P, Foglio MA, Mazzola PG. Plant-based active photoprotectants for sunscreens. Int J Cosmet Sci. 2016;38(4):346-353. doi:10.1111/ics.12316
34. Korać RR, Khambholja KM. Potential of herbs in skin protection from ultraviolet radiation. Pharmacogn Rev. 2011;5(10):164-173. doi:10.4103/0973-7847.91114
35. Lim HW, Arellano-Mendoza MI, Stengel F. Current challenges in photoprotection. J Am Acad Dermatol. 2017;76(3S1):S91-S99. doi:10.1016/j.jaad.2016.09.040
36. Badea G, Lăcătuşu I, Badea N, Ott C, Meghea A. Use of various vegetable oils in designing photoprotective nanostructured formulations for UV protection and antioxidant activity. Ind Crops Prod. 2015;67:18-24. doi:10.1016/j.indcrop.2014.12.049
37. Basile A, Ferrara L, Pezzo MD, et al. Antibacterial and antioxidant activities of ethanol extract from Paullinia cupana Mart. J Ethnopharmacol. 2005;102(1):32-36. doi:10.1016/j.jep.2005.05.038
38. Mattei R, Dias RF, Espínola EB, Carlini EA, Barros SB. Guarana (*Paullinia cupana*): toxic behavioral effects in laboratory animals and antioxidants activity in vitro. J Ethnopharmacol. 1998;60(2):111-116. doi:10.1016/s0378-8741(97)00141-4
39. Peixoto H, Roxo M, Röhrig T, Richling E, Wang X, Wink M. Anti-aging and antioxidant potential of *Paullinia cupana* var. *sorbilis*: findings in *Caenorhabditis elegans* indicate a new utilization for roasted seeds of guarana. Medicines (Basel). 2017;4(3). doi:10.3390/medicines4030061
40. Wróblewska KB, Baby AR, Grombone Guaratini MT, Moreno PRH. In vitro antioxidant and photoprotective activity of five native Brazilian bamboo species. Ind Crops Prod. 2019;130:208-215. doi:10.1016/j.indcrop.2018.12.081
41. Dalonso N, Petkowicz CL. Guarana powder polysaccharides: characterisation and evaluation of the antioxidant activity of a pectic fraction. Food Chem. 2012;134(4):1804-1812. doi:10.1016/j.foodchem.2012.03.088
42. Chu YF, Chen Y, Brown PH, et al. Bioactivities of crude caffeine: antioxidant activity, cyclooxygenase-2 inhibition, and enhanced glucose uptake. Food Chemistry. 2012;131(2):564-568. doi:10.1016/j.foodchem.2011.09.024
43. Whitney AK, Weir TL. Interaction of caffeine with the SOS response pathway in *Escherichia coli*. Gut Pathog. 2015;7:21. doi:10.1186/s13099-015-0069-x
44. Lele OH, Maniar JA, Chakravorty RL, Vaidya SP, Chowdhary AS. Assessment of biological activities of

caffeine. Int J Curr Microbiol Appl Sci. 2016;5(5):45-53. doi:10.20546/ijcmas.2016.505.005

45. Carvalho LV, Cordeiro MF, TU EL, et al. Evaluation of antibacterial, antineoplastic, and immunomodulatory activity of *Paullinia cupana* seeds crude extract and ethyl-acetate fraction. Evid Based Complement Alternat Med. 2016;2016:1203274. doi:10.1155/2016/1203274
46. Majhenič L, Škerget M, Knez Ž. Antioxidant and antimicrobial activity of guarana seed extracts. Food Chem. 2007;104(3):1258-1268. doi:10.1016/j.foodchem.2007.01.074
47. Antonelli-Ushirobira TM, Kaneshima EN, Gabriel M, Audi EA, Marques LC, Mello JC. Acute and subchronic toxicological evaluation of the semipurified extract of seeds of guaraná (*Paullinia cupana*) in rodents. Food Chem Toxicol. 2010;48(7):1817-1820. doi:10.1016/j.fct.2010.04.013
48. Hamilton T, de Gannes GC. Allergic contact dermatitis to preservatives and fragrances in cosmetics. Skin Therapy Lett. 2011;16(4):1-4.
49. Bednarczuk VO, Verdam MCS, Miguel MD, Miguel OG. [Tests in vitro and in vivo used in the toxicological screening of natural products]. Visão Acadêmica. 2010;11(2):43-50. doi:10.5380/acd.v11i2.21366
50. Nguta JM, Mbaria JM, Gakuya DW, Gathumbi PK, Kabasa JD, Kiama SG. Biological screeing of Kenya medicinal plants using *Artemia salina* L. (Artemiidae). Pharmacologyonline. 2011;2:458-478.
51. da Fonseca CA, Leal J, Costa SS, Leitão AC. Genotoxic and mutagenic effects of guarana (*Paullinia cupana*) in prokaryotic organisms. Mutat Res. 1994;321(3):165-173. doi:10.1016/0165-1218(94)90041-8
52. Fukumasu H, da Silva TC, Avanzo JL, et al. Chemopreventive effects of *Paullinia cupana* Mart *var*. *sorbilis*, the guaraná, on mouse hepatocarcinogenesis. Cancer Lett. 2006;233(1):158-164. doi:10.1016/j.canlet.2005.03.007

© 2020 The Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.