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RESEARCH

Food Extract of Purple Yam (*Dioscorea trifida* L.f.) from Brazil: Optimization of Extraction Method, Characterization, In Vivo Toxicity, and Antimicrobial Activity

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Abstract

Purple yam (*Dioscorea trifida*) has high agricultural productivity in the Amazon region but has not been much investigated. Multivariate strategies were employed to optimize the method to obtain a food extract rich in functional compounds. The optimal conditions showed that the combination of 0.2 g of dried purple yam with 15 mL of citric acid (1%) under agitation in a water bath at 36 °C for 4 min yields an extract with a high content of peonidin-3-O-glucoside-5-O-glucoside, peonidin-3-O-feruloylglycosideum-5-O-glycosideum, peonidin-3-Op-coumaroylglycosideum-5-O-glycoside, quinic acid, apigenin 8-C-xyloside-6-C-glycoside (vicenin 3), quinic acid, apigenin 6-C-xyloside-8-C-glycoside (vicenin 1), and isorhamnetin-O-dihexoside. Our extract also presented 56.91 ± 0.76 mg 100 g^{-1} of total anthocyanins, 417.05 ± 11.37 mg 100 g^{-1} total carotenoids, and 493.09 ± 6.38 mg GAE 100 g^{-1} of total phenolic compounds. In a *Galleria mellonella*, in vivo model consumption safety was for up to 150 g by 70 kg of body weight. In addition, it inhibited the growth of Gram-negative bacteria (*Salmonella typhimurium* and *Escherichia coli*). The simple, fast, and ecofriendly extraction conditions, combined with the biological effects of our extract, gives us a great potential for its application in food or packaging technologies.

Keywords Bioactive compound · Green solvent · Tuberous roots · Simple extraction method · Multivariate design

Introduction

The yam (*Dioscorea trifida*) is a tuberous root native to the African and Asian continents (Costa et al. 2020[a]). *Dioscorea trifida* L.f. has been domesticated by indigenous peoples in America (Nascimento et al. 2015; SiBBr 2024). The species *Dioscorea trifida* L.f. has local ethnovarieties around the world, especially in the interior regions of Amazonas. Variations are found under the popular names "cará-branco," "cará-pata-de-onça," "cará-macaxeira," "cará-inhame," "cará-miguel," "cará-rabo-de mucura," "cará-ovo-de-cavalo," "cará-roxo," "cará-durão," and "cará-roxão" (Couto and Fraga 2024; Coradin et al. 2022). Purple yam ("cará-roxo") is a tuber and plays a significant cultural and socioeconomic contribution in the north of Brazil.

Its production is carried out in a sustainable way by indigenous peoples, riverine caboclo, and family farmers in the floodplain areas of the Amazon basin. It is easy to grow in warm, humid climates and makes a significant contribution to the income and employment in the small-scale agriculture of countries where the climate and soil are suitable (Lima et al. 2022; Meinhart et al. 2019; Teixeira et al. 2016). Yam is a natural source of carbohydrates, mainly starch, which is the main energy reserve of vegetables (Nascimento et al. 2015; SiBBr, 2024). It also contains proteins, minerals (phosphorus, calcium, and iron), vitamins A and C, B vitamins, and anthocyanins,

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which give it its purple color (Lima et al. 2022; Meinhart et al. 2019; Teixeira et al. 2016).

Anthocyanins are water-soluble pigments responsible for a wide variety of colors present in flowers, fruits, stems, leaves, and roots of plants. Such compounds have proven antioxidant effects in the literature (Chen et al. 2021; Pires et al. 2021; Xue et al. 2022). Several studies correlate anthocyanins with a wide range of health benefits such as improved vision intensity, effectiveness in the treatment of various disorders blood circulation, vasoprotective effects, maintenance of normal vascular permeability, and diabetes control (Oliveira Filho et al. 2021; Santhakumar et al. 2015; Vugic et al. 2020). Due to their beneficial functions to the human body, anthocyanins have been studied for use in functional foods, showing anti-inflammatory properties, antineoplastic effect, antioxidant, antimicrobial effects, and activities against foodborne bacterial pathogens (Nascimento 2022; Ma et al. 2019).

Despite their stability, anthocyanins present restrictions. Indeed, they can be degraded by heat, presence of oxygen, light exposure, and pH of the environment. These parameters are important in the extraction, purification, and application processes in food (Oliveira Filho et al. 2021; Phan et al. 2021). However, there is a need to research extraction methods of anthocyanin in order to improve the yield of each specific matrix to Kurambhatti et al. (2020), as well as to employ ecofriendly methods, resulting is an edible extract. Several extraction methods for anthocyanin can be used, depending on the sample and the specific conditions of each laboratory. The most common methods include solid–liquid or liquid–liquid extraction, using solvents and/or a mixture of them, such as ethanol, methanol, acetonitrile, and/or water, in acidified media. The solvent is added to the matrix, and both are subjected to stirring processes with or without heating, maceration, reflux, and ultrasound, among others (Tena and Asuero 2022; Zhang et al. 2022). Extraction by steam distillation, high-pressure extraction, deep eutectic solvent utilization, and enzymatic extraction are also successfully employed, but heir implementation is more costly (Tan et al. 2022).

In this context, the aim of this study is to optimize the extraction of bioactive compounds from purple yam through a simple method, utilizing solid–liquid extraction in a water bath with mechanical agitation, with the advantage of using edible solvents. In addition, the extract was evaluated in terms of composition, in vivo toxicity, and antimicrobial activity, aiming to shed light on new applications for the purple yam in the food and packaging industry (for example, in the segment of natural colorants and bioactive packaging such as hydrogel and biofilm).

Materials and Methods

Reagents

Methanol P.A. was obtained from Synth (Diadema, SP, Brazil), chloridric acid P.A. from Neon (Suzano, SP, Brazil), citric acid P.A. from Perfyl Tech (São Paulo, SP, Brazil), dynamic gallic acid from Indaiatuba (SP, Brazil), anhydrous soda ash P.A from Synth (Diadema, SP, Brazil), Folin-Ciocalteu reagent from Êxodo Scientific (Sumaré, SP, Brazil), potassium persulfate from Synth (Indaiatuba, SP, Brazil), ethyl alcohol P.A. from ACS (Indaiatuba, SP, Brazil), and trifluoroacetic acid (TFA), formic acid grade LC–MS, and acetonitrile grade LC–MS from Fischer Scientific (Pittsburgh, Pennsylvania). The analytical standards of cyanidin-3-glucoside, chlorogenic acid, gallic acid, apigenin-7-glucoside, and quecetin-3-O-glucoside were obtained from Sigma-Aldrich.

Sample and Raw Material Preparation

Purple yam (*Dioscorea trifida* L.f.) was purchased from a local fair trade in Manaus (−3.14021900; −60.01874100), Amazonas, Brazil. Approximately 1200 g of purple yam was washed with potable water, sanitized in sodium hypochlorite solution (150 ppm), and infused for 10 min. Then, they were peeled and manually cut into 1-cm^3 cubes, frozen an ultra-freezer (−70 °C) for 24 h and freeze-dried (Liotop K 108) with parameters of 63 μHg, −60 °C, 206 Vca, and 72 h. Then, the sample was grounded in a coffee grinder (Oster OMDR100. Brazil) for 5 min to homogenize the particles and sieved in sieve through a 200 *mesh*. The ground samples were stored in a freezer (−18 °C) and in plastic bottles, protected from light and moisture, until the analysis was carried out (Zenebon and Pascuet 2008).

Experimental Design

To optimize the production of the food extract rich in bioactive compounds, a multivariate central composite complete factorial 2^3 design was used, with axial and central points, carried out in duplicate, totaling 34 experiments (Table 1). To obtain optimal extraction conditions, the variables investigated were the volume of extracted solution (from 5 at 15 mL, values corresponding to axial points −1.68 and +1.68, respectively), water bath temperature (from 30 at 60 °C), and the agitation time in a water bath (from 2 at 30 min). The experiments were carried out randomly.

Optimization of Extraction

For this, 0.2 g of lyophilized purple yam was added to a solution of citric acid (1%) (in volumes according to the experimental design matrix). Citric acid was chosen as

Table 1 Variables and responses of multivariate design 2^3 to optimize the production of purple yam food extract

Experiment	Coded variables and levels			Decoded variables and levels			Responses	
	A	*B*	*C*	Volume of extracting solution(mL)	Temperature water bath (°C)	Stirring time (min)	Total anthocyanins (mg 100 g^{-1} dry sample)	Total carotenoids (mg 100 g^{-1} dry sample)	Total phenolic compounds (mg GAE 100 g^{-1} dry sample)
1A	−1	−1	−1	7	36.1	7.7	27.90	346.08	249.46
1B	−1	−1	−1	7	36.1	7.7	27.96	321.47	267.24
2A	1	−1	−1	13	36.1	7.7	48.31	437.76	404.59
2B	1	−1	−1	13	36.1	7.7	46.68	416.61	421.01
3A	−1	1	−1	7	53.9	7.7	44.79	342.92	289.97
3B	−1	1	−1	7	53.9	7.7	43.80	331.31	306.12
4A	1	1	−1	13	53.9	7.7	48.88	350.95	432.38
4B	1	1	−1	13	53.9	7.7	50.69	364.67	440.09
5A	−1	−1	1	7	36.1	24.3	44.59	345.43	321.42
5B	−1	−1	1	7	36.1	24.3	45.07	351.04	316.57
6A	1	−1	1	13	36.1	24.3	46.88	355.26	443.10
6B	1	−1	1	13	36.1	24.3	48.01	363.20	455.80
7A	−1	1	1	7	53.9	24.3	53.34	375.84	184.76
7B	−1	1	1	7	53.9	24.3	52.02	372.00	190.18
8A	1	1	1	13	53.9	24.3	50.92	376.28	415.19
8B	1	1	1	13	53.9	24.3	51.79	383.11	415.35
9A	0	0	0	10	45	16	47.57	357.50	408.29
9B	0	0	0	10	45	16	47.60	352.47	373.76
10A	0	0	0	10	45	16	48.04	317.99	487.95
10B	0	0	0	10	45	16	48.26	352.65	498.75
11A	0	0	0	10	45	16	47.57	360.37	314.31
11B	0	0	0	10	45	16	47.23	365.10	277.83
12A	−1.68	0	0	5	45	16	44.40	372.48	171.47
12B	−1.68	0	0	5	45	16	43.81	318.61	182.29
13A	1.68	0	0	15	45	16	51.53	316.10	447.16
13B	1.68	0	0	15	45	16	50.95	359.16	485.59
14A	0	−1.68	0	10	30	16	41.68	352.28	314.15
14 B	0	−1.68	0	10	30	16	43.61	343.10	328.55
15A	0	1.68	0	10	60	16	49.74	371.60	373.76
15B	0	1.68	0	10	60	16	49.78	372.65	377.90
16A	0	0	−1.68	10	45	2	45.26	329.59	265.66
16B	0	0	−1.68	10	45	2	45.36	331.01	300.48
17A	0	0	1.68	10	45	30	41.98	330.77	363.20
17 B	0	0	1.68	10	45	30	41.69	308.45	359.31

solvent due to its solubility in water and its property as an acidulant agent, which contributes to preserving the stability of anthocyanins during the extraction process.

Then, the solutions were transferred to the water bath (Velp Scientifica, Enzymatic Digester-GDE, Italy), with agitation at 300 rpm, at temperature and times according to the experimental matrix. Subsequently, the solutions were centrifuged (Eppendorf Centrifuge 5430 R, Germany) at 4000 rpm, for 20 min at 4 °C. The liquid extracts were filtered through qualitative filter paper with a pore size of 180 µm and taken for analysis.

The evaluated parameters included the quantification of total monomeric anthocyanins (Lee, et al. 2005a, b), total carotenoids (Lee et al. 2005a, b), and determination of total phenolic compounds according by Folin-Ciocalteu method (Singleton et al. 1999).

These data were evaluated to determine the feasibility of establishing mathematical models to predict the optimal

extraction conditions. The models were combined, using the Derringer-Suich desirability function (Derringer and Suich 1980). To set the optimal condition, the predicted condition was then performed experimentally, in triplicate, and the predicted values were compared to the observed values. The data were treated using the Statistica 7.0 and Design Expert 6.0 software through analysis of variance with 95% statistical confidence.

Comparison of the Optimum Point with Conventional Extraction Method

A conventional extraction method consolidated in the literature was used to compare the extraction obtained with the of the optimal extraction condition were compared. The content of total anthocyanins, total carotenoids, and total phenolic compounds measured in the methanolic extracts (equivalent to 100%) was compared with the amount of these compounds in the aqueous extracts. In this way, it was possible to estimate the percentage of total anthocyanins, total carotenoids, and total phenolic compounds that migrated from the matrix to the aqueous extract during the agitation time. The conventional method is about extracting with solvent containing hydrochloric acidic acid methanol (HCl), as described by Lee et al. (2005a, b), with some adaptations. Briefly, 0.2 g freeze-dried purple yam was added from 10 mL of extraction solution (methanol/HCl 1.5 M (85:15)), stirred in a water bath for 1 h at 30 °C, followed by centrifugation at 4000 rpm for 20 min at 4 °C.

Characterization of the Extract Obtained at the Optimum Point

The extract obtained under optimal conditions (882 mL) was lyophilized (Liotop K108), using the parameters set to 63 µHg, − 60 °C, and 206 Vac for 96 h, which yielded 90.5 g of lyophilized extract. It was stored in a freezer (− 18 °C), in a plastic bottle, protected from light and moisture, until the characterization of bioactive compounds by HPLC–MS, in vivo toxicity testing and antimicrobial activity assessment. Before analysis, the extract was quantitatively resuspended in ultra-pure water. Control experiments were using citric acid solution.

After mathematical modeling to obtain the optimum extract condition, 882 mL of extract was prepared following the session (Optimization of extraction), obtaining 90.5 g of freeze-dried extract from 100 g of freeze-dried tuber. The extract was stored in a freezer (− 18 °C), in a plastic bottle, protected from light and humidity, until the bioactive compounds were characterized; the in vivo toxicity test was carried out, and the antimicrobial activity was assessed. Before analysis, the extract was quantitatively resuspended in ultrapure water. Control experiments were carried out with citric acid solution.

Identification and Quantification of Bioactive Compounds by High-Performance Liquid Chromatography (HPLC) Coupled to Mass Spectrometry (MS)

The analysis of anthocyanin and non-anthocyanin compounds by HPLC–MS was performed according to the method described by Gonçalves et al. (2024). Chromatographic separation of anthocyanins was performed using a Dionex Ultimate 3000 UHPLC device (Thermo Scientific, San Jose, CA, USA), equipped with a diode array detector and coupled with an electrospray ionization mass detector (LC-DAD-ESI/MSn), quaternary pump, autosampler (kept at 5 °C), degasser, and thermostated speaker compartment (in 35 °C). A column C18 (5 µm, 4,6 mm × 150 mm, Phenomenex, CA, USA) was used. The mobile phase contained (A) 0.1% trifluoracetic acid in water and (B) acetonitrile. The gradient started with 10% of B and was maintained at that level until 3 min; then, there was a linear increase up to 15% of B (reached at 18 min), which was maintained at 15% B until 23 min. From there, there was another linear increase up to 18% of B (completed at 28 min), 30% of B (at 48 min), and 35% of B (at 52 min). Then, the spine was reconditioned for the next race. The flow rate was 0.5 mL/min. The Mass Spectrometer Linear Ion Trap LTQ XL (Thermo Finnigan, San Jose, CA, USA), equipped with an ESI source, was operated in positive mode. The ionization conditions were as follows: nitrogen at 50 psi, 4.8 kV, ion source temperature of 320 °C, and capillary voltage of 14 V. The displacement of the tube lens has been maintained at the voltage of 75 V. The full sweep covers the mass range of *m*/*z* 100 a 2000. Collision energy used has been normalized (30%). Data acquisition was performed using a data system Xcalibur® (A Thermo Finnigan, San Jose, CA, USA).

The identification of phenolic compounds was carried out in a Dionex Ultimate 3000 UHPLC (Thermo Scientific, San Jose, CA, USA), equipped with a diode array detector, coupled to an electrospray ionization mass detector (LC-DAD-ESI/MSn), quaternary pump, autosampler (kept at 5 °C), a degasser, and thermostatic column compartment. Chromatographic separation was performed at the same time 35 °C using a Waters Column Spherisorb S3 ODS-2C18 (3 µm, 4.6 mm × 150 mm, Waters, Milford, MA, USA). The mobile phase solvents were as follows: (A) 0.1% formic acid in water and (B) acetonitrile. The analysis started with 15% of B and was maintained isocratically for up to 5 min; then, there was a linear increase in the gradient up to 20% of B (at 10 min), followed by a linear increase in B until it reaches 25% (at 20 min), up to 35% in B (at 30 min), 50% in B (at 20 min), 35% in B (at 30 min), 50% in B (at 30 min), a 50%

increase in B (at 30 min), and a 50% increase in B (at 3 40 min). And then, the spine was reconditioned for the next analysis. The flow rate was 0.5 mL/min. The detection of MS was performed in negative mode, using a Linear Ion Trap LTQ XL mass spectrometer (Thermo Finnigan, San Jose, CA, USA) equipped with an ESI source. Nitrogen employed at 50 psi was used in the ionization source; the system was operated at 5 kV, with temperature of 325 °C and voltage of − 20 V in the capillary. The lens shift of the tube was maintained at a voltage of − 66 V. The full scan covers the mass range of *m*/*z* 100–2000. Collision energy used was normalized (30%). Data acquisition was performed using the data system Xcalibur® (Thermo Finnigan, San Jose, CA, USA).

The identification of the compounds was performed through the obtained fragmentations, retention times, and scanning spectrum in the UV/Visible region. These data were compared with manuscripts disponible in the scientific literature for confirmation of compound identities.

For the quantitative analysis, the identified anthocyanin compounds (1, 3, 4, 5, 6, and 7; Table 3) were quantified based on the cyanidin-3-glucoside analytical curve (reading at 520 nm from the diode array detector). On the other hand, non-anthocyanin compounds were quantified as follows: compound 1 quantified with the analytical curve of chlorogenic acid (330 nm); compound 2 with gallic acid (280 nm); compounds 11, 12, and 13 with apigenin-7-glucoside (370 nm); and compound 14 with quecetin-3-O-glucoside (330 nm).

In Vivo Toxicity Test in a *Galleria mellonella*

Galleria mellonella has an immune system similar to the innate immune response of mammals, without the interference of acquired immunity (Browne et al. 2013; Kwadha et al. 2017). They are fast and inexpensive to create and are an in vivo model that results in safe and LD_{50} values similar to other mammalian models. They are a suitable size for manipulation, reduced biological risk, as well as low rearing costs. The use of larvae has been an excellent alternative for in vivo toxicological tests in an in vivo model to study the immune response to pathogens, antibiotics, and drugs, the toxicity of compounds, and food preservatives (Allegra et al. 2018; Dolan et al. 2016; Megaw et al. 2015; Moya-Andérico et al. 2021; Vellé et al. 2017). In a study using eight food preservatives, there was a positive correlation in the LD_{50} values in the *G. mellonella* larvae model and in mammalian models.

For the determination of the lethal dose that kills 50% of organisms (LD_{50}) and the safe dose, toxicity tests were performed using the in vivo model of *G. mellonella*, as described by Sardi et al. (2017), with minor adaptations. The lyophilized extract of purple yam was diluted at a concentration of 100 mg mL^{-1} in ultrapure water, vortexed, homogenized for 30 s, and filtered on a qualitative paper filter a pore size of 180 µm. From this solution, eight concentrations were prepared: 0 mg/mL (control, ultra-pure water), 10, 25, 40, 55, 70, 85, and 100 mg mL^{-1}.

The different concentrations were injected into larvae of *G. mellonella*, weighing between 0.2 and 0.3 g, with no signs of melanization, randomly selected. A total of 15 larvae from a single group were evenly distributed across sterile plates, with each plate containing 5 larvae. A volume of 10 µL of each concentration was inoculated into the abdominal hematocele (body cavity present in insects of the order Lepidoptera, where blood flow occurs) of the larvae of *G. mellonella* through the last left pseudopath, with the aid of a Hamilton syringe of 10 µL (Hamilton, Reno, NV). The larvae were incubated in BOD (biochemical oxygen demand) incubator, at 30 °C, in the dark, without nutrition, and monitored for survival count after 24 h, 48 h, and 72 h. Larvae that did not show touch movements and/or were melanized were considered dead. The number of *G. mellonella* deaths was recorded for survival curve analysis. The survival percentage was plotted as a function of the extract concentration using a polynomial model expressed in grams of extract per kilogram of body *G. mellonella*. Figures 1 and 2 of the supplementary material show images of the test in *G. mellonella.*

Antimicrobial Activity

Microorganisms are known to cause foodborne illnesses (DTAs) in humans. *Staphylococcus aureus* can produce toxins that lead to food poisoning, while *Salmonella typhimurium* and *Escherichia coli* can cause gastrointestinal infections such as vomiting and diarrhea. Evaluating the safety of the extract and its ability to inhibit the growth of these pathogenic bacteria is possible to determine whether the extract has antimicrobial properties.

Therefore, the lyophilized extract of purple yam was diluted in sterile distilled water at a concentration of 1000 mg mL^{-1}. Antimicrobial activity was tested against three important foodborne pathogens: a Gram-positive bacterium (*Staphylococcus aureus* ATCC 25923) and two Gram-negative bacteria (*Salmonella* Typhimurium ATCC 14028 and *Escherichia coli* ATCC 25922), following the protocol recommended by the Institute of Clinical and Laboratory Standards (CLSI, 2018). The strains were cultured on tryptone soy agar (TSA, Acumedia, USA) and incubated under aerobic conditions at 37 °C, for 24 h. The concentration of the inoculum was standardized on the turbidity scale 0.5 of McFarland (10^8 UFC mL^{-1}). Subsequently, it was seeded onto Petri dishes containing Mueller Hinton agar (MHA, Usina, Brazil). Sterile filter paper discs (6 mm) were placed in the center of the Petri dishes, which had been previously

seeded with the inoculum, and immediately impregnated with 20 µL of purple yam extract. The dishes were then incubated at 37 °C for 24 h under aerobic conditions. Streptomycin disc (10 µg) was used as a positive control, while discs impregnated with sterile water and 1% citric acid were used as a negative control. The test was performed in duplicate, and the antibacterial activity was confirmed through the formation of an inhibition halo around the discs impregnated with the evaluated extract. The results were expressed as the average halo diameter of the inhibition zones.

To determine the minimum inhibitory concentration (MIC), a bacterial suspension was prepared according to the 0.5 scale of McFarland (10^8 UFC mL^{-1}). Subsequently, suspensions were prepared corresponding to 10^6 UFC mL^{-1} in a broth Mueller Hinton (MHB, Kasvi, Brazil) sterile. From this inoculum, 50 µL was added to each well of a 96-well microplate and the extract was tested at different concentrations (25%, 12.5%, 6.25%, 3.125%, 1.562%, 0.78%, 0.39%, and 0.19%, in relation to the initial concentration of the extract of 1000 mg mL^{-1}). Thus, in each well, 50 µL of the MHB was added, followed by the addition of 50 µL of the extract, and dilutions were performed. Finally, 50 µL of the standardized inoculum was added, totaling 150 µL per well.

The MIC is defined as the lowest concentration of an antimicrobial compound capable of inhibiting visible bacterial growth after 24 h of incubation. To determine the minimum bactericidal concentration (MBC), suspensions that showed no visible bacterial multiplication were collected and seeded in MHA. The plates were incubated under aerobic conditions for 24 h at 37 °C. Afterwards, the presence of bacterial multiplication in the plates was assessed. Wells where no bacterial growth was observed indicated the absence of viable cells, and the bactericidal concentration was stipulated. The MBC is defined as the lowest concentration in which 99.9% of the cells initially inoculated were inactivated. Both experiments were carried out in triplicate and analyzed later.

Results and Discussion

Multivariate Optimization of Extract Production

Table 1 shows the results of the multivariate design aimed at obtaining the edible extract from purple yam. Table 2 shows the mathematical models, the significant coefficients, as well as the test of lack of fit the significance of the regressions.

The model for total anthocyanins exhibited a significant lack of adjustment, as evidenced by the *F* value obtained was 27 times higher than the critical *F*. The regression itself was significant, since the *F* value obtained was higher than the critical value, indicating that the effects of the significant coefficients differ from the residuals (Table 2). Despite the lack of adjustment, significant coefficients indicate that

Table 2 Significant coefficients for the mathematical models that describe the dependent variables for the optimization of the extraction of the food extract obtained from purple yam, *F* values for model adjustment, and regression significance

Compounds	Model	Significant coefficients (error)*									Adjustment model (*F*) (Critical *F*)	Significance regression (*F*) (Critical *F*)
		Interceptar		*C*	*A2*	*B2*	*C2*	*AB*	Alternating current	AC		
Total anthocyanins	Modified	46.11	2.81	3.11	1.54			− 2.25	− 2.97		66.12	14.42
		(0.51)	(0.57)	(0.57)	(0.57)			(0.75)	(0.75)		(*F*8.19: 2.48)	(*F*6.27: 2.46)
Total carotenoids	Modified	354.29			− 0.94				− 12.17	14.02	4.97	2.74
		(4.05)			(4.52)				(5.90)	(5.90)	(*F*8.19: 2.48)	(*F*6.27: 2.46)
Total phenolic compounds	Linear	349.52	83.35								1.94	21.23
		(9.39)	(10.48)								(*F*11.19: 2..38)	(*F*3.30: 2.92)

A volume of extractor solution, *B* water bath temperature, *C* stirring time

the volume of the extraction solution (A), temperature (B), and time (C) showed positive linear effects, resulting in an increase in the concentration of compounds during the extraction process.

This observed effect is evident in the experimental results obtained (experiments 1 and 2 for volume, 1 and 3 for temperature, 1 and 5 for time). However, when both volume and temperature (A and B, experiment 4) and volume and time (A and C, experiment 6) were simultaneously increased, a reduction in anthocyanin extraction was observed. Second, Li and collaborators (2013) show that while optimizing the extraction of purple sweet potato, an initial increase in yield is observed with increasing temperature and extraction time, followed by a subsequent decrease.

On the other hand, the model for total carotenoids showed a slight lack of adjustment (with *F* obtained only two times above the critical one) and the regression was significant. The most significant effect was observed when the temperature and time variables (B and C) were simultaneously increased, impacting the increase in extraction. Also, a simultaneous increase in the volume and agitation time (A and C) resulted in a reduction in extraction. Similarly, the linear effect of stirring time showed a negative impact on these compounds. The relationship between time and temperature is not perfectly linear; however, increasing the temperature can speed up the extraction process to a certain extent. This is because higher temperatures increase the kinetic energy of the molecules involved, making them move faster and facilitating contact between the solvent and the target component. However, this has limitations. Excessively high temperatures can degrade the target component or the solvent, reducing the extraction yield. Authors such as Ulyarti and Lisani (2021) have observed that time and temperature are among the factors influencing the extraction of anthocyanins. The model for total phenolic compounds demonstrated satisfactory adjustment and a significant regression. The only variable that influenced this response was the volume of extractor solution (A), where an increase led to enhanced extraction.

To define the optimal extraction condition using the Derringer and Suich (1980), the criteria presented in Table 1 of the Supplementary Material were established. It can be observed that for the responses of total anthocyanins and total carotenoids, the goal was to increase the response, with an importance of 3 (due to the fit weaknesses of the models). Conversely, the total phenolic compounds were given an importance of 5, since it is a response that had a well-adjusted model and represents approximately the total composition of the compounds with total phenolic compounds present in the sample.

Through the combination of the models, it was possible to conclude (with 97.3% of the desirability met) that the best combination of the variables consists of 15 mL of 1% citric acid solution (pH = 3.5) to 0.2 g of dried purple yam in a water bath at 36 °C for 4 min; then, the sample is centrifuged at 4000 rpm for 20 min and filtered. From 100 g of purple yam, the yield was 90.56 g of dried extract acid. The optimal condition was performed experimentally, in triplicate, and presented results statistically similar to the predicted condition (Table 1 of the Supplementary Material), with 56.91 ± 0.76 mg 100 g^{-1} of total anthocyanins, 417.05 ± 11.37 mg 100 g^{-1} carotenoids, and 493.09 ± 6.38 mg GAE 100 g^{-1} of total phenolic compounds.

The extract obtained under the optimal conditions resulted in the extraction of total anthocyanins from 56.91 mg 100 g^{-1} dry specimen, with an extraction yield 32% higher when compared to the conventional method (with methanol and hydrochloric acid as the extraction solution), as shown in Table 1 of the Supplementary Material. The results obtained for carotenoids and total phenolic compounds were higher in extraction with the conventional method, in + 31% and + 18%, respectively. Such effects can be attributed to the higher dissociation constant of hydrochloric acid, which provides greater cleavage of the vacuolar cell wall, resulting in greater extraction of some groups of compounds (Thakur et al. 2013). No data specific to the yam variety studied in this research were found in the literature. However, in comparison, the fleshed potato cultivars had an average total carotenoid content of red/purple (5.69 µg/g MS) and the levels of phenolic compounds (originally 209 mg GAE/100 g FW) (Vellé et al. 2017).

Nonetheless, the proposed method's optimal condition, using 1% citric acid as the extraction solution, is noteworthy for its eco-friendliness and rapidity, producing an extract suitable for application in food and food packaging.

Characterizations of the Extract Obtained at the Optimum Extraction Point

Identification of Bioactive Compounds by HPLC–MS/MS in Purple Yam Extract

Table 3 shows the identified and quantified anthocyanin and non-anthocyanin compounds in the extract obtained from the sample. Chromatograms for the analysis of anthocyanin and non-anthocyanin compounds are presented in Figs. 3 and 4 of the Supplementary Material.

Compound 1 is possibly a derivative of peonine, since one of the fragments corresponds to *m/z* of this aglycone. Compound 2 could not be identified. Compound 3 refers to peonidin. Compounds 4, 6, and 7 were identified as peonidin-3-O-glucoside-5-O-glucoside, peonidin-3-O-feruloylglucoside-5-O-glucoside, and peonidin-3-Op-coumaroylglucoside-5-O-glucoside, respectively. Compound 4 (molecular ion in *m/z* $[M^+]$ 625) showed a larger fragment ion in the MS^2 in *m/z* 301 (peonidin), a smaller one in *m/z*

Table 3 Identification and quantification of bioactive compounds in purple yam (mg 100 g^{-1} lyophilized sample)

Anthocyanin compounds

Peak	Tr (min.)	Lambda maximum (mn)	Molecular formula	Majority ion (M^+)	Fragment of ion (MS^2)	Fragment of ion (MS^3)	Identification	Concentration mg 100^{-1} of dried yam
1	4.0	222, 263, 503		407.0	389, 215, 371, 301	301, 327, 290, 239	Unidentified	
2	8.1	202, 282, 535		162.0	106, 121, 122, 135, 145		Unidentified	
3	17.9	282, 250, 533		301.0	283, 179, 189, 241		Unidentified	
4	18.0	513, 282, 250	$C_{34}H_{43}O_{21}^+$	625.0	301, 463	286, 258, 227	Peonidin-3-O-glycosideo-5-O-glycoside	2.39
5	25.7	291, 370, 534		180.0	161, 135, 121, 137, 163		Unidentified	
6	27.9	526, 282, 250	$C_{44}H_{51}O_{24}^+$	801.0	301, 544, 285, 638		Peonidin-3-O-feruloylglycosideum-5-O-glycosidium	4.43
7	29.4	526, 285, 319	$C_{43}H_{49}O_{22}^+$	771.0	301, 286, 463, 610, 753	286, 258, 230, 165	Peonidin-3-Op-coumaroylglycosideo-5-O-glycosidium	22.92

Non-anthocyanin compounds

Peak	Tr (min.)	Lambda maximum (mn)	Molecular formula	Majority ion (M^-)	Fragment of ion (MS^2)	Fragment of ion (MS^3)	Identification	Concentration mg 100^{-1} of dried yam
1	3.0	192		515.2	173, 453,		Unidentified	
2	3.2	223	$C_7H_{12}O_6$	191.0	111, 173, 87, 155		Quinic acid	3.43
3	3.3	192		190.9	111, 131, 173, 85		Unidentified	
4	4.4	253, 344		1112.8	1089, 731, 1328		Unidentified	
5	5.3	192, 221, 275, 327		1108.9	856, 766		Unidentified	
6	6.7	280, 320, 348		900.6	1343.5		Unidentified	
7	7.3	195, 281, 303, 338, 388		265.0	169, 221, 113, 171, 191		Unidentified	
8	8.0	196, 282, 299, 331, 361, 415		382.7	289, 269, 195		Unidentified	
9	9.1	193, 210, 229, 251,283, 315, 344, 390		922.9	877	62	Unidentified	
10	11.7	223, 280, 296, 326, 358, 390		768.9	519, 675, 615, 425		Unidentified	
11	14.0	229, 272, 333	$C_{26}H_{28}O_{14}$	563.2	473, 443, 353, 384, 414	353, 383, 233, 417	Apigenin 8-*C*-xyloside-6-*C*-glucoside (vicenin 3)	0.4
12	15.4	219, 278, 328	$C_{26}H_{28}O_{14}$	563.7	443, 353, 455, 473	353, 383	Apigenin 6-*C*-xyloside-8-*C*-glucoside (vicenin 1)	5.12
13	17.7	243, 269, 280, 317, 328, 341, 360, 390	$C_{42}H_{45}O_{22}$	609.3	285, 286, 229, 267, 301, 343, 196	267, 243, 213, 185, 241, 229, 195	Kaempferol-*O*-dihesoxide	0.14
14	18.7	253, 357, 543		639.5	315, 330, 607, 227	300, 287, 272, 151	Isorhamnetin-*O*-dihexosideo	1.25
15	19.1	242, 280		641.7	299, 517, 597, 623	284, 257, 149	Unidentified	
16	33.6	239, 285, 317		723.6	678, 661	451, 225, 660	Unidentified	
17	35.3	260		1350	1530		Unidentified	
18	42.0	262		675	551		Unidentified	
19	44.7	277, 312		152.9	113		Unidentified	

463 (162 glucose loss mass value), and in the MS^3 at *m/z* 286 and 258. It is possibly derived from the degradation of the aglycone, while the minority sign of 642 corresponds to bonding with a water molecule ($M+H_2O$). Second, Ramos-Escudero et al. (2010) demonstrated through this fragmentation pattern that glucose residues are located at different positions on the aglycone (like a disaccharide).

Compounds 6 and 7 correspond to acylated peonidins. Compound 6 has a molecular ion at *m/z* 801 [M^+], with MS^2 fragments at 301 (aglycone) and 638 [(M – H) – 337]. The *m/z* 337 fragment corresponds to the molecular ion of O-feruloyl glucosyl. Compound 7 has a molecular ion at *m/z* 771 [M^+], with MS^2 fragments at 301 (aglycone) and 463 that indicate the loss of feruloyl glucosyl or coumaroyl glucosyl residues. That loss is also observed by Ramos-Escudero et al. (2010).

For non-anthocyanic compounds, compounds 2, 11, 12, and 14 were identified as quinic acid, apigenin 8-C-xyloside-6-glucoside (vicenin 3), apigenin 6-C-xyloside-8-C-glycoside (vicenin 1), and isorhamnetin-O-dihexoside. Compound 1 with *m/z* [M^-] of 515 and fragments of 173 and 453 in MS^2 cold be the dicaffeoylquinic acid; however, it was not possible to identify the isomers of caffeoylquinic acids. According to Lorini et al. (2021), the fragment *m/z* 173 corresponds to dehydrated quinic acid (M-H_2O-H), while the fragment *m/z* 453 corresponds to [(M – H) – 358 – 95], with *m/z* 358 (dicaffeoylquinic acid) and 95 (possibly fragmentation of quinic acid in C_6H_9O). Compound 2 exhibited an ion *m/z* [M^-] in 191 (quinic acid, M-H); its fragmentation MS^2 showed ion *m/z* 173 (M-H-H_2O).

Compound 11 had *m/z* [M^-] at 563 with MS^2 fragmentation at 473 [(M – H) – 90], corresponding to C-hexosyl flavones: 443 [(M – H) – 120], 353 [(M – H) – 120 – 90], 384 [(M – H) – 120 – 60], 413 [(M – H) – 90 – 60], and 546 [(M – H) – H_2O] which are named isomers of 6-C-pentosyl-8-C glucosyl apigenin or 6-C-glucosyl-8-C-pentosyl apigenin. The C-6 isomer is more susceptible to fragmentation in relation to the C-8 isomer. The $[(M-H)-60]^-$ ion is characteristic of pentose derivatives, with isomers containing pentose in position 6 being more common than in the position 8. The absorption spectra at 229, 272, and 333 nm (Llorent-Martínez et al. 2015; Benayad et al. 2014) report that these neutral losses are characteristic of glycosyl and pentosyl residues. Compound 12, with the same molecular ion, presents the molecular ion 443 [(M – H) – 120] corresponding to C-hexosyl flavones, but with its absorption spectra at 219, 278, and 328 nm (Grati 2022).

Compound 14 with the *m/z* [M^-] 639 and fragmentation MS^2 at 315, 330, 607, and 227, respectively, possibly indicated the loss of different glycosidic moieties resulting in isorhamnetin aglycone (*m/z* 315). The common features of flavonoid aglycone fragmentation are ionic fragmentation as a result of the cleavage of CH_3 (– 15, – 30 u) (Spínola et al. 2015). It was not possible to identify the other compounds.

In the databases consulted by the authors, only two studies were found that identified the compounds of *Dioscorea trifida*. Carreno-Diaz and Grau (1977) identified by paper chromatography of the compounds peonidin 3,5-diglycoside and malvidin 3,5-diglycoside acylated with ferulic acid in samples of purple yam from Indonesia. Indeed, Ramos-Escudero et al. (2010) identified the same peonidins mentioned here in the samples from Peru by means of an LC-ESY-MS–MS, but they did not identify malvidin. None of the studies performed quantification.

In Vivo Toxicity in a Model of *Galleria mellonella*

Acute systemic toxicity was evaluated using the *G. mellonella* model. Figure 1 shows the 72-h death rate after

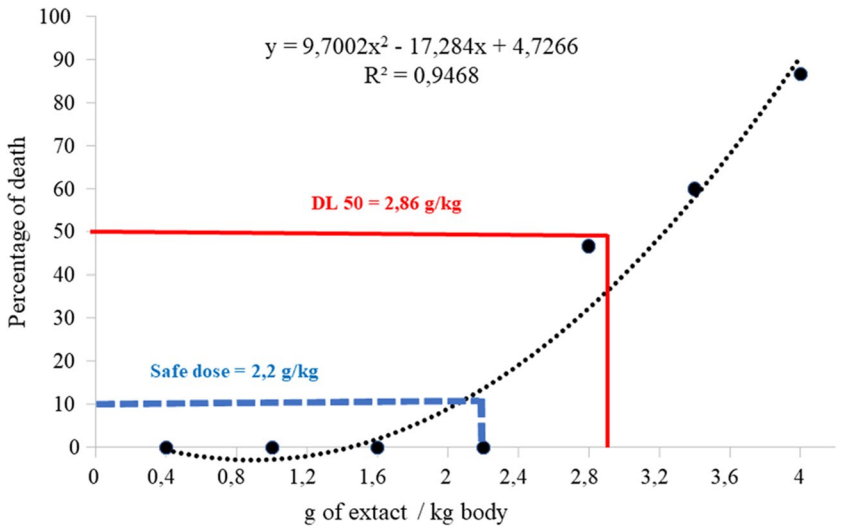

Fig. 1 Toxicity graph of purple yam extract in a model of *Galleria mellonella*

Table 4 Antibacterial action, minimum inhibitory concentration, and minimum bactericidal concentration of the extract of purple yam extract against food-borne pathogens

Pathogens	Size (mm) Halos	Controls			CIM	CBM
		Water	Citric acid 1%	Gentamicin	mg mL^{-1}	
Staphylococcus aureus	24	0	0	23	3.9	31.25
Salmonella Typhimurium	13	0	0	13	7.8	62.5
Escherichia coli	14	0	0	17	3.9	7.8

inoculation. No deaths were observed when administered at a concentration of 2.2 g of extract per kg of body weight, which is equivalent to 154 g of extract for a 70-kg individual. In the control group, all larvae survived. Utilizing the polynomial equation, which had a 94.68% correlation between the death rate and the concentration, the DL_{50} resulted in 2.86 g of extract per kg of body. Arsene et al. (2021) studied the relative toxicity of hydroalcoholic and aqueous extracts of seven Cameroonian medicinal plants, in *G. mellonella*, and obtained LD_{50} of 19.01 mg/mL g and 6.01 mg/mL of extract per kg body for Azadirachta indica seed extracts, respectively (Arsene et al. 2021).

Antimicrobial Activity of Purple Yam Extract

Bacterial resistance is currently a growing and worrisome problem worldwide (GLASS, 2022). Therefore, the search for alternative methods of controlling pathogenic bacteria is necessary in order to prevent the spread of resistant isolates, thus ensuring food safety. Wu et al. (2023) also emphasize the importance of promoting the diversification of strategies to combat pathogens, minimizing risks to public health. In addition, the utilization of extracts as natural preservatives in foods can not only aid in controlling bacterial contamination, but also provide bioactive compounds that confer health benefits to consumers (Mounika et al. 2022). It is worth mentioning the need to intensify the search for methods to control *S. aureus, Salmonella*, and *E. coli* in the food chain, since more than 53% of the outbreaks of foodborne diseases in Brazil, in the period from 2012 to 2021, were caused by these pathogens (Gargiulo et al. 2022).

There are limited studies about the antimicrobial activity of purple yam extract against foodborne bacteria, but the antibacterial potential of tuber extracts has been reported by several studies (Chandrasekara and Kumar 2016). In the present study, it was possible to verify that all bacterial strains tested were susceptible to 1% acidified purple tree extract (Table 4), according to the formation of an inhibition halo around the discs. In the disk diffusion assay, the Gram-positive bacteria *S. aureus* was more susceptible to the extract (24 mm) than Gram-negative bacteria *S. typhimurium* and *E. coli*, with 13 mm and 14 mm, respectively. This result is consistent with findings in the literature, where Gram-positive bacteria are more affected by plant extracts, possibly due to their cell wall structure, which favors the action of the compounds (Gonçalves et al. 2024).

The antibacterial potential of yam extract is possibly due to its constituents; Nurfitriani et al. (2021) evaluated the antibacterial action of purple yam sap extract against *E. coli* and *S. aureus*, and inhibition halos of 9.3 mm and 8.62 mm in diameter, respectively, were obtained. According to the authors, purple sap extract contains components such as phenols, tannins, steroids/saponins, and flavonoids, which act as effective active substances to inhibit bacterial multiplication. Also, a study that developed the formulation of a film based on *Dioscorea alata* susceptibility was identified only to *S. aureus* and not to *Salmonella* spp. and *E. coli* (Istiqomah et al. 2022).

According to the results shown in Table 4, the extract showed an inhibitory effect against both Gram-negative and Gram-positive pathogens. Nevertheless, *E. coli* was the bacterium most susceptible to purple yam extract, with a MIC of 3.9 mg mL^{-1} and CBM of 7.8 mg mL^{-1} (Table 4). This can be attributed to the differences in the cellular structure of Gram-positive and Gram-negative bacteria. The cell wall of Gram-positive cells facilitates the penetration of molecules, while Gram-negative cells have an outer membrane with lipopolysaccharides that confer resistance to antimicrobial extracts (Sharma et al. 2024; GLASS, 2022; Wu et al. 2023; Mounika et al. 2022).

Conclusion

This study is unprecedented in terms of the characterization of *Dioscorea trifida* L.f. produced in Manaus, Brazil. We proposed an unprecedented method to obtain a food extract, rich in bioactive compounds, with fast, simple, ecofriendly extraction, and easy industrial expansion, from a matrix that has been underexplored in the food industry. The optimum conditions for extraction with the parameters of the best combination of variables consisted of 15 mL of 1% citric acid solution for 0.2 g of purple yam dried in a water bath at 36 °C for 4 min. The extract obtained under the optimal conditions resulted in the extraction of 56.91 mg from total anthocyanins per 100 g of dry sample (32% higher when compared to the conventional method). The extract yielded three identified anthocyanin

compounds (peoinidin-3-O-glucoside, peonidin-3-O-feruloylglucoside-5-O-glucoside, peonidin-3-Op-coumaroylglucospide-5-O-glucoside) and four non-anthocyanin compounds (quinic acid, apigenin 8-C-xyloside-6-C-glycoside (vicenin 3), apigenin 6-cxyloside-8 C-glycoside (vicenin 1), and isorhamnetin-O-dihexoside), as well as 20 unidentified compounds. The extract showed low toxicity, in *an* in vivo *test*, and was safe for consumption in up to 154 g of lyophilized extract per 70 kg of body; it was able to inhibit pathogenic bacteria that cause foodborne diseases *E. coli* and *S. aureus*. These results suggest that the purple yam, produced in Brazil, may have added value when applied in food, active and intelligent packaging, and quality monitoring in the food industry.

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Declarations

Competing interests The authors declare no competing interests.

Ethics Approval The research was registered with the Ministry of the Environment of Brazil, in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge under number no. A937529. This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of Interest The authors declare they have no financial interests.

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