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MICROBIOLOGY

Optimized microwave-assisted extraction of polyphenols and tannins from *Syzygium cumini* (L.) Skeels leaves through an experimental design coupled to a desirability approach

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Abstract: The present study consisted in optimizing the extractive method of polyphenols and total tannins of leaves of *Syzygium cumini* (L) Skeels assisted by microwaves to potentiate the antimicrobial activity of the dried extract of *S. cumini* against sensitive and resistant strains. A Box-Behnken design that consisted of 27 experimental runs coupled with a desirability function for multiple response optimization was employed to optimize the total polyphenols content and total tannins content. Antimicrobial sensitivity tests were evaluated by obtaining the minimum inhibitory concentration, minimum fungicidal concentration and minimum bactericidal concentration in 96-well petri dishes. The optimal extraction conditions were found to be 8 min of extraction, under 300 w of microwave power, using a 1:34 g/mL solid/solvent ratio and 38% of ethanol concentration as extraction solvent. The parameter with the greatest influence in the extraction was primarily the time, followed by the potency and proportion solid/solvent. This yielded a total polyphenol content of 87.37 ± 1.85 mg TAE g^{-1}ext and a total tannin content of 79.68 ± 1.64 mg TAE g^{-1} ext. All tested microorganisms were sensitive to the extract, evidencing the effectiveness of the extraction method optimization.

Key words: Antibacterial, antifungal, experimental design microwave-assisted extraction, *Syzigium cumini*.

INTRODUCTION

The problem of resistance of pathogenic microorganisms to innumerous drugs is currently recognized (WHO 2014, Padiyara et al. 2018). Research centers around the world have reported the antimicrobial activities of natural products, and many compound group swith these properties include phenolic substances, polyphenols, tannins, terpenoids etc, which can be extracted from plants (Ahmad & Aqil 2007, De Souza et al. 2018). These secondary metabolites can work as new antimicrobial agents while reducing side effects that are usually part of conventional antimicrobial therapy.

Syzygium cumini (L.) Skeels (synonyms: *Eugenia cumini* (L.) Druce, *Syzygium jambolanum* DC) belongs to the Myrtaceae family and is popularly known as jambolana, jamun or jambul in India and indian blackberry in America. *S. cumini* is widely known for presenting many therapeutic appliances (Srivastava & Chandra 2013). Among them, antimicrobial activity has been recently reported mainly associated to its polyphenols and tannins content as secondary metabolites (Migliato et al. 2010, Ugbabe et al. 2010, Bag et al. 2012, Singh et al. 2016, Khan et al. 2017), although other metabolites have also been reported as antimicrobial resource for *S. cumini* (Shafi et al. 2002).

In recent years, microwave-assisted extraction (MAE) has stood out among other extraction methods because it offers a reduction of extraction time and a low solvent consumption while reaching high extraction rates and better biological results with lower costs and production waste (Panja 2018, De Hoyos-Martínez et al. 2019). However, due to many parameters that influence MAE, it is often necessary to optimize the extraction process in order to obtain the maximum amount of bioactive compounds (Angoy et al. 2018, Vázquez-Espinosa et al. 2018). The design of experiments (DOE) is an effective method for optimization of extraction procedures, since it can combine several independent factors while taking into account possible interrelations among them, reducing the number of required experimental tests and allowing a considerable reduction of cost and operational time (Das et al. 2014, Naima et al. 2019).

Considering that Myrtaceae family has the advantage of being spread around the globe and can be easily cultivated for phytotherapy purposes, the objectives of the present study were: (i) to optimize the extraction of polyphenols and total tannins from the leaves of *S. cumini* assisted by microwave and (ii) to evaluate the antifungal and antibacterial activity against sensitive and resistant strains of the dried extract of the leaves of *S. cumini* obtained by the optimized extraction method.

MATERIALS AND METHODS

Chemicals

Pure Tannic Acid (Vetec®), Sodium carbonate (Na_2CO_3) (Dinâmica®), Casein (Dinâmica®), Folin-Ciocalteu Phenol reagent. Culture medium was RPMI 1640 (Sigma-Aldrich®) and Mueller-Hinton broth (Sigma-Aldrich®).

Plant material

Flowering branches of *Syzygium cumini* (L.) Skeels were collected at the county of Cabo de Santo Agostinho (8°29'86,07 "S and 35°06'45,29" W) and sent to the Agronomic Institute of Pernambuco (IPA), where they were identified by the Ms. Rita de Cássia Pereira, under the overturning number 90672. The samples were collected in the seasons of the year (spring, summer, autumn and winter).

Optimization of extractive method and determination of TPC and TTC

Extraction procedures and experimental design

An experimental design was planned to optimize the extraction of polyphenols and tannins from leaves of *S. cumini* through MAE. At first, the raw plant material was prepared by treating samples of the plant leaves with alcohol 70% (v/v) and putting them into a circulating air oven (Ethiktechnology®) for 40 h at 40 °C. After drying, the material was pulverized in a knife mill (SL-31, Solab®) with a 20-mesh (0.84 mm) sieve, weighed and packed into a glass vessel, suitably sealed and maintained in the absence of light. This prepared raw plant material was further used for MAE in a Microwave Synthesizer (Discover system, CEM®).

Extraction was performed in 30s cycles of irradiation and a cooling time of 10 min to reach a temperature of 25 °C. The method of operation of the equipment was that of fixed power, in which the selected power remained the same throughout the extraction, respecting the tested conditions of 50, 150 and 300 w. Extractions were performed at controlled temperature (30 °C) to avoid thermal degradation of the compounds.

Extraction time (X_1, min), microwave power (X_2, w), solid/solvent ratio (X_3, g/mL) and ethanol concentration (X_4, %) were the independent

factors chosen for this study. These factors were studied at three levels, in a Box-Behnken design (Table I) that consisted of 27 extractions. The impact of the independent factors on responses of total polyphenols content (TPC, Y_1) and total tannins content (TTC, Y_2) were analyzed. Both responses were measured in milligram tannic acid equivalent (TAE) per gram of crude extract (mg TAE g^{-1}ext). All experimental levels were selected based on previous studies involving similar extractions (Dahmoune et al. 2015, Naima et al. 2015, 2019).

Effects were estimated by least squares regression and analyzed by ANOVA (95% confidence level) and the combination of linear and quadratic effects were observed. The best extraction condition was chosen based on the prediction of responses through a general desirability approach.

The dried extract of *S. cumini* was obtained by lyophilization during 72 hours from the fluid solution optimized by MAE. The determination of TPC and TTC in the dried extracts were carried out by dissolving 142 mg of the dry extract in 100 mL ethanol 38% and proceeding according to a modified method of Folin-Ciocalteu (Amorim et al. 2008).

Determination of total polyphenols content and total tannins content

The TPC and TTC in the hydroethanolic extracts were determined by a modified method of Folin-Ciocalteu (Amorim et al. 2008). Initially, 10mL of the extractive solution was diluted 10 times in a volumetric flask. An aliquot of 0.33mL of the diluted extractive solution was homogenized with 0.7mL of the Folin-Ciocalteu solution (10% v/v) and 1 mL. of sodium carbonate (7.5% v/v) in a 10mL volumetric flask that was completed with deionized water. Solutions were incubated at 27°C for 30 min absorbances were measured at 706 nm (UV/Vis-mini-1240, Shimadzu®). Absorbances were compared to an analytical curve of tannic acid and TPC was determined.

For TTC, 1g casein was complexed with 6mL of the diluted extract solution and another 12mL of water in an Erlenmeyer flask during 30 minutes under constant stirring. The complex was filtered and 0.3 mL of the supernatant was homogenized with 0.7 mL of the Folin-Ciocalteu

Table I. Independent factors and levels employed in the Box-Behnken experimental design for optimizing TPC and TTC (mg TAE g^{-1}ext) in the liquid extract from *S. cumini* leaves through microwave-assisted extraction.

	Code	Factors	Levels		
			Low (-1)	Mid (0)	High (1)
Independent factors	X_1	Extraction time (min)	1	5	10
	X_2	Microwave power (w)	50	150	300
	X_3	Solid/solvent ratio (g/mL)	1/10	1/25	1/40
	X_4	Ethanol concentration (%)	20	50	80
Responses	Y_1	TPC (mg TAE g^{-1}ext)			
	Y_2	TTC (mg TAE g^{-1}ext)			

TAE = Tannic Acid Equivalent; TPC = Total Polyphenols Content; TTC = Total Tannins Content.

solution (10% v/v) and 1 mL of the sodium carbonate solution (7.5% v/v) in a volumetric flask of 10mL and completed with water. The solutions were incubated at 27°C for 30 min and absorbances were measured at 706 nm in a spectrophotometer (UV/Vis-mini-1240, Shimadzu®).

In vitro antifungal sensitivity test of the dried extract of *S. cumini*

In vitro antifungal sensitivity of the dried extracts was evaluated according to the protocol described in document M27–A3 by the Clinical and Laboratory Standards Institute (CLSI) (CLSI 2008). As a control, *Candida parapsilosis* standard American Type Culture Collection (ATCC) 22019 with echinocandin sensitive profile was used. The extracts were diluted in water and the tested concentrations ranged from 640 µg/mL to 1.25 µg/mL.

Clinical isolates from the blood of patients from the Intensive Care Unit (ICU) of the Clinics Hospital of Pernambuco were used for the test. The yeasts that presented resistance to echinocandins were *Candida albicans, C. glabrata* and *C. haemulonii*. Yeasts were kept in Sabouraud Agar Dextrose medium (SDA), incubated at 35°C for 48h. The inoculum volume was adjusted to 5.0 mL of sterile saline solution and, subsequently, diluted in RPMI 1640 to a concentration of 2–5x10^3 cells/mL.

For the sensitivity tests, 96–well flat microtiter plates (TPP; Trasadingen, Switzerland) were used. The determination of the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of optimized dried extract (see Optimization of extractive conditions.) were performed with 100% inhibition concerning the control well. MFC was confirmed by the absence of fungal growth.

In vitro antibacterial sensitivity test of the dried extract of *S. cumini*

For the antibacterial sensitivity test, five strains of *Staphylococcus aureus* were used: LPBM OXA1, LPBM 17 (clinical isolates of blood culture) LPBM 16 (clinical isolate of sputum), LPBM 31 (clinical isolate of urine) and LPBM 18 (clinical isolate of iliac secretion) and five *Enterococcus faecalis* strains (LPBM 918, LPBM 189, LPBM 335, LPBM 421, LPBM 240) isolated from clinical samples of blood culture. The resistance phenotype of these microorganisms to β-lactams, macrolides, aminoglycosides and fluoroquinolones had previously been determined by the solid–media diffusion method. The reference strains *S. aureus* (ATCC 335921 and ATCC 25923) and *E. faecalis* (ATCC 51299 and ATCC 27212) were also inserted to the study. These microorganisms are kept in the Laboratory of Physiology and Biochemistry of Microorganisms, Department of Antibiotics at the Federal University of Pernambuco.

Microorganisms were preserved under mineral oil and reactivated by transferring part of the culture into tubes containing Mueller-Hinton broth and incubated at 37°C for 24 hours. These cultures were then seeded in Mueller–Hinton Agar and re-incubated under the same conditions. With the aid of a calibrated loop, two colonies of approximately 2 mm in diameter were inoculated into 10 mL of Mueller–Hinton broth and tubes were incubated at 37°C for 24 hours. These cultures were diluted and their turbidity compared to the 0.5 tube of the Mac Farland scale, which corresponds to 10^8 CFU/mL. A dilution (1:100) was performed in order to obtain an inoculum of 10^6 CFU/mL.

The optimized dried extract (see Optimization of extractive conditions.) of *S. cumini* was analytically weighed and solubilized in ethanol:distilled water (4:6) to obtain a standardized solution of 2560 µg/mL. This stock solution was sterilized by filtration under

Milipore® membrane (0.22 µm porosity). An antimicrobial evaluation of the solvent was conducted to ensure that this system does not have intrinsic activity on the evaluated microorganisms.

To determine the MIC of the dried extract of *S. cumini*, the microdilution method recommended by CLSI (CLSI 2017) was employed with some modifications. In order to do this, 100 µL of the standardized extract solution was dispensed into wells 1 through 7 of line A. In the other wells, they were pipetted with 100 µL of Mueller-Hinton broth. A 100 µL aliquot of the contents of each well of line A was transferred to line B and the procedure was repeated until line H. At the end, the concentration of the extract ranged from 1280 to 20 µg/mL. Subsequently, a volume of 5 µL of standardized cultures at 10^6 CFU/mL were inoculated into all wells to obtain a concentration of 10^4 CFU/mL of microorganisms per well. Plates were incubated in a bacteriological oven at 37°C for 24 hours. MIC was defined as the lowest concentration of antimicrobial agent expressed in micrograms/milliliter capable of preventing bacterial growth.

RESULTS AND DISCUSSION

Optimization of extractive conditions

Table II brings the response values obtained through the Box Behnken design for optimizing the extraction of polyphenols and total tannins from *S. cumini* leaves.

Sample 4 resulted in the highest concentration for both TPC (76.32 mg TAE g^{-1}ext) and TTC (68.73 mg TAE g^{-1}ext). This condition presented the highest tested values of extraction time (10 min) and microwave power (300 w) while solid/solvent ratio (1/25 g/mL) and ethanol concentration (50%) were at the medium points. These results are in agreement with previous studies that investigated similar conditions for extracting polyphenols and tannins (Medouni-Adrar et al. 2015, Naima et al. 2015).

The effects of independent factor's coefficients were evaluated, where a high significance (95% confidence level) of all factors was observed for both metabolites responses (Table III). Additionally, ANOVA estimated a non-significant lack of fit, indicating that the proposed model was adequate explaining the variations. The R^2 varied between 0.86 for polyphenols and 0.85 for total tannins, indicating a strong correlation between independent factors and responses. Removing non-significant effects, the equations for estimating TPC (Eq. 1) and TTC (Eq. 2) are as follows:

$$Y_1 = 35.39 + 11.33X_1 + 7.06X_2 - 9.32X_4 + 4.94X_1^2 + 7.01X_3^2 + 5.89X_4^2 \quad (1)$$

$$Y_2 = 29.89 + 9.37X_1 + 4.85X_2 - 8.34X_3^2 + 5.32X_4^2 \; 11.83X_1X_3 + 8.78X_2X_3 - 6.74X_3X_4 \quad (2)$$

The most relevant positive effect was the extraction time, with higher responses being reached at a longer period of extraction. It is quite common that extraction time should improve analyte's concentration, usually with limits where there is exhaustion or loss by degradation (Dahmoune et al. 2015). Microwave power was also shown to be significant for a higher concentration of metabolites, with the highest power being linearly associated tohigher recoveries. The solid/solvent ratio was also revealed as a positive factor, and the best results were obtained when there was more solvent concerning the drug mass. This can be associated to the gradient within the analytes and solvent, which usually helps in the extraction process (Camel 2000, Kullu et al. 2014).

Significant second order effects were observed when there was a combination of higher solid/solvent ratio and longer time, microwave power or ethanol concentration in

Table II. Responses obtained from the Box-Behnken experimental design for optimizing TPC and TTC (mg TAE g^{-1}ext) in the extract from *S. cumini* leaves through microwave-assisted extraction. The bold line represents the best condition.

Sample	Coded factors[a]				Responses	
	X_1	X_2	X_3	X_4	TPC (mg TAE g^{-1}ext)	TTC (mg TAE g^{-1}ext)
1	-1	-1	0	0	28.9523	25.3930
2	1	-1	0	0	57.8202	53.5398
3	-1	1	0	0	51.8741	48.8469
4	**1**	**1**	**0**	**0**	**76.3192**	**68.7275**
5	0	0	-1	-1	31.0562	24.6902
6	0	0	1	-1	56.8358	50.1279
7	0	0	-1	1	31.3865	27.3168
8	0	0	1	1	30.4087	25.7978
9	0	0	0	0	63.3809	58.0808
10	-1	0	0	-1	39.5953	36.1469
11	1	0	0	-1	53.6872	48.6968
12	-1	0	0	1	12.2060	9.57010
13	1	0	0	1	42.8798	38.7276
14	0	-1	-1	0	31.5194	27.8737
15	0	1	-1	0	35.0371	8.39150
16	0	-1	1	0	48.6883	45.2234
17	0	1	1	0	65.3020	60.8490
18	0	0	0	0	55.1706	53.7903
19	-1	0	-1	0	32.5047	30.8097
20	1	0	-1	0	35.2996	18.5142
21	-1	0	1	0	10.2922	10.3011
22	1	0	1	0	45.3859	45.3293
23	0	-1	0	-1	50.7380	50.5696
24	0	1	0	-1	59.8093	59.8121
25	0	-1	0	1	24.4435	24.4353
26	0	1	0	1	38.5407	38.5734
27	0	0	0	0	58.2157	58.2277

[a]**For real factor levels. please refer to Table I; TAE: Tannic Acid Equivalent; TPC: Total Polyphenols Content; TTC: Total Tannins Content; X_1: Extraction Time (min); X_2: Microwave Power (w); X_3: Solid/Solvent Ratio (g/mL); X_4: Ethanol Concentration (%).**

TTC (Y_2) extraction. The ethanol concentration was negative when combined with a higher solid/solvent ratio, indicating that a higher amount of ethanol was unfavorable for both extractions. Ethanol concentration had different behaviors in linear and quadratic effects. Linearly, the increase of ethanol above 50% caused a reduction in the extraction of the metabolites, with the ratio of 50/50 being the one that produced the best recovery results. However, when considering the difference in the coefficient signals, it was observed that values within the range of the tested levels could

Table III. Estimated effects and analysis of variance (ANOVA) of the Box-Behnken design for optimizing TPC and TTC in the liquid extract from *S. cumini* leaves through microwave-assisted extraction. Significant values (p < 0,05) are in bold.

Factors	Coefficients	
	(Y_1) TPC (mg TAE g^{-1}ext)	(Y_2) TTC (mg TAE g^{-1}ext)
Intercept	**35.39154**	**29.89255**
(X_1) Extraction time (min) (L)	**11.33061**	**9.37228**
(X_1) Extraction time (min) (Q)	**4.94169**	**5.30938**
(X_2) Microwave power (w) (L)	**7.06006**	**4.84714**
(X_2) Microwave power (w) (Q)	-0.19753	0.45735
(X_3) Solid/solvent ratio (g/mL) (L)	5.00912	**8.33603**
(X_3) Solid/solvent ratio (g/mL) (Q)	**7.01044**	**9.02052**
(X_4) Ethanol concentration (%) (L)	**-9.32137**	**-8.80187**
(X_4) Ethanol concentration (%) (Q)	**5.89353**	**5.31803**
X_1 (L)^XX_2 (L)	-1.10572	-2.06654
X_1 (L)^XX_3 (L)	8.07468	**11.83094**
X_1 (L)^XX_4 (L)	4.14548	4.15190
X_2 (L)^XX_3 (L)	3.27400	**8.77698**
X_2 (L)^XX_4 (L)	1.25650	1.22392
X_3 (L)^XX_4 (L)	-6.68935	**-6.73917**
ANOVA		
Pure error	34.454	12.707
Lack of fit	892.253	1127.539
R^2	0.86327	0.85082

(L): linear; (Q): quadratic; TAE: Tannic Acid Equivalent; TPC: Total Polyphenols Content; TTC: Total Tannins Content.

produce better results. This was explored in the next session.

Optimization through a desirability function

After fitting an adequate model for explaining the variations of both TPC and TTC through a quadratic model, the choice for the best extractive conditions occurred through the application of a global desirability (*D*) proposed by Derringer & Suich (1980) that varies between 0 and 1, and can be expressed by (Eq. 3):

$$D = \left(d_1\left(Y_1\right) \times d_2\left(Y_2\right)\right)^n \quad (3)$$

Where d_i is the individual desirability of each response Y_i and *n* is the number of responses (in this case, 2). If a response is considered completely undesirable, then the *d* value would be 0, as for 1 being the most desirable situation. To estimate *d* values, one must decide

whether each response Y_i is to be maximized of minimized. In our case, both TPC and TTC should be maximized, thus *d* values can be calculated by (Eq. 4):

$$d_i(Y_i) = \left(\frac{Y_i - L_i}{H_i - L_i} \right)^s \quad (4)$$

Where L_i is the lower response, H_i is the higher response and *s* the curvature at which the target d_i is constructed (i.e., how important the higher response is considered).

Eq. 1 and 2 were used to estimate responses between the lower and upper levels (-1 to +1) of independent factors at a step size of 0.2, then for each response Y, a desirability value was assigned and contours for response desirability were analyzed (Figure 1). The optimum desirability was shown to be with X_1 = 0.6 (8 min), X_2 = 1 (300 w), X_3 = 0.6 (1/34 g/mL) and X_4 = −0.4 (38%) at which *D* was equal to 0.98. These coded values were then transformed into real extraction conditions and were further tested to obtain better results, which in fact led to a higher extraction yield when compared to the extraction conditions previously tested (Table IV). This was the final experimental condition adopted for the extraction method which produced the extract used for antifungal and antibacterial sensitivity tests.

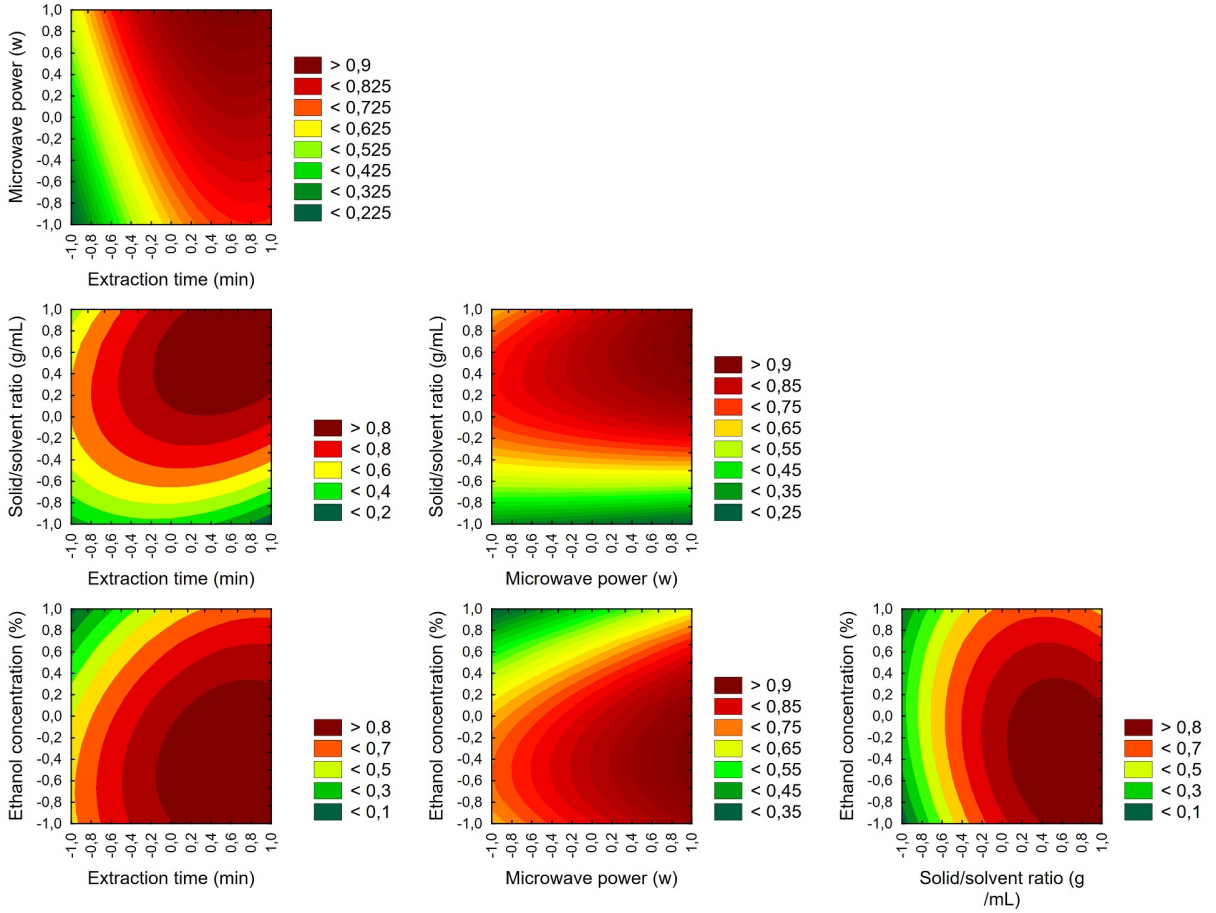

Figure 1. Contours of response surfaces obtained for polyphenol and total tannin's extraction optimization from *S. cumini* leaves through microwave-assisted extraction. Contour colors are associated to the global desirability between 0 and 1.

In vitro antifungal sensitivity test of the dried extract *S. cumini*

The results of the analyses indicated sensitivity in all tested strains (Table V). Results for minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were the same, since the same dosage that inhibited fungal growth also killed them. In most reports, only *Candida albicans* is usually employed, although other species of the same genus are also among the main pathogenic species such as *Candida parapsilosis*, *Candida glabrata* and *Candida haemulonii*, from which are emerging multidrug resistant pathogens (Ben-Ami et al. 2017).

Researchers suggest that a MIC up to 500 µg/mL is considered as potent inhibitors; MICs between 600–1500 µg/mL are moderate inhibitors and MIC above 1600 µg/mL are weak inhibitors (Aligiannis et al. 2001). Therefore, the standardized dried extract of *S. cumini* was considered a potent growth inhibitor in all tested species, including *Candida haemulonii*.

In addition, researchers also tested the antifungal activity of the hydroalcoholic extract from the leaves of *S. cumini* against the same species through the microdilution technique in solid agar medium (disk and wells), MICs ranged from 70 to 200 µg/mL (Oliveira et al. 2007). These MICs were less potent than those presented in this study, evidencing the efficiency of the optimization of the extraction by the microwave-assisted method.

Table IV. Values estimated by the desirability function compared to the experimental results (n=3) at the optimized condition where X_1 = 0.6 (8 min), X_2 = 1 (300 w), X_3 = 0.6 (1/34 g/mL) and X_4 = –0.4 (38%). Confidence level was established as 95%.

		TPC (mg TAE g^{-1}ext)	TTC (mg TAE g^{-1}ext)
Desirability function	Predicted	73.73	71.18
	-95% CI	60.78	63.31
	+95% CI	86.69	79.04
Observed (n=3)	1	85.28	77.84
	2	87.99	80.21
	3	88.83	81.00
Mean		87.37	79.68
SD		1.85	1.64
RSD (%)		2.12	2.06

Work data; TAE: Tannic Acid Equivalent; TPC: Total Polyphenols Content; TTC: Total Tannins Content; X_1: Extraction Time (min); X_2: Microwave Power (w); X_3: Solid/Solvent Ratio (g/mL); X_4: Ethanol Concentration (%).

In vitro antibacterial sensitivity test of the dried extract of *S. cumini*

The results of the MICs of the *S. cumini* extract are presented in table VI. All microorganisms were sensitive to the standardized extract of the leaves of *S. cumini*, of which MICs and MBCs ranged from 80 to 1280 µg/mL and 160 to 1280 µg/mL, respectively. *Enterococcus faecalis* LPBM 918 and the reference strain *E. faecalis* ATCC 51299 were shown to be more sensitive to the extract than to vancomycin, the antimicrobial used as standard. Similar results were observed for *Staphylococcus aureus* isolates LPBM OXA 1 and LPBM 16, of which MIC values (160 µg/mL) of the *S. cumini* extract were lower than that obtained for oxacillin.

The results of the antimicrobial activity of the *S. cumini* extract for *S. aureus* presented in this work agrees with Albuquerque et al. (2017), where they evaluated the antibacterial activity of the aqueous extract of leaves of *S. cumini* (1:10 w/v) standardized at 100 mg/mL on *S. aureus* and *E. faecalis* isolates, using the diffusion method in Mueller-Hinton Agar, which presented inhibition halos for *S. aureus* of 10 mm. However, there was no inhibition for *E. faecalis*, presenting 0 mm of halo.

Table V. Antifungal activity of the dried extract of *S. cumini* measured by the minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC).

Isolated species	MIC/MFC (µg/mL)	MIC (µg/mL)		
	Extract	Anidula	Caspo	Mica
C. glabrata	1.25/1.25	I. 0.25	S: 0.031	S: 0.031
C. haemulonii	1.25/1.25	S: 0.016	S: 0.016	S: 0.016
C. albicans	1.25/1.25	S: 0.031	S: 0.031	S: 0.031
C. parapsilosis ATCC 22019	1.25/1.25	S: 1.0	S: 1.0	S: 0.5

ATCC – American Type Culture Collection; Extract– *S. cumini* extract; Anidula – Anidulafungine; Caspo – Caspofungine; Mica – Micafungine; I: intermediary; S: sensitive; R: resistant; Interpretation criteria: CLSI 2012.

Table VI. Antibacterial activity of the dried extract of *S. cumini* measured by the minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

Isolated species	MIC/MBC (µg/mL)		
	Extract	Oxacillin*	Vancomycin*
Enterococcus faecalis ATCC51299	640/≥1280	-	1024/≥1024
Enterococcus faecalis ATCC 27212	160/320	-	4/8
Enterococcus faecalis LPBM 918	80/160	-	128/256
Enterococcus faecalis LPBM 189	160/1280	-	128/256
Enterococcus faecalis LPBM 335	160/1280	-	512/1024
Enterococcus faecalis LPBM 421	160/1280	-	128/256
Enterococcus faecalis LPBM 240	160/1280	-	128/256
Staphylococcus aureus LPBM OXA 1	160/640	256/≥256	-
Staphylococcus aureus LPBM 16	160/640	256/≥256	-
Staphylococcus aureus LPBM 17	320/1280	0.25/1.0	-
Staphylococcus aureus LPBM 31	320/1280	16/16	-
Staphylococcus aureus LPBM 18	160/640	128/256	-
Staphylococcus aureus ATCC 25923	160/640	0.125/0.25	-
Staphylococcus aureus ATCC 335921	160/640	64/128	-

ATCC – American Type Culture Collection; LPBM – Laboratory of Physiology and Biochemistry of Microorganisms; Extract – *S. cumini* extract; Interpretation criterion – MIC:Oxacillin (≥ 4.0, resistant) for *S. aureus.* Vancomycin (≥ 32.0, resistant) for *E. faecalis*. Source: CLSI 2015; *previously determined values.

Previous studies confirmed the antibacterial activity of the 10% hydroalcoholic extract of the leaves of *S. cumini* against cocci, Gram-positive and Gram-negative bacilli, including *S. aureus* and observed that the sensitivity of these microorganisms was similar regardless of whether they were Gram positive or Gram negative (Loguercio et al. 2005).

The antimicrobial activity of the leaves of *S. cumini* presented in this study is probably due to the presence of tannins and flavonoids. Moreover, flavonoids have the ability to complex with proteins and bacterial cells forming irreversible complexes, mainly with nucleophilic amino acids. This complex usually leads to protein inactivation and loss of function (Cowan 1999). Tannins present antimicrobial activity due to their binding to proteins and adhesins, inhibiting various enzymes, their complexation with the cell wall and metal ions, destabilizing it, as well as they can lyse the cytoplasmic membrane.

CONCLUSION

The optimization of the extraction method of polyphenols and total tannins assisted by microwave showed that the parameter with the greatest influence was primarily the time, followed by the potency and proportion solid/solvent, furthermore, it also showed that the concentration of ethanol in high proportion behaves in a negative way to extract the metabolites of interest.

All isolates tested for fungi and bacteria were sensitive to the dried extract at concentrations much lower than those previously published with the same species. Evidencing the potency antifungal and antibacterial activity of polyphenols and total tannins presents on the leaves of *S. cumini* and the importance of the optimization of the extractive method as well as the choice of the design for the development of the study.

The antimicrobial potential of the standardized dried extract of *S. cumini* leaves and the effectiveness in the optimization of the microwave-assisted extraction method presented in this study are an interesting alternative for the therapy of infectious diseases caused by resistant multi-drug microorganisms. However, in-depth studies on in vivo toxicity need to be performed.

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REFERENCES

AHMAD I & AQIL F. 2007. *In vitro* efficacy of bioactive extracts of 15 medicinal plants against ESβL-producing multidrug-resistant enteric bacteria. Microbiol Res 162(3): 264-275.

ALBUQUERQUE FHC, SOARES KDS & OLIVEIRA MAS. 2017. Atividade antimicrobiana *in vitro* dos extratos aquosos, hidroalcoólicos e alcoólicos das folhas de espécies da família Myrtaceae frente à cepas de bactérias de interesse. Rev Ciênc Méd Biol 16(2): 139-145.

ALIGIANNIS N, KALPOUTZAKIS E, MITAKU S & CHINOU IB. 2001. Composition and antimicrobial activity of the essential oils of two Origanum species. J Agric Food Chem 49(9): 4168-4170.

AMORIM ELC, NASCIMENTO JE, MONTEIRO JM, PEIXOTO-SOBRINHO TJS, ARAÚJO TAS & ALBUQUERQUE UP. 2008. A Simple and Accurate Procedure for the Determination of Tannin and Flavonoid Levels and Some Applications in Ethnobotany and Ethnopharmacology. Funct Ecosyst Communities 2(1): 88-94.

ANGOY A, VALAT M, GINISTY P, SOMMIER A, GOUPY P, CARIS-VEYRAT C & CHEMAT F. 2018. Development of microwave-assisted dynamic extraction by combination with centrifugal

force for polyphenols extraction from lettuce. LWT 98: 283-290.

BAG A, BHATTACHARYYA SK, PAL NK & CHATTOPADHYAY RR. 2012. *In vitro* antibacterial potential of *Eugenia jambolana* seed extracts against multidrug-resistant human bacterial pathogens. Microbiol Res 167(6): 352-357.

BEN-AMI R ET AL. 2017. Multidrug-Resistant *Candida haemulonii* and *C. auris*, Tel Aviv, Israel. Emerg Infect Dis 23(1): 195-203.

CAMEL V. 2000. Microwave-assisted solvent extraction of environmental samples. TrAC Trends Anal Chem 19(4): 229-248.

CLSI. 2008. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard—Third Edition. CLSI document M27-A3. Wayne, PA: doi:10.1007/SpringerReference_5244.

CLSI. 2017. Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI supplement M100. Wayne, PA.

COWAN MM. 1999. Plant products as antimicrobial agents. Clin Microbiol Rev 12(4): 564-582.

DAHMOUNE F, NAYAK B, MOUSSI K, REMINI H & MADANI K. 2015. Optimization of microwave-assisted extraction of polyphenols from *Myrtus communis* L. leaves. Food Chem 166: 585-595.

DAS AK, MANDAL V & MANDAL SC. 2014. A Brief Understanding of Process Optimisation in Microwave-assisted Extraction of Botanical Materials: Options and Opportunities with Chemometric Tools. Phytochem Anal 25(1): 1-12.

DE HOYOS-MARTÍNEZ PL, MERLE J, LABIDI J & CHARRIER - EL BOUHTOURY F. 2019. Tannins extraction: A key point for their valorization and cleaner production. J Clean Prod 206: 1138-1155.

DERRINGER G & SUICH R. 1980. Simultaneous Optimization of Several Response Variables. J Qual Technol 12(4): 214-219.

DE SOUZA A, DE OLIVEIRA C, DE OLIVEIRA V, BETIM F, MIGUEL O & MIGUEL M. 2018. Traditional Uses, Phytochemistry, and Antimicrobial Activities of Eugenia Species - A Review. Planta Med 84(17): 1232-1248.

KHAN S, IMRAN M, IMRAN M & PINDARI N. 2017. Antimicrobial activity of various ethanolic plant extracts against pathogenic multi drug resistant *Candida* spp. Bioinformation 13(3): 67-72.

KULLU J, DUTTA A, CONSTALES D, CHAUDHURI S & DUTTA D. 2014. Experimental and modeling studies on microwave-assisted extraction of mangiferin from *Curcuma amada*. 3 Biotech 4(2): 107-120.

LOGUERCIO AP, BATTISTIN A, VARGAS AC DE, HENZEL A & WITT NM. 2005. Atividade antibacteriana deextrato hidro-alcoólico de folhas de jambolão (*Syzygium cumini* (L.) Skells). Ciênc Rural 35(2): 371-376.

MEDOUNI-ADRAR S, BOULEKBACHE-MAKHLOUF L, CADOT Y, MEDOUNI-HAROUNE L, DAHMOUNE F, MAKHOUKHE A & MADANI K. 2015. Optimization of the recovery of phenolic compounds from Algerian grape by-products. Ind Crops Prod 77: 123-132.

MIGLIATO KF, MELLO JCP, HIGA OZ, RODAS ACD, CORRÊA MA, MENDES-GIANNINI MJS, FUSCO-ALMEIDA AM, PIZZOLITTO AC & SALGADO HRN. 2010. Antimicrobial and Cytotoxic Activity of Fruit Extract from *Syzygium cumini* (L.) Skeels. Lat Am J Pharm 29(5): 725-730.

NAIMA R, HANNACHE H, OUMAM MM, SESBOU A, CHARRIER B, PIZZI AP & CHARRIER-EL BOUHTOURY F. 2019. Green extraction process of tannins obtained from Moroccan *Acacia mollissima* barks by microwave: Modeling and optimization of the process using the response surface methodology RSM. Arab J Chem 12(8): 2668-2684.

NAIMA R, OUMAM MM, HANNACHE H, SESBOU A, CHARRIER B, PIZZI AP & CHARRIER-EL BOUHTOURY F. 2015. Comparison of the impact of different extraction methods on polyphenols yields and tannins extracted from Moroccan *Acacia mollissima* barks. Ind Crops Prod 70: 245-252.

OLIVEIRA GF DE, FURTADO NAJC, SILVA FILHO AA DA, MARTINS CHG, BASTOS JK, CUNHA WR & SILVA MLA. 2007. Antimicrobial activity of *Syzygium cumini* (Myrtaceae) leaves extract. Braz J Microbiol 38(2): 381-384.

PADIYARA P, INOUE H & SPRENGER M. 2018. Global Governance Mechanisms to Address Antimicrobial Resistance. Infect Dis Res Treat 11: 1-4.

PANJA P. 2018. Green extraction methods of food polyphenols from vegetable materials. Curr Opin Food Sci 23: 173-182.

SHAFI P, ROSAMMA M, JAMIL K & REDDY P. 2002. Antibacterial activity of *Syzygium cumini* and *Syzygium travancoricum* leaf essential oils. Fitoterapia 73(5): 414-416.

SINGH JP, KAUR A, SINGH N, NIM L, SHEVKANI K, KAUR H & ARORA DS. 2016. *In vitro* antioxidant and antimicrobial properties of jambolan (*Syzygium cumini*) fruit polyphenols. LWT - Food Sci Technol 65: 1025-1030.

SRIVASTAVA S & CHANDRA D. 2013. Pharmacological potentials of *Syzygium cumini*: A review. J Sci Food Agric 93(9): 2084-2093.

UGBABE G, EZEUNALA M, EDMOND I, APEV J & SALAWU O. 2010. Preliminary Phytochemical, Antimicrobial and Acute Toxicity Studies of the Stem, bark and the Leaves of a cultivated *Syzygium cumini* Linn. (Family: Myrtaceae) in Nigeria. African J Biotechnol 9(41): 6943-6947.

VÁZQUEZ-ESPINOSA M, ESPADA-BELLIDO E, PEREDO AVG, FERREIRO-GONZÁLEZ M, CARRERA C, PALMA M, BARROSO CG & BARBERO GF. 2018. Optimization of Microwave-Assisted

Extraction for the Recovery of Bioactive Compounds from the Chilean Superfruit (*Aristotelia chilensis* (Mol.) Stuntz). Agronomy 8: 240.

WHO. 2014. Antimicrobial resistance: global report on surveillance.

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