







## Extraction of antioxidant and antimicrobial phytochemicals from corn stigma: a promising alternative to valorization of agricultural residues

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**ABSTRACT:** *The discovery of new natural additives from agro-industrial waste is considered an important research topic. This study investigated the feasibility of ultrasound-assisted extraction (UAE) of antioxidant compounds from corn stigma (CS) and the effect of independent variables (time and solid-solvent ratio) and their interaction in the extraction of CS. Results indicated that the UAE method increases the antioxidant activity and reduces the extraction time by 67%. Optimized conditions for the simultaneous extraction of antioxidants and polyphenols from CS were obtained using 5 min and a solid-solvent ratio of 0.05 g mL<sup>-1</sup>. The CS extract obtained by UAE was characterized by ESI-ToF-MS and 27 phytochemicals were reported. The extract showed promising antifungal and antibacterial activities against 23 of the studied microorganisms. Therefore, the CS extract obtained by the UAE can be used as a source of bioactive and antimicrobial compounds for use as a functional ingredient in the food and pharmaceutical industry.*

**Key words:** corn stigma, ultrasound-assisted extraction, antifungal activity, antibacterial activity.

## Extração de fitoquímicos antioxidantes e antimicrobianos do estigma do milho: uma alternativa promissora para valorização de resíduos agrícolas

**RESUMO:** *A descoberta de novos aditivos naturais a partir de resíduos agroindustriais é considerada um importante tópico de pesquisa. Este estudo teve como objetivo investigar a viabilidade da extração assistida por ultrassom (EAU) de compostos antioxidantes do estigma do milho (EM) e o efeito de variáveis independentes (tempo e relação sólido-solvente) e sua interação na extração de EM. Os resultados indicaram que a EUA aumenta a atividade antioxidante e reduz o tempo de extração em 67%. Condições otimizadas para a extração simultânea de antioxidantes e polifenóis do EM foram obtidas com 5 min e uma relação sólido-solvente de 0,05 g mL<sup>-1</sup>. O extrato de EM obtido pela EUA foi caracterizado por ESI-ToF-MS e 27 fitoquímicos foram encontrados. O extrato apresentou atividades antifúngicas e antibacterianas promissoras contra 23 dos micro-organismos estudados. Portanto, o extrato de EM obtido pela extração assistida por ultrassom pode ser utilizado como fonte de compostos bioativos e antimicrobianos para uso como ingrediente funcional na indústria alimentícia e farmacêutica.*

**Palavras-chave:** estigma do milho, extração assistida por ultrassom, atividade antifúngica, atividade antibacteriana.

## INTRODUCTION

Food wastes are produced in several steps of the food life cycle, such as agricultural production, industrial processing, and market distribution. In the agricultural production, it is estimated that at least 40% of the initial feedstock mass is discarded as waste. Several studies proposed the valorization of food wastes, the extraction of value-added compounds from agricultural residues have been reported as a potential alternative to obtain nutraceuticals and functional ingredients (VODNAR et al., 2017).

Corn stigma (CS) has been used as a therapeutic medicine in many countries, such as

Brazil, China, Turkey, United States, and France for the diseases treatment. The CS is a potential renewable source of phenolic compounds, and flavonoids, it is also composed by proteins, vitamins, carbohydrates, macronutrients, volatile oils, steroids, alkaloids and saponins. Pharmacological studies suggested that CS extracts and its bioactive components offer many benefits that can be used to ensure human health (CHAIITTIANAN et al., 2017). As it is an agro-industrial by-product available in abundance and without commercial cost, CS becomes a potential food additive, but for this detailed information about its phytochemical composition is needed to obtain an adequate functional ingredient.

Many extraction procedures also called “conventional extraction” have been proposed for the extraction of bioactive compounds from agricultural residues. These methods generally require the use of high amounts of organic solvents, high energy consumption, and long extraction times. Ultrasound-assisted extraction (UAE) has been reported as a potential alternative to increase the extraction yields of bioactive compounds from fruits and vegetable residues. The use of UAE promotes the exhaustive extraction of active ingredients from plants with relatively short time and high extraction efficiency when compared to conventional procedures (CHEMAT et al., 2017).

In this sense, the present research was proposed to investigate the effect of ultrasound as a feasible way for the extraction of bioactives compounds from CS. To ensure the applicability of CS extract in food processing, the antimicrobial action against 14 bacteria and 9 fungi was investigated. Additionally, the UAE extract was characterized by electrospray ionization with high resolution time-of-flight mass spectrometry (ESI-ToF-MS) and the compounds identified were directly related to the antioxidant activity demonstrated in vitro.

## MATERIALS AND METHODS

The plant material used in this experiment was purchased from rural producers in Santa Maria (Brazil). The CS was dried in an oven at 45 °C ( $\pm$  5) until constant weight and after crushed in a Willey knife mill (MA - 680; Marconi Ltda., Piracicaba, Brazil) using a 20 mesh sieve. The material was kept at a temperature of -18 °C in an airtight container and protected from light until the analyzes were performed.

A factorial design 2<sup>2</sup> was proposed to investigate the effect of extraction time and the solid-solvent relationship on the dependent variables (ORAC and TPC). The CS was added to a solution of 70% ethanol at 60 °C, as proposed by JABBAR et al. (2014). The extractions were performed in different periods of time ( $X_1$ ), with different solid-solvent ratios ( $X_2$ ) (Table 1). The UAE experiments were performed using an ultrasonic probe (VC-750; Sonics & Materials, Inc., Newtown, CT, USA) operating at 20 kHz with 70% of US amplitude and 750 W of nominal power (SAEEDUDDIN et al., 2015). The shaking extraction (SE) procedures were carried out in an ultra-thermostatic bath (Marconi, model MA-184, São Paulo, Brazil) with constant agitation at 100 g the agitator (Marconi MA-039, São Paulo, Brazil).

The CS extracts were then centrifuged at 202 g for 15 min (Centrifuga Coleman Model 90-1, São Paulo, Brazil) and the supernatants were collected as the extracts. Three repetitions were performed in each experiment. The extracts were stored at -18 °C for further analysis.

The determination of total phenolic compounds (TPC) was performed by the Folin-Ciocalteu method described by ROESLER et al. (2007). The content of total phenolic compounds was expressed in milligrams of gallic acid per gram of dry sample (mg GAE g<sup>-1</sup>).

The oxygen radical absorbance capacity (ORAC) was analysed as proposed by DÁVALOS et al (2004). Results were expressed in  $\mu$ mol Trolox equivalents per gram of dry sample ( $\mu$ mol Trolox/g).

The extract obtained by UAE was submitted for the screening analysis by high-resolution electrospray ionization time-of-flight mass spectrometry (ESI-ToF-MS, model Xevo G2 Qtof, Waters Inc., Milford, USA), as proposed by BOEIRA et al. (2020).

For UAE extract, the antibacterial and antifungal activities were assayed using the broth microdilution method (NCCLS, 2017, 2018). A collection of twenty-three microorganisms was used, including five Gram-positive bacteria: *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11778), *Enterococcus faecalis* (ATCC 19433), *Enterococcus ssp.* (ATCC 6589), *Bacillus subtilis* (ATCC 19659), ten Gram-negative bacteria: *Salmonella enteric serovar Typhimurium*, (ATCC 14028), *Escherichia coli* (ATCC 25922), *Shigella sonnei* (ATCC 25931), *Enterobacter aerogenes* (ATCC 13048), *Salmonella enteritidis* (ATCC 13076), *Shigella flexneri* (ATCC 12022), *Pseudomonas aeruginosa* (ATCC 27853), *Morganella morganii* (ATCC 25829), *Proteus mirabilis* (ATCC 25933), *Klebsiella pneumoniae* (clinical isolate) and nine yeasts: *Candida parapsilosis* (ATCC 22019), *Candida tropicalis* (ATCC 750), *Candida albicans* (ATCC 10231), *Candida glabrata* (ATCC 2001), *Candida dubliniensis* (ATCC MYA-577), *Candida krusei* (ATCC 6258), *Cryptococcus gatti* (ATCC 56990), *Cryptococcus neoformans* (ATCC 28952), *Saccharomyces cerevisiae* (ATCC 2601). Standard strains of the microorganisms were obtained from American Type Culture Collection (ATCC), and standard antibiotics chloramphenicol, ampicillin, fluconazole and nystatin were used in order to control the sensitivity of the microbial test. The minimal inhibitory concentration (MIC) was determined on 96-well culture plates by a micro dilution method using a microorganism suspension

Table 1 - Experimental runs using coded levels of time (min.  $X_1$ ), and with different solid-solvent ratios ( $\text{g mL}^{-1}$   $X_2$ ) according to the factorial design  $2^2$ .

-----Coded variables levels-----		
Runs	$X_1$ (min)	$X_2$ ( $\text{g mL}^{-1}$ )
1	5 (-1)	0.05 (-1)
2	15 (1)	0.05 (-1)
3	5 (-1)	0.1 (1)
4	15 (1)	0.1 (1)
5	10 (0)	0.075 (0)
6	10 (0)	0.075 (0)
7	10 (0)	0.075 (0)

at a density of  $10^5$  CFU  $\text{mL}^{-1}$  with Casein Soy Broth incubated for 24 h at  $37^\circ\text{C}$  for bacteria and Sabouraud Broth incubated for 48 h at  $25^\circ\text{C}$  for yeasts (NCCLS, 2017, 2018). The cultures that did not present growth were used to inoculate plates again (Casein Soy Broth and Sabouraud), in order to determine the minimal lethal concentration (MLC). Proper blanks were simultaneously assayed, and all samples were tested in triplicate.

The experiment was carried out in triplicate. The experimental data were submitted to analysis of variance (ANOVA) and the means were compared using the Tukey test through the statistical software STATISTICA® 10.0 (StatSoft, Inc., Tulsa, OK 74104, EUA), with a 95% significance level.

## RESULTS AND DISCUSSION

The TPC and ORAC results for all conditions evaluated using ultrasound or shaking are shown in table 2. For shaking extraction (SE), the  $R^2$  coefficients reached 0.841 for TPC and 0.854 for ORAC. Calculated  $R^2$  values of the models for TPC and ORAC obtained by the UAE were 0.993 and 0.942, respectively.

For SE, it is observed that the highest yield of TPC ( $57.20$  mg GAE  $\text{g}^{-1}$ ) and ORAC ( $80.82$   $\mu\text{mol}$  Trolox  $\text{g}^{-1}$ ) was obtained in the condition 15 min/ $0.05$   $\text{g mL}^{-1}$ . Some authors studied the extraction of bioactive compounds from CS, the use of long periods of extraction, exceeding 24 h of process combined with high temperatures, increase the possibility of loss of thermolabile compounds, resulting in the degradation of the phenolic compounds of interest (WONG-PAZ et al., 2017).

For UAE, using 5 min of extraction time and a solid-solvent ratio of  $0.05$   $\text{g mL}^{-1}$ , the highest yields of TPC ( $65.31$  mg GAE  $\text{g}^{-1}$ ) and ORAC ( $124.44$   $\mu\text{mol}$  Trolox  $\text{g}^{-1}$ ) were obtained. The ultrasound application increased the extraction rates with 67% less time when compared to SE. SILVA et al. (2020) when evaluating the use of ultrasound to extract bioactive compounds from acerola residue, emphasized that the UAE allows extraction times up to 100 times shorter than necessary when using conventional methods. This is because the use of UAE increases mass transfer, reduces extraction time, and increases the solubilization of target compounds. This phenomenon can be explained by the action of cavitation bubbles in the liquid medium during ultrasound propagation. These effects provide a vibratory effect on plant cells, capable of breaking the cell wall and successfully applying to the processes of extracting components from natural products (CHEMAT et al., 2017).

The influence of extraction time and the solid-solvent ratio for TPC and ORAC, can be reported in table 2. Results demonstrated that both conventional extraction and ultrasound showed significant effects ( $P < 0.05$ ), indicating that the model is descriptive for the extraction.

For SE, the interaction between the two variables (time and solid-solvent ratio) was significant ( $P < 0.05$ ) and negative. The isolated effect of time on TPC and ORAC was also significant ( $P < 0.05$ ) and positive, while the solid-solvent ratio had significant effect ( $P < 0.05$ ), but negative. It was observed that for TPC and ORAC showed similar behavior (significant,  $P < 0.05$ ).

Figure 1 shows the analysis of residues and represents a comparative analysis between the values

Table 2 - Values of TPC and ORAC after SE and UAE and the effect of the studied variables.

-----Analytical results-----						
-----Shaking extraction (SE)-----			-----Ultrasound-assisted extraction (UAE)-----			
Runs	TPC (mg GAE g <sup>-1</sup> )	ORAC (μmol Trolox g <sup>-1</sup> )	TPC (mg GAE g <sup>-1</sup> )	ORAC (μmol Trolox g <sup>-1</sup> )		
1	47.14±1.58	68.18±0.83	65.31±2.25	124.44±1.43		
2	57.20±1.58	80.82±1.29	50.03±1.09	72.49±0.89		
3	32.07±2.12	38.06±0.92	45.82±1.44	65.84±1.12		
4	39.79±0.92	43.71 ±0.33	37.73±1.45	33.64 ±1.37		
5	50.24±0.58	46.27±0.84	48.57±0.44	62.73±0.78		
6	50.17±0.44	46.81±1.12	48.05±2.54	61.76±0.92		
7	50.20±0.94	46.99±0.94	49.46±0.29	61.16±0.64		
-----Effects-----						
-----Shaking extraction (SE)-----			-----Ultrasound-assisted extraction (UAE)-----			
-----TPC-----			-----TPC-----			
	Effect	Standard Error	P	Effect	Standard Error	P
Mean/Interaction	46.689	0.014	< 0.001*	49.287	0.269	< 0.001*
(X <sub>1</sub> ) Time	8.889	0.037	< 0.001*	-11.680	0.711	0.003*
(X <sub>2</sub> ) Solid-solvent ratios	-16.239	0.037	< 0.001*	-15.891	0.711	0.002*
X <sub>1</sub> by X <sub>2</sub>	-1.1670	0.037	0.001*	3.597	0.711	0.036*
-----R <sup>2</sup> = 0.841-----			-----R <sup>2</sup> = 0.993-----			
-----Adj-R <sup>2</sup> = 0.682-----			-----Adj-R <sup>2</sup> = 0.986-----			
-----ORAC-----			-----ORAC-----			
	Effect	Standard Error	P	Effect	Standard Error	P
Mean/Interaction	52.980	0.142	< 0.001*	68.865	0.299	< 0.001*
(X <sub>1</sub> ) Time	9.149	0.376	0.001*	-42.075	0.792	< 0.001*
(X <sub>2</sub> ) Solid-solvent ratios	-33.613	0.376	< 0.001*	-48.725	0.792	< 0.001*
X <sub>1</sub> by X <sub>2</sub>	-3.494	0.376	0.011*	9.875	0.792	0.006*
-----R <sup>2</sup> = 0.854-----			-----R <sup>2</sup> = 0.942-----			
-----Adj-R <sup>2</sup> = 0.709-----			-----Adj-R <sup>2</sup> = 0.885-----			

Results are expressed as Mean±SD (n=3); TPC=Total phenolic content ORAC=Oxygen radical absorption capacity (\*)There was significant effect considering 95% significance.

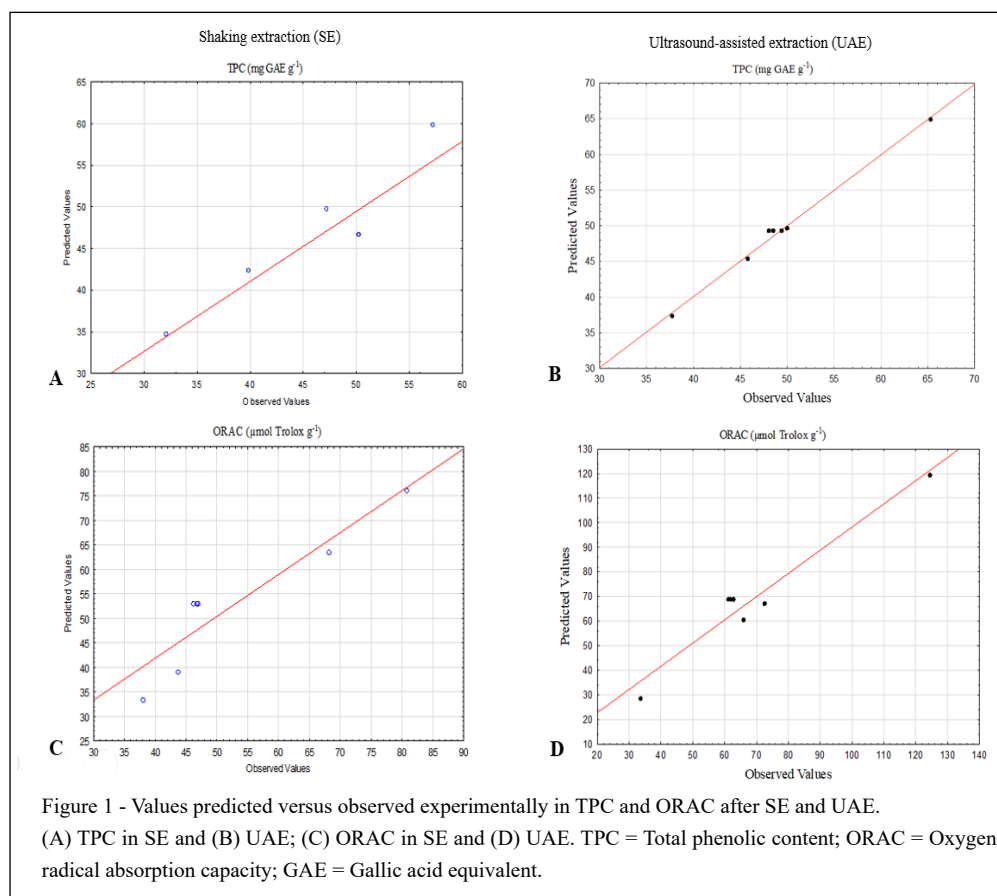
of TPC and ORAC predicted by the model and those observed experimentally. The results showed that the responses are aligned, indicating the adequacy of the predicted data to the experimental values. Thus, the proposed model proved to be adequate to predict the extraction of bioactive compounds from CS.

The optimized conditions for the simultaneous extraction of antioxidants and polyphenols from CS was 15 minutes for SE and 5 minutes for UAE. The solid-solvent ratio was

selected as 0.05 g mL<sup>-1</sup> for both extraction methods. Considering the significant reduction of required extraction time by UAE, the optimized extract was evaluated for antimicrobial capacity, as well as for the chemical characterization of the phytochemicals present by ESI-ToF-MS.

The extract obtained by UAE, was submitted for the screening analysis by ESI-ToF-MS. The chemical structures were tentatively determined based on reference mass spectral data, and





fragmentation pathways. An overview of all compounds identified in the extract is shown in table 3. Based on a multiple data processing approach, 50 ratio  $m/z$  in the CS extract were detected. Among them, 27 compounds belonging to different classes were identified, including 1 alkaloid, 1 carbohydrate, 1 amino acid, 3 phenolics, 3 flavonoids, 3 terpenes, 6 fatty acids, 6 organic acids, and 3 other compounds. As far as we know, most of these compounds (21 of them) were reported here in the CS extract for the first time. Only valine, disaccharide, tetracosanoic acid, 9-oxo-10E, 12Z octadecadienoic acid, linoleic acid, and palmitic acid had already been identified in CS.

The largest class of compounds reported in this study was fatty acids such as (linoleic acid, palmitic acid, DL-Cerebronic acid, and tetracosanoic acid). These compounds are produced by plants, which are responsible for the production of most biological lipids in the world. Organic acids, the second largest class observed in

CS extract, are naturally present in vegetables, in minimal amounts or accumulating in certain species (MARTIN et al., 2006). In the present study, citric acid, trans-acotinic acid, D-xylonate, malic acid, 1-propanedioic acid-4-caffeoylquinic acid, and 2,4,5,6-tetrahydroxyhexanoic acid were found.

The antioxidant and bioactive activity shown by CS, in in vitro tests, may be related to the presence of phytochemicals in the class of phenolics, flavonoids, terpenes, and alkaloids. Previous studies have reported that the pharmacological effect of CS, such as antioxidants, anti-inflammatories, and diuretic activity, is attributed to the presence of these compounds (LIU et al., 2011). In the present study, 10 phytochemical compounds were reported.

The terpenoid compound Nardoaristolone A is described by MATOS et al. (2015), as an important precursor of secondary metabolites of flavonoids and isoflavonoids in plants. Its inhibitory effects on acute inflammation and chronic pain stand

Table 3 - Identification of the main chemical compounds of the extract of CS by ESI-ToF-MS.

No.	Experimental mass (m/z)	Theoretical mass (m/z)	Error (ppm)	Possible molecular structure	Compounds	Classification
-----Positive mode [M+H] <sup>+</sup> -----						
1	629.1295	629.1280	2.4	C <sub>33</sub> H <sub>25</sub> O <sub>13</sub>	Acremoxanthone D	Phenolic
2	589.4257	589.4248	1.5	C <sub>39</sub> H <sub>56</sub> O <sub>4</sub>	Lupeol caffeate	Terpene
3	543.1350	543.1344	1.1	C <sub>23</sub> H <sub>27</sub> O <sub>15</sub>	5,6-O-β-D-Diglucoopyranosylangelicin	Flavonoid
4	531.2383	531.2401	3.4	C <sub>32</sub> H <sub>35</sub> O <sub>7</sub>	Nardoaristolone A	Terpene
5	465.2125	465.2111	3.0	C <sub>24</sub> H <sub>33</sub> O <sub>9</sub>	Caesalpinin MH	Alkaloid
6	381.0822	381.0801	5.5	C <sub>17</sub> H <sub>17</sub> O <sub>10</sub>	Unknown phenolic glycoside	Phenolic
7	333.1855	333.1832	6.9	C <sub>23</sub> H <sub>25</sub> O <sub>2</sub>	((3-(Benzyloxy)propoxy)methylene) dibenzene	Other compound
8	293.0661	293.0656	1.7	C <sub>14</sub> H <sub>13</sub> O <sub>7</sub>	Planchol E	Phenolic
9	156.0421				Valine	Amino acid
-----Negative mode [M-H] <sup>-</sup> -----						
10	607.1630	607.1604	4.3	C <sub>35</sub> H <sub>27</sub> O <sub>10</sub>	Australisine A	Terpene
11	575.1401	575.1394	1.2	C <sub>27</sub> H <sub>27</sub> O <sub>14</sub>	Luteolin-8-C-6''deoxy-3''-hexoside-2''-O-ramnoside	Flavonoid
12	563.1401	563.1381	3.4	C <sub>26</sub> H <sub>28</sub> O <sub>14</sub>	Schaftoside	Flavonoid
13	439.0877	439.0890	3.0	C <sub>19</sub> H <sub>20</sub> O <sub>12</sub>	1-propanedioic acid-4-caffeoylquinic acid	Organic acid
14	411.0716	411.0741	6.1	C <sub>21</sub> H <sub>14</sub> O <sub>9</sub>	Acetone adduct from polyozellic acid	Other compound
15	383.3525	383.3532	1.8	C <sub>24</sub> H <sub>48</sub> O <sub>3</sub>	DL-Cerebronic acid	Fatty acid
16	367.3576	367.3545	7.8	C <sub>24</sub> H <sub>48</sub> O <sub>2</sub>	Tetracosanoic acid	Fatty acid
17	341.1084	341.1076	2.3	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	Disaccharides	Carbohydrate
18	327.2899	327.2909	3.1	C <sub>20</sub> H <sub>39</sub> O <sub>3</sub>	Ethyl 2-Acetylhexadecanoate	Other compound
19	311.2222	311.2237	4.9	C <sub>18</sub> H <sub>32</sub> O <sub>4</sub>	(Z)-6-Hydroxy-4-oxo-octadec-11-enoic acid	Fatty acid
20	293.2117	293.2116	0.3	C <sub>18</sub> H <sub>30</sub> O <sub>3</sub>	9-oxo-10E,12Z octadecadienoic acid	Fatty acid
21	279.2324	279.2344	7.2	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	Linoleic acid	Fatty acid
22	255.2324	255.2316	3.1	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	Palmitic acid	Fatty acid
23	195.0505	195.0506	0.5	C <sub>6</sub> H <sub>12</sub> O <sub>7</sub>	2,4,5,6-tetrahydroxyhexanoic acid	Organic acid
24	191.0192	191.0185	3.7	C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>	Citric acid	Organic acid
25	173.0086	173.0100	8.0	C <sub>6</sub> H <sub>5</sub> O <sub>6</sub>	Trans-Acotinic Acid	Organic acid
26	165.0399	165.0413	8.0	C <sub>5</sub> H <sub>9</sub> O <sub>6</sub>	D-xylonate	Organic acid
27	133.0137	133.0146	6.8	C <sub>4</sub> H <sub>6</sub> O <sub>5</sub>	Malic acid	Organic acid

Mode negative: 100 µL CS extract diluted in 1.5 mL of methanol with 20 µL of ammonium hydroxide (100 mmolL<sup>-1</sup>) / Mode positive: 100 µL CS extract diluted in 1.5 mL of methanol with 20 µL of formic acid

out, with significant activity in the treatment of rheumatoid arthritis. Likewise, a study on the alkaloid Caesalpinin MH, demonstrated its anti-inflammatory actions as a selective modulator of glucocorticoids and having great potential for therapeutic use in inflammatory diseases (XIANG et al., 2018).

The screening by ESI-ToF-MS analysis, allowed the recovery and characterization of a large number of phytochemicals, many of which were not previously reported in the literature for this raw material. Previous studies confirm the bioactivity of corn stigma. ŽILIC et al. (2016) report the abundance

of bioactive compounds, mainly hydroxycinnamic acid esters and luteolin derivatives. LI et al. (2019) found that CS tea reduced systolic blood pressure levels in hypertensive rats and inhibited the activity of the angiotensin-converting enzyme. In addition, HO et al. (2017) discovered a peptide with CS anti-inflammatory abilities. CS can be considered a safe ingredient for food application, according to data obtained by WANG

et al. (2011). When performing subchronic toxicity tests, they reported that CS has no adverse effects and supports its safety for use in humans.

After the screening by ESI-ToF-MS, experiments regarding to antimicrobial activity of CS extract were performed. The results obtained in the screening to determine the antifungal and antibacterial activities are shown in table 4.

Table 4 - Minimum inhibitory and lethal concentrations for bacteria and fungi from CS extract obtained by UAE.

Bacteria	-----Fractions (MIC 50/MLC $\mu\text{g mL}^{-1}$ )-----					
	-----Extract-----		-----Chloramphenicol-----		-----Ampicillin-----	
	MIC	MLC	MIC	MLC	MIC	MLC
Gram positive						
<i>Bacillus cereus</i>	62.5	500	3.12	12.5	200	200
<i>Bacillus subtilis</i>	62.5	>500	6.25	50	100	200
<i>Staphylococcus aureus</i>	125	500	1.56	6.25	200	200
<i>Enterococcus faecalis</i>	62.5	>500	3.12	12.5	1.56	12.5
-----Gram negative-----						
<i>Pseudomonas aeruginosa</i>	125	>500	3.12	12.5	50	200
<i>Shigella flexneri</i>	125	>500	1.56	3.12	12.5	200
<i>Proteus mirabilis</i>	250	>500	6.25	25	25	200
<i>Escherichia coli</i>	62.5	500	3.12	100	200	200
<i>Shigella sonnei</i>	62.5	500	1.56	3.12	25	200
<i>Salmonella typhimurium</i>	62.5	500	3.12	200	200	200
<i>Salmonella enteritidis</i>	125	>500	1.56	12.5	3.12	100
<i>Klebsiella pneumoniae</i>	125	500	6.25	200	100	200
<i>Morganella morganii</i>	62.5	500	6.25	50	200	200
<i>Enterobacter aerogenes</i>	125	>500	1.56	12.5	200	200
Fungal						
	-----Fractions (MIC 50/MLC $\mu\text{g mL}^{-1}$ )-----					
	-----Extract-----		-----Fluconazole-----		-----Nystati-----	
	MIC	MLC	MIC	MLC	MIC	MLC
<i>Candida albicans</i>	125	500	25	100	50	100
<i>Candida parapslosis</i>	250	>500	1.56	25	1.56	100
<i>Candida krusei</i>	125	500	25	200	12.5	50
<i>Candida tropicalis</i>	500	500	50	200	100	200
<i>Cryptococcus neoformans</i>	125	500	3.12	12.5	25	100
<i>Cryptococcus gatti</i>	125	500	3.12	25	25	100
<i>Candida dubliniensis</i>	250	>500	3.12	12.5	50	100
<i>Candida glabrata</i>	500	>500	3.12	200	50	100
<i>Saccharomyces cerevisiae</i>	250	>500	1.56	25	1.56	3.12

MIC 50: minimum inhibitory concentration capable of inhibiting 50% of the microorganism growth ( $\mu\text{g mL}^{-1}$ ); MLC: minimum lethal concentration ( $\mu\text{g mL}^{-1}$ ); Chloramphenicol, Ampicillin, Fluconazole and Nystatin: positive controls.

The MIC and MLC values of each of the tested fractions were compared with positive controls. The extract obtained by UAE exhibited significant inhibitory activity against all evaluated bacteria, with MIC values between 62.5 and 250  $\mu\text{g}\cdot\text{mL}^{-1}$ , with Gram-positive bacteria *Bacillus cereus*, *Bacillus subtilis* and *Enterococcus faecalis* (MIC of 62.5  $\mu\text{g}\cdot\text{mL}^{-1}$ ) and the Gram-negative bacteria *Escherichia coli*, *Shigella sonnei*, *Salmonella typhimurium* and *Morganella morganii* (MIC of 62.5  $\mu\text{g}\cdot\text{mL}^{-1}$ ) the most sensitive. The CS extract showed antifungal potential, inhibiting the growth of all evaluated fungi, with MIC values between 125 and 500  $\mu\text{g}\cdot\text{mL}^{-1}$ , with *Candida albicans*, *Candida krusei*, *Cryptococcus neoformans* and *Cryptococcus gatti* (MIC of 125  $\mu\text{g}\cdot\text{mL}^{-1}$ ) the most sensitive.

The extract exhibited superior bactericidal potential compared to positive ampicillin control, for 7 of the 14 bacteria tested. Phytochemicals that present in MIC susceptibility tests between 100-1000  $\mu\text{g}\cdot\text{mL}^{-1}$  in vitro, can be classified as antimicrobials (DOS REIS et al., 2019). Therefore, the extract obtained by ultrasound of corn stigma evaluated in this study can be considered a substance with antimicrobial potential ( $\text{MIC} \leq 500 \mu\text{g}\cdot\text{mL}^{-1}$ ).

The antibacterial and antifungal effect observed in CS, is directly associated with its phytochemical composition. According to data obtained in the ESI-ToF-MS analysis, the presence of fatty acids was reported, among which linoleic acid, palmitic acid, DL-cerebronic acid and tetracosanoic acid stand out, which are described in the literature for having strong antimicrobial properties, acting against Gram-positive organisms. In addition, previous studies reveal that organic acids, the second largest class of compounds present in CS, also have action against different types of pathogens. These constituents of CS, act in reducing the internal pH of the microbial cell by ionization of acid molecules, not dissociated, and interrupting the transport of substrate by altering the permeability of the cell membrane or reduction of proton motive force (FENG et al., 2011).

## CONCLUSION

The results of the study indicated that the ultrasound-assisted extraction (UAE) of corn stigma (CS) has the ability to increase the total phenolic compounds (TPC) and oxygen radical absorbance capacity (ORAC) values and reduce the extraction time by 67% when compared to the conventional method. The optimal condition for simultaneous extraction of antioxidants and polyphenols from CS

by UAE was 5 minutes at a solid-solvent ratio of 0.05  $\text{g mL}^{-1}$ . Using the screening by ESI-ToF-MS, it was possible identified 27 phytochemical compounds, of which 21 have not yet been described in the literature for CS. In addition, results also showed that CS has a more active and efficient bacteriostatic potential than the positive ampicillin control for 7 of the 14 bacteria studied. The results compiled in this study prove that corn stigma can be considered a source of bioactive metabolites with antioxidant and antimicrobial functions. This agricultural residue can be used in the food industry as a natural antioxidant ingredient, or it can be applied in bioactive packaging to extend the shelf life of food products.

## ACKNOWLEDGEMENTS

The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Grant nr. 309549/2016-7, 309297/2016-8, 313786/2019-4) and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS, Grant nr. 16/2551-0000226-6) for supporting this study. This study was partly financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) Brasil - Finance code 001.

## DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

## AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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