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Chemical constituents, antioxidant potential, antibacterial study and photoprotective activity of Brazilian corn silk extract

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Abstract

The aim of this work was to determine the total phenolic content and evaluate the antioxidant, antibacterial, photoprotective and cytotoxic properties of corn silk ethanol extract prepared from corn silk that was air-dried at room temperature. Corn silk ethanol extract has a high content of phenolic compounds. The concentrations of ethanol extract required to produce 50% of maximal effect (EC_{50}) in DPPH and ABTS radical scavenging assays were 489.0 and 166.1 µg/mL respectively, indicating low activity when compared to the positive control. The ethanolic extract of corn silk showed antibacterial activity, mainly on Gram-positive bacteria. The *in vitro* sun protection factor (SPF) of the ethanolic extract of corn silk incorporated into sunscreen UVA-UVB 5% gel with Pemulen TR-1® was evaluated for the first time. A relative increase in SPF efficacy was observed for the extract when the sunscreen was incorporated. The ethanolic extract of corn silk and the gel were considered non-cytotoxic. The corn silk ethanolic extract was characterized using ultra performance liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry (UPLC-QTOF-MS/MS). Eleven compounds including flavonoids, coumaric acid derivatives, terpenoid, hydroxycinnamic acid derivative and quinic acid derivative were suggested based on their accurate mass and MS/MS spectra.

Keywords: corn silk; ethanol extract; phenolic compounds; sun protection factor; sunscreen UVA-UVB 5% gel with Pemulen TR-1.

Practical Application: This research investigated the antioxidant, antibacterial, photoprotective and cytotoxic properties of corn silk ethanol extract. This is the first study on the photoprotective effect of ethanolic extract from corn silk incorporated into a formulation containing the sunscreen UVA-UVB 5% gel with Pemulen TR-1®. UPLC-QTOF-MS/MS method was used to analyze the chemical profile of the crude ethanol extract from corn silk. The study is important in terms of updating our information about the properties of corn silk.

1 Introduction

The corn silk (Stigma maydis) is the elongated stigma of the female flower of the corn (*Zea mays* L.), which is soft and smooth, and looks like a thread or yellowish hair. The function of the corn silk is to capture pollen for pollination. Corn silk elongates beyond the cob covering the edible part of the plant, the length can reach 30 cm or more (Haslina et al., 2017; Sani, 2016). Corn silk has a long history of use as a traditional medicine in many parts of the world (Hasanudin et al., 2012). In China, corn silk has been reported to have the function of inducing diuresis (Wang & Zhao, 2019). Studies with Wistar rats have shown that the aqueous extract of corn silk has a diuretic effect, but does not act as a loop diuretic (diuretics that act at the ascending limb of the loop of Henle in the kidney) since it did not lead to potassium loss or marked sodium loss, compared to furosemide drug (Pinheiro et al., 2011). Also in China, people have been using corn silk decoction for diabetes therapy for decades (Zhao et al., 2012). Research outcomes of the ethanol extract of corn silk suggested that the antioxidant activities of corn silk could contribute, at least in part, to its traditionally claimed therapeutic benefits on diabetes mellitus and diabetic nephropathy (Wang & Zhao, 2019). In addition, corn silk is well known in treating infection and cystitis, kidney stone, nephritis and other kidney diseases (Sani, 2016). It is also used in the treatment of jaundice, measles, edema, gout, celiac hematuria, prostatitis and other diseases (Ting & Hongli, 2018; Catap et al., 2015). Its potential for use is closely related to its properties and the mechanism of action of its bioactive chemical constituents such as alkaloids, flavonoids, saponins, tannins, organic acids, phytosterols, allantoin and so on (Ren et al., 2013; Hasanudin et al., 2012). Corn silk also contains Ca^{2+}, Mg^{2+}, K^{+} and Na^{+} salts, vitamin C and K, proteins,

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carbohydrates, and volatile oils (Ebrahimzadeh et al., 2008; Wan Ishak & Mat Ali, 2016). The potential healthcare applications of corn silk are very much related to its chemical composition and mechanism of action of its bioactive constituents such as phenols, terpenoids, polysaccharides and glycoproteins (Žilić et al., 2016). The present study was carried out to determine the total phenolic content, as well as the antioxidant, antibacterial, photoprotective, and cytotoxic properties of corn silk. The photoprotective action of corn silk ethanol extract incorporated into formulations is unprecedented in the literature. Therefore, UPLC-QTOF-MS/MS was used for rapid global chemical profiling of the corn silk ethanol extract.

2 Materials and methods

2.1 Materials

Absorbance readings were performed on a Genesys 105 UV-VIS spectrophotometer equipped with 1 cm quartz cell. The sunscreen UVA-UVB 5% gel with Pemulen TR-1® (polymeric emulsifier) contains 2-phenyl-benzimidazole-5-sulfonic acid (Eusolex 232®) and 2-hydroxy-4-methoxybenzophenone, commercially available, as UVA/UVB sunscreen agents and it was obtained from BioFarma.

2.2 Extraction

Corncobs (*Zea mays*) were purchased at a local market in October 2018, in the city of Ouro Preto, State of Minas Gerais, Brazil. After husks removal, corn silk samples were separate from their fruits and air-dried at room temperature for a week, yielding 70.89 g, which were subjected to successive extraction (3 x 500 mL) rounds with absolute ethanol (99.8% Neon Comercial Ltd.) by maceration at room temperature. Solvent was removed using a rotary flash evaporator to give 2.52 g of crude extract.

2.3 Phytochemical screening

Qualitative phytochemical screening was carried on the corn silk ethanol extract to identify its phytoconstituents, i.e., alkaloids, flavonoids, phenols/tannins, saponins, terpenoids, carbohydrates and anthraquinones, using standard procedures (Edeoga et al., 2005; Egwaikhide & Gimba, 2007; Abu-Qaoud et al., 2018; Usman et al., 2009) with few modifications.

Test for alkaloids (Dragendorff's test)

The dry crude extract (10 mg) was dissolved in 0.5 mL of ethanol and the ethanolic solution was applied to a TLC plate along with a positive alkaloid (quinine) pattern. The development was carried out with the application of a specific reagent for alkaloids (Dragendorff's reagent), followed by heating the plate to 100 °C. The appearance of a red-orange spot in the Dragendorff test was considered a positive result for the presence of alkaloids.

Test for flavonoids (alkaline reagent test)

Ten milligrams (10 mg) of the dry crude extract were dissolved in 2 mL of 2% sodium hydroxide (NaOH). An intense yellow color indicated the presence of flavonoids.

Test for phenols/tannins (ferric chloride test)

Ten milligrams (10 mg) of the crude extract were stirred with 2 mL of distilled water. It was filtered and a few drops of 2% ferric chloride ($FeCl_3$) were added and observed for coloration. Brownish green or a blue-black color indicated the presence of tannins and phenols.

Test for saponins (froth test)

Five milliliters (5 mL) of distilled water were added to 10 mg ethanolic crude extract and shaken vigorously for a stable persistent froth.

Test for terpenoids (Salkowski test)

The dry crude extract (10 mg) was dissolved in 2 mL of chloroform ($CHCl_3$) and then 3 mL of concentrated sulfuric acid (H_2SO_4) were added to form a layer. A reddish brown coloration of the interface was formed to indicate positive results for the presence of terpenoids.

Test for carbohydrate (Iodine test)

Ten milligrams (10 mg) of the dry crude extract were mixed with 2 mL of iodine solution. A dark blue/purple coloration indicates the presence of carbohydrates.

Test for anthraquinones (Borntrager's test)

Ten milligrams (10 mg) of the dry crude extract were shaken with 2 mL benzene and 1 mL of ammonia solution was added and shaken. The presence of a pink, red or violet color in the ammoniacal (lower) phase indicates the presence of free anthraquinones.

2.4 Analysis of chemical components in the corn silk ethanol extract by UPLC-QTOF-MS/MS

Liquid chromatography coupled to mass spectrometry (LC-MS) analysis was performed to characterize the chemical profile of the corn silk ethanol extract. The sample was prepared in acetonitrile (200 ppm), then it was filtered through syringe filters with a nylon membrane (0.45 µm). The chromatographic separation was carried out on a Zorbax Eclipse C18 column (2.1 x 50 mm, 1.8 µm), maintained at 30 °C, after injection of 3 µL of the sample. Elution was performed in gradient mode at a flow rate of 0.350 mL/min, using a system consisting of eluents A (H_2O acidified with 0.1% formic acid) and B (acetonitrile acidified with 0.1% formic acid) in the following proportion: 5-100% B for 22 min and 100-5% B for 3 min. MS^1 and MS^2 data were acquired in negative and positive mode by Agilent 6545 Q-TOF (Agilent Technologies Inc., Santa Clara, CA) equipped with electrospray ionization (ESI) source. The operational source parameters for TOF-MS mode were performed at three power levels: low (capillary voltage: 2200 V, fragmentation voltage: 110 V, nozzle voltage: 300 V), medium (capillary voltage: 2500 V, fragmentation voltage: 120 V, nozzle voltage: 500 V) and high (capillary voltage: 3000 V, fragmentation voltage: 130 V, nozzle voltage: 600 V). Auto MS/MS mode was performed using collision energy table

with the following values: 150-500 Da (20-25 eV), 500-1000 Da (25-50eV) and 1000-1500 (50-60 eV). For data processing, the MS-Dial 4.60 software was used, then the data were submitted to analysis using the GNPS (Global Natural Products Network) platform, the MS-Finder software and the MSP library available for MS-Dial to perform the compounds annotation.

2.5 Determination of total phenolic content

The Folin-Ciocalteu method was adapted from Bonoli et al. (2004) and used to measure the total phenolic content. 80 µL of corn silk ethanol extract (10 mg/mL in ethanol 95%) were transferred to a 96-well plate, and 60 µL of water and 10 µL of Folin-Ciocalteu (Cromoline) were added. Next, the plate was agitated for 1 min and 40 µL of a sodium carbonate solution (15% w/v) were added. Subsequently, the plate was agitated for 30 s and 10 µL of water were added. After incubation for 2 h, absorbance readings were carried out at 650 nm in a microplate reader (Molecular Devices). The total phenolic content was quantified by using a calibration curve for standard gallic acid (10-320 µg/mL; $r^2 = 0.9993$; $y = 0.0030x + 0.0956$). The experiment was performed in triplicate and the results were expressed as mg of gallic acid equivalents (GAE) per g of sample (mgGAE/g).

2.6 Antioxidant activity assay

DPPH radical scavenging assay

Corn silk ethanol extract and quercetin (standard) were respectively solubilized in ethanol to obtain stock solutions of 320.0 µg/mL. Different aliquots were pipetted and diluted to give final solutions from 0.1 to 10.0 mg/mL. Then 100 µL of DPPH solution at 0.008% w/v in ethanol were added to each of these samples. The final volume was adjusted to 240 µL with ethanol. The negative control was obtained from 100 µL of the DPPH and 140 µL of the ethanol, which was used to calculate the inhibition percentage of free radicals. Then, all the samples were incubated for 30 minutes at room temperature (25 ± 2 °C), protected from light. The absorbance (Abs) reading was carried out in a plate-reader at a wavelength of 490 nm. The test was performed in triplicate and the ability to scavenge free radicals was evaluated by the free radical scavenging percentage (% I), calculated using the formula: $\% I = (Abs_{control} - Abs_{sample}/Abs_{control}) \times 100$ (Sousa et al., 2007). The concentration required to produce 50% antioxidant effect (EC_{50}) was calculated by linear regression.

ABTS radical scavenging assay

The working solution was prepared by mixing ABTS (7.4 mmol/L) and potassium persulfate (2.6 mmol/L), and keeping it for 12-16 h at room temperature, protected from light. On the day of analysis, this solution was diluted in ethanol to give an absorbance of 0.70 (± 0.02) at 650 nm. The concentrations of corn silk ethanol extract and gallic acid were obtained in the same manner as described in the previous test (0.1 to 10.0 mg/mL). Then, 120 µL of ABTS were added to these samples. The final volume was adjusted to 150 µL by adding ethanol. The negative control was prepared by mixing 120 µL of ABTS and 30 µL of ethanol. All the samples were incubated for 6 min at room temperature (25 ± 2 °C), protected from light (Li et al., 2009). The readings were performed at 650 nm and the % scavenging activity of the sample and the EC_{50} were calculated as previously described.

2.7 Antibacterial activity assay

The antibacterial activity of the corn silk ethanol extract was evaluated by microdilution assay (Clinical and Laboratory Standards Institute, 2012), using 4 bacteria strains: Gram-positive bacteria *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228; Gram-negative bacteria *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. These bacteria were cultivated in Müeller-Hinton medium (Himedia®) during 24 h/37 °C. All inoculums were prepared using the direct colony suspension method, with a saline suspension (0.9% NaCl) of the colonies selected from a 24 h agar plate, before each assay. The suspension was adjusted to achieve a turbidity equivalent to a 0.5 McFarland standard (about 1 x 10^8 CFU/mL). The suspension was diluted (1:100) in Müeller-Hinton broth to obtain a final assay of 5 x 10^5 CFU/mL. The extract was dissolved in Müeller-Hinton broth, with 1% Tween 80, at a concentration of 100 mg/mL. An aliquot (100.0 µL) of the sample solution was added into the first well of a 96-well microtiter plate and broth (50.0 µL) was added into the other wells. Serial dilutions (1:2) were prepared by removing 50 µL from the first well and transferring them to the next well to obtain extract concentrations from 50.000 to 0.024 mg/mL. Only 50.0 µL of broth were added into the grown control well. Tetracycline (100.0 µg/mL) was used as positive control and the control of the culture medium was prepared using only broth. Next, 50.0 µL of diluted inoculums were added (except for medium control) into the wells. The capped plates were incubated for 24 h at 37 °C. After the incubation period, 30 µL of triphenyl tetrazolium chloride (TTC) (2.0 mg/mL) (Neon®) were added and the plates were incubated for an additional period of three hours. The bacteria from wells displaying no visible color were peaked in Petri dishes using an inoculation loop and incubated for 24 h. The minimal inhibition concentration (MIC) was established as the smallest concentration at which no color was observed in TTC. The minimal bactericidal concentration (MBC) was defined as the smallest concentration at which no microbial growth was observed in Petri dishes. MBC/MIC ratio was calculated to define the antibiosis mode of the fractions: bactericidal when MBC/MIC ≤ 4; and bacteriostatic when MBC/MIC > 4 (Ayala-Núñez et al., 2009).

2.8 Photoprotective activity assay

In vitro determination of the Sun Protection Factor (SPF) of the ethanol extract

Dry corn silk ethanol extract was diluted in absolute methanol to give the concentration of 0.1 mg/mL. The scanning was performed in wavelengths from 200 to 800 nm in the UV spectrophotometer for the extract solution. The purpose of the scan was to check the absorptions in the ultraviolet region B

(UVB). Absolute methanol was used as a white in the respective reading. Through the Mansur method equation, it was possible to determine the value of the Sun Protection Factor (SPF) for this concentration (Mansur et al., 1986). The absorption readings were taken between 290 and 320 nm (UVB region). The experiment was performed in triplicate.

In vitro determination of the Sun Protection Factor (SPF) after incorporation of the ethanol extract into the sunscreen

The ethanol extract of the corn silk was incorporated in the photoprotective formulation with the sunscreen UVA-UVB 5% gel with Pemulen TR-1®. The sunscreen UVA-UVB 5% gel with Pemulen TR-1® (1 g) and 1 ml of the corn silk ethanol extract solution (1 mg/mL) were added to a 100 mL beaker. The mixture was maintained under stirring for 30 min at room temperature and then stored (Nascimento et al., 2009).

Portions containing 1 g of the sunscreen UVA-UVB 5% gel with Pemulen TR-1® (incorporated with the corn silk ethanol extract) and the sunscreen UVA-UVB 5% gel with Pemulen TR-1® alone (positive control) were weighed. Subsequently, dilutions of this material in 70% ethanol were performed in triplicate to obtain a concentration of 0.2 µL/mL. The scanning of solutions was performed in triplicate in wavelengths from 200 to 800 nm in the UV spectrophotometer, using ethanol as white in the respective reading. Using the equation of the method by Mansur in the absorption readings from 290 to 320 nm, with intervals of 5 nm, it was possible to determine the Sun Protection Factor (SPF) value of each solution (Mansur et al., 1986).

2.9 Cytotoxicity

Human fibroblast MRC-5 cells cultivated in RPMI 1640 medium (Sigma®) were distributed in a 96-well microtiter plate using a density of 5 x 10^4 cell/well, and after that, they were incubated at 37 °C with 5% CO_2 for 24 h. Cells were treated with the sample dissolved in RPMI with 2% DMSO, at concentrations ranging from 1000 to 62 µg/mL. Cell viability was evaluated using the sulforhodamine B assay (SRB) (Skehan et al., 1990). After 24 h incubation, media was removed and the cells were fixed using cold 20% trichloroacetic acid for 1 h at 4 °C. The microtiter plate was washed with distilled water and air dried. Thereafter, fixed cells were stained for 30 min with 0.1% SRB dissolved in 1% acetic acid. The plate was washed again with 1% acetic acid and subjected to air drying. Then, 200 µL of 10 mM TRIS buffer (pH 10.5) were added to the stain solubilization at room temperature for ~30 min. The absorbance readings of the samples were performed in the spectrophotometer (490 nm) and the results were expressed as percentage of viable cells over untreated cells.

3 Results and discussion

3.1 Phytochemical screening

The results are summarized below (Table 1). The ethanolic extract from corn silk showed a positive result for flavonoids, phenols/tannins and terpenoids. The test was based on the color changes after the reaction of the extract with standard reagents to detect secondary metabolites.

Table 1. Phytochemical analysis of the ethanolic extract from corn silk.

Phytochemicals	Ethanolic Extract
Alkaloids (Dragendorff's test)	Reddish brown (–)
Flavonoids (alkaline reagent test)	Yellow coloration (+)
Phenols/Tannins (ferric chloride test)	Brownish green (+)
Saponins (froth test)	Formation of froth (–)
Terpenoids (Salkoski test)	Reddish brown coloration of the interface (+)
Carbohydrate (Iodine test)	Color change from clear blue to brick-red precipitate (–)
Anthraquinones (Borntrager's test)	Rose pink coloration (–)

Key: + = present; – = absent.

3.2 Analysis of chemical components in the corn silk ethanol extract by UPLC-QTOF-MS/MS

The chemical profile of the ethanolic extract from corn silk was performed using UPLC-QTOF-MS/MS analysis and the molecular formula of precursor ions (MS^1 spectra) was calculated (accurate 5 ppm). Then, ions-fragment analysis (MS^2 spectra) was performed using molecular annotation from different programs (GNPS, MS-Dial and MS-Finder) based on different metabolite databases and confidence literature (Cheiran et al., 2019; Zhang et al., 2018; Ee et al., 2009; Kang et al., 2018). According to Table 2, eleven compounds were suggested and their chemical structures are represented in the Figure 1. The characterized compounds were classified into five groups: quinic acid derivative, coumaric acid derivatives, hydroxycinnamic acid derivative, flavonoids and terpenoid. These results corroborate the data found in the phytochemical screening assays.

3.3 Determination of total phenolic content

The total content of phenolic compounds of the corn silk ethanol extract was investigated using the modified Folin-Ciocalteu assay, which is sensitive to phenol and polyphenol entities and other electron donating antioxidants, such as ascorbic acid (Alzahrani et al., 2012). The mean value and standard deviation are shown in Table 3.

The total phenol content was calculated by Folin-Ciocalteu reagent in terms of gallic acid equivalent. The result of total phenol content obtained, 5291.80 ± 66.80 mgGAE/g, was higher than the value previously reported for other corn silk extract. In the study published by Ebrahimzadeh et al. (2008), in which an ethanol-water (1:1) extraction was performed to prepare the corn silk extract, a lower total phenolic concentration was found, 118.94 ± 2.78 mgGAE/g. The phenolic compounds concentration in corn silk is dependent on its origin and variety. Haslina et al. (2017) demonstrated that corn silk powder from three different local varieties have differences in terms of total phenolic content. The processing method also affects the level of total phenols. The content of phenolic compounds is highly susceptible to

Table 2. Detected compounds from ethanolic extract from corn silk UPLC-QTOF-MS/MS.

Nº	t_R (min)	Precursor ion (*m/z*)	Molecular formula	Adduct	Error (ppm)	Ions-fragment (*m/z*)	Annotation	Class	Reference
1	4.361	367.1045	$C_{17}H_{20}O_9$	$[M-H]^-$	-4.4	193.0509; 173.0453; 149.0609; 134.0374	3-O-Feruloylquinic acid	QD	Cheiran et al. (2019)
2	5.210	563.1425	$C_{26}H_{28}O_{14}$	$[M-H]^-$	-4.3	473.1102; 443.0992; 383.0784; 353.0679	8-[3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-5,7-dihydroxy-2-(4-hydroxyphenyl)-6-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl] chromen-4-one	FL	Mass Bank of Europe (2022)
3	5.235	565.1555	$C_{26}H_{28}O_{14}$	$[M+H]^+$	0.4	409.0918; 391.0813; 379.0815; 325.0710	Corymboside	FL	Mass Bank of North America (2022)
4	5.773	579.1720	$C_{27}H_{30}O_{14}$	$[M+H]^+$	-1.0	379.0821; 337.0719; 313.0723; 283.0615	Vitexin-2"-O-rhamnoside	FL	Mass Bank of Europe (2022)
5	5.909	433.1144	$C_{21}H_{20}O_{10}$	$[M+H]^+$	-2.1	397.0917; 337.0726; 313.0715; 283.0608	Vitexin	FL	Zhang et al. (2018)
6	7.392	561.1613	$C_{27}H_{28}O_{13}$	$[M+H]^+$	-0.9	397.0945; 353.0665; 313.0723; 283.0607	5,7-dihydroxy-6-[(2S,3R,4R,6R)-4-hydroxy-6-methyl-5-oxo-3-[(2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl] oxyoxan-2-yl]-2-(4-hydroxyphenyl) chromen-4-one	FL	Mass Bank of North America (2022)
7	7.594	563.1769	$C_{27}H_{30}O_{13}$	$[M+H]^+$	-0.7	381.0976; 337.0716; 313.0724; 283.0614	6-[4,5-dihydroxy-6-methyl-3-[(2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl] oxyoxan-2-yl]-5,7-dihydroxy-2-(4-hydroxyphenyl) chromen-4-one	FL	Mass Bank of North America (2022)
8	7.728	284.1294	$C_{17}H_{17}NO_3$	$[M+H]^+$	-2.5	147.0441; 121.0648; 93.0700	Paprazine	CD	Ee et al. (2009)
9	7.930	314.1396	$C_{18}H_{19}NO_4$	$[M+H]^+$	-1.3	177.0552; 145.0287; 121.0648	Moupinamide	HD	Kang et al. (2018)
10	13.324	432.2399	$C_{22}H_{30}O_6$	$[M+ACN+H]^+$	-3.0	135.0810; 119.0859; 107.0859	7b,9-Dihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-5-oxo-1,1a,1b,4,4a,5,7a,7b,8,9-decahydro-9aH-cyclopropa[3,4]benzo[1,2-e] azulen-9a-yl acetate	TE	Mass Bank of North America (2022)
11	20.270	161.0603	$C_{10}H_{10}O_3$	$[M+H]^+$	0.0	133.0654; 118.0419; 103.0545; 90.0465	3-Methoxycinnamic acid	CD	Mass Bank of North America (2022)

Coumaric acid derivative (CD); Flavonoid (FL); Hydroxycinnamic acid derivative (HD); Quinic acid derivative (QD); Terpenoid (TE).

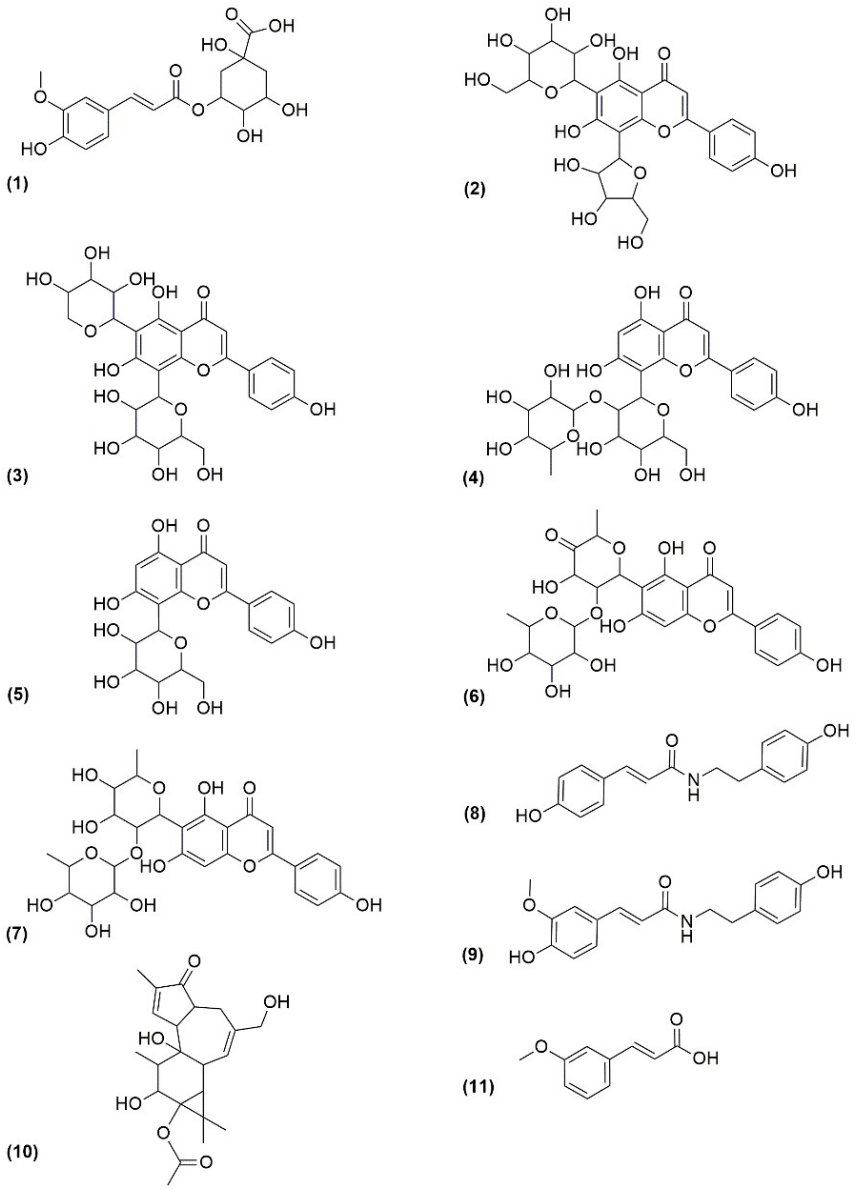

Figure 1. Chemical structures of the proposed compounds 1-11 present in the corn silk ethanol extract.

Table 3. Total phenolic content of the corn silk ethanol extract.

Sample	Total phenolic content (mgGAE/g)[1]
Corn silk ethanol extract	5291.80 ± 66.80

[1]mg of gallic acid equivalents per g of sample. Results expressed as mean ± standard deviation.

degradation, which can be caused by temperature, oxygen level and light. In this way, there are differences between the phenol content of the extract derived from fresh and dry samples due to the drying process. Increased drying temperatures will reduce the total content of phenolic compounds, particularly if the drying process is carried out at temperatures higher than 60 °C (Haslina et al., 2017). In the present study, corn silk was dried at room temperature for one week. The ethanolic extract from corn silk presented a positive result for phenols in the phytochemical screening and also were suggested using UPLC-QTOF-MS/MS analysis (compounds 1-9, Figure 1).

3.4 Antioxidant activity assay

DPPH and ABTS radical scavenging assays

The concentrations required to produce 50% of maximal effect (EC_{50}) of the corn silk ethanol extract in DPPH and ABTS radical scavenging assays are shown in Table 4.

The smaller the EC_{50} value, the greater the antioxidant capacity on DPPH and ABTS radicals. The EC_{50} values of the corn silk ethanol extract for DPPH radical scavenging activity and ABTS radical scavenging activity were 489.0 and 166.1 µg/mL, respectively. Therefore, the ethanol extract presented a lower potency than the control (quercetin) in this study.

Wang & Zhao (2019) showed that the corn silk ethanol extract displayed appreciable DPPH radical scavenging activity with an EC_{50} value equal to 116.2 μg/mL. However, the ethanol extract was prepared with 80% ethanol, unlike our study, whose extract was prepared with absolute ethanol (99.8%).

The results of different antioxidant assays in previous studies have revealed the potential use of corn silk extracts as an important bioactive source of natural antioxidants (Hasanudin et al., 2012). These findings suggest that the phenol content should be considered as an important feature of corn silk because the antioxidant activity could be attributed to the presence of these constituents, which contain hydroxyl groups and are considered responsible for the radical scavenging effect in plants (Ebrahimzadeh et al., 2008). The catechol, having two hydroxyl groups in the ortho position, it is considered the main group functional for antioxidant action and is present in many natural phenolic compounds (Anunciação et al., 2020). According to our study, these phytochemicals were suggested in the corn silk ethanolic extract by the analysis by UPLC-QTOF-MS/MS (compounds **1**-**9**, Figure 1), however, they do not have a catechol group and this may be one of the reasons for its low antioxidant activity.

3.5 Antibacterial activity assay

The results of the antibacterial activity test are shown in Table 5.

Corn silk ethanol extract showed antibacterial activity against all bacteria tested, with Gram-positive strains (*S. aureus* and *S. epidermidis*) being the most sensitive ones. The prevalence of staphylococcal resistance to conventional antibiotics associated with skin and soft-tissue infections, necrotizing pneumonia, osteomyelitis and sepsis, has become a global epidemic, and research on new antimicrobials is important (Mohammad et al., 2015). MIC values indicate a weak antibacterial activity; however, the antibiosis action of the extract was classified as bactericidal (MBC/MIC ≤ 4). The use of bactericidal antimicrobials might mean a possibility to reduce the selection of resistant microorganisms (Ayala-Núñez et al., 2009).

Table 4. Antioxidant activity of the corn silk ethanol extract.

Samples	EC_{50} (μg/mL)	
	DPPH	ABTS
Extract	489.0 ± 5.0	166.1 ± 25.00
Quercetin (positive control)	2.9 ± 0.1	2.0 ± 0.7

EC_{50}: concentration required to produce 50% of antioxidant effect. Results expressed as mean ± standard deviation.

Table 5. Minimal Inhibition Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of the corn silk ethanol extract.

Bacteria	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC
S. aureus	12.5	50.0	4
S. epidermidis	12.5	50.0	4
E. coli	50.0	50.0	1
P. aeruginosa	25.0	50.0	2

In the study by Morshed & Islam (2015), several organic solvents were used for screening the antimicrobial activity of corn silk extract, showing sensitive responses in methanol and ethanol based extracts against 11 of bacteria strains out of the 12 strains tested. In the study by Nessa et al. (2012), a corn silk methanol extract also was active against 11 bacteria strains out of 12 bacteria strains tested and it showed a higher activity, in comparison with the use of gentamycin, against 7 bacteria strains. In both studies mentioned above, the less sensitive bacteria strain was *E. coli*, the same result found in our study.

Phenolic compounds in the corn silk ethanol extract may have been responsible for the antibacterial activity. Phenols exhibit antimicrobial activity against broad spectrum of bacteria. They possess antibacterial activity thanks to the ability to inhibit bacterial virulence factors such as enzymes and toxins, interact with cytoplasmic membrane and suppress biofilm formation (Miklasińska-Majdanik et al., 2018). Adamczak et al. (2020) showed that the glycosides of flavonoids, vitexin-2"-O-rhamnoside (compound **4**) and vitexin (compound **5**) have antibacterial effects. The vitexin has also been tested in order to study its effect on bacterial surface hydrophobicity and biofilm formation. In this study the vitexin reduced the hydrophobicity of cell surface and membrane permeability of *S. aureus* and also showed antibiofilm activity and bactericidal effect (Das et al., 2018). The vitexin also exerted antibacterial properties against *P. aeruginosa*, and exhibited moderate antibiofilm activity (Das et al., 2016).

3.6 Photoprotective activity assay

In vitro determination of the Sun Protection Factor (SPF) of the ethanol extract

The results of the *in vitro* determination of the SPF values of the corn silk ethanol extract and the positive control (sunscreen UVA-UVB 5% gel with Pemulen TR-1®) in the concentration of 0.1 mg/mL are shown in Table 6.

It can be observed that the SPF value of the corn silk ethanol extract was lower than the SPF value of the positive control. Thus, this extract did not exhibit photoprotective action, as a sunscreen must have at least SPF 6 to be considered a true sunscreen, according to a Brazilian resolution (Brasil, 2012).

In vitro determination of the Sun Protection Factor (SPF) after incorporation of the ethanol extract into the sunscreen

The results of the *in vitro* determination of SPF values of the corn silk ethanol extract incorporated into the 5% gel and into the 5% sunscreen are shown in Table 7.

Table 6. SPF values of the corn silk ethanol extract and the positive control (sunscreen UVA-UVB 5% gel with Pemulen TR-1®) in the concentration of 0.1 mg/mL.

Sample	SPF in Region UVB (290-320 nm)
Corn silk ethanol extract	2.41 ± 0.02
Sunscreen UVA-UVB 5% gel with Pemulen TR-1® (positive control)	14.42 ± 4.58

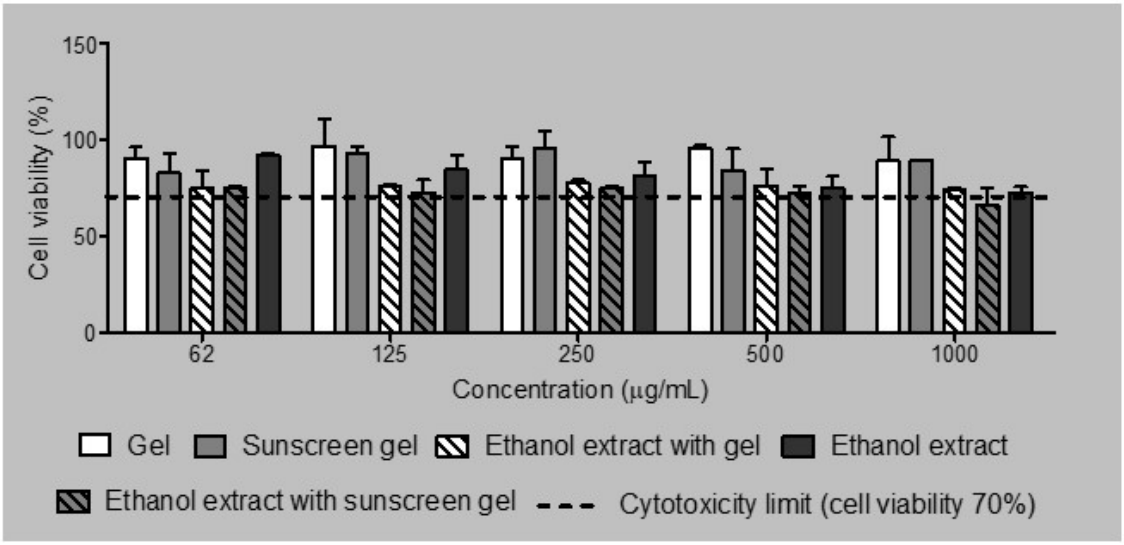

Figure 2. Cell viability determined by sulforhodamine B assay of human fibroblast MRC-5 cells exposed to different concentrations of gel without sunscreen, gel with sunscreen, gel with corn silk ethanol extract without sunscreen, ethanol extract, and gel with corn silk ethanol extract with sunscreen.

Table 7. SPF values of the corn silk ethanol extract incorporated with 5% gel and with sunscreen UVA-UVB 5% gel with Pemulen TR-1® in the concentration of 0.2 µL/mL.

Sample	SPF in Region UVB (290-320 nm)
Corn silk ethanol extract with 5% gel	0.14 ± 0.13
Corn silk ethanol extract with sunscreen UVA-UVB 5% gel with Pemulen TR-1®	21.73 ± 3.55
Sunscreen UVA-UVB 5% gel with Pemulen TR-1® (positive control)	14.42 ± 4.58

The result observed after its incorporation into sunscreen show that the corn silk ethanol extract enhanced the SPF through a synergistic action with the sunscreen. Polyphenols are among the active substances present in plants that have been added to formulations to produce a broader skin photoprotection (Souza et al., 2013). Phenolic compounds present in the ethanolic extract from corn silk could be responsible for the intensification of the photoprotective effect presented in this study, observed after incorporation of the extract into sunscreen. Galanakis et al. (2018) investigated the application of phenols (recovered from olive mill wastewater) as a UV filter in comparison with other natural antioxidants (ascorbic acid and α-tocopherol). Olive phenols were more active as UV filters in a broader region of UVB and UVA when compared with ascorbic acid and α-tocopherol, suggesting their application as a UV protection booster in particular cases to enhance the absorption of synthetic sunscreen agents. Phenolic compounds, especially flavonoids and tannins, have the potential as sunscreen agents because of the presence of chromophore groups (conjugated single double bonds) which can absorb UV light, both UVA and UVB (Shovyana & Zulkarnain, 2013). Detection of flavonoids (compounds **2-7**, Figure 1) in the ethanolic extract from corn silk, using UPLC-QTOF-MS/MS, corroborates the correlation between phenolic compounds and photoprotective activity.

In two other studies, it has been shown that the methanol extract from corn silk can be used alone as a sunscreen (Ebrahimzadeh et al., 2014; Laeliocattleya, 2019). The ability of corn silk ethanol extract to exhibit photoprotective activity when added to formulations has not been reported so far.

3.7 Cytotoxicity

One of the objectives in photoprotection research is the development of photoprotective formulations using natural substances rather than synthetic sunscreens (Mansur et al., 2016). In this way, assays were performed to evaluate the cytotoxic potential of the corn silk ethanolic extract and gels against MRC-5 human fibroblast cell line in the sulforhodamine B assay. The results were evaluated according to the ISO 10993-5:2009, which describes that a substance is considered cytotoxic when it produces a cell viability less than or equal to 70% (Gonçalves et al., 2019). The results show that the ethanolic extract from Brazilian corn silk has a low cytotoxicity since it presented a cell viability above 70%, even when its highest concentration was tested (1000 µg/mL) (Figure 2). The assay also provided data related to gel without sunscreen, gel with sunscreen, gel with corn silk ethanol extract without sunscreen, gel with corn silk ethanol extract with sunscreen, and ethanol extract, as shown in Figure 2. All gels can be considered to have low toxicity. Only the ethanol extract with sunscreen gel was toxic at the highest concentration tested (1000 µg/mL), but this concentration is 5000 times higher than that used in the SPF test (0.2 µg/mL).

4 Conclusion

In this study, UPLC-QTOF-MS/MS was used for the full chemical characterization of the corn silk ethanol extract in

both positive and negative ion modes. A total of 11 compounds were suggested. The results indicates that the corn silk ethanol extract possess important phenolic compounds (compounds **1**-**9**) which are the main biologically active constituents present. These components are the responsible for its antibacterial activity and for its photoprotective properties, as observed in the assays when the extract was incorporated into the sunscreen UVA-UVB 5% gel. As far as we know, this is the first study on the photoprotective effect of ethanolic extract from corn silk incorporated into a formulation containing sunscreen UVA-UVB 5% gel with Pemulen TR-1®. The antibacterial and photoprotective activities and the low cytotoxicity exhibited by the ethanolic extract of corn silk in this study suggest that it is a promising source of natural compounds for the development of new formulations and that it is worthy of further studies on the elucidation of the mechanisms of action responsible for such properties.

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