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ORIGINAL ARTICLE

UPLC–MS–QTOF analysis and antifungal activity of Cumaru (*Amburana cearensis*)

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Abstract

This study was aimed at investigating the phytochemical constituents, antifungal properties and antibiotic-modifying activity of the aqueous crude extract and fractions of *Amburana cearensis* seeds (CEFAC). The CEFAC were chemically characterized by LC–MS/MS–QTOF. In addition, the antifungal activity was assayed by the microdilution method against strains of *Candida albicans*. The phytochemical profile of CEFAC exhibited phenolic compounds, organic acids, and polyphenols. The results of the assessment of antifungal activity reveled an IC_{50} ranging from 45.6 to 2048 µg/mL. Interestingly, when CEFAC was associated with Fluconazole, we evidenced a decreased IC_{50} (1.81–11.9 µg/mL), suggesting a synergism with antibiotic. It was possible to identify in the crude extract and fractions several phenolic compounds, organic acids, and some polyphenols in positive ionization mode. These results suggest that CEFAC may present compounds with the ability to interact and act synergistically with antimicrobial drugs, highlighting its potential as an alternative source for the development of new antimicrobial agents.

Keywords *Amburana cearensis* · *Candida* spp. · Fungistatic · Fluconazole

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Introduction

The fungi are part of the human microbiota (Siqueira and Sen 2004) and important etiological agents of human diseases, due to its ability to modulate the expression of virulence factors such as host cell adhesion, hyphae formation, phenotypic plasticity and production of hydrolytic enzymes. The genus Candida is the most widespread opportunistic fungal pathogen in the human body, causing mucosal and systemic infections, especially among immunosuppressed and hospitalized patients (Goulart et al. 2018). Due to the wide use of antifungal agents, drug resistance of *Candida albicans* is increasing, which poses a serious threat to antifungal therapy (Ksiezopolska and Gabaldõn 2018). Therefore, exploring new effective antifungal agents is urgently needed.

In this sense, natural products have been reported as sources of various bioactive molecules that may pave the way for novel antifungal agents (Ksouri et al. 2017; Zida et al. 2017). In addition, studies are carried out to promote the combination of antifungal drugs and natural products against resistant Candida

strains (Calixto Jr. et al. 2015), which can be a strongly promise to face this challenge.

Amburana cearensis (*Allemao*) *A. C. Sm.*, *Fabaceae* family (*Papilionoideae, Leguminosae*) is naturally widespread in Caatinga, northeastern Brazil (Bravo et al. 1999). The medicinal use of *A. cearensis* is common, especially in the treatment of headaches, muscle aches, constipation, and urinary tract infections (Leal et al. 2003; Agra et al. 2007; Lima et al. 2013). Several compounds with antioxidant and antimicrobial properties have been isolated from *A. cearensis* seeds, including coumarins, methyl esters, phytosterols (γ-sitosterol, stigmasterol, and campesterol) (Calixto Jr. et al. 2015; Pereira et al. 2017; Zida et al. 2017). Thus, the aqueous crude extract and fractions of *A. cearensis* seeds were subjected to chemical characterization and antifungal assessments to verify whether they present any potential to induce a synergistic effect when combined with Fluconazole against *C. albicans*.

Materials and methods

Plant material

Amburana cearensis seeds were identified and provided by Floresta Nacional de Nísia, a Conservation Unit managed by the Chico Mendes Institute for Biodiversity Conservation (ICMBio), located in the municipality of Nísia Floresta, state of the Rio Grande do Norte, Brazil, and the Caatinga Seed Network (UNIVASF).

Extraction and fractionation

The extraction was performed by homogenizing the seed powder of *A. cearensis* with 50 mM Tris–HCl solution, pH 7.5, ratio 1:10 (m/v), under constant agitation for 4 h at 4 °C and then centrifuged at 10,000×*g* for 30 min at the same temperature. The material was filtered, and the supernatant was identified as crude extract (CE). Then, CE was fractionated into three ammonium sulfate concentration ranges: 0–30% (FR-1), 30–60% (FR-2) and 60–90% (FR-3) of saturation. After each precipitation step, the sample was maintained at 4 °C for approximately 16 h and then centrifuged at 10,000×*g* for 30 min at 4 °C. The fractions were then resuspended and dialyzed for 20 h using distilled water. After dialysis, the fractions were lyophilized and stored at room temperature.

Identification of compounds by ultra-performance liquid chromatography-quadrupole\time-of-flight (UPLC–QTOF) system

The analysis was performed on ACQUITY UPLC (Waters), coupled to quadrupole/time-of-flight system (QTOF, Waters) by Brazilian Agricultural Research Corporation (EMBRAPA). The chromatographs were performed on a column Waters ACQUITY UPLC BEH (150 × 2.1 mm, 1.7 µm), fixed temperature of 40 °C, mobile water phases with 0.1% of formic acid (A) and acetonitrile with 0.1% formic acid (B), gradient ranging from 2 to 95% B (15 min), 0.4 mL/min flow and 5-µL injection volume. The ESI- mode was purchased in the 110–1180 Da, fixed source temperature at 120 °C, desolvation temperature at 350 °C, desolvation gas flow at 500 L/h, extraction cone of 0.5 V and 2.6 kV of capillary voltage. The ESI + mode was purchased in the 110–1180 Da, fixed source temperature at 120 °C, desolvation temperature at 350 °C, desolvation gas flow at 500 L/h and 3.2 kV of capillary voltage. Encephalin leucine was used as lock mass. The acquisition mode was MS^E. The instrument was controlled by the software Masslynx 4.1 (Waters Corporation).

Antifungal assays

Strains and culture medium

The strains used in this study were standard type and isolated from Cultura Oswaldo Cruz (FIOCRUZ), Instituto Nacional de Controle de Qualidade em Saúde (INCQS), specifically CA INCQS 40006 and CA URM 4125, standard and isolated strain, respectively. The strains were inoculated on Sabouraud Dextrose Agar (SDA, KASVI) and incubated for 24 h at 37 °C. Subsequently, aliquots of the yeast were transported to test tubes, each containing 3 mL of sterile saline (0.9%). Inoculum concentration was standardized by comparison with the 0.5 McFarland scale (barium chloride) (NCCLS 2012). Sabouraud Dextrose (SD, HIMEDIA) in double concentration was used in the microdilution assay.

Chemicals and reagents

Dimethyl sulfoxide (DMSO, Merck, Germany) was used to fluidize the CE and fractions, while Fluconazole (Capsule—Prati Donaduzzi, Brazil) was diluted with distilled water and used as reference drug. The test solution was prepared by weighing 0.15 g of the CE and fractions and diluting with 1 mL of DMSO to obtain the desired concentration. Samples were diluted in sterile distilled water (16.384 µg/mL) such that DMSO had no activity on the tested cells (Stoppa et al. 2009).

IC_{50} and cell viability

To determine the concentration capable of inhibiting 50% of yeast growth (IC_{50}), the CE and fractions isolated or combined with Fluconazole were tested by microdilution method in Sabouraud Dextrose broth in 96-well microplates with

initial concentration of 16.384 µg/mL, and serially diluted to a final concentration of 8 µg/mL (Javadpour et al. 1996; Morais-Braga et al. 2016). Controls were also prepared for the diluents using 0.9% sodium chloride solution instead of inoculum. All tests were performed in quadruplicates. The plates were incubated at 37 °C for 24 h and then read on a spectrophotometer ELISA (Thermoplate®) at 630 nm.

Determination of minimum fungicidal concentration (MFC)

To verify the MFC, the tip of a sterile rod was inserted into each well of the previously tested plate. After inserting into the middle of each well, the rod was taken to a Petri dish containing SD with the aid of a guide plate attached to the bottom of the plate for yeast subculture and cell viability evaluation. After 24 h of incubation, the plates were analyzed for colony formation (Ernst et al. 1999). The concentration in which there was no growth of fungal colony was considered the MFC.

Evaluation of the modifying effect on Fluconazole action

First, the intrinsic action of CE, fractions and Fluconazole on yeast growth were verified. Then, it was evaluated if the antifungal action of Fluconazole was modulated by the CE and fractions. Therefore, CE and fractions were used at sub-inhibitory concentrations (MFC/16) according to Coutinho et al. (2008) with minor modifications from Morais-Braga et al. (2016). If the CE or fractions potentiates the action of the Fluconazole, the verified effect was considered synergic type. If it interfered with the action of the Fluconazole, the verified effect was considered antagonistic type. The plates were filled with 100 µL of medium + inoculum + sample followed by microdilution with 100 µL of Fluconazole at concentration of 8192 µg/mL. The mixture was added to the first well of the plate to be subjected to serial dilutions (1:1), ranging from 8192 to 8 µg/mL. The last well was used as control. The plates were incubated at 37 °C for 24 h. The reading was performed on an ELISA spectrophotometer (Thermoplate®).

Statistical analysis

GraphPad Prism software v.5.0 was used for statistical analysis. The data obtained were verified for their normal distribution using a two-way ANOVA ($P < 0.05$; $^{*}P < 0.1$; $^{****}P < 0.0001$) and Bonferroni post hoc test. The IC_{50} values were obtained by nonlinear regression analysis with interpolation of the unknown standard curve obtained from fungal growth assays as a function of extract concentration and expressed in µg/mL.

Results

Characterization of CE and fractions from *A. cearensis* seeds

Figures 1, 2, 3 and 4 show the composition of the CE and fractions of *A. cearensis* seeds determined by LC–MS/MS–QTOF. The parameters analyzed were molecular weight, ionic mass, retention time, fragmentation pattern and compared with the available literature. In this study, we identified seven compounds in the CE (Table 1), six compounds in FR-1 (Table 2), eight compounds in FR-2 (Table 3) and five compounds in FR-3 (Table 4), varying from flavonoids, phenolic acids, flavan derivatives, organic acids and phenolic compounds. The chemical structures of the compounds are shown in Fig. 5.

In addition, it was possible to identify the presence of some organic acids such as cinnamic acid and some possible *trans*-isomers in FR-1 and FR-2 (Tables 2 and 3, respectively). We evidenced the presence of some polyphenols as 4′- or 5′-*O*-methyl-(epi)catechin I in CE, and a polyphenol monomer trigalloyl hexoside in FR-3. The presence of the flavonoid icariin glucuronide was observed in all samples, as well as dihydroxymethoxyisoflavone, 7,8,3′-trihydroxy-4′-methoxyisoflavone and trihydroxyflavanone.

Antifungal activity of CE and fractions of *A. cearensis* seeds

All samples used alone induced inhibition growth of *C. albicans* strains (Figs. 6, 7, 8, 9), and FR-3 of *A. cearensis* seeds showed the lowest IC_{50} (123.5 µg/mL) against INCQS 40.006 (Table 5). However, Fluconazole + FR-2 showed the lowest IC_{50} (2.10 and 1.81 µg/mL), indicating that the combination of antifungal drug and FR-2 has synergistic effect against *C. albicans* (Table 5). Similar effects have been evidenced for the CE and FR-1 combined with Fluconazole against URM 4127 strain. On the other hand, we evidenced antagonistic effect when CE, FR-1 and FR-3 were combined with Fluconazole (Table 5).

Fungicidal activity

The MFC for all samples tested alone was ≥ 16.384 µg/mL for INCQS 40.006. However, fluconazole + FR-2 showed the lowest MFC (128 µg/mL) against URM 4127 strain (Table 6), which indicated a synergic effect of antifungal drug and FR-2. Conversely, CE and FR-1 combined with antifungal drug showed antagonistic effect against URM 4127 strain (Table 6).

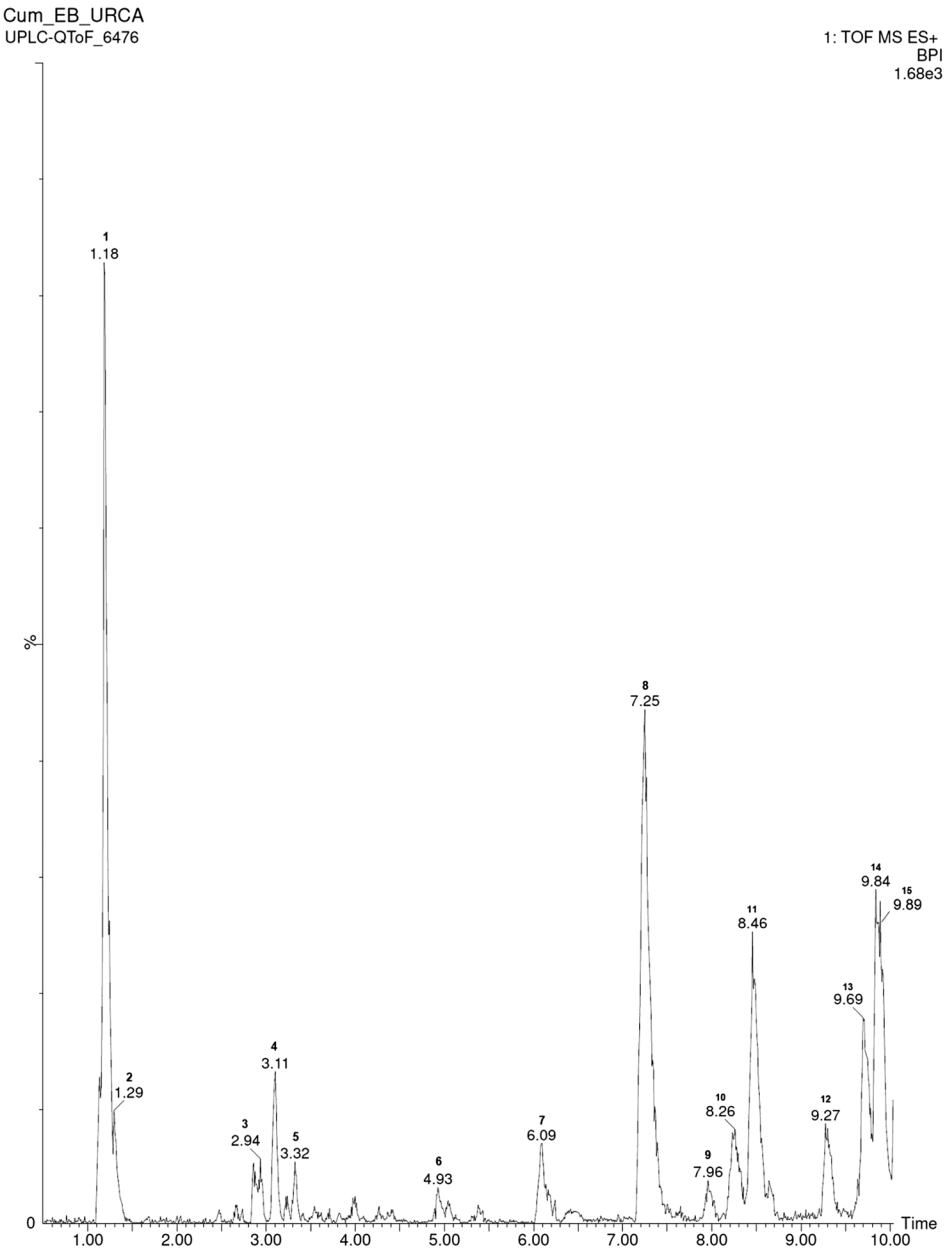

Fig. 1 Ultra-performance liquid chromatography with high-definition mass spectrometry (UPLC–MS) chromatography of CE from *A. cearensis* seeds

Discussion

Amburana cearensis characterization

Polyphenols are secondary metabolites ubiquitously distributed among plants. They are divided in phenolic compounds, phenolic acids (cinnamic acid derivatives) and glycosidic phenylpropanoid esters (Ferreira et al. 2010). Phenolic compounds were previously reported as having antimicrobial and anti-inflammatory activities (Klančnik et al. 2010).

In addition, they exhibit antioxidant properties (Moure et al. 2001; Giada 2013; Flores et al. 2013), due to hydroxyls attached to aromatic rings. These molecules include flavonoids, phenolic acids, tannins, and tocopherols (Soares 2002; Angelo and Jorge 2007) that act as effective scavengers of reactive oxygen species, and chelating Fe^{3+} that catalyze lipid peroxidation (Andrade et al. 2007).

On the other hand, flavonoids represent a group of plant pigments abundantly distributed in nature. Its presence in plants may have a relationship with defensive ability, such as protection against UV radiation, antifungal and antibacterial actions, as well as attracting pollinators (Lavola 1998). These compounds have two aromatic rings connected by a bridge of three carbon atoms ($C_6C_3C_6$) (Simões et al. 2007).

Icariin glucuronide is categorized as a flavonol, one of the subclasses of flavonoid, which have ability to induce bone

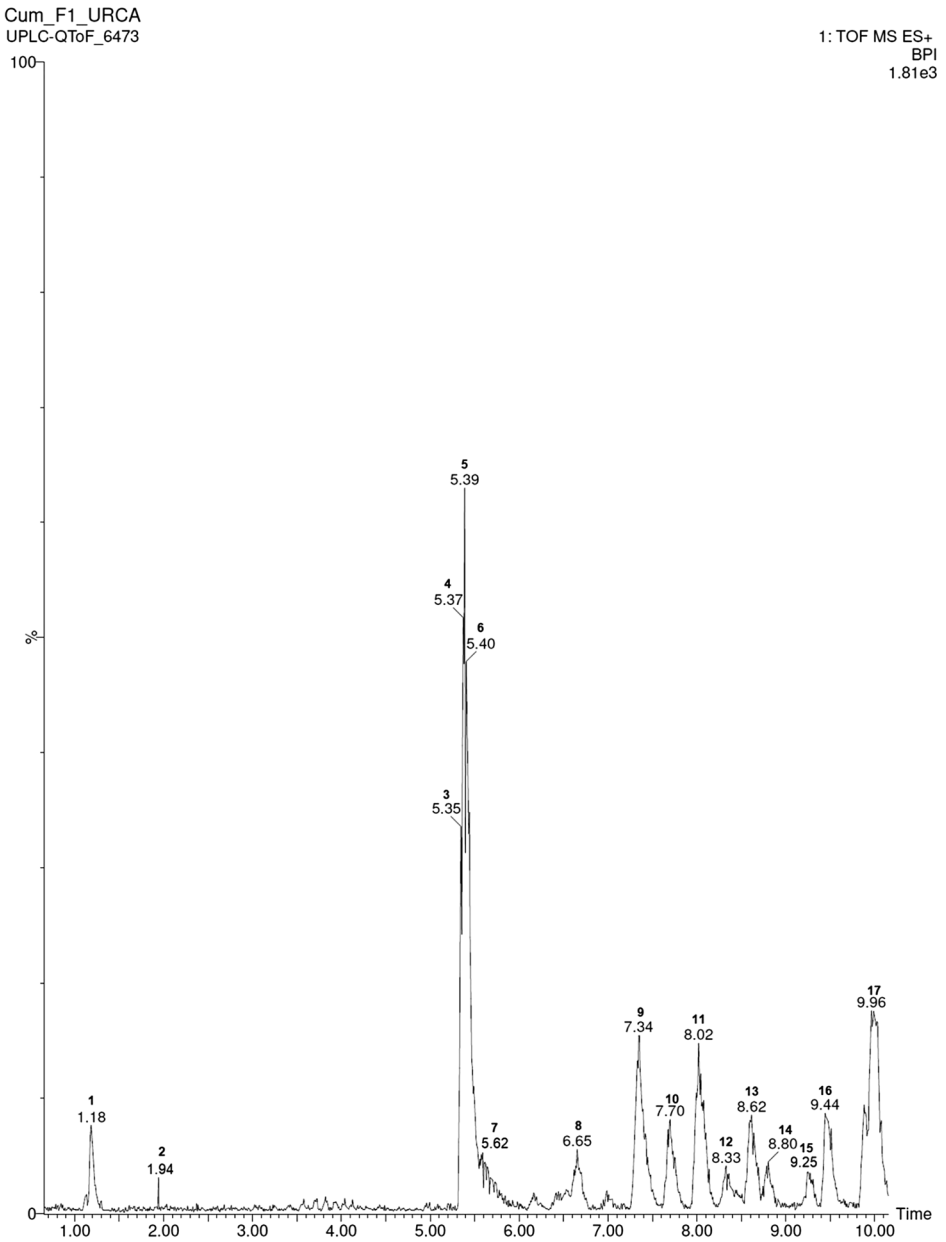

Fig. 2 Ultra-performance liquid chromatography with high-definition mass spectrometry (UPLC–MS) chromatography of FR-1 from *A. cearensis* seeds

tissue repair (Wei et al. 2011), human stem cell proliferation (Fan et al. 2011), immunoregulation (Kim et al. 2001), increases of cGMP in smooth cavernous muscle cells (Ning et al. 2006), increases of nitric oxide (Bin and Huang 2007), and mimics testosterone's effects (Zhang and Yang 2006).

Cinnamic acid is a naturally occurring aromatic carboxylic acid whose molecular structure is usually presented in *trans*-form. It is part of the auxin group, plant hormones that regulate cell growth and differentiation, and some of its derivatives play an important role in plant defense against pest attack (Niero 2010). It should be noted that its derivatives exhibit antioxidant (Hussain et al. 2014), antibacterial (Chiriac et al. 2005), anticancer (Ekmekcioglu et al. 1998), antifungal (Sadeghi et al. 2013), antitumor (Lee et al. 2003) and phytotoxic effects (Nishikawa et al. 2013).

Coumarins are derived from phenylalanine metabolism, being one of the early precursors of p-hydroxycinnamic acid (p-coumaric acid), which is hydroxylated at the C-2′ (ortho-hydroxylation). The ortho-hydroxylated derivative undergoes a photocatalyzed double-bond (EZ) isomerization. The Z isomer spontaneously lactonizes, producing umbelliferone. Prenylation of the benzene ring at positions 6 or 8 of the 7-hydroxy coumarin derivative is the first step in furan- and pyranocoumarin biogenesis. Cyclization of 6- or 8-isoprenylcoumarin derivatives occurs by nucleophilic attack of the C-7 hydroxyl group to the epoxide formed by oxidation of

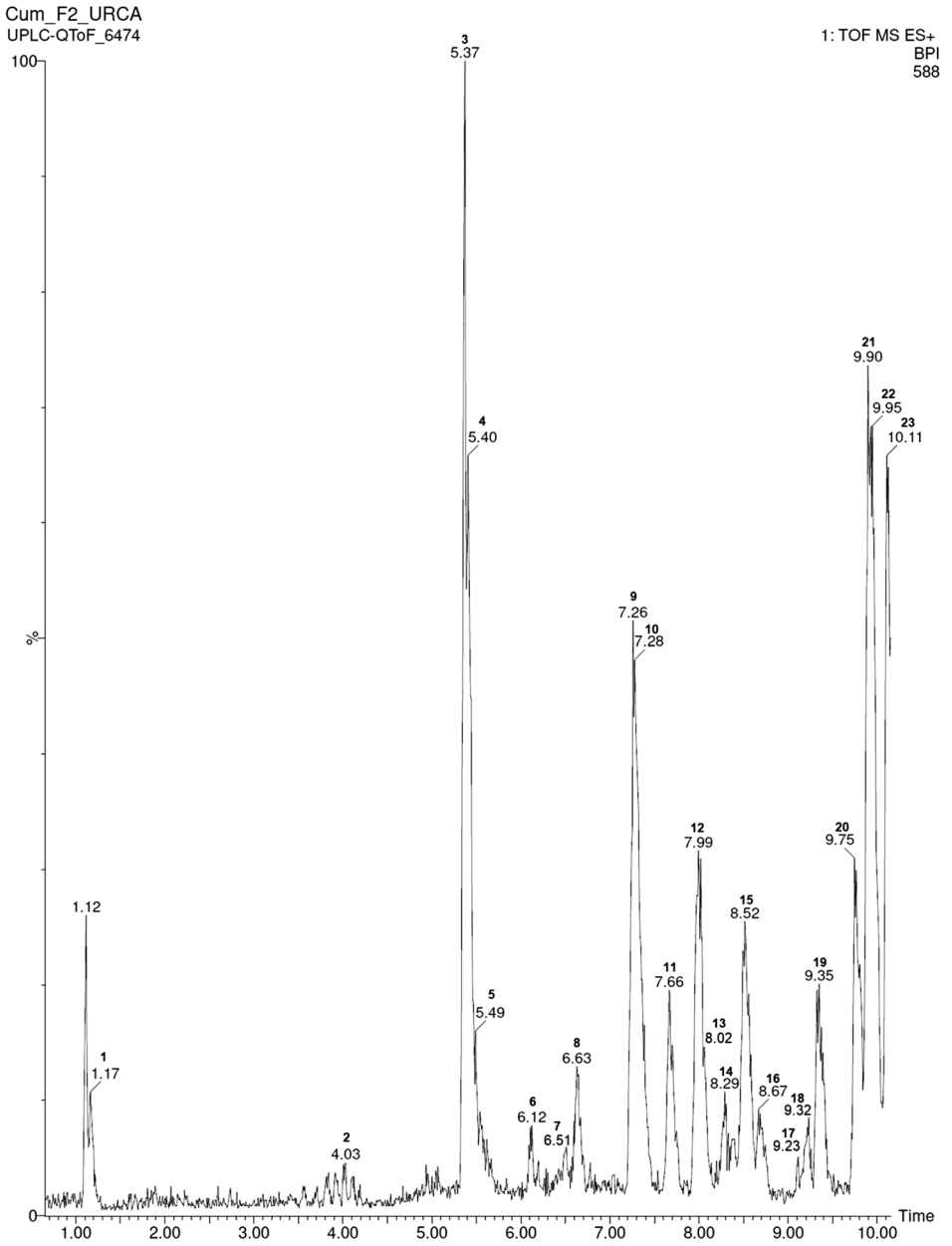

Fig. 3 Ultra-performance liquid chromatography with high-definition mass spectrometry (UPLC–MS) chromatography of FR-2 from *A. cearensis* seeds

the double bond of the isopentenyl residue. Based on the orientation of the nucleophilic attack, the product will be hydroxyisopropyl dihydrofuranocoumarin or hydroxy dimethyl dihydropyranocoumarin (Bourgaud et al. 2006).

Most coumarins are biogenetically derived from the shikimic acid pathway, but a significant number of them appear to derive from a mixed pathway (shikimic acid and acetate) such as phenylcoumarin. 4-*n*-Propylcoumarin, e.g., are entirely derived from the acetate pathway (Strack 1997). Coumarin synthesis can be induced by a response to biotic and abiotic stress, nutritional deficiency, chemical messengers such as plant hormones and other external metabolites (Cabello-Hurtado et al. 1998; Dewick 2009; Bosqueiro 1996; Haida et al. 2007). Coumarins have a variety of pharmacological properties, especially anti-inflammatory, antioxidant, antibacterial, antiviral, antithrombotic, antimutagenic and antihypertensive activities (Hoult and Payá 1996).

Characterization studies performed on stem bark extracts of *A. cearensis*, evidenced coumarin (Carvalho 1994), responsible for its characteristic odor, isokaempferide (Maia 2004), kaempferol and afromosin, amburoside A (Leal et al. 1997) and B (Leal et al. 2003), vanillic acid (Bravo et al. 1999) and protocatechuic acid (Canuto et al. 2004), and large amount of sucrose. In addition, other studies show that coumarin (Carvalho 1994), isokaempferide (Maia 2004) and amburoside A (Leal et al. 1997) have anti-inflammatory,

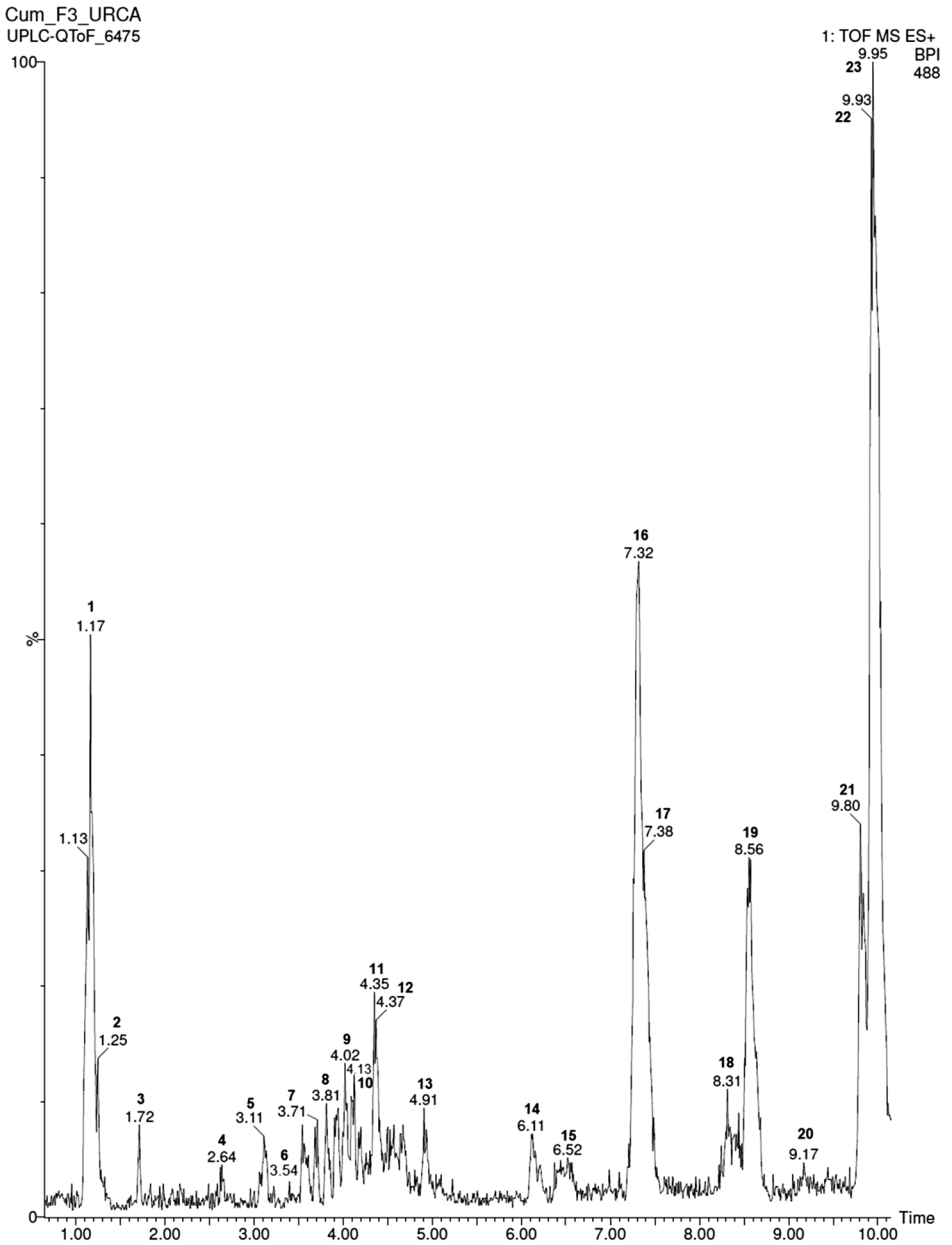

Fig. 4 Ultra-performance liquid chromatography with high-definition mass spectrometry (UPLC–MS) chromatography of FR-3 from *A. cearensis* seeds

antioxidant, and bronchodilator effects (Leal et al. 2008). Of which some coincide with precursors of those found in our study. However, it should be noted that some identified compounds in this study are not yet reported for its biological activity.

Antifungal, fungicidal, and modulating antifungal activity

It is noteworthy that the indiscriminate and increasing use of azole antifungal drugs, both to prevent and treat active infections, has increased the prevalence of Fluconazole-resistant strains of Candida (Day et al. 2013). In United States, an annual increase of > 3400 cases of antifungal resistance were identified, as reported by the Center for Disease Control and Prevention, thus making fluconazole-resistant Candida a serious threat (Gajdács 2019).

Fluconazole acts by preventing the biosynthesis pathway of the main constituent of the fungal cell membrane, ergosterol. It inhibits the enzyme lanosterol 14-α demethylase in the fungal cytochrome P-450 enzyme system, which is encoded by the *ERG11* gene. Thus, lanosterol cannot be converted to ergosterol and consequently the accumulation of precursors occurs causing fungal membrane instability (Carrillo-Muñoz et al. 2006; Menozzi et al. 2017).

Table 1 Compounds identified by UPLC-ESI-TOFMS/MS in positive mode in the CE of *A. cearensis* seeds

Peak no.	Retention time (min)	$[M-H]^-$ observed	$[M-H]^-$ calculated	Product ions (MS/MS)	Empirical formula	Parts per million (error)	Putative name	References
1	1.18	689.2051	689.2082	689.2087, 367.1008, 352.8637	$C_{33}H_{37}O_{16}$	4.5	Icariin glucuronide	Shunjun et al. (2017)
2	1.29	527.1564	527.1553	202.1848, 184.9314, 178.0209	$C_{27}H_{27}O_{11}$	2.1	QQCA (quinic-quinic-caffeic acid ester)	Plazonić et al. (2009)
3	2.94	467.1732	467.1706	329.0866, 261.0790, 179.0338, 137.0256	$C_{21}H_{23}O_{12}$	2.2	(Epi)gallocatechin hexose II	Ibrahim et al. 2014
4	3.11	305.0735	305.0720	179.0339, 167.0341, 137.0252, 125.0241	$C_{15}H_{13}O_7$	4.9	Gallocatechin	Ibrahim et al. (2014)
5	3.32	172.0376	172.0372	242.9876, 144.0423, 116.0501	$C_6H_2N_7$	2.3	No identified	–
6	4.93	218.2143	218.2120	177.0513, 145. 0170, 225.9959	$C_{12}H_{28}NO_2$	10.5	No identified	–
7	6.09	246.2422	246.2433	258.8799, 184.0619, 156.0619	$C_{14}H_{32}NO_2$	4.5	No identified	–
8	7.25	247.2733	247.2746	275.2783, 256.2661, 230.2536	$C_{16}H_{36}NO_2$	4.7	No identified	–
9	7.96	283.1002	283.1029	295.0207, 240.0207, 133.0802	$C_{16}H_{12}O_5$	9.5	Dihydroxymethoxyisoflavone	Oliveira et al. (2017)
10	8.26	119.0829	119.0821	437.2041, 185.1608, 120.0852	$C_4H_{11}N_2O_2$	6.7	No identified	–
11	8.46	303.0885	303.0874	137.0244	$C_{16}H_{15}O_6$	3.6	4′- or 5′-*O*-Methyl-(epi)catechin I	Ibrahim et al. (2014)
12	9.27	393.3636	393.3639	334.3075, 249.1913,133.0866	$C_{13}H_{41}N_{14}$	0.8	No identified	–
13	9.69	332.3412	332.28	330.3311, 270.9351, 150.7104		1.0	Gallic-caffeic acid ester	Plazonić et al. (2009)
14	9.84	457.2762	457.2743	155.0720, 458.2881	$C_{31}H_{37}O_3$	4.2	No identified	–
15	9.89	457.2829	457.2801	155.0675, 458.2868	$C_{24}H_{41}O_8$	6.1	No identified	–

Alternatives for treating Candida infections are considered limited and many existing antifungal drugs have undesirable side effects such as high toxicity, especially in immunosuppressed individuals (Fica 2004). For these patients, antifungal therapies are a challenging situation, which represent an urgent need to research and develop novel antifungal drugs more effective and less toxic (Calabrese et al. 2013).

Bravo et al. (1999) already shown that the main component of *A. cearensis* was coumarin, mainly responsible for bronchodilator activity. It also highlights the presence of phenolic glycosides that presented antimalarial, antiprotozoal, antifungal, and antibacterial activity in vitro. In addition, Salas et al. (2011) using natural and enzymatically modified flavonoids from plants against Penicillium, Aspergillus and Fusarium species evidenced antifungal activity. However, Steiner et al. (2008) showed that antifungal activity induced by isoflavone, especially genistein, demonstrated a better effect, but without a clear mechanism of action.

The combined use of drugs that can achieve synergistic results, showing increased of therapeutic effect, decreased minimal inhibitory concentration, regression of antibiotic resistance development, and a decrease in host toxicity has become increasingly studied as an alternative for the treatment of infections (Silva et al. 2015).

Some phenolic compounds such as gallic acid, catechin, luteolin and quercetin demonstrated in vitro antifungal activity against different Candida species, including *C. albicans* and *C. tropicalis* (Alves et al. 2014), corroborating with this study. In addition, Silva et al. (2014) evaluated the flavonoid combined with Fluconazole in in vitro microdilution against Fluconazole-resistant *C. tropicalis*, and observed a

Table 2 Compounds identified by UPLC–ESI–TOFMS/MS in positive mode in the FR-1 of *A. cearensis* seeds

Peak no.	Retention time (min)	$[M-H]^-$ observed	$[M-H]^-$ calculated	Product ions (MS/MS)	Empirical formula	Parts per million (error)	Putative name	References
1	1.18	689.2130	689.2117	689.2087, 367.1008, 352.8637	$C_{33}H_{37}O_{16}$	4.5	Icariin glucuronide	Shunjun et al. (2017)
2	1.94	453.7779	453.7796	454.7751, 451.7924, 449.7784	$C_6NO_{11}S_6$	3.7	No identified	–
3	5.35	147.0405	147.0446	147.0417, 62.1057	$C_9H_8O_2$	−27.0	*Trans*-cinnamic acid	Ibrahim et al. (2014)
4	5.37	147.0408	147.0446	147.0417, 62.1057	$C_9H_8O_2$	−25.0	Possible isomer *trans*-cinnamic acid	Ibrahim et al. (2014)
5	5.39	147.0407	147.0446	147.0417, 62.1057	$C_9H_8O_2$		Possible isomer *trans*-cinnamic acid	Ibrahim et al. (2014)
6	5.40	147.0417	147.0446	147.0417, 62.1057	$C_9H_8O_2$	−19.0	Possible isomer *trans*-cinnamic acid	Ibrahim et al. (2014)
7	5.62	147.0417	147.0446	147.0417, 62.1057	$C_9H_8O_2$	−19.0	Possible isomer *trans*-cinnamic acid	Ibrahim et al. (2014)
8	6.65	299.0892	299.0919	284.0323,256.0673,184.0652	$C_{17}H_{15}O_5$	9.0	7,8,3′-Trihydroxy-4′-methoxyisoflavone	Oliveira et al. (2017)
9	7.34	274.2764	274.2746	318.3075, 274.2844, 256.2657	$C_{16}H_{36}NO_2$	6.6	No identified	–
10	7.70	313.1020	313.1017	269.0793, 252.0761, 184.0693	$C_{18}H_{17}O_5$	1.0	7,3′-Dihydroxy-8,4′-dimethoxyisoflavone	Oliveira et al. (2017)
11	8.02	283.0938	283.0970	240.0811, 211.0820, 197.0556	$C_{17}H_{15}O_4$	11.3	Dihydroxymethoxyisoflavone	Oliveira et al. (2017)
12	8.33	119.0855	119.0861	113.0861, 117.1045	C_9H_{11}	−5.0	No identified	–
13	8.62	302.3014	302.3059	184.0782, 177.1111, 113.0846	$C_{18}H_{40}NO_2$	−6.0	No identified	–
14	8.80	391.3424	391.3424	331.2966, 177.1157, 133.0889	$C_{22}H_{47}O_5$	0.0	No identified	–
15	9.25	323.2748	323.2797	373.1831, 339.2472,133.0887	$C_{17}H_{39}O_5$	−15.2	No identified	–
16	9.44	393.3588	393.3588	393.3560, 334.3107, 177.1114	$C_{22}H_{49}O_5$	2.0	No identified	–
17	9.96	330.3365	330.3372	258.0484, 312.3296, 331.3336	$C_{20}H_{44}NO_2$	−2.1	No identified	–

Table 3 Compounds identified by UPLC–ESI–TOFMS/MS in positive mode in the FR-2 of *A. cearensis* seeds

Peak no	Retention time (min)	$[M-H]^-$ observed	$[M-H]^-$ calculated	Product ions (MS/MS)	Empirical formula	Parts per million (error)	Putative name	References
1	1.17	689.2051	689.2082	689.2087, 367.1008, 352.8637	$C_{33}H_{37}O_{16}$	4.5	Icariin glucuronide	Shunjun et al. (2017)
2	4.03	591.3693	591.3686	401.1527, 172.8673, 133.0836	$C_{37}H_{51}O_6$	1.2	No identified	–
3	5.37	147.0419	147.0446	147.0422, 62.2985	$C_9H_7O_2$	− 18.4	*Trans*-cinnamic acid	Ibrahim et al. (2014)
4	5.40	147.0412	147.0446	147.0422, 62.2985	$C_9H_7O_2$	− 23.1	Possible isomer *trans*-cinnamic acid	Ibrahim et al. (2014)
5	5.49	147.0408	147.0446	147.0422, 62.2985	$C_9H_7O_2$	− 23.1	Possible isomer *trans*-cinnamic acid	Ibrahim et al. (2014)
6	6.12	246.2432	246.2433	247.4212, 184.0706, 156.0435	$C_{14}H_{32}NO_2$	− 0.4	No identified	Ibrahim et al. (2014)
7	6.51	285.2693	285.2700	269.7, 163.8, 150.5	$C_{15}H_{10}O_6$	− 2.5	Hydroxygenistein	Schmeda-Hirschmann et al. (2019)
8	6.63	299.0910	299.0919	283.7, 230.7	$C_{17}H_{15}O_5$	− 3.0	Methoxy trihydroxy flavanone	Schmeda-Hirschmann et al. (2019)
9	7.26	274.2721	274.2719	275.2858, 256.2728, 184.0647	$C_{12}H_{32}N_7$	0.7	No identified	–
10	7.28	274.2730	274.2719	275.2858, 256.2728, 184.0647	$C_{12}H_{32}N_7$	4.0	No identified	–
11	7.66	313.1066	313.1076	269.0793, 252.0761, 184.0693	$C_{18}H_{17}O_5$	− 3.2	7,3′-Dihydroxy-8,4′-dimethoxyisoflavone	Oliveira et al. (2017)
12	7.99	283.0953	283.0970	240.0811, 211.0820, 197.0556	$C_{17}H_{15}O_4$	− 6.0	Dihydroxymethoxyisoflavone	Oliveira et al. (2017)
13	8.02	283.0921	283.0970	240.0811, 211.0820, 197.0556	$C_{17}H_{15}O_4$	− 17.3	Dihydroxymethoxyisoflavone possible isomer	Oliveira et al. (2017)
14	8.29	119.0850	119.0861	113.0861, 117.1045	C_9H_{11}	− 9.2	No identified	–
15	8.52	302.3076	302.3059	184.0782, 177.1111, 113.0846	$C_{18}H_{40}NO_2$	5.6	No identified	–
16	8.67	391.3500	391.3509	184.0709, 177.1171	$C_{17}H_{43}N_8O_2$	− 2.3	No identified	–
17	9.23	323.2783	323.2797	373.1831, 339.2472,133.0887	$C_{17}H_{39}O_5$	− 4.3	No identified	–
18	9.32	393.3557	393.3580	393.3560, 334.3107, 177.1114	$C_{22}H_{49}O_5$	− 5.8	No identified	–
19	9.35	393.3589	393.3580	393.3560, 334.3107, 177.1114	$C_{22}H_{49}O_5$	2.3	No identified	–
20	9.75	330.3365	330.3372	258.0484, 312.3296, 331.3336	$C_{20}H_{44}NO_2$	− 2.1	No identified	–
21	9.90	457.2744	457.2743	155.0720, 458.2881	$C_{31}H_{37}O_3$	0.2	No identified	–
22	9.95	457.2778	457.2743	155.0720, 458.2881	$C_{31}H_{37}O_3$	7.7	No identified	–
23	10.11	395.3740	395.3750	335.3429, 184.0704, 133.0864	$C_{23}H_{47}N_4O$	− 2.5	No identified	–

Table 4 Compounds identified by UPLC–ESI–TOFMS/MS in positive mode in the FR-3 of *A. cearensis* seeds

Peak no	Retention time (min)	$[M-H]^-$ observed	$[M-H]^-$ calculated	Product ions (MS/MS)	Empirical formula	Parts per million (error)	Putative name	References
1	1.17	689.2147	689.2140	689.2087, 367.1008, 352.8637	$C_{33}H_{37}O_{16}$	1.0	Icariin glucuronide	Shunjun et al. (2017)
2	1.25	136.0607	136.0610	152.0563, 136.0607	$C_4H_{10}NO_4$	−2.2	No identified	–
3	1.72	136.0612	136.0610	152.0563, 136.0607	$C_4H_{10}NO_4$	1.5	No identified	–
4	2.64	172.0363	172.0372	242.9876, 144.0423, 116.0501	$C_6H_2N_7$	−5.2	No identified	–
5	3.11	433.1133	433.1135	300.9686	$C_{21}H_{21}O_{10}$	−0.5	Ellagic acid pentoside 1	Schmeda-Hirschmann et al. (2019)
6	3.54	287.0533	287.0556	287.0538, 241.0332, 175.0282	$C_{15}H_{11}O_6$	8.0	Luteolin	Ibrahim et al. (2014)
7	3.71	133.0901	133.0865	207.0626, 151.0330, 133.0813	$C_6H_{13}O_3$	2.7	No identified	–
8	3.81	133.0860	133.0865	207.0626, 151.0330, 133.0813	$C_6H_{13}O_3$	−3.5	No identified	–
9	4.02	591.3591	591.3592	207,0608, 151.0291 133.0836	$C_{26}H_{55}O_{14}$	−0.2	No identified	–
10	4.13	635.3927	635.3927	465.0265	$C_{39}H_{55}O_7$	−3.3	Trigalloyl hexoside	Schmeda-Hirschmann et al. (2019)
11	4.35	207.0683	207.0657	369.1195, 207.0621, 175.0858	$C_{11}H_{11}O_4$	12.6	No identified	–
12	4.37	207.0658	207.0657	369.1195, 207.0621, 175.0858	$C_{11}H_{11}O_4$	0.5	No identified	–
13	4.91	207.0661	207.0657	240.0811, 211.0820, 197.0556	$C_{11}H_{11}O_4$	1.9	No identified	–
14	6.11	246.2453	246.2433	258.8799, 184.0619, 156.0619	$C_{14}H_{32}NO_2$	8.1	No identified	–
15	6.52	283.0364	283.1029	295.0207, 240.0207, 133.0802	$C_{16}H_{12}O_5$	9.5	Dihydroxymethoxyisoflavone	Oliveira et al. (2017)
16	7.32	274.2742	274.2746	318.3075, 274.2844, 256.2657	$C_{16}H_{36}NO_2$	−1.5	No identified	–
17	7.38	318.2995	318.2981	274.2706. 256.2648	$C_{14}H_{36}N_7O$	4.4	No identified	–
18	8.31	119.0870	119.0821	437.2041, 185.1608, 120.0852	$C_4H_{11}N_2O_2$	6.7	No identified	–
19	8.56	302.3055	302.3059	184.0782, 177.1111, 113.0846	$C_{18}H_{40}NO_2$	−1.3	No identified	–

Table 4 (continued)

Peak no	Retention time (min)	$[M-H]^-$ observed	$[M-H]^-$ calculated	Product ions (MS/MS)	Empirical formula	Parts per million (error)	Putative name	References
20	9.17	304.3001	304.3004	212.2469, 133.0871	$C_{21}H_{38}N$	−1.0	No identified	–
21	9.80	330.3346	330.3372	258.0484, 312.3296, 331.3336	$C_{20}H_{44}NO_2$	−2.1	No identified	–
22	9.93	457.2736	457.2743	155.0720, 458.2881	$C_{31}H_{37}O_3$	−1.5	No identified	–
23	9.95	457.2711	457.2743	155.0720, 458.2881	$C_{31}H_{37}O_3$	−7.0	No identified	

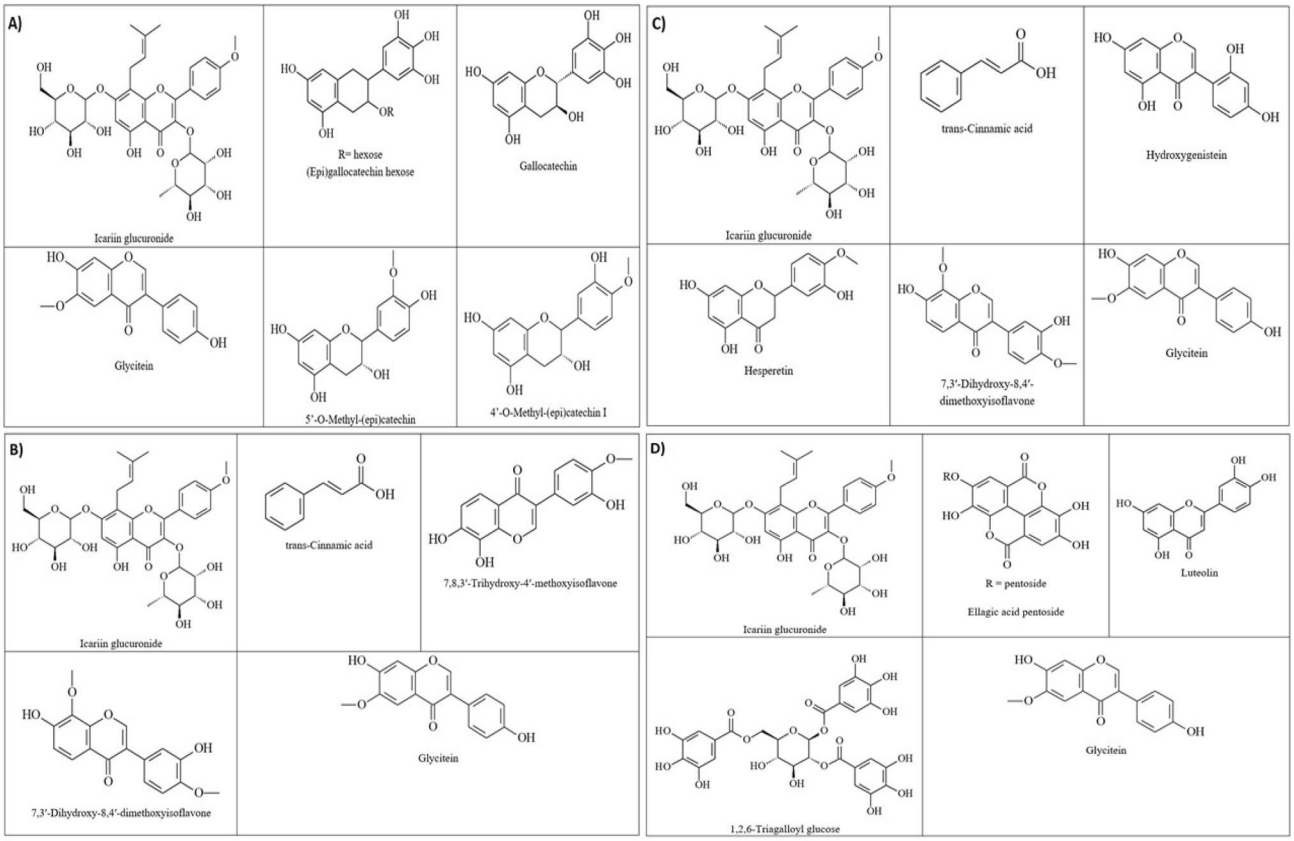

Fig. 5 Main compounds identified in CE, FR-1, FR-2 and FR-3 of *A. cearensis* seeds. **a** aqueous crude extract; **b** fraction 1; **c** fraction 2; and **d** fraction 3

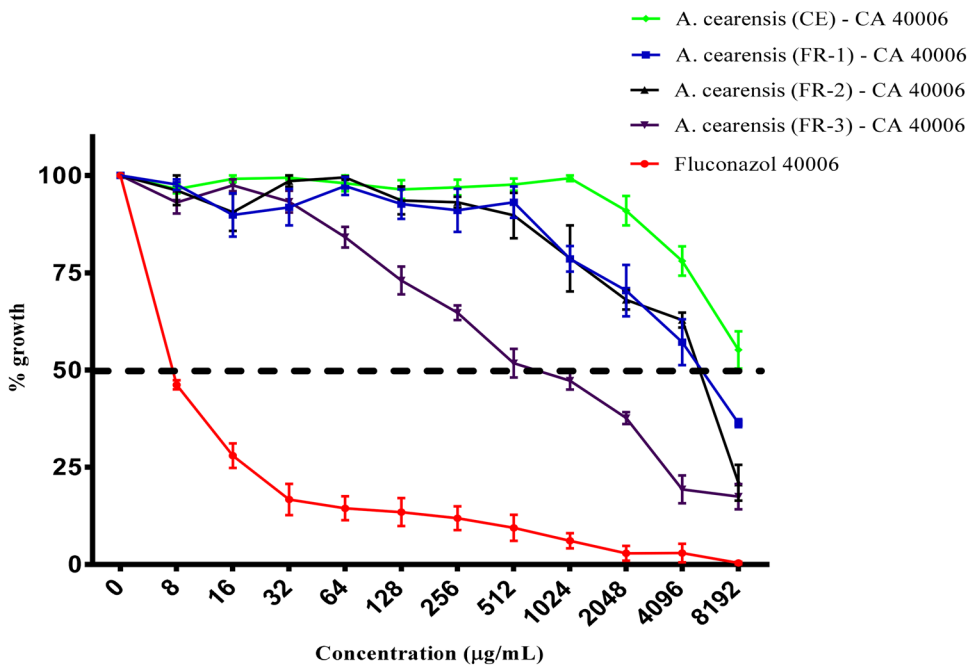

Fig. 6 Antifungal effects (µg/mL) of aqueous crude extract and fractions of *A. cearensis* seeds against *C. albicans* 40006. Fluconazole was used as a control against *C. albicans*

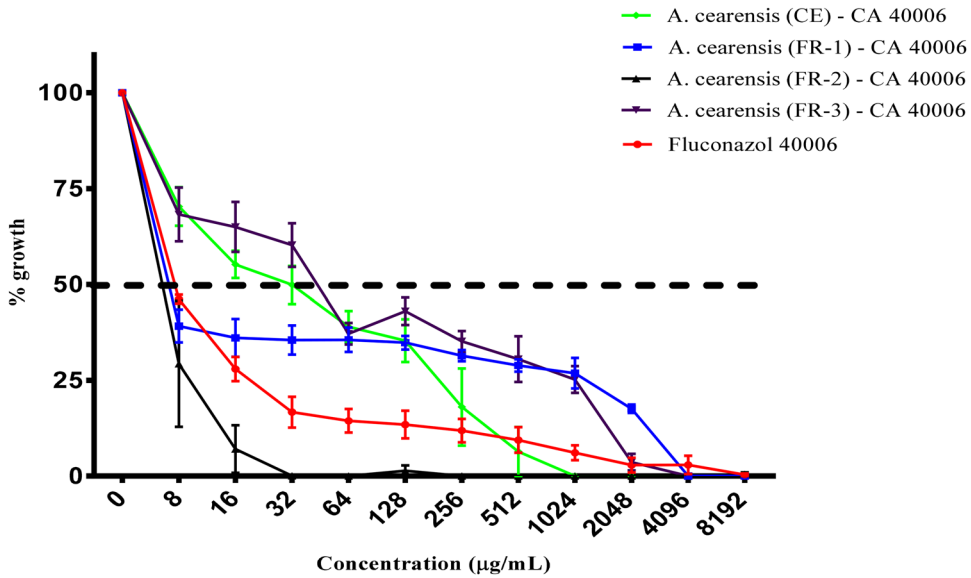

Fig. 7 Antifungal effects (µg/mL) of aqueous crude extract and fractions of *A. cearensis* seeds combined with fluconazole against *C. albicans* 40006. Fluconazole was also used as a control against *C. albicans*

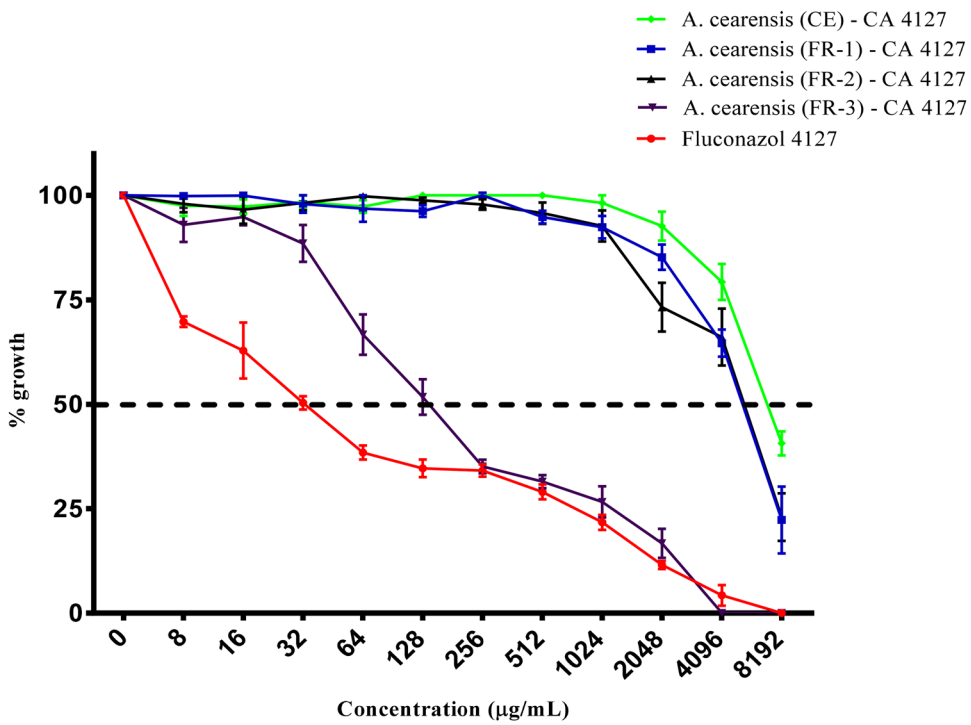

Fig. 8 Antifungal effects (µg/mL) of aqueous crude extract and fractions of *A. cearensis* seeds against *C. albicans* 4127. Fluconazole was used as a control against *C. albicans*

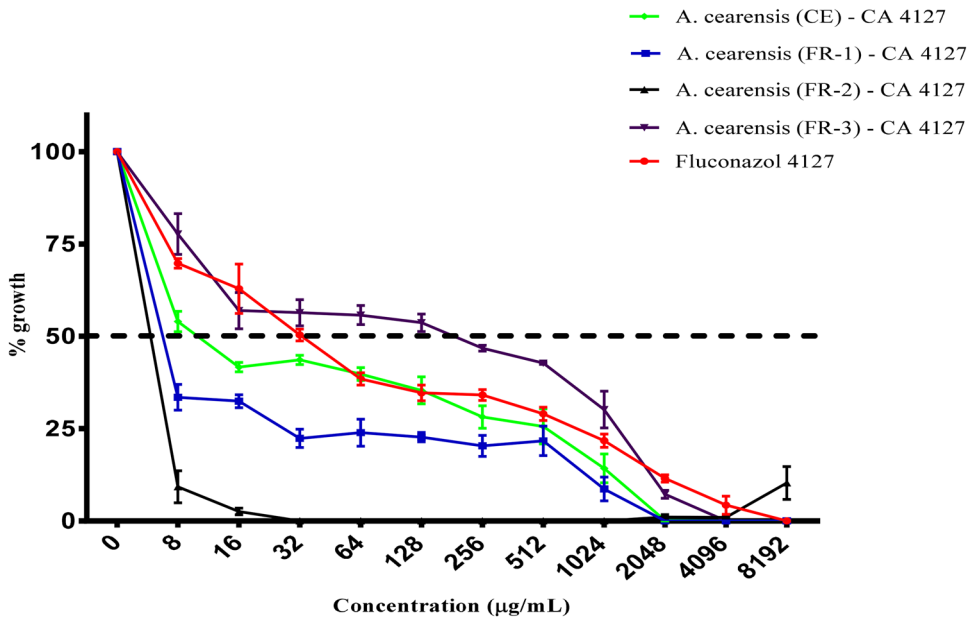

Fig. 9 Antifungal effects (µg/mL) of aqueous crude extract and fractions of *A. cearensis* seeds combined with fluconazole against *C. albicans* 4127. Fluconazole was also used as a control against *C. albicans*

Table 5 IC_{50} (µg/mL) of CE and fractions from *A. cearensis* seeds against *C. albicans strains*

Evaluated combination	CA INCQS 40006	CA URM 4127
FCZ	2.4 ± 1.2	7.68 ± 4.8
CE—*A. cearensis*	> 2048 ± 3.6	> 2048 ± 3.2
FR-1—*A. cearensis*	1968.6 ± 9.5	1138.4 ± 4.8
FR-2—*A. cearensis*	1173.3 ± 8.1	1273.3 ± 6.3
FR-3—*A. cearensis*	123.5 ± 5.5	45.6 ± 6.1
CE—*A. cearensis* + FCZ	9.54 ± 4.0	3.14 ± 2.0
FR-1—*A. cearensis* + FCZ	3.51 ± 2.5	2.41 ± 1.8
FR-2—*A. cearensis* + FCZ	2.1 ± 0.99	1.81 ± 0.8
FR-3—*A. cearensis* + FCZ	11.9 ± 8.7	18.8 ± 7.4

CA Candida albicans, *FCZ* fluconazole, *CE* aqueous crude extract, *FR-1* fraction 1, *FR-2* fraction 2, *FR-3* fraction 3, *MIC* minimum inhibitory concentration, *INCQS* National Institute of Health Control, *URM* University Recife Mycology—$P \leq 0.05$ in relation to FCZ control

Table 6 Minimal fungicidal concentrations (MFC) in µg/mL to fluconazole (FCZ) alone and combined with CE and fractions from *A. cearensis* seeds

	CA URM 4127
Fluconazole	2048
A. cearensis CE + FCZ	2048
A. cearensis FR-1 + FCZ	8192
A. cearensis FR-2 + FCZ	128
A. cearensis FR-3 + FCZ	8192

considerable synergistic effect, reducing the MICs of the flavonoids from 64 to 0.25 µg/mL, similar to our results against *C. albicans*.

Conclusion

CE, FR-1, FR-2 and FR-3 of *A. cearensis* seeds presented phenolic compounds, organic acids, and polyphenols, that contributed to its antifungal capacity and synergistic effects. Our results show that *A. cearensis* seeds can be considered as promising source of antifungal agents and modulators of antifungal activity. However, further studies are needed to identify the active compounds and to prove their efficacy, safety, and mechanism of action.

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Author contributions Methodology—antimicrobial assays (MTAO; MVOBA and GMMM); methodology—chemical analysis (PRVR; ESB and EOS); methodology—statistical analysis (IRAM; JCA and EAS); supervision o f work—(HDMC and AFU); resources (VPAL and JIOC).

Compliance with ethical standards

Ethical statements This article is according to the international, national and institutional rules considering biodiversity rights.

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