Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org
Original Research

Phytochemical characterization of the *Ziziphus joazeiro* Mart. metabolites by UPLC-QTOF and antifungal activity evaluation

Jacqueline C. Andrade[1,2], Antônia T.L. dos Santos[3], Ana R.P. da Silva[1], Maria A. Freitas[1], Muhammad Inam Afzal[4], Maria I.P. Gonçalo[2], Victor J. A. Fonseca[3], Maria do S. Costa[1], Joara N.P. Carneiro[3], Erlânio O. Sousa[1], Yedda M.L.S. de Matos[1], Thiago S. de Freitas[1], Amanda K. de Sousa[1], Maria F.B. Morais-Braga[3], Henrique D.M. Coutinho[1], Shinawar Waseem Ali[5*], Bahare Salehi[6*], Muhammad Imran[7], Muhammad Umer[4], Ehsan-ul-Haque[8], William N. Setzer[9,10], Javad Sharifi-Rad[11*]

[1] Laboratório de Microbiologia e Biologia Molecular- LMBM, Universidade Regional do Cariri- URCA, Crato (CE), Brasil
[2] Laboratório de Bioensaios – LABIO, Universidade Federal do Cariri - UFCA, Instituto de Formação de Educadores, Campus Brejo Santo (CE), Brasil
[3] Laboratório de Micologia Aplicada do Cariri – LMAC, Universidade Regional do Cariri- URCA, Crato (CE), Brasil
[4] Department of Biosciences, COMSATS University Islamabad, Park Road, Tarlai kalan, Islamabad, 45550, Pakistan
[5] Institute of Agricultural Sciences, University of the Punjab, Quaid-i-Azam Campus, Lahore-54590, Pakistan
[6] Student Research Committee, School of Medicine, Bam University of Medical Sciences, Bam, Iran
[7] University Institute of Diet and Nutritional Sciences, Faculty of Allied Health Sciences, The University of Lahore-Lahore, Pakistan
[8] CITRUS Research Institute, Sargodha-Pakistan
[9] Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL, 35899, USA
[10] Aromatic Plant Research Center, 230 N 1200 E, Suite 100, Lehi, UT, 84043, USA
[11] Phytochemistry Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[*]**Correspondence to:** shinawar.iags@pu.edu.pk; bahar.salehi007@gmail.com; javad.sharifirad@gmail.com

Received April 9, 2020; **Accepted** May 2, 2020; **Published** June 25, 2020

Doi: http://dx.doi.org/10.14715/cmb/2020.66.4.17

Abstract: The aim of this study was to evaluate the antifungal and modulatory potential of the *Ziziphus joazeiro* bark and leaf extracts, both in isolation and in association with fluconazole, against resistant species from the *Candida* genus. Antifungal assays were used to determine the half maximal inhibitory concentration (IC_{50}) of the extract in isolation and in combination with fluconazole using the broth microdilution method and spectrophotometric readings, followed by verification of the minimum fungicidal concentration by solid medium subculture. According to the cell viability curve, both extracts inhibited fungal growth in a concentration dependent manner, in addition to showing inhibitory concentrations similar to fluconazole. However, the extracts behaved in a fungistatic manner with minimum inhibitory concentration > 8.19 mg/mL and IC_{50} values ranging from 0.450 mg/mL to 9 mg/mL. The minimum inhibitory concentration for both extracts decreased when in combination with fluconazole, with the AEL standing out against *Candida albicans* URM 4387, displaying an IC_{50} equal to that of fluconazole (0.002 mg/mL). Nevertheless, fluconazole antagonism was observed against the tested strains. Overall, the evaluation of both extracts against *Candida* spp. presented inhibitory concentration values greater than fluconazole. Moreover, despite these being chemically complex crude extracts, they did demonstrate antifungal effects and properties that concur with their ethno-biological aspect.

Key words: Fungistatic; Opportunistic pathogens; Joazeiro.

Introduction

Yeasts from the *Candida* genus are present in the normal human microbiota and become pathogenic in immunocompromised patients. These are responsible for candidiasis, one of the most common opportunistic fungal infections with variable clinical conditions diagnosed as mild, acute or chronic. The genus containst roughly 200 species with *Candida albicans* being one of the most virulent, followed by other non-*albicans* species (1,2).

Fungal infection occurrence is ever more observed, causing morbidity and mortality, even when therapeutic resources exist, where this situation occurs mainly due to commercialized drug inactivity. Resistance by species from the *Candida* genus may be intrinsic or acquired with treatment, where resistance mechanisms such as biofilm, pseudo-hyphae or hyphae development, efflux pumps and mutant transcription factors contribute to the expansion of microbiological resistance against antifungal agents (3,4).

Prescribing fluconazole was the main fungal treatment of choice for a long time, where the abusive use of this drug is considered to be responsible for the increase in *Candida* species with adaptive responses against this treatment, thus reducing drug sensitivity (5). In this manner, studies focusing on the development of new substances which are effective against candidiasis are imperative.

Plant extracts, from a pharmacological perspective,

are important in the search for active principles with biological effects, which may lead to the development of new drugs. *Ziziphus joazeiro* Mart. is an endemic species from the caatinga, belonging to the Rhamnaceae family, popularly known as "Joazeiro", "Juá-babão", "Juá de Boi", "Juazeiro" and "Juá" (6,7). *Ziziphus joazeiro* is commonly used in oral hygiene and gingivitis treatment (8), in addition to having several proven biological activities such as analgesic (9), gastroprotective (10,11), antipyretic (12), antioxidant, antibacterial (13), antifungal (14) and antiparasitic (15) activities.

The aim of this study was to verify the antifungal activity of extracts in isolation and in combination with fluconazole to evaluate their modifying potential of the aforementioned antifungal against resistant species from the *Candida* genus.

Materials and Methods

Collection area and plant material

The leaves and stem bark were collected from eight *Ziziphus joareizo* Mart. specimens located in the Sítio Ipueiras in the rural area of the Brejo Santo municipality, south of Ceará, Brazil, at the foot of the Chapada do Araripe (elevation and geographical coordinates, south latitude and west longitude of Greenwich:
1: 442m, 07º28′54.4″S/39º01′47.2″W;
2: 431m, 07º28′53.3″S/39º01′46.1″W;
3: 436m, 07º28′50.5″S/39º01′57.6″W;
4: 440m, 07º28′42.8″S/39º02′10.2″W;
5: 447m, 07º28′48.5″S/39º02′12.0″W;
6: 441m, 07º28′51.4″S/39º02′16.0″W;
7: 439m, 07º28′54.6″S/39º02′07.6″W;
8: 436m, 07º28′58.6″S/39º01′48.8″W).

The exsiccate produced was deposited in the Herbarium Dárdano de Andrade Lima of the Regional University of Cariri - URCA under n° 13.346 and identified as *Ziziphus joazeiro* Mart. Material collection took place during the month of February in 2017, from 7:30 to 9:00 in the morning. The plant material was sent to the laboratory, cleaned and weighed. Plant material colletion was authorized by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) with the SISBIO number 55064, registered on the SISGEN system (SISGEN No. A9250A4).

Extract preparation

The *Z. joazeiro* leaf and stem bark aqueous extracts (AEL and AEB, respectively) were prepared by cold extraction maceration (16). Fresh leaves were cut to increase their surface area, while the stem barks were dried at room temperature and ground in a mechanical mill. Subsequently, both were added to distilled sterile water and kept in a container protected from light and air. After 72 hours, the extracts were filtered, frozen and taken to a lyophilizer (-60 °C) producing crude extracts of 39.9 g and 111.58 g, respectively.

Qualitative Phytochemical Prospection

Phytochemical prospection aimed to identify the presence of secondary metabolites in the *Z. joazeiro* extract. To this end, the methodology proposed by Matos (17), which detects the presence of steroids, quinones, organic acids, triterpenes, coumarins and alkaloids, was used. The test is based on visual colorimetric observations or precipitate formations after the addition of specific reagents and induction of pH and temperature variations.

Compound identification through Ultra-efficient Liquid Chromatography coupled to Quadrupole/Time of Flight (UPLC-QTOF)

Identification of the compounds present in the extracts was performed in an Acquity® UPLC system coupled to a Quadrupole/Time of Flight (QTOF) system (Waters Corporation, Milford, USA) kindly provided by the Chemistry and Natural Products Laboratory, Embrapa Tropical Agroindustry (Fortaleza, Ceará). Chromatographic runs were performed on Waters Acquity UPLC BEH columns (150 × 2.1 mm; 1.7 µm) with 40°C fixed temperature and mobile phases: water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B), using a gradient ranging from 2% to 95% B (15 min) at a 0.4 mL/min flow rate and 5 µl injection volume. The ESI mode was acquired in the 110-1180 Da range with 120 °C fixed source temperature, 350 °C desolvation temperature, 500 L/h desolvation gas flow, 0.5 V extraction cone and 2.6 kV capillary voltage. The ESI+ mode was acquired in the 110-1180 Da range with 120 °C fixed source temperature, 350 °C desolvation temperature, 500 L/h desolvation gas flow and 3.2 kV capillary voltage. Leucine enkephalin was used as the lock mass. MSE (high energy mass spectrometry) was the mode of acquisition used. The instrument was controlled by the Masslynx® 4.1 software (Waters Corporation, Milford, USA).

Antifungal assays

The assayed fungal strains, methodology, culture reagents, maintenance, viability curve and IC_{50} determinations, as well as fluconazole activity modulation by the AEL and AEB were performed as previously described (18-24).

Statistical analysis

The data obtained were checked for their normal distribution and analyzed using a Two-Way ANOVA (*P<0.05 and ****P<0.0001) comparing the values for each concentration of the extract, point by point, with Bonferroni's post hoc test. The IC_{50} values were obtained by non-linear regression analysis with interpolation of standard curve unknowns obtained from fungal growth assays as a function of the extract concentration and expressed in µg/mL. For statistical analysis, the software GraphPad Prism, v.5.0 was used.

Results

Phytochemical characterization of the *Ziziphus joazeiro* aqueous extracts showed the presence of secondary metabolite classes such as phenols, flavonoids and triterpenoids (Table 1).

In studies performed with UPLC-ESI-QTOF-MS in the negative ionic mode, ten flavonoids (catechin, myricetin-*O*-rutinoside, myricetin-*O*-hexoside, rutin, quercetin-*O*-glucoside, quercetin-*O*-hexoside, isorhamnetin-*O*-rutinoside, isorhamnetin-*O*-hexoside, kaempferol-*O*-(sinapoyl)-sophoroside, kaempferol-3-*O*-(feruloyl)-

Table 1. Qualitative phytochemical prospection of the aqueous extracts of *Ziziphus joazeiro*.

Extracts	Metabollites														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
AEL	+	-	+	-	+	+	+	-	+	+	-	-	+	+	+
AEB	-	-	-	-	+	+	+	-	+	+	-	-	+	-	+

1- Phenols; 2- Tannin pyrogallates; 3- Condensed tannins; 4- Anthocyanidins; 5- Leucoanthocyanidins; 6 - Flavones; 7 - Flavonols; 8 - Flavononols; 9 - Flavonones; 10 - Xantones; 11 - Aurones; 12 - Chalcones; 13 - Catechins; 14 - Alkaloids; 15 – Steroids and/or triterpenoids; (+) presence; (-) absense; AEB: Aqueous extract of the stem bark of Ziziphus joazeiro; AEL: Aqueous extract of the leaves of Ziziphus joazeiro

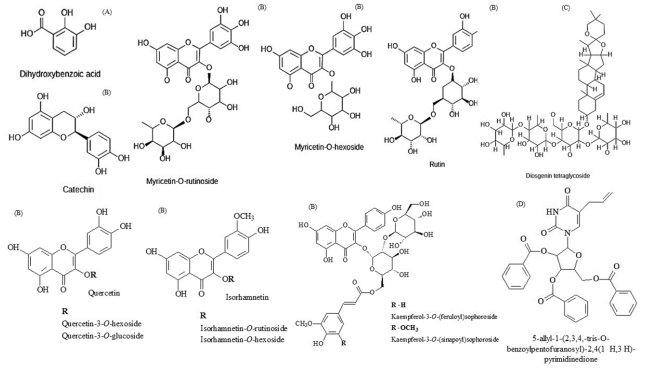

Figure 1. Chemical structure of the main compounds of classes (A) Phenolic Acids (B) Flavonoids (C) Saponin and (D) nitrogen compound present in the phytochemical composition of the aqueous extracts of *Ziziphus joazeiro.*

sophoroside), four saponins (diosgenin tetraglycoside and saponin derivatives), one phenolic acid (dihydroxybenzoic acid pentoside) and one nitrogen compound (5-allyl-1-(2,3,4-tris-*O*-benzoylpentafuranosyl)-2,4(1*H*,3*H*)–pyrimidinedione) were identified in the *Ziziphus joazeiro* aqueous leaf extract. As for the *Ziziphus joazeiro* aqueous stem bark extract, three saponin derivatives were identified. Figures 1 and 2 show the chemical structures of the main compounds present in the extracts' phytochemical composition.

Both extracts presented fungal growth inhibition which increased with increasing extract concentrations in the *Candida albicans* INCQS 40006 and URM 4387 antifungal assays as shown by the cellular viability curve (Figure 3).

In a comparative analysis, a more significant AEB action was observed against *Candida albicans* INCQS 40006 where the extract showed greater than 50% inhibition at a concentration of 0.512 mg/mL, this result being similar to that of fluconazole at a concentration of 1.024 mg/mL. However, at the same concentration, the AEL did not obtain the same result with the extract approaching 50% inhibition only at the highest concentration used (8.192 mg/mL) against both strains.

The results for the *Candida albicans* URM 4387 assays were similar where the AEB showed significant inhibition at a concentration of 0.512 mg/mL, presenting the same inhibitory effect as fluconazole at this concentration.

With respect to the minimal fungicidal concentration, none of the evaluated extracts presented a fungicidal effect since inhibition did not occur, instead a decrease in *Candida* fungal growth was observed. Thus, the antifungal effect is denominated as fungistatic with a minimum concentration ≥ 8.192 mg/mL.

The AEB stands out compared to the AEL against

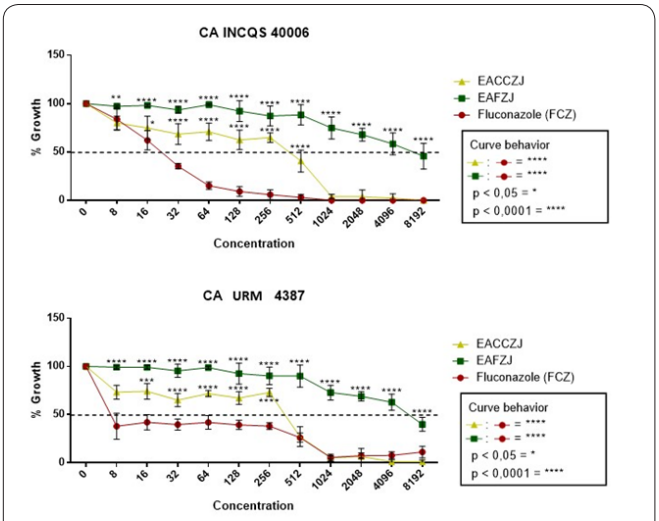

Figure 2. Viability curve of the *Candida albicans* strains under the effect of the aqueous extracts of *Ziziphus joazeiro* Mart. *AEB=EACCZJ: Aqueous extract of the stem bark of *Ziziphus joazeiro*; AEL=EAFZJ: Aqueous extract of the leaves of *Ziziphus joazeiro*; FCZ: Fluconazole; CA: *Candida albicans*; INCQS: National Institute of Quality Control in Health; *(p < 0,05); ****(p < 0,0001) in comparision with the control.

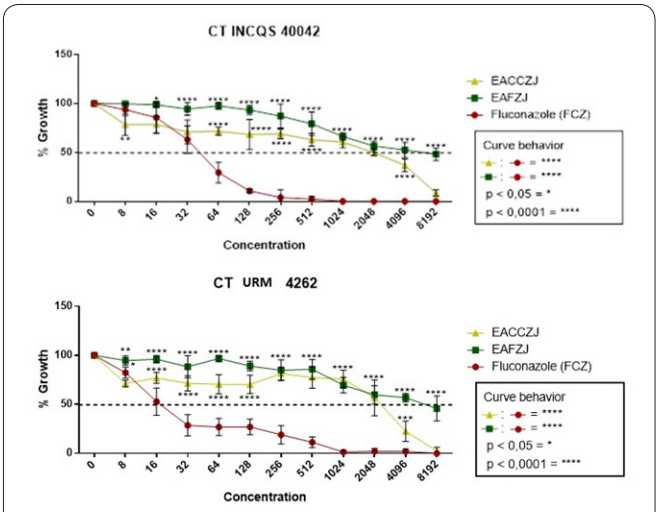

Figure 3. Viability curve of the *Candida tropicalis* strains under the effect of the aqueous extracts of *Ziziphus joazeiro* Mart.*AEB=EACCZJ: Aqueous extract of the stem bark of *Ziziphus joazeiro*; AEL=EAFZJ: Aqueous extract of the leaves of *Ziziphus joazeiro*; FCZ: Fluconazole; CA: *Candida albicans*; INCQS: National Institute of Quality Control in Health; *(p < 0.05); ****(p < 0.0001) in comparision with the control.

Candida tropicalis (Figure 4) strains. However, the AEB showed only a 50% decrease in cell viability at a concentration of 4.096 mg/mL against these strains. On another hand, fluconazole presented significant inhibition at 0.064 mg/mL for *Candida tropicalis* INSQC

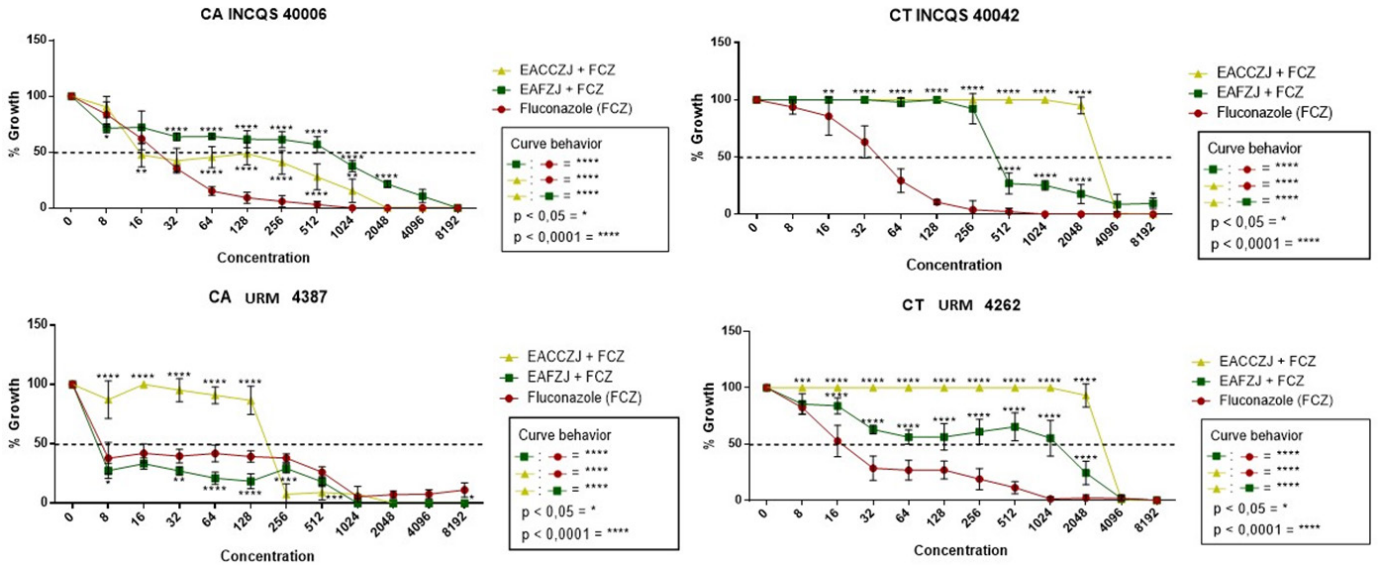

Figure 4. Viability curve of the *Candida* spp. strains under the effect of the aqueous extracts of *Ziziphus joazeiro* Mart. In association with fluconazole.*AEB=EACCZJ: Aqueous extract of the stem bark of *Ziziphus joazeiro*; AEL=EAFZJ: Aqueous extract of the leaves of *Ziziphus joazeiro*; FCZ: Fluconazole; CA: *Candida albicans*; INCQS: National Institute of Quality Control in Health; *($p < 0.05$); ****($p < 0.0001$) in comparision with the control.

40042 and 0.032 mg/mL for URM 4262.

When evaluating the antifungal modulatory potential of the extracts over fluconazole activity against *Candida* strains (Figure 5), the combination of the reference drug with the extracts at sub-inhibitory concentrations did not demonstrate a significant effect when compared to the drug alone against CA 40006. In contrast, the AEL modulatory effect over fluconazole activity presented a similar behavior to the isolated drug against CA URM 4387, potentiating the action of concentrations ranging from 0.032 to 0.256 µg/mL.

The antifungal modulatory action against *Candida tropicalis* (Fig. 4) did not present fluconazole potentiation when combined with the extracts, where a decrease in fluconazole action was instead observed. In a comparative analysis, the AEB combination presented an increase in the cellular variability curve at concentrations ranging from 0.008 to 2.048 mg/mL against CT INCQS 40042 and CT URM 4262, this being characterized as an antagonism.

Table 2 presents IC_{50} extract values where the AEL and EASCCZJ isolated extracts possessed a high inhibitory concentration compared to fluconazole, with IC_{50} values ranging from 0.450 to 9 mg/mL. When combined with fluconazole, a decrease in minimum inhibitory concentration for both extracts was observed, with the AEL presenting an IC_{50} equal to that of fluconazole (0.002 mg/mL) against CA URM 4387.

Discussion

Considerable quantities of flavonoids, steroids, tannins, alkaloids and saponins were identified in the *Z. joazeiro* leaf, bark and heartwood essential oil phytochemical characterisations (25).

Previous studies have performed Ultra-High-Performance Liquid Chromatography (UPLC) analyses with species from the *Ziziphus* genus of the Rhamnaceae family, where some of these corroborate with the chemical composition found in the extracts herein, such as *Ziziphus mauritiana* (26,27), *Ziziphus jujuba* (28) and *Ziziphus jujuba* (29,30).

Triterpenoids and flavonoids have been indicated as substances with antifungal potential from the metabolites found in the extracts' chemical characterizations (31,32). Terpenoid antifungal activity, such as that of saponins, is associated with membrane disorganization leading to cell lysis, in addition to interacting with steroids (33). Saponins isolated from the *Ziziphus joazeiro* stem presented antifungal activity against *Candida albicans* ATCC 10231, obtaining a minimum inhibitory concentration of 156 µg/mL (25).

Flavonoids on the other hand, probably have the ability to destabilize fungal membranes due to their lipophilic nature, in addition to being able to form complexes with soluble proteins present in the fungal cell wall (34-37).

Table 2. IC_{50} (mg/mL) of the extracts from *Ziziphus joazeiro* alone or in association with fluconazole.

Products	*Candida albicans*		*Candida tropicalis*	
	INCQS 40006	URM 4387	INCQS 40042	URM 4262
Fluconazole	0.021	0.002	0.041	0.014
AEL	5.889	6.035	9.040	5.453
AEB	0.480	0.450	2.193	3.026
AEL+FCZ	0.506	0.002	0.378	0.937
AEB+FCZ	0.053	0.173	2.654	2.632

*AEB=EACCZJ: Aqueous extract of the stem bark of *Ziziphus joazeiro*; AEL=EAFZJ: Aqueous extract of the leaves of *Ziziphus joazeiro*; FCZ: Fluconazole; INCQS: National Institute of Quality Control in Health.

The antifungal action of the *Ziziphus joazeiro* aqueous bark extract was evaluated in a study using non-*albicans Candida* species such as *Trichophyton rubrum* and *Candida guilliermondii* presenting results similar to amphotericin B, used as the reference drug (14).

The AEL modulatory effect over fluconazole may be due to its polar characteristics, which are a consequence of the flavonoids present in its phytochemical composition, in addition to possessing flavonoid classes associated with antifungal activities including flavonols, which are said to possess activity against *C. albicans*, in addition to other non-*albicans Candida* species (38).

Reports addressing the modulatory effect of extracts over fluconazole activity against *C. tropicalis* exist (31). However, the synergistic combination of the isolated flavonoids and fluconazole obtained different results from those cited in this study, demonstrating flavonoids have a potential as fluconazole synergistic effectors.

Overall, this study highlights the antifungal action of extracts used against *Candida* spp., which obtained MIC values greater than the reference antifungal, fluconazole. The crude extracts here studied, which possess a rich chemical complexity, presented antifungal effects and properties that consolidate the *Ziziphus joazeiro* ethno-biological aspect. In a comparative evaluation between the extracts, the AEB presented greater antifungal activity when used in isolation. On the other hand, the AEL stood out when used in combination with fluconazole, presenting itself as a synergistic effector for fluconazole against *Candida albicans*. Both extracts presented antagonism against *Candida tropicalis*. In light of the observed data, further studies are needed to understand the mechanistic interactions between extracts and drugs.

Conflicts of Interest

The authors have no conflicts of interest to disclose.

References

1. Rohilla S, Bhatt DC, Gupta A. Therapeutic potential of phytomedicines and novel polymeric strategies for significant management of candidiasis. Curr Pharm De 2018; 25-27.

2. Hacioglu M, Guzel CB, Savage PB, Tan ASB. Antifungal susceptibilities, in vitro production of virulence factors and activities of ceragenins against *Candida* spp. isolated from vulvovaginal candidiasis. Med Mycol 2019; 57:291-99.

3. Vieira AJH, Santos JI. Mecanismos de resistência de *Candida albicans* aos antifúngicos anfotericina B, fluconazol e caspofungina. Rev bras anal clin 2017; 49: 235-39.

4. Morais-Braga MFB, Carneiro JNP, Machado AJT, Sales DL, Santos ATL, Boligon AA *et al.* Phenolic composition and medicinal usage of *Psidium guajava* Linn.: Antifungal activity or inhibition of virulence? Saudi J Biol Sci 2017; 24: 302–13.

5. Linares CE, Giacomelli SR, Altenhofen D, Alves SH, Morsch VM, Schetinger MR. Fluconazole and amphoterecin-B resistance are associated with increased catalase and superoxide dismutase activity in *Candida albicans* and *Candida dubliniensis*. Rev Soc Bras Med Trop 2013;46: 752-58.

6. Carvalho Per. Juazeiro *Ziziphus joazeiro*. Embrapa, Circular Técnico 139. Colombo, PR, 2007.

7. Sousa FC, Martins JJA, Rocha APT, Gomes JP, Pessoa T, Martins JN, 2015. Predição de modelos sobre a cinética de secagem de folhas de *Ziziphus joazeiro* Mart. Rev Bras Pl Med 2015; 17: 195-200.

8. Almeida CFCBR, Amorim ELC, Albuquerque UP, Maia MB, 2006. Medicinal plants popularly used in the Xingo regiona semiarid location in Northeastern Brazil. J Ethnobiol Ethnomed 2006; 15: 1–7.

9. Schühly W, Heilmann J, Calis I, Sticher O. New triterpenoids with antibacterial activity from *Zizyphus joazeiro*. Planta Med.1999; 65:740-43.

10. Washida B, Abderrahman B, Nabil C, 2007. Antiulcerogenic activity of *Zizyphus lotus* (L.) extracts. J Ethnopharmacol 2007;112:228-31.

11. Romão MODC, Costa AMDD, Terra FDS, Boriollo MFG, Soares EA. Assessment of gastric protective of rasp juá extract. Rev Soc Bras Clín Méd 2010; 8: 222–27.

12. Nunes PHM, Marinho LC, Nunes MLRL, Soares EO. Antipyretic activity of an aqueous extract of *Zizyphus joazeiro* Mart (Rhamnaceae). Braz J Med Biol Res. 1987; 20: 599-601.

13. Alviano WS, Alviano DS, Diniz CG, Antoniolli AR, Alviano C, Farias LM *et al*. In vitro antioxidant potential of medicinal plant extracts and their activities against oral bacteria based on Brazilian folk medicine. Arch Oral Biol 2008; 53: 545-52.

14. Cruz MCS, Santos PO, Barbosa Jr AM, Melo D LFM, Alviano CS, Antoniolli AR *et al*. Antifungal activity of Brazilian medicinal plants involved in popular treatment of mycoses**.** J Ethnopharmacol 2007; 111:409-12.

15. Gomes DC, Lima HG, Vaza AV, Santosa NS, Santos FO, Dias ÊR *et al.* In vitro anthelmintic activity of the *Zizyphus joazeiro* bark against gastrointestinal nematodes of goats and its cytotoxicity on Vero cells. Vet. Parasitol 2016; 15: 10-16.

16. Matos FJA, 2009. Introduction to Experimental Phytochemical, 3th edn, Fortaleza: Publisher UFC, Português.

17. Matos FJA, 2002. Farmácias vivas. 4 ed. Fortaleza: Editora UFC. P. 2002. 36-40.

18. Campbell MK, Shawn OF, 2005. 2005. Biochemistry. (4th ed.) Singapore: Thomson Asia pte Ltd.

19. NCCLS Norma M27–A2, 2002. Método de Referência para Testes de Diluicão em Caldo para Determinacão da Sensibilidade à Terapia Antifúngica das leveduras; Norma Aprovada – Segunda Ed. Norma M27–A2 do NCCLS (ISBN 1-56238-469-4). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania. 2002, 19087-1898.

20. Stoppa MA, Casemiro LA, Vinholis AHC, Cunha WR, Silva MLA, Martins CHG *et al*. Estudo comparativo entre as metodologias preconizadas pelo CLSI e pelo EUCAST para avaliação da atividade antifúngica. Quím Nova 2009; 498–502.

21. Javadpour M.M, et al. De novo antimicrobial peptides with low mammalian cell toxicity. J Med Chem 1996; 39: 3107–13.

22. Morais-Braga M FB, Sales DL, Carneiro JNP, Machado AJT, Santos ATL, Freitas, MA *et al*. *Psidium guajava L*. and *Psidium brownianum* Mart ex DC.: Chemical composition and anti–Candida effect in association with fluconazole. Microb Pathog 2016; 95: 200-07.

23. Ernst EJ, Klepser ME, Ernst ME, Messer SA, Pfaller MA. In vitro pharmacodynamic properties of MK-0991 determined by time-kill methods. Diagn Microbiol Infect Dis 1999; 33: 75–80.

24. Coutinho HDM, Costa JGM, Lima EO, Falcão-Silva VS, Siqueira-Junior JP. In vitro interference of *Momordica charantia* and chlorpromazine in the resistance to aminoglycosides, Pharm Biol 2009; 47: 1056–59.

25. Ribeiro BD, Coelho MAZ, Marrucho IM. Extraction of saponins from sisal (*Agave sisalana*) and juá (*Ziziphus joazeiro*) with cholinium-based ionic liquids and deep eutectic solvents. Eur Food Res Technol 2013; 237: 965–75.

26. Nag TN, Chouhan N. 2009. Secondary metabolites from tissue cultures and plant parts of *Ziziphus mauritiana* cultivars Gola and

Seb. Acta Hortic 2009; 840: 327-34.
27. Memona AA, Memon N, Bhanger M, Luthria DL. 2013. Assay of phenolic compounds from four species of ber (*Ziziphus mauritiana* L.) fruits: Comparison of three base hydrolysis procedure for quantification of total phenolic acids. Food Chem 2013; 139: 496–502.
28. Wojdyło A, Carbonell-Barrachina AA, Legua P, Hernández F. Phenolic Composition, ascorbic acid content, and antioxidant capacity of Spanish jujube (*Ziziphus jujube* Mill.) fruits. Food Chem 2016; 201: 307–14.
29. Choi J, An X, Lee BH, Lee JS , Heo HJ, Kim T *et al*. Protective Effects of Bioactive Phenolics from Jujube (*Ziziphus jujuba*) Seeds against H2O2–induced Oxidative Stress in Neuronal PC-12 Cells. Food Sci Biotechnol 2015; 24: 2219-27.
30. Pu Y, Ding T, Zhang N, Jiang P, Liu D. Identification of bitter compounds from dried fruit of *Ziziphus jujuba* cv. Junzao. Int 2017; 01-34.
31. Serpa R, França E, Maia L, Andrade C, Diniz A, Furlaneto M. In vitro antifungal activity of the flavonoid baicalein against *Candida* species. J Med Microbiol 2012; 61: 1704 – 09.
32. Simões CMO, Schenkel EP, Gosmann G, Mello JCP, Mentz LA, Petrovick PR(Orgs), 2017. Farmacognosia: da planta ao medicamento. 6.ed. Porto Alegre: Editora da UFRGS: Florianópolis: Editora da UFSC. 1104.
33. Cunha AP and Roque OR, 2005. Esteróis e triterpenos: ácidos biliares, precursores da vitamina D e fitosteróides, cardiotónicos, hormonas esteróides, matérias-primas de núcleo esteróide usadas em sínteses parciais e saponósidos. In: CUNHA, A. P. Farmacognosia e fitoquímica. Lisboa: Fundação Calouste Gulbenkian, 2005, 432-482.
34. Arif T, Mandal TK, Dabur R. Natural products: Anti – fungal agents derived from plants. Opportunity, Challenge and Scope of Natural Products in Medicinal Chemistry. 2011; 81:283 – 311.
35. Salas PM, Céliz G, Geronazzo H, Daz M, Resnik SL. Antifungal activity and enzymatically – modified flavonoids isolated from citrus species. Food Chem 2011; 124: 1411 – 15.
36. Chua LS. A review on plant-based rutin extraction methods and its pharmacological activities. J Ethnopharmacol 2013; 150: 805 – 17.
37. Weissman Z, Pinsky M, Wolfgeher DJ, Kron SJ, Truman AW, Kornitzer D. Genetic analysis of Hsp70 phosphorylation sites reveals a role in *Candida albicans* cell and colony morphogenesis. BBA-Biomembranes 2018; 1-30.
38. Scorzoni L, Sangalli-Leite F, Lacorte SJ, Paula SAC, Costa-Orlandi CB, Fusco-Almeida AM *et al*. Searching new antifungals: The use of in vitro and in vivo methods for evaluation of natural compounds. J Microbiol Methods 2016; 123: 68-78.