**An Acad Bras Cienc (2024) 96(Suppl. 1): e20230420 DOI 10.1590/0001-3765202420230420**
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

MICROBIOLOGY

# Unusual plant-extract based media for the differentiation between species in the *Candida albicans* complex: A comparative study

SORAYA MORALES-LÓPEZ, LISAHIDY MACÍAS, DANNA ELLES & JAYR YEPES

**Abstract:** *Candida albicans* is the most common agent in human fungal infections; nevertheless, in the last decades, the closely related yeasts *Candida dubliniensis* and *Candida africana* have emerged as pathogens. The purpose of this study was to compare tobacco agar with another five agars prepared from plant extracts (*Origanum vulgare*, *Rosmarinus officinalis*, *Solanum rudepannum*, *Solanum oblongifolium* and *Brugmansia arborea*) on the differentiation of *C. albicans* complex. The hyphae and chlamyconidia formation and the color and margin of the colonies of 200 clinical isolates of *C. albicans*, *C. dubliniensis* and *C. africana* were evaluated. After seven days of incubation at 28 °C, Tobacco agar, *S. rudepannum* and *B. arborea* agars allowed the differentiation of 100 % *C. dubliniensis*. Additionally, 24% of *C. africana* isolates produced brownish colonies in the medium prepared from *Rosmarinus officinalis* (rosemary) extract. These results indicate that *S. rudepannun, B. arborea* and rosemary agar could be used as screening for the phenotypic differentiation between the species of *C. albicans* complex. Rosemary agar could be used to aid in the differentiation of *C. albicans* from *C. africana*. These culture media based on plants, could be used as simple and inexpensive screening methods in the phenotypic differentiation of *C. dubliniensis* and *C. africana*.

**Key words:** *Brugmansia arborea, Candida africana, Candida albicans, Candida dubliniensis, Solanum oblongifolium, Solanum rudepannum.*

## INTRODUCTION

The taxonomy of the genus *Candida* has changed revealing that the most significant species of the genus (*Candida albicans*, *Candida parapsilopsis* and *Candida glabrata*) are closely related to other cryptic species and are therefore recognized as "species complexes" (Nnadi et al. 2012). These new sister-yeasts were reported as "atypical species", since they shared phenotypic characteristics with the type species. The routine discrimination between the closely related species has been problematic, and the most accurate method for their identification are the PCR-based tests (Arastehfar et al. 2018, Theill et al. 2016).

Currently, *C. albicans complex* includes *Candida albicans* sensu stricto; *C. africana* and *C. stellatoidea*, biovars of *C. albicans* (Tietz et al. 2001, Langeron & Guerra 1939, Jacobsen et al. 2008); and *Candida dubliniensis* (Sullivan et al. 1995), recognized as a genetically and phenotypically differentiable specie of *C. albicans* (Hu et al. 2015, Romeo et al. 2013). While *C. albicans* is the main fungal pathogen, the true occurrence of *C. africana*, *C. stellatoidea* and *C. dubliniensis* might be underestimated (Jorgensen & Pfaller 2015).

*C. dubliniensis* has demonstrated enhanced resistance to fluconazole, out of all available drugs and isolates that were sensitive to fluconazole would go on to generate resistant

derivatives (Reginato el al. 2016, Sullivan et al. 2004). The primary mechanism of fluconazole resistance, which involves the upregulation of the key facilitator efflux pumps MDR1 and CDR1, is comparable to that of *C. albicans* (Pristov & Ghannoum 2019). Meanwhile, *C africana* isolates are still susceptible to widely used antifungal agents despite developing an increasing resistance to them. (Romeo & Criseo 2010).

A small number of selective complex media have been designed to distinguish members of the *C. albicans* complex based on colony morphology and physiological assimilation tests. On some of them, such as rosemary agar and oregano agar, *C. dubliniensis* isolates formed rough colonies with peripheral hyphal fringes and abundant chlamydospores after 24 to 48 h of incubation at 25 °C, but on which *C. albicans* isolates grew as smooth colonies without chlamydospores (Loreto et al. 2008).

On media such as *Staib agar (Guizotia abyssinica*), Sunflower (*Helianthus annuus*) and Tobacco agar (*Nicotiana tabacum*), it is also expressed, the property of producing brown colonies is associated with the action of the laccase enzyme on melanin substrates (Al Mosaid et al. 2001, Khan et al. 2005, 2004).

All these media have proven to be inexpensive, simple, and could potentially allow an early detection of *C. dubliniensis* colonies; however, several authors have reported atypical isolates. Furthermore, none of these media has been used to differentiate isolates of *C. africana* and the use of new substrates (based on other solanaceous plants) has not been studied either.

The aim of this work is to compare the differentiation of *C. albicans*, *C. dubliniensis* and *C. africana* on Tobacco extract agar and another five plant extracts: *Origanum vulgare*, *Rosmarinus officinalis*, *Solanum rudepannum*, *Solanum oblongifolium* and *Brugmansia arborea*. Isolates of *Candida stellatoidea* are scarce and they were not included in this study.

## MATERIALS AND METHODS

### Strains

A total of 200 clinical isolates were used in this study, including *C. albicans* (n: 146), *C. dubliniensis* (n: 25) and *C. africana* (n: 25). Four atypical *Candida albicans* (with phenotypical characteristics compatible with *C. africana*, but with HWP1 sequences consistent with *C. albicans*) were included too.

All *C. albicans* and *C. dubliniensis* isolates came from vaginal and oropharyngeal samples from Colombia and they were obtained from the culture collection of the Microbiology Laboratory of Universidad Popular del Cesar (Valledupar, Colombia); and *C. africana* isolates came from vaginal samples from different geographical origins, including America, Europe and Asia. Atypical *C. albicans* were previously described by Rodríguez-Leguizamón (Bogotá, Colombia) (Rodriguez-leguizamon et al. 2015). All the strains were previously identified by phenotypic, proteomic and molecular methods (Lachance et al. 2011, Romeo & Criseo 2008, Roberts et al. 2016).

*C. albicans* ATCC 68548, ATCC 28367, ATCC 90028 and *C. dubliniensis* CBS 7987 were used as control strains. Besides, 100 clinical isolates including *Cryptococcus neoformans* (10), *Nakaseomyces glabratus (C. glabrata) (10), C. parapsilosis (10), C. tropicalis (10), C. auris (30), C. pseudohaemulonii* (10), *Clavispora lusitaniae (C. lusitaniae) (5), C. metapsilosis* (5), *Kluyveromyces marxianus (C. kefyr)* (5), and *Meyerozyma guilliermondii (C. guilliermondii)* (5) were used.

### Plants

All plants were acquired from Mogambo Sendero Ambiental (http://mogambosenderoambiental.

com) and voucher specimens were deposited at Herbario Nacional de Colombia. Plant material was dried at 50 °C in a convection oven and stored at room temperature (25 °C).

*N. tabacum, R. officinalis* (rosemary) and *O. vulgare* (oregano) agar have been used before by other authors (Khan et al. 2004, De Loreto et al. 2008). *S. rudepannum* (turkey berry), *S. oblongifolium* (cucubo) and *B. arborea* (white angel's trumpet) were used as they are plants of the Solanacea family, as is *N. tabacum.*

### Culture media

Tobacco agar was prepared according to Khan et al. 2004, and rosemary agar and oregano agar were prepared according to De Loreto et al. 2008. Three more agars were prepared from the extracts of plants in the *Solanaceae* family: turkey berry, cucubo and white angel's trumpet, which were obtained by 2 h of boiling using a Clevenger apparaturs (200 g of dried stems and leaves per liter of destilled water) that reduced the loss of water by condensation of water vapor. Later, filtration through several layers of gauze was done and 200 mL of each extract were added to 800 mL of a basal medium (dextrose 1%; creatinine 0.078%; agar 2%, and 800 mL of distilled water) and sucrose 5 g/L was added. The solutions were autoclaved separately at 121 °C for 15 min and then allowed to cool to 50 – 55 °C. These conditions are similar to those used by De Loreto et al. 2008.

Finally, all the media were dispensed in 25 mL into Petri dishes (90 mm-diameter) and the agar plates were inoculated with 48-hour-old culture (Sabouraud agar) *C. albicans*, *C. dubliniensis* and *C. africana* isolates; followed by incubation at 28 °C and 37 °C for 7 days. 28° C (25 °C – 30 °C) is the temperature used in previous studies; but 37ºC is the most used in clinical laboratories, and in fact, many laboratories only have one incubator, which is usually used at 37° C.

Plates were examined daily looking for variations in macromorphology (color and margin of the colony) and micromorphology (single conidia, blastoconidia, chlamydoconidia and hyphae). Analysis was carried out three times on different days. The microscopic characteristics were observed under 100 and 400 x magnifications.

The authors declare that in this work no experiments were carried out in humans or animals.

## RESULTS

*C. albicans* and *C. dubliniensis* isolates grew well on all the culture media and at both incubation temperatures; however, *C. africana* isolates grew poorly in all media when the incubation temperature was 28 °C.

In both temperatures and after 7 days of incubation, *C. albicans* isolates (typical and atypical) showed white-to-cream color colonies in all media (Figure 1a-b, A); *C. dubliniensis* isolates showed white-to-cream color colonies in all media (Figure 1a-1b, B), except Tobacco agar, where it produced yellowish brown colonies; and *C. africana* presented white-to-cream colonies in media prepared from oregano and white angel's trumpet (Figure 1a, C). Six strains of *C. africana,* showed brown colonies in media prepared from rosemary (Figure 1b, C).

In all culture, *C. dubliniensis* isolates showed conidia, blastoconidia and pseudomycelia; while *C. albicans* and *C. africana* showed solitary conidia and few blastoconidia. Chlamydoconidia were absent in *C. albicans* and *C. africana* isolates and *C. dubliniensis* produced chlamydoconidia on media prepared from tobacco, turkey berry and white angel's trumpet. In terms of colony appearance, production of conidia and micromorphology, no differences were observed


**Figure 1.** ***C. albicans*** **(A),** ***C. dubliniensis*** **(B) and** ***C. africana*** **(C) isolates in media prepared from a)** ***Solanum oblongifolium*** **(cucubo) and b)** ***Rosmarinus officinalis*** **(rosemary) after 7 days of incubation.**

between isolates of the same species. (Figure 2 and Table I).

*Cryptococcus neoformans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. auris*, *C. pseudohaemulonii*, *C. lusitaniae*, *C. metapsilosis*, *C. kefyr*, and *C. guilliermondii* isolates grew poorly at 28 °C. At 37 °C the growth was abundant.

*Candida glabrata*, *C. auris*, *C. tropicalis*, *C. lusitaniae*, *C. parapsilosis* and *C. metapsilosis* produced white-to-cream color colonies in all media. In Tobacco agar, *C. neoformans* and *C. guilliermondii* produced brownish colonies, and *C. pseudohaemulonii* produced khaki color colonies.

Brown color colonies were also produced by *C. neoformans* in oregano, rosemary (figure 3a), borrachero and cucubo agar, while khaki color colonies were also produced in oregano and cucubo agar by *C. guilliermondii* and *C. kefyr*; in turkey berry agar by *C. guilliermondii*, *C. kefyr* and *C. pseudohaemulonii*, and in rosemary agar by *C. kefyr* (figure 3f), *C. guilliermondii* and *C. pseudohaemulonii* (figure 3d). Isolates did not produce chlamydospore on any media. *C. parapsilosis* (figure 3b), *C. africana* (figure 3c) and *C. glabrata* (figure 3e), isolates are showed in Rosemary agar at 37 °C.

## DISCUSSION

Tobacco agar allowed the differentiation of *C. dubliniensis* isolates. Similar results were reported by other authors (Bosco-Borgeat et al. 2011, Loreto et al. 2010, Liverio et al. 2017). *S. rudepannun*, *B. arborea* agar also allowed the differentiation of this specie. These two plant-extract based agar have not been reported before as culture media. On the other hand, although the taxonomic status of *C. africana* has been controversial, this yeast is recognized as a varietal distinction of *C. albicans* (Hu et al. 2015).

*Candida africana* has been linked to various human pathologies and has been isolated mainly from vaginal samples (Borman et al. 2013). Clinical isolates were reported in China (Shan et al. 2014), Argentina (Theill et al. 2016), India (Sharma et al. 2014), Iran (Khedri et al. 2018, Nikmanesh et al. 2020, Majdabadi et al.

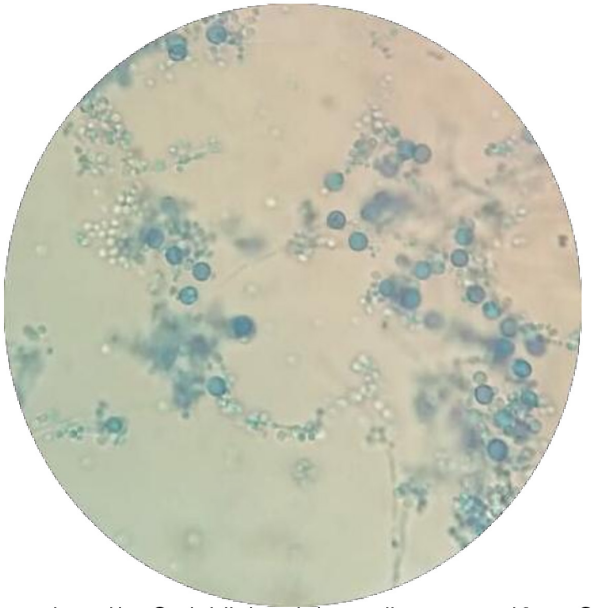

**Figure 2. Chlamydoconidias produced by *C. dubliniensis* in media prepared from *Solanum rudepannum* (friegaplatos) (Lactophenol cottom blue, 400x).**

2018, Naeimi et al. 2018, Yazdanparast et al. 2015), Africa (Nnadi et al. 2012, Tietz et al. 2001, Ngouana et al. 2015), USA (Romeo et al. 2013), Chile (Odds et al. 2007, Odds & Jacobsen 2008) and Europe (Borman et al. 2013, Romeo & Criseo 2009, Alonso-Vargas et al. 2008, Mendling et al. 2004).

At present, it is difficult to distinguish *C. africana* from *C. albicans* using routine laboratory methods and this circumstance has prevented the epidemiology to be well defined. Furthermore, recent genomic analyses have suggested that *C. stellatoidea* like *C. africana*, descended from the same hybrid ancestor as other *C. albicans* strains and has undergone a parallel massive loss of heterozygosity (Mixao et al. 2021). The agar media prepared from rosemary allowed to differenciate some strains of *C. africana* from *C. dubliniensis* and *C. albicans*. *C. africana* was the only specie that produced brown colonies on this medium, but only six strains of twenty-five did so.

Rosemary extract agar and oregano extract agar had previously been reported by De Loreto for the differentiation of *C. dubliniensis* and *C. albicans*. These authors described that in both media, *C. dubliniensis* isolates formed rough colonies with hyphal fringes and abundant chlamydospores after 24 to 48 h of incubation at 25 °C. In our case, *C. dubliniensis* isolates formed smooth colonies with no hyphal fringes or chlamydospores.

Considering that the chemical composition of plant extracts is influenced by several factors, information about the chemical composition of the extracts would provide valuable information to understand its influence on the culture media; however, this information is not available and could be a limitation in this study like the small sample size of *C. africana*.

De Loreto provide no information regarding the differentiation of *C. africana* of *C. albicans*. Nevertheless, in our study, 24 % *C. africana* isolates produced brown colonies on rosemary agar. In this work, incubation lasted for up to a week, which could explain the differences observed in the color of *C. africana* colonies compared to that report.

Media containing plant or seed extracts such as niger (*Guizottia abyssinica*), sunflower, sesame seed and tomato juice, have also been used as differential culture media for the *Candida albicans* complex; however, all of them have been used for the discrimination of *C. dubliniensis* and *C. albicans* (Alves et al. 2006, Al Mosaid et al. 2003, Staib & Morschhauser 1999). Another media as DRBC (Dichloran-rose bengal-chloramphenicol) agar and Sabouraud-triphenyltetrazolium agar also allow the differentiation between *C. dubliniensis* and *C. albicans* (Theill et al. 2016).

Although Tobacco agar is widely known as a medium for the differentiation of *C. albicans* and *C. dubliniensis* (Bosco-Borgeat et al. 2011,

**Table I. Macroscopic and microscopic characteristics of *Candida albicans* complex in different plant extract media, after incubation at 28 °C.**

| Plant extract | Characteristics | *C. albicans* (n: 146) | *C. africana(n: 25)** | *C. dubliniensis(n: 25)* |
|---|---|---|---|---|
| *Brugmansia arborea* (borrachero) | Colony color | White to cream | White to cream | White to cream |
| | Colony margin | Entire | Entire | **Scalloped** |
| | Chlamydoconidia | Absent | Absent | **Present** |
| *Nicotiana tabacum* (tobacco) | Colony color | White to cream | White to cream | Brown |
| | Colony margin | Entire | Entire | **Scalloped** |
| | Chlamydoconidia | Absent | Absent | **Present** |
| *Rosmarinus officinalis* (rosemary) | Colony color | White to cream | White to cream (76 % ) and **Brown** (24 %) | White to cream |
| | Colony margin | Entire | Entire | **Scalloped** |
| | Chlamydoconidia | Absent | Absent | Absent |
| *Origanum vulgare* (oregano) | Colony color | White to cream | White to cream | White to cream |
| | Colony margin | Entire | Entire | Scalloped |
| | Chlamydoconidia | Absent | Absent | Absent |
| *Solanum rudepannum.* (turkey berry) | Colony color | White to cream | White to cream | White to cream |
| | Colony margin | Entire | Entire | **Scalloped** |
| | Chlamydoconidia | Absent | Absent | **Present** |
| *Solanum oblongifolium* ("cucubo") | Colony color | White to cream | White to cream | White to cream |
| | Colony margin | Entire | Entire | **Scalloped** |
| | Chlamydoconidia | Absent | Absent | Absent |

**The differential characteristics are marked in bold type.**
***C. africana* shows poor growth (less than 15 CFU per plate) at 28 °C.**

Liverio et al. 2017, Monteiro et al. 2011) there are no reports on the growth description of *C. africana* in this medium. Furthermore, to our knowledge, the solanaceous plants turkey berry, cucubo and white angel's trumpet had not been previously described for the production of agar media.

None of the isolates of *C. africana* grew abundantly on the media at 28 °C. In addition, at 37 °C, chlamydospores formation by *C. dubliniensis* was reduced. De Loreto et al. 2008, found that the temperature was a determinant factor to produce chlamydospores in *C. dubliniensis* isolates on rosemary and oregano extract agar.

## CONCLUSIONS

In conclusion, appropriate differentiation of cryptic species is clinically relevant. *C. dubliniensis* and *C. albicans* var. africana may be underreported in clinical samples because most identification methods fail in their recognition. The antifungal susceptibility profiles, phenotypic, clinical, and ecologic similarity data for non-*Candida albicans* to help better understand the pathogenic mechanisms and

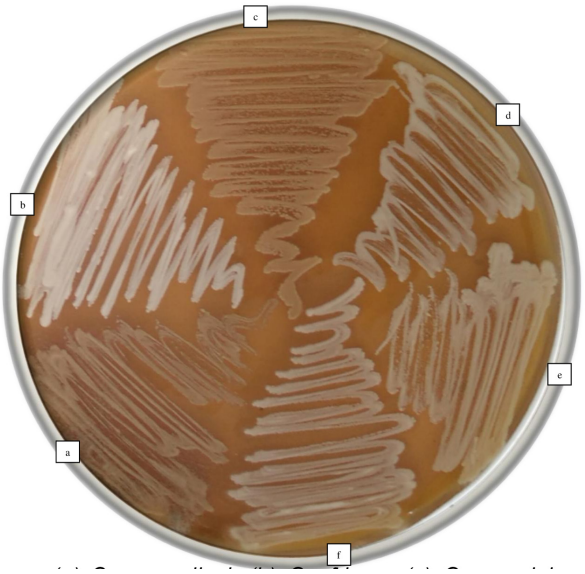


**Figure 3.** ***Cryptococcus neoformans*** **(a),** ***C. parapsilosis*** **(b),** ***C. africana*** **(c),** ***C. pseudohaemulonii*** **(d),** ***C. glabrata*****(e),** ***C. kefyr*** **(f), isolates in Rosemary agar at 37 °C.**

best treatment options. Here, we showed that previously reported rosemary extract agar is an easy, simple to prepare and inexpensive method that could also be used as a further test in the presumptive differentiation between *C. africana* and *C. albicans*. However, further studies, including a larger number of *C. africana* isolates, are crucial to confirm the present results.

## Acknowledgments

Authors acknowledge Claudia Parra and Andrés Ceballos (Laboratory of proteomics from Universidad Javeriana, Colombia), Guillermo García Effron (Laboratory for Mycology and Molecular Diagnosis from Universidad Nacional del Litoral, Argentina), Patrice Le Pape (Laboratoire de Parasitologie-Mycologie et immunologie parasitaire Institut de Biologie- CHU de Nantes, France), Shangrong Fan (Dept of Obstetrics and Gynecology, Peking University Shenzhen Hospital, China), Sebastien Bertout (Laboratoire de Parasitologie et Mycologie Medicale UFR Pharmacie, Universite de Montpellier), Andrew Borman (UK National Mycology Reference Laboratory from Public Health England South West Laboratory, England) and Jacques Meis (Centre of Expertise in Mycology Radboudumc/CWZ, The Netherlands) for providing Candida africana isolates. This study was financed by the call 891 de MINCIENCIAS (Colombia) and the financing agreement of research projects number 169 of the Universidad Popular del Cesar (Valledupar, Colombia).

## REFERENCES

AL MOSAID A, SULLIVAN D & COLEMAN DC. 2003. Differentiation of Candida dubliniensis from Candida albicans on Pal's agar. J Clin Microbiol 32: 1923-1929.

AL MOSAID A, SULLIVAN D, SALKIN IF, SHANLEY D & COLEMAN DC. 2001. Differentiation of Candida dubliniensis from Candida albicans on Staib agar and caffeic acid-ferric citrate agar. J Clin Microbiol 39: 323-327.

ALONSO-VARGAS R, ELORDUY L, ERASO E, CANO JF, GUARRO J, PONTÓN J & QUINDÓS G. 2008. Isolation of Candida africana, probable atypical strains of Candida albicans, from a patient with vaginitis. Med Mycol 46: 167-170.

ALVES SH, DE LORETO ES, LINARES CE, SILVEIRA CP, SCHEID LA, PEREIRA DI & SANTUARIO JM. 2006. Comparison among tomato juice agar with other three media for differentiation of Candida dubliniensis from Candida albicans. Rev Inst Med Trop Sao Paulo 48: 119-121.

ARASTEHFAR A, FANG W, PAN W, LIAO W, YAN L & BOEKHOUT T. 2018. Identification of nine cryptic species of *Candida albicans*, *C. glabrata*, and *C. parapsilosis* complexes using one-step multiplex PCR. BMC Infect Dis 18: 1-9.

BORMAN AM, SZEKELY A, LINTON CJ, PALMER MD, BROWN P & JOHNSON EM. 2013. Epidemiology, antifungal susceptibility, and pathogenicity of Candida africana isolates from the United Kingdom. J Clin Microbiol 5: 967-972.

BOSCO-BORGEAT ME, TAVERNA CG, CORDOBA S, ISLA MG, MURISENGO OA, SZUSZ W, VIVOT W & DAVEL G. Prevalence of Candida dubliniensis Fungemia in Argentina: Identification by a Novel Multiplex PCR and Comparison of Different Phenotypic Methods. Mycopathologia 172: 407-414.

HU Y, YU A, CHEN X, WANG G & FENG X. 2015. Molecular Characterization of Candida africana in Genital Specimens in Shanghai, China. Biomed Res Int 2015: 185387.

JACOBSEN MD, BOEKHOUT T & ODDS FC. 2008. Multilocus sequence typing con¢rms synonymy but highlights differences between *Candida albicans* and *Candida stellatoidea*. FEMS Yeast Res 8: 764-770.

JORGENSEN JH & PFALLER MA. 2015. Introduction to the 11th Edition of the Manual of Clinical Microbiology. In: JORGENSEN JH, CARROLL KC, FUNKE G, PFALLER MA, LANDRY ML, RICHTER SS, WARNOCK DW, RICHTER SS

& PATEL R (Eds), Manual of Clinical Microbiology, p. 1984-2014.

KHAN ZU, AHMAD S, MOKADDAS E, AL-SWEIH N & CHANDY R. 2005. Sunflower seed husk agar: a new medium for the differentiation of *Candida dubliniensis* from *Candida albicans*. Indian J Med Microbiol 23: 182-185.

KHAN ZU, AHMAD S, MOKADDAS E & CHANDY R. 2004. Tobacco agar, a new medium for differentiating Candida dubliniensis from Candida albicans. J Clin Microbiol 42: 4796-4798.

KHEDRI S, ROUDBARY M, HADIGHI R, FARAHYAR S, KHOSHMIRSAFA M & KALANTARI S. 2018. Running Iranian HIV/AIDS patients with oropharyngeal candidiasis: identification, prevalence and antifungal susceptibility of Candida species. Lett Appl Microbiol 67: 392-399.

LACHANCE MA, BOEKHOUT T, SCORZETTI G, FELL JW & KURTZMAN CP. 2011. The yeasts a taxonomic study. 5th ed., London: Elsevier.

LANGERON M & GUERRA P. 1939. Remarques sur le Candida stellatoidea (Jones et Martin 1938). Ann Parasitole Hum Comp 17: 257-260.

LIVÉRIO HO, RUIZ LDS, FREITAS RS, NISHIKAKU A, SOUZA AC, PAULAD CR & DOMANESCHI C. 2017. Phenotypic and genotypic detection of Candida albicans and Candida dubliniensis strains isolated from oral mucosa of AIDS pediatric patients. Revista do Inst Med Trop do Sao Paulo 1: 35-42.

LORETO S, POZZATTI P, ALVES LA, SANTURIO D, SANTURIO JM & HARTZ S. 2008. Differentiation of Candida dubliniensis from Candida albicans on Rosemary Extract Agar and Oregano Extract Agar. J Clin Alb Anal 22: 172-177.

LORETO S, SCHEID LA, NOGUEIRA, CW, ZENI G, SANTURIO JM & ALVES SH. 2010. Candida dubliniensis: epidemiology and phenotypic methods for identification. Mycopathologia 169: 431-443.

MAJDABADI N, FALAHATI M, HEIDARIE-KOHAN F, FARAHYAR S, RAHIMI-MOGHADDAM P, ASHRAFI-KHOZANI M, RAZAVI T & MOHAMMADNEJAD S. 2018. Effect of 2-Phenylethanol as Antifungal Agent and Common Antifungals (Amphotericin B, Fluconazole, and Itraconazole) on Candida Species Isolated from Chronic and Recurrent Cases of Candidal Vulvovaginitis. Assay Drug Devel Technol 16: 141-149.

MENDLING W, KRAUSS C & FLADUNG B. 2004. A clinical multicenter study comparing efficacy and tolerability of topical combination therapy with clotrimazole (Canesten, two formats) with oral single dose fluconazole. Mycoses 47: 136-142.

MIXAO V, SAUS E, BOEKHOUT T & GABALDÓN T. 2021. Extreme diversification driven by parallel events of massive loss of heterozygosity in the hybrid lineage of Candida albicans. Genetics 217(2): iyaa004.

MONTEIRO P, RODRIGUES-QUERIDO R, NUEREMBERG G, MOTA AJ, KOGA-ITO C & CARDOSO AO. 2011. Research on Candida dubliniensis in a Brazilian yeast collection obtained from cardiac transplant, tuberculosis, and HIV-positive patients, and evaluation of phenotypic tests using agar screening methods. Diagn Microbiol Infect Dis 71: 81-86.

NAEIMI B, MIRHENDI H, KHAMISIPOUR G, SADEGHZADEH F & AHMADI B. 2018. Candida africana in recurrent vulvovaginal candidiasis (RVVC) patients: frequency and phenotypic and genotypic characteristics. J Med Microbiol 67: 1601-1607.

NGOUANA TK ET AL. 2015. Investigation of minor species Candida africana, Candida stellatoidea and Candida dubliniensis in the Candida albicans complex among Yaoundé (Cameroon) HIV-infected patients. Mycoses 58: 33-39.

NIKMANESH B, AHMADIKIA K, GETSO MI, GHAREHBOLAGH SA, ABOUTALEBIAN S, MIRHENDI H & MAHMOUDI S. 2020. Candida africana and Candida dubliniensis as causes of pediatric candiduria: A study using HWP1 gene size polymorphism. AIMS Microbiology 6: 272-279.

NNADI NE, AYANBIMPE GM, SCORDINO F, OKOLO MO, ENWEANI IB, CRISEO G & ROMEO O. 2012. Isolation and molecular characterization of Candida africana from Jos, Nigeria. Med Mycol 50: 765-767.

ODDS FC ET AL. 2007. Molecular phylogenetics of Candida albicans. Eukaryot Cell 6: 1041-1052.

ODDS & FC JACOBSEN MD. 2008. Multilocus sequence typing of pathogenic Candida species. Eukaryot Cell 7: 1075-1084.

PRISTOV KE & GHANNOUM MA. 2019. Resistance of Candida to azoles and echinocandins worldwide. Clin Microbiol Infect 25: 792-798.

REGINATO CF, BANDEIRA LA, ZANETTE RA, SANTURIO JM, ALVES SH & DANESI CC. 2016. Antifungal activity of synthetic antiseptics and natural compounds against Candida dubliniensis before and after in vitro fluconazole exposure. Rev Soc Bras Med Trop 50: 75e9.

ROBERTS AL, ALELEW A & IWEN PC. 2016. Evaluation of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry to differentiate between Candida albicans and Candida dubliniensis. Diagn Microbiol Infect Dis 85: 73-76.

RODRÍGUEZ-LEGUIZAMÓN G, FIORI A, LÓPEZ LF, GÓMEZ BL, PARRA-GIRALDO CM, GÓMEZ-LÓPEZ A, SUÁREZ C, CEBALLOS A,

VAN-DIJCK P & PATARROYO M. 2015. Characterising atypical Candida albicans clinical isolates from six third-level hospitals in Bogotá, Colombia. BMC Microbiol 15: 1-10.

ROMEO O & CRISEO G. 2008. First molecular method for discriminating between Candida africana, Candida albicans, and Candida dubliniensis by using hwp1 gene. Diagn Microbiol Infect Dis 6: 230-233.

ROMEO O & CRISEO G. 2009. Morphological, biochemical and molecular characterisation of the first Italian Candida africana isolate. Mycoses 52: 454-457.

ROMEO O & CRISEO G. 2010. Candida africana and its closest relatives. Mycoses 54: 475-486.

ROMEO O, TIETZ HJ & CRISEO G. 2013. Candida Africana: Is it a fungal pathogen? Curr Fungal Infect Rep 7: 192-197.

SHAN Y, FAN S, LIU X & LI J. 2014. Prevalence of Candida albicans-closely related yeasts, Candida africana and Candida dubliniensis, in vulvovaginal candidiasis. Med Mycol 52: 636-640.

SHARMA C, MURALIDHAR S, XU J, MEIS JF & CHOWDHARY A. 2014. Multilocus sequence typing of Candida africana from patients with vulvovaginal candidiasis in New Delhi, India. Mycoses 5: 544-552.

STAIB P & MORSCHHAUSER J. 1999. Chlamydospore formation on Staib agar as a species- specific characteristic of Candida dubliniensis. Mycoses 42: 521-524.

SULLIVAN DJ, MORAN GP, PINJON E, AL-MOSAID A, STOKES C, VAUGHAN C & COLEMAN DC. 2004. Comparison of epidemiology, drug resistance mechanisms, and virulence of Candida dubliniensis and Candida albicans. FEMS Yeast Rev 4: 369e76.

SULLIVAN DJ, WESTERNENG J, HAINES KA, BENNETT DS & COLEMAN DC. 1995. Candida dubliniensis sp. nov.: phenotypic and Molecular characterization of a novel species associated with oral candidosis in HIV-infected individuals. Microbiology 141: 1507-1521.

THEILL L, DUDIUK C, MORANO S, GAMARRA S, NARDIN ME, MÉNDEZ E & GARCIA- EFFRON G. 2016. Prevalence and antifungal susceptibility of Candida albicans and its related species Candida dubliniensis and Candida africana isolated from vulvovaginal samples in a hospital of Argentina. Rev Argent Microbiol 48: 43-49.

TIETZ H, HOPP M, SCHMALRECK A, STERRY W & CZAIKA V. 2001. Candida africana sp. nov., a new human pathogen or a variant of Candida albicans ? Candida africana sp. nov., eine neue humanpathogene Art oder eine Variante von Candida albicans? Mycoses 44: 437-454.

YAZDANPARAST SA, KHODAVAISY S, FAKHIM H, SHOKOHI T, HAGHANI I, NABILI M, GHOLAMI H, AHMADI I & BADALI H. 2015. Molecular Characterization of Highly Susceptible Candida africana from Vulvovaginal Candidiasis. Mycopathologia 180: 317-323.

**How to cite**

MORALES-LÓPEZ S, MACÍAS L, ELLES D & YEPES J. 2024. Unusual plant-extract based media for the differentiation between species in the *Candida albicans* complex: A comparative study. An Acad Bras Cienc 96: e20230420. DOI 10.1590/0001-3765202420230420.

*Manuscript received on May 27, 2023;*
*accepted for publication on March 14, 2024*

**SORAYA MORALES-LÓPEZ**[1,2]
https://orcid.org/0000-0003-4402-041X

**LISAHIDY MACÍAS**[2]
https://orcid.org/0000-0003-2090-2266

**DANA ELLES**[2]
https://orcid.org/0000-0003-3103-3297

**JAYR YEPES**[2]
https://orcid.org/0000-0002-0730-6921

[1]Laboratorios Nancy Flórez García S.A.S.,
200001, Valledupar, Colombia

[2]Universidad Popular del Cesar, Grupo CINBIOS, Programa de Microbiología, Avenida Sabanas, 20004, Valledupar, Colombia

Correspondence to: **Soraya Morales-López**
*E-mail: sorayaeugeniam@hotmail.com*

## Author contributions

The Study conception and design: SML. Data collection, analysis and interpretation of results: SML, LM, DE and JY. Draft manuscript preparation: SML and JY. Guide and review research: SML. All authors have contributed to editing this paper; they have approved this final submission