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Alpinia conchigera Antifungal Activity against Oral *Candida*: An *in vitro* Study

(Aktiviti Antikulat *Alpinia conchigera* terhadap *Candida* Oral: Satu Kajian *in vitro*)

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ABSTRACT

Candida species are the dominant inhabitants in the oral cavity and can potentially develop into opportunistic pathogens in immunocompromised individuals, leading to oral infections. The emergence of *Candida* resistance against antifungal drugs has called for an alternative. This study aimed to evaluate the antifungal efficacy of *n*-hexane extract and its bioactive compound, 1'-*S*-1'-acetoxychavicol acetate (ACA), isolated from the rhizome of *Alpinia conchigera* against oral *Candida*. A crude *n*-hexane extract (HE) was obtained from the dried, ground *A. conchigera* rhizome using the maceration technique. The HE was subjected to column chromatography to isolate ACA, followed by structural elucidation and purity confirmation using nuclear magnetic resonance (NMR) and high-performance liquid chromatography (HPLC). Oral *Candida* species were retrieved from the oral rinses of the older adults. The antifungal effects of HE and ACA against oral *Candida* isolates were screened using the disc diffusion assay, followed by an evaluation of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). A total of 21.391 g of HE was obtained, yielding 3.7573 mg of ACA with a purity of 93.67%. The largest inhibition zones were observed for HE and ACA, both at a concentration of 100 mg/mL against *C. albicans* (12.0 ± 0.8 mm) and *C. glabrata* (10.0 ± 0.8 mm), respectively. The MIC and MFC values of HE ranged from 0.7 to 12.5 mg/mL and from 0.7 to 25 mg/mL, respectively. Meanwhile, for ACA, both MIC and MFC values are within the range of 0.7 to 25 mg/mL. This *in vitro* study showed that both HE and ACA possess antifungal properties against *Candida* isolates, which highlights their potential as natural antifungal agents for incorporation into oral care products.

Keywords: Fungal; herbal plant; natural compound; oral health; 1'-*S*-1'-acetoxychavicol acetate

ABSTRAK

Candida merupakan spesies yang dominan di dalam rongga mulut dan berpotensi menjadi patogen bagi individu yang mempunyai sistem imun yang lemah, serta boleh menyebabkan jangkitan oral. Insiden kerintangan *Candida* terhadap ubat antikulat juga memerlukan penyelesaian alternatif. Kajian ini bertujuan untuk menilai keberkesanan antikulat ekstrak *n*-heksana dan sebatian bioaktifnya, 1'-*S*-1'-asetoksikavikol asetat (ACA) yang diekstrak daripada rizom *Alpinia conchigera* terhadap *Candida* oral. Ekstrak *n*-heksana (HE) diperoleh daripada serbuk rizom *A. conchigera* yang kering melalui teknik maserasi. ACA kemudiannya diekstrak daripada HE melalui teknik kromatografi, diikuti oleh penentuan struktur dan pengesahan ketulenan menggunakan resonans magnet nukleus (NMR) dan kromatografi cecair prestasi tinggi (HPLC). Spesies *Candida* oral telah diperoleh daripada bilasan mulut warga emas. Kesan antikulat HE dan ACA terhadap *Candida* oral diuji melalui asai resapan cakera, diikuti dengan penentuan kepekatan rencatan minimum (MIC) dan kepekatan fungisid minimum (MFC). Sebanyak 21.391 g HE telah diperoleh dan menghasilkan 3.7573 mg ACA dengan ketulenan 93.67%. Zon perencatan terbesar dicatatkan untuk HE dan ACA pada kepekatan 100 mg/mL masing-masing terhadap *C. albicans* (12.0 ± 0.8 mm) dan *C. glabrata* (10.0 ± 0.8 mm). Nilai MIC dan MFC HE berada dalam julat 0.7 hingga 12.5 mg/mL dan 0.7 hingga 25 mg/mL. Manakala bagi ACA, kedua-dua nilai MIC dan MFC berada dalam julat 0.7 hingga 25 mg/mL. Kajian *in vitro* ini menunjukkan bahawa HE dan ACA mempunyai sifat antikulat terhadap *Candida*, yang menunjukkan potensinya sebagai agen antikulat semula jadi untuk dimasukkan ke dalam produk penjagaan mulut.

Kata kunci: Kesihatan mulut; kulat; sebatian semula jadi; tumbuhan herba; 1'-*S*-1'-asetoksikavikol asetat

INTRODUCTION

Candida overgrowth poses a major challenge to general health. It is a ubiquitous commensal microflora that resides throughout the body. This species is prevalent in the oral cavity of immunocompromised individuals, notably older adults (Deeiam et al. 2023; Matsumura et al. 2020; Sato et al. 2017; Thiyahuddin et al. 2019). *Candida albicans*, along with non-*albicans* species like *C. glabrata* and *C. tropicalis*, are notorious inhabitants of the oral cavity (Kibwana et al. 2023; Lemberg et al. 2022; Patel 2022). The impairment of the immune system causes these species to develop into opportunistic pathogens. Aided by their virulence factors, such as the formation of biofilms, morphological shifting from yeast to hyphae and hydrolytic enzyme secretion, they have caused numerous oral diseases (Pathakumari, Liang & Liu 2020). For instance, *C. tropicalis* invades host tissues by secreting aspartyl proteinase, phospholipase, and esterase (Aparna et al. 2023). Meanwhile, *C. albicans* and *C. glabrata* secrete extracellular enzymes such as candidalysin, resulting in the degradation of oral tissues, disruption of the epithelial layer, activation of several proinflammatory cytokines, and potential exacerbation of oral health conditions (Duggan & Usher 2023; Ho et al. 2019; Naglik, Gaffen & Hube 2019; Talapko et al. 2021). These virulent characteristics proposed by *Candida* species have contributed to the cause of oral diseases, including oral candidiasis.

Moreover, the emergence of antifungal resistance among *Candida* strains limits the effectiveness of conventional antifungal drugs. For decades, antifungal drugs, particularly those from the azole group, have been the primary treatment for controlling *Candida* overgrowth. However, recently, the azole group has become less efficient in combating several *Candida* species, as well as *C. albicans*, *C. tropicalis,* and *C. glabrata* (Bohner, Papp & Gácser 2022). These species are capable of undergoing genetic mutation to maintain the cell membrane functionality and to pump out the drugs, thus causing unresponsive antifungal treatment (Pristov & Ghannoum 2019). This incidence has elevated the public health burden, and yet, the Centre for Disease Control and Prevention (CDC) has listed *Candida* under serious antimicrobial resistance (AMR) (CDC 2019).

Nonetheless, nystatin, a polyene antifungal agent, is widely used for treating fungal infections, especially oral candidiasis. However, its application is accompanied by several downsides that limit its effectiveness and safety. These concerns include its toxicity, poor solubility and low chemical stability. Nystatin exerts its antifungal effect by binding to sterols in the cell membrane, forming pores that can also cause haemolytic effects in host cells (Tevyashova et al. 2023). Due to its amphipathic polyene structure, nystatin exhibits poor solubility in both polar and non-polar solvents, resulting in low bioavailability and limited applicability for drug delivery systems (Sousa et al. 2023). Additionally, it is prone to degradation upon exposure to sunlight, heat and oxygen while offering a bitter taste, possibly reducing patient compliance, particularly in oral formulations (de Aguiar et al. 2023). Thus, such drawbacks significantly compromise nystatin's clinical utility, prompting the exploration of safer and more effective antifungal agents.

Additionally, to prevent oral disease caused by *Candida*, a highly demanded antimicrobial agent such as chlorhexidine has been recommended. It provides a broad-spectrum action against microbes (El-Rabbany et al. 2015). Chlorhexidine has been actively incorporated into oral hygiene products such as mouthwash and oral gel (Fiorillo 2019) and have been shown to reduced the microbes counts (Brookes et al. 2021; Ab Malik et al. 2017). However, the long-term use of chlorhexidine may cause teeth staining, numbness and discolouration of the tongue, and promote xerostomia, hypogeusia and supragingival calculus formation (Deus & Ouanounou 2022). Therefore, the presence of opportunistic oral *Candida* limits antifungal efficacy, whereas the side effects of chlorhexidine pose significant challenges in managing oral health, especially among older adults or immunocompromised individuals.

Plants and their derivative compounds have been used for therapeutic purposes since ancient times because they are safe, economical, effective and easily available. *Alpinia conchigera* is an herbal tree native to Asian countries, including Bangladesh, India, Indonesia, Cambodia, Laos, Thailand and Vietnam (Ibrahim et al. 2012). This species can also be found on the east coast of Peninsular Malaysia, specifically in Kelantan (Appalasamy et al. 2022). It is known as a local medicinal plant with diverse pharmacological potential, especially the rhizome part. The rhizome parts are consumed as postpartum medicine, while the young shoots are used in food preparation. *A. conchigera* has been demonstrated to have antimicrobial activities, anti-inflammatory effects and anti-cancer properties (Anuar et al. 2020; Liew et al. 2017; Sulaiman et al. 2010). Its major bioactive compound, acetoxychavicol acetate (ACA), has been demonstrated to exhibit a potent antibacterial property against methicillin-resistant *Staphylococcus aureus* (MRSA) (Aziz et al. 2013; Taib et al. 2020). However, most of the studies have primarily focused on dermatophyte pathogens. Therefore, it is an opportunity to explore its potential for oral health benefits. The study on the antifungal properties of *A. conchigera* rhizome extract against oral pathogens, specifically *Candida*, is still limited. Thus, this study reports the *in vitro* antifungal effect of *A. conchigera* HE and ACA against oral *Candida* isolates, consisting of *C. albicans*, *C. glabrata*, and *C. tropicalis*.

MATERIALS AND METHODS

EXTRACTION OF *A. conchigera* RHIZOME AND ISOLATION OF ACA

The extraction method was performed according to Taib et al. (2020). The dried and ground *A. conchigera* rhizome was acquired from Hulu Langat, Selangor. A total of 10 kg

of dried ground *A. conchigera* rhizome was soaked in 15 L of *n*-hexane solvent (Revlogi Materials, Malaysia) for three days. The residue underwent two further extraction cycles (three cycles in total) to increase the extraction yield. The liquid extract was filtered and dried using a rotary evaporator (Eyela, USA) at room temperature. The extract was left in the fume hood until it achieved a constant weight to confirm a complete elimination of *n*-hexane solvent residue, thus producing the crude *n*-hexane extract (HE) of *A. conchigera*.

The HE (15 g) was further subjected to fractionation using silica gel column chromatography (CC). The HE was wet-packed with *n*-hexane solvent and loaded into a column packed with silica gel (0.040-0.063 mm) (Merck, Germany), with a ratio of HE to silica gel of 1:30. The prepared column was eluted using an *n*-hexane:ethyl acetate gradient solvent with ratios of 1:0 to 4:1. The eluates were collected in the conical flask. The conical flasks that had an identical thin layer chromatography (TLC) profile were combined, labelled as fraction 1, and further purified to obtain the bioactive compound, ACA. The ACA was subjected to the spectroscopic method, Nuclear Magnetic Resonance (NMR) (Bruker 400 MHz), and high-performance liquid chromatography (HPLC) to confirm the structural elucidation and the purity of isolated ACA by comparing it with the literature values (Taib et al. 2020).

The HPLC analysis was carried out using a Shimadzu HPLC-LC20AT system, equipped with a binary pump, an automatic injector, and a photodiode array detector (SPD-M20A). Separation was performed on a Shim-Pack C18 column (250 mm × 4.6 mm, 5 µm) using an isocratic solvent system composed of 20% acetonitrile and 80% double-distilled water with 0.1% formic acid over 10 min. The flow rate was maintained at 0.8 mL/min with absorbance monitored at 254 nm. Prior to injection, the solutions were filtered through a 13 mm, 0.2 µm Whatman nylon membrane filter. The injection volume was 10 µL (Chua et al. 2017).

PREPARATION FOR *Candida* ISOLATES

ORAL RINSE COLLECTION

Clinical samples were obtained from the residents of old folks' homes with ethical approval (USIM/JKEP/2021-184) from the Ethics Committee, Universiti Sains Islam Malaysia (USIM). Twelve subjects were recruited through convenience sampling, a non-probability sampling method, for oral rinse collection to retrieve the interested pathogens. Inclusion criteria were as follows: individuals aged over 60 years old, not edentulous and with no history of antibiotic usage in the past three months. Exclusion criteria included the presence of a systemic condition predisposing to oral candidiasis, current use of medications associated with increased susceptibility to oral candidiasis and the use of removable dental prostheses such as partial dentures. The subjects were briefed about the study, and written consents were obtained. Then, they were asked to rinse their mouth gently using sterile water, followed by 10 mL of sterile phosphate buffer saline (PBS) (0.1 M, pH 7.2) (R&M Chemicals, Malaysia) solution for 60 s. The collected samples were placed in an icebox and transported within 6 h to the microbiological laboratory of the Faculty of Dentistry, USIM, for processing and subsequent procedures.

SAMPLE PROCESSING

The oral rinse samples were centrifuged at 4000 rpm at 4 °C for 15 min. The supernatant was removed, and the pellets obtained were suspended in 1 mL of sterile PBS to prepare the main stocks. A 100 µL aliquot from the main stock was then used to dilute the samples using a serial dilution technique. The samples were diluted by a factor of 10 four times (10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}).

ISOLATION OF *Candida* SPECIES

A volume of 50 µL aliquot of each dilution (main stock, 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}) was inoculated on differential-selective growth media, which was HiChrome *Candida* Differential Agar (Himedia, India), and incubated for 24 h at 37 °C to isolate the pure colonies of *Candida* species. The colours of colonies formed on HiChrome *Candida* Differential Agar were used to differentiate the different species of *Candida* by comparing them with the technical data provided along with the HiCrome *Candida* Differential Agar. The representatives of different species of *Candida* were further confirmed for identification using 18S rRNA sequencing provided by 1st Base, Singapore. The *Candida* that was included in the study were *C. albicans*, *C. glabrata*, and *C. tropicalis*. All *Candida* were grown and cultured on Potato Dextrose Agar (PDA) (Himedia, India) and Potato Dextrose Broth (PDB) (Himedia, India) for 24 h at 37 °C.

ANTIFUNGAL ASSAY

DISC DIFFUSION ASSAY (DDA)

The DDA was used to screen the antifungal properties of *n*-hexane extract and ACA. This method was employed according to Gothai et al. (2018). Each *Candida* isolate was inoculated in PDB for 24 h at 37 °C, and the turbidity was modified to 0.5 McFarland (Oxoid, UK). Then, 100 µL of the modified culture was inoculated onto PDA, followed by the application of 5 × 6 mm paper discs (Whatman No. 1, UK) impregnated with 10 µL of *n*-hexane extract or ACA at concentrations ranging from 25 to 100 mg/mL. Both the *n*-hexane and ACA were dissolved in 10% dimethyl sulfoxide (DMSO) (Merck, Germany) due to the non-polar nature of the extract and ACA. A negative control using 10% DMSO and a positive control using 0.12% w/v chlorhexidine mouthwash (CHX) (Oradex, Malaysia) were also used. The susceptibility of *n*-hexane

extract and ACA against *Candida* isolates was evaluated by measuring the diameter of the inhibition zone surrounding the disc after incubating the agar plate at 37 °C for 24 h. An inhibition zone of more than 6 mm signifies that *Candida* was susceptible to *n*-hexane extract and ACA. The DDA was carried out in three replicates.

MINIMUM INHIBITORY CONCENTRATION (MIC) ASSAY

The MIC assay was conducted to evaluate the lowest concentration of *n*-hexane extract and ACA to inhibit the growth of *Candida* isolates using a broth microdilution assay according to Nordin et al. (2022). Standard chlorhexidine mouthwash (0.12% w/v) was included as a positive control, while 10% DMSO was included as a negative control. Two-fold serial dilutions of *n*-hexane extract and ACA, ranging from 0.2 to 25 mg/mL, were set in a sterile 96-well plate using PDB as the diluent. The suspension of each *Candida* isolate was modified to a final concentration of 0.5 McFarland. A 100 µL of modified *Candida* suspension was added to each well. The prepared plate was then incubated for 24 h at 37 °C. An aliquot of 10 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Merk, Germany) (3 mg/mL) solution was added and further incubated in the dark at 37 °C for 2 h. The lowest concentration of *n*-hexane extract and ACA that showed no changes after adding the MTT solution was noted as MIC. This procedure was carried out in three replicates.

MINIMUM FUNGICIDAL CONCENTRATION (MFC) ASSAY

The MFC assay was carried out to evaluate the lowest concentration of *n*-hexane extract and ACA to kill the *Candida* isolates. Before the MTT solution was added, 10 µL of each dilution (0.2 to 25 mg/mL) of each well from MIC determination was streaked on the PDA. The plate was labelled with different concentrations and incubated for 24 h at 37 °C. The growth of *Candida* isolates on each plate was observed. The lowest concentration of *n*-hexane extract and ACA that killed the fungus was noted as MFC. This procedure was conducted in three replicates.

RESULTS AND DISCUSSION

EXTRACTION OF *n*-HEXANE AND ISOLATION OF ACA FROM *A. conchigera* RHIZOME

A total of 21.391 g of *n*-hexane extract was obtained from the rhizome of *A. conchigera*. Out of this, 15 g of the HE (Fraction 1) was subjected to column chromatography eluting 3.7573 mg of ACA. The remaining 6.391 g of HE was retained for antifungal testing. The fractionation of HE in column chromatography was monitored using the TLC profile. The TLC profiles were compared with a reference standard of ACA (Figure 1). The purity of the isolated ACA was further confirmed using HPLC, showing a purity of 93.67%, which is shown in Figure 2.

The structural elucidation of ACA was conducted using ^{1}H NMR and ^{13}C NMR spectroscopy, which are

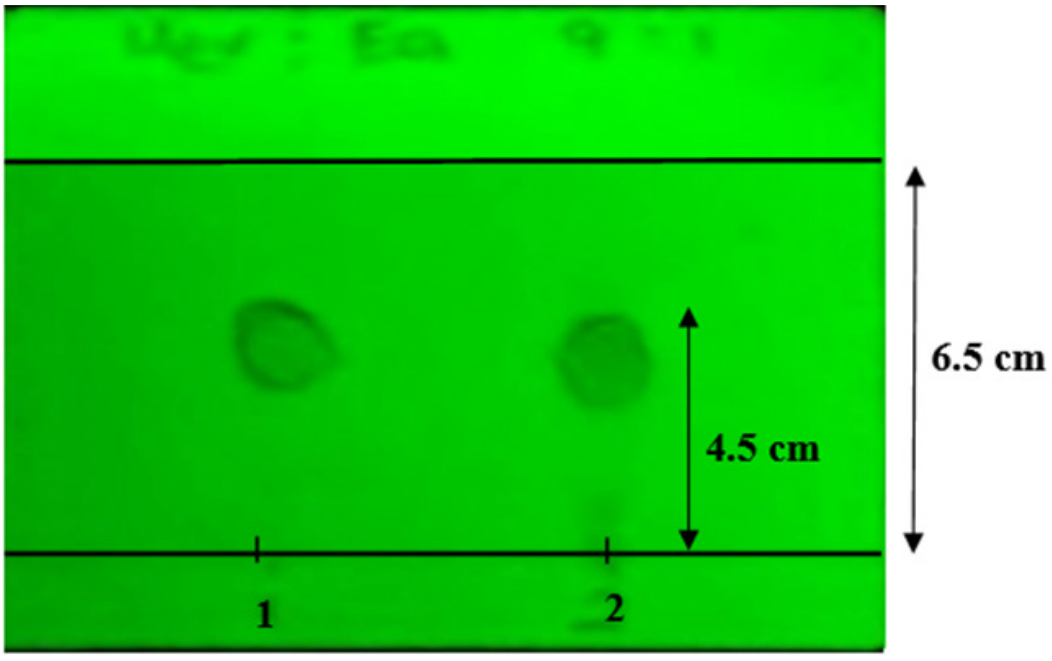

FIGURE 1. TLC profile, stationary phase = silica gel F_{254}, solvent system =*n*-hexane: ethyl acetate (90:10), viewed under UV light, shortwave, 254 nm. (1) represents the TLC profile of the ACA reference and (2) represents the TLC profile of the isolated ACA. The R_f value was 0.7

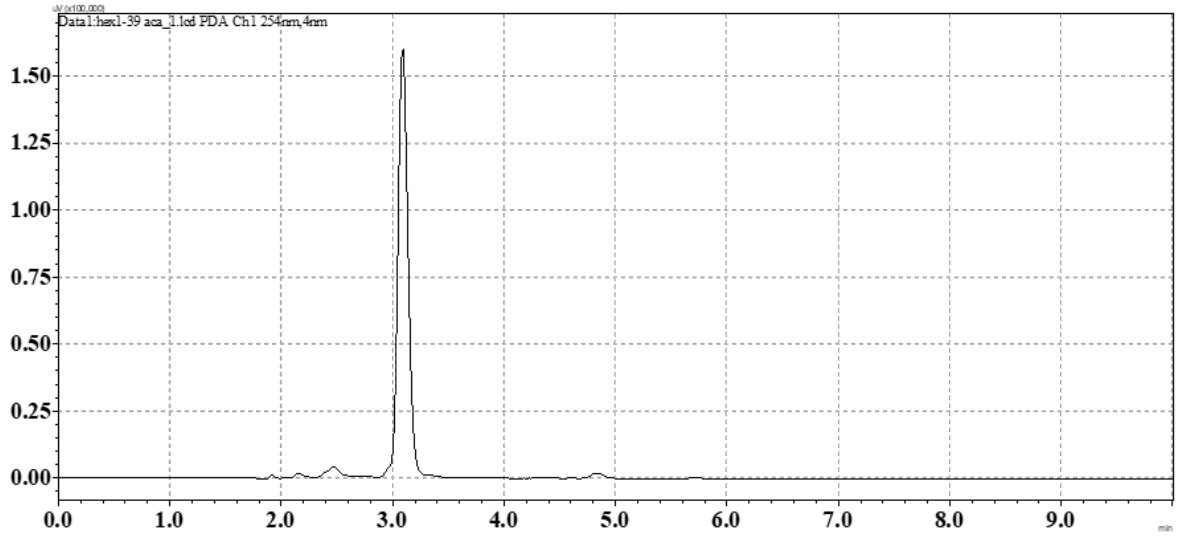

FIGURE 2. The HPLC chromatogram of ACA isolated from *A. conchigera* rhizome *n*-hexane extract. The sample was analysed at 254 nm, showing a prominent peak at 3.089 min, indicating the presence of ACA. The purity of the isolated ACA was 93.67%

shown in Figure 3(A) and 3(B), respectively. The ^{1}H NMR spectrum (400 MHz, CDCL3, δ ppm) displayed the following chemical shifts: 2.10 (3H, s, H5'), 2.29 (3H, s, H2''), 5.26 (2H, dd, *J*=17.28, 10.52, H3'), 5.98 (1H, m, H2'), 6.26 (1H, d, *J* = 5.76, H1), 7.09 (2H, d, *J* = 7.92, H3, H5), 7.36 (2H, d, *J* = 7.96, H2, H6). Meanwhile, the ^{13}C NMR spectrum (100 MHz, CDCL3, δ ppm) showed the following chemical shifts: 21.2 (C5'), 21.3 (C2''), 75.6 (C1'), 117.2 (C3'), 121.8 (C3, C5), 128.5 (C2, C6), 136.1 (C2'), 136.5 (C1), 150.5 (C4), 169.5 (C1''), 170.02 (C4').

CLINICAL *Candida* ISOLATES

A total of eight *Candida* isolates were successfully retrieved on HiChrome *Candida* Differential Agar from 12 oral rinse samples. Out of the eight species of retrieved isolates, four isolates (50%) were *C. albicans* (labelled as *C. albicans* 1, 2, 3 and 4), three isolates (37.5%) were *C. glabrata* (labelled as *C. glabrata* 1, 2 and 3), and one isolate (12.5%) was *C. tropicalis* (labelled as *C. tropicalis* 1). *C. albicans* was seen as light green coloured colonies on HiChrome *Candida* Differential Agar (Figure 4). Meanwhile, *C. glabrata* and *C. tropicalis* appeared as cream- to white- and blue-metallic coloured colonies, as shown in Figures 5 and 6, respectively.

ANTIFUNGAL EFFECT OF *n*-HEXANE EXTRACT AND ACA

The DDA was conducted to screen the antifungal activity of HE and ACA against various oral *Candida* species, including four isolates of *C. albicans*, three isolates of *C. glabrata* and an isolate of *C. tropicalis*. Chlorhexidine (CHX) at 0.12% served as a positive control and DMSO (10%) as a negative control. The inhibition diameters are presented as mean ± standard deviation (SD). Table 1 provides the summary of the inhibition zone diameters for both HE and ACA against oral *Candida*.

Most of the *Candida* isolates were susceptible to all concentrations used for both HE and ACA. Overall, the diameter of the inhibition zone was decreased along with the decreasing concentration of HE and ACA. The 100 mg/mL of HE showed inhibitory activity against *C. albicans*, *C. glabrata*, and *C. tropicalis* with inhibition zone diameters ranging from 7.0 to 12.0 mm, 10.3 to 11.7 mm, and 10.7 mm, respectively. On the other hand, the ACA (100 mg/mL) also showed inhibitory activity against *C. albicans*, *C. glabrata*, and *C. tropicalis*, but with smaller inhibition zone diameters ranging from 6.3 to 9.0 mm, 7.7 to 10.0 mm, and 9.3 mm, respectively. The positive control, 0.12% w/v chlorhexidine, was susceptible to *C. albicans*, *C. glabrata,* and *C. tropicalis,* with inhibition zone diameters ranging from 7.3 to 8.0 mm, 7.8 to 8.2 mm and 9.3 mm, respectively. No inhibition diameter zone was observed in the negative control, 10% DMSO.

THE MIC AND MFC OF *n*-HEXANE EXTRACT AND ACA

The MIC and MFC values for HE and ACA against various *Candida* isolates are presented in Table 2. The HE exhibited MIC and MFC values ranging from 0.7 to 12.5 mg/mL across the *Candida* species. Notably, the HE demonstrated the lowest MIC and MFC value (0.7 mg/mL) against *C. glabrata* 1, while the highest MIC value (12.5 mg/mL) was observed against *C. glabrata* 2 and *C. glabrata* 3. The HE exhibited the same MIC and MFC values against most of the *Candida* isolates except for *C. glabrata* 2.

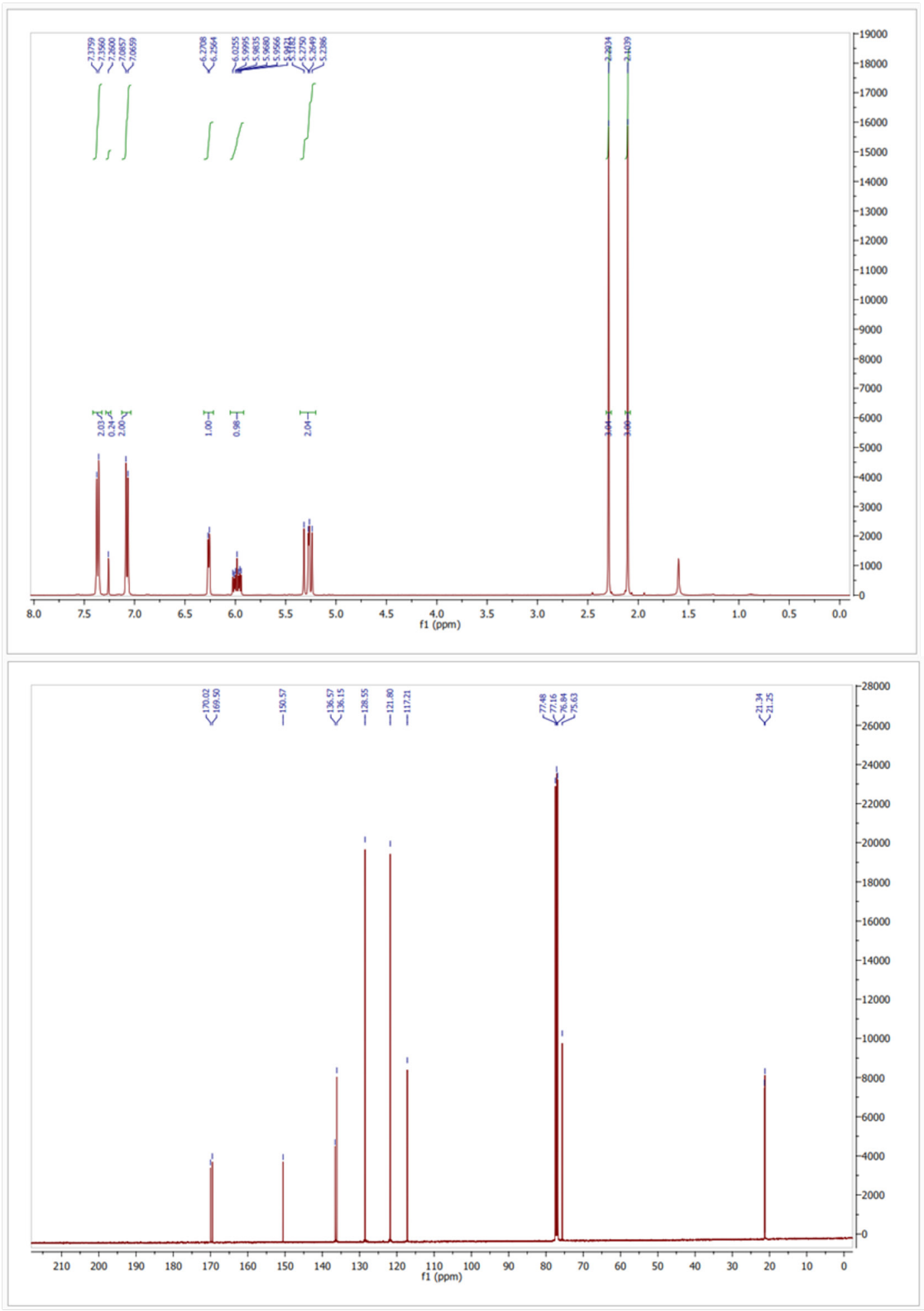

FIGURE 3. (A) The ^{1}H NMR spectrum of isolated ACA, (B) The ^{13}C NMR spectrum of isolated ACA

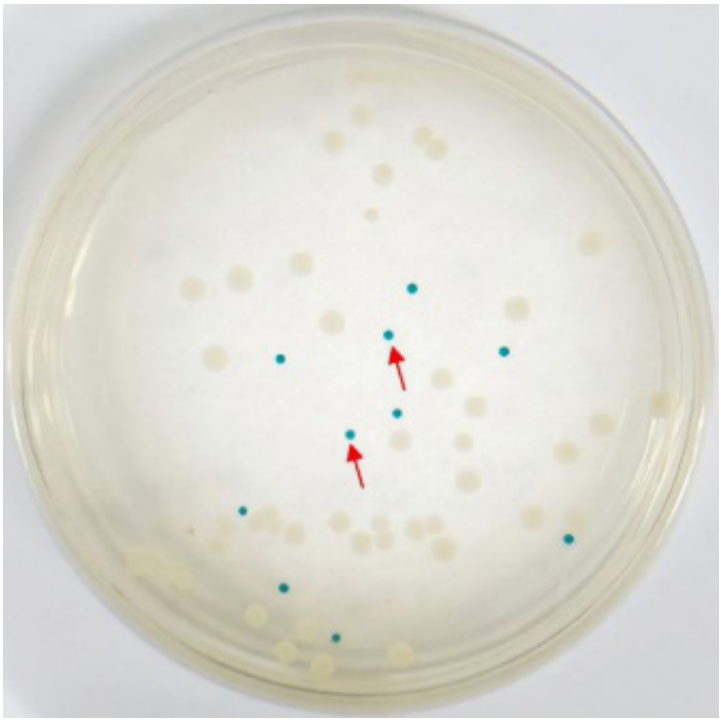

FIGURE 4. The colonies growth on HiChrome *Candida* differential agar. The red arrow shows the representative light green, smooth colonies which are identified as *C. albicans*

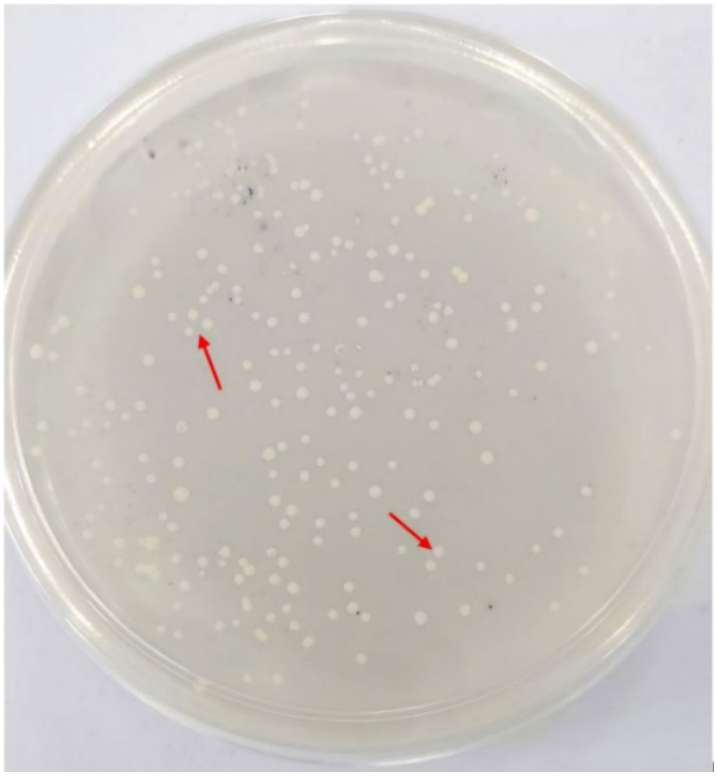

FIGURE 5. The colonies growth on HiChrome *Candida* Differential Agar. The red arrow shows the representative cream to white, smooth colonies which are identified as *C. glabrata*

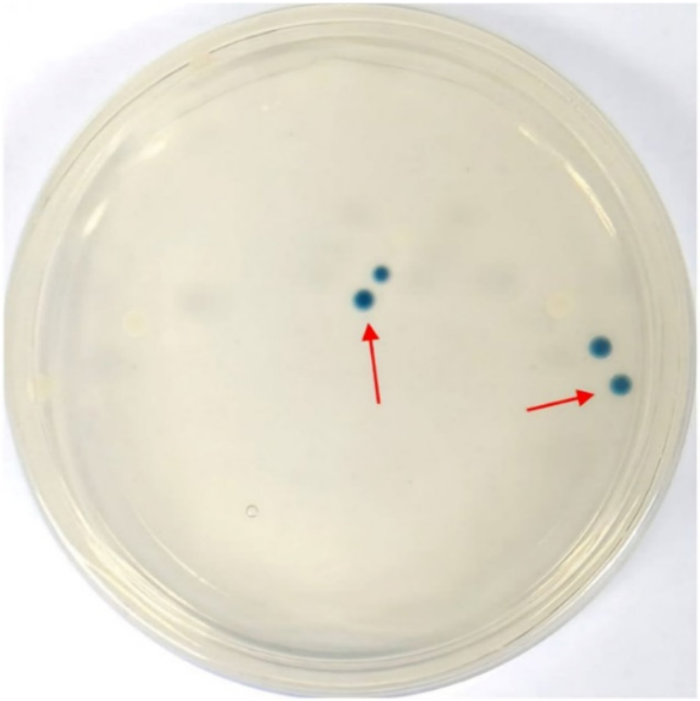

FIGURE 6. The colonies growth on HiChrome *Candida* Differential Agar. The red arrow shows the representative blue metallic, raised colonies which are identified as *C. tropicalis*

TABLE 1. Inhibition zone of *n*-hexane extract and ACA against oral *Candida* isolates

		Inhibition diameter mean ± SD							
Samples	Concentration (mg/mL)	*C. albicans* 1	*C. albicans* 2	*C. albicans* 3	*C. albicans* 4	*C. glabrata* 1	*C. glabrata* 2	*C. glabrata* 3	*C. tropicalis* 1
HE	25	7.3 ± 0.5	7.3 ± 0.5	6.0 ± 0.0	6.0 ± 0.0	6.3 ± 0.2	6.7 ± 0.2	6.3 ± 0.2	8.7 ± 0.9
	50	9.3 ± 0.5	7.7 ± 0.5	6.0 ± 0.0	6.3 ± 0.2	7.7 ± 0.5	8.0 ± 0.0	6.5 ± 0.0	8.7 ± 0.9
	100	12.0 ± 0.8	10.0 ± 0.8	7.3 ± 0.5	7.0 ± 0.0	11 ± 0.8	11.7 ± 0.5	10.3 ± 0.5	10.7 ± 0.5
ACA	25	7.0 ± 0.0	6.5 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.3 ± 0.2	6.5 ± 0.0	6.2 ± 0.2	6.8 ± 0.2
	50	7.8 ± 0.2	6.8 ± 0.2	6.2 ± 0.2	6.0 ± 0.0	7.3 ± 0.5	7.3 ± 0.5	7.0 ± 0.8	7.8 ± 0.6
	100	9.0 ± 0.0	8.0 ± 0.0	6.3 ± 0.2	6.3 ± 0.2	7.7 ± 0.5	7.7 ± 0.5	10.0 ± 0.8	9.3 ± 0.5
CHX	0.12%	8.0 ± 0.0	7.3 ± 0.5	7.7 ± 0.5	8.0 ± 0.0	7.8 ± 0.2	8.0 ± 0.0	8.2 ± 0.2	9.3 ± 0.5
DMSO	10%	6.0 ± 0.0							

ACA, on the other hand, displayed more potent antifungal activity with MIC values ranging from 1.56 to 25 mg/mL. The lowest MIC (1.56 mg/mL) for ACA was recorded against *C. albicans* 1 and *C. glabrata* 2, while the highest MIC (25 mg/mL) was observed against *C. albicans* 4. The ACA exhibited the same MIC and MFC values against most of the *Candida* isolates except for *C. glabrata* 3. The 0.12% w/v chlorhexidine, used as a positive control, showed MIC and MFC values in the range of 0.0038 to 0.03% w/v. The negative control, 10% DMSO, recorded no MIC and MFC value.

The MIC and MFC assays showed a contrasting trend with DDA. The ACA demonstrates better antifungal efficacy compared to HE. For example, ACA exhibited lower MIC and MFC values (1.56 mg/mL) against *C. glabrata* 2 than the HE (12.5 mg/mL (MIC) and 25 mg/mL (MFC)), indicating that ACA was more effective in inhibiting the growth and killing *Candida* at lower concentrations. This discrepancy between DDA and MIC/MFC results may be attributed to the inherent limitation of the DDA method when testing non-polar compounds like ACA and HE. The polarity of compounds can affect

TABLE 2. MIC and MFC values of *n*-hexane extract and ACA against *Candida* isolates

Extract	HE		ACA		CHX	
Species	MIC (mg/mL)	MFC (mg/mL)	MIC (mg/mL)	MFC (mg/mL)	MIC (% w/v)	MFC (% w/v)
C. albicans 1	6.25	6.25	1.56	1.56	0.03	0.03
C. albicans 2	6.25	6.25	12.5	12.5	0.0075	0.0075
C. albicans 3	3.13	3.13	12.5	12.5	0.0075	0.0075
C. albicans 4	3.13	3.13	25.0	25.0	0.015	0.015
C. glabrata 1	0.7	0.7	12.5	12.5	0.015	0.015
C. glabrata 2	12.5	25	1.56	1.56	0.0038	0.0038
C. glabrata 3	12.5	12.5	3.13	6.25	0.015	0.015
C. tropicalis 1	6.25	6.25	3.13	3.13	0.0038	0.0038

the diffusion rates into the agar medium, leading to potentially misleading results in DDA, especially for non-polar substances (Bubonja-Šonje, Knezević & Abram 2020). Therefore, while the DDA serves as an important preliminary screening tool, the MIC and MFC results provide a more reliable measure of antifungal potency for non-polar plant-derived compounds.

The antifungal potential of ACA was particularly notable, with the lowest MIC and MFC value observed against *C. albicans* 1 (1.56 mg/mL), a finding that aligns with studies reporting ACA's antimicrobial activity against Methicillin-Resistant *Staphylococcus aureus* (MRSA), multidrug-resistant *S. aureus* and dermatophyte pathogens including *Trichophyton mentagrophytes*, *T. rubrum,* and *Aspergillus niger* (Janssen & Scheffer 1985; Taib et al. 2020; Zhang et al. 2021). The potent activity of ACA is likely due to its structural features, including a carbonyl ester moiety that is directly attached to the phenyl ring, positioned at C-4 and C-1' and the presence of a 2'-3'-double bond and methoxyl group at C-3 and C-5, which may contribute to its ability to disrupt the cell membrane integrity of the pathogens (Anuar et al. 2020; Zhang et al. 2021).

The findings of this study also align with Aziz et al. (2013), who reported that ACA has potent antifungal properties against *C. albicans*, although the MIC values reported in this study were lower for certain isolates, such as *C. albicans* 1 (1.56 mg/mL vs. 2.5 mg/mL). This discrepancy may be due to the difference in the genetic profiles of the *Candida* isolates used, with Aziz et al. (2013) testing a laboratory strain of *C. albicans* from the American Type Culture Collection (ATCC 10231), whereas the current study utilised clinical isolates obtained from oral rinse samples. Variations in the antifungal susceptibility of *Candida* species can be influenced by numerous factors, including subjects' medication history, which may affect the pathogenicity and drug resistance profiles of the clinical isolates (Tamai, Pakbin & Fasaei 2021).

CONCLUSIONS

In conclusion, this study explored the potential of the HE of *A. conchigera* rhizome and ACA as alternative antifungal agents for the treatment of oral *Candida* infections. Although both HE and ACA exhibited antifungal activity, ACA demonstrated greater potency in the MIC and MFC assays, indicating its potential as a targeted antifungal compound. Further research is required to identify the synergistic effect of ACA with other bioactive compounds in the HE to evaluate its efficacy in the clinical setting.

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