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Original Research

Tropical Medicinal Plant Extracts from Indonesia as Antifungal Agents against *Candida Albicans*

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Abstract

Background: *Candida albicans* is responsible for a wide range of medical ailments, from harmless cutaneous to life-threatening bloodstream infections. Growing cases of antifungal-drug resistance strains of *C. albicans* become a rationale to explore and develop novel anti-candida agents. In this paper, we assessed the anti-candida activity of the methanolic extracts of various tropical medicinal plants from Myrtaceae, Poaceae, and Zingiberaceae, commonly used in Indonesia to treat fungal infections. **Methods**: *Candida albicans* strain ATCC 10231 was used as a subject to assess the anti-Candida activities of plant methanolic extracts through disc diffusion assay. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) were observed. **Results**: All plant extracts in this study showed antifungal activities against *C. albicans*. Among them, *Cymbopogon citratus, Curcuma xanthorrhiza, Curcuma aeruginosa*, and *Zingiber officinale* var. *rubrum* showed the lowest MIC and MFC value of 3.8 mg/mL. **Conclusions**: The growth inhibition of *C. albicans* on disc diffusion assay was demonstrated by *Z. officinale* var. *rubrum* and *C. longa*, which were comparable to antifungal nystatin. Further investigation of the chemical constituents of the extracts and the cytotoxicity test is needed to further develop plant-derived anti-candida agents.

Keywords: health care; tropical medicinal plants; *Candida albicans*; methanolic extract; minimum inhibitory concentration; minimum fungicidal concentration

1. Introduction

Candidiasis refers to infections caused by fungi of the genus *Candida* [1,2]. These infections may be cutaneous, mucosal (oral, oesophageal, and vulvovaginal candidiasis), or invasive (bloodstream and deep-seated candidiasis) [3–5]. The clinical spectrum of candidiasis varies from mild mucocutaneous infections to life-threatening candidemia (bloodstream infections), brain abscess, and endocarditis (infection of heart's inner lining) [6,7]. Candidiasis is recognized as one of the most common infections, with the global burden for superficial cutaneous and mucosal candidiasis being over 1,000,000,000 and 130,000,000 annual cases, respectively [8,9]. Furthermore, the estimated annual invasive candidiasis are 750,000 cases with a mortality rate of 40%–55%, especially in patients with risk factors such as immunodeficiency, advanced age, exposure to broad-spectrum antibiotics, cancer chemotherapy, organ transplantation, and prolonged stay in an intensive care setting [8,10,11].

Candida albicans, an opportunistic pathogen which is also a part of the normal human microflora, is the most common causing agent of candidiasis [12,13]. *C. albicans* is responsible for >90% and >40% of mucosal and invasive candidiasis cases in various countries, respectively [14–16]. Current therapies for *C. albicans* infection mainly involve administering antifungal drugs such as polyenes, azoles, echinocandins, and 5-Flucytosine (5FC) [17,18]. Unfortunately, as in the case of antibiotics for bacterial infection, there are rising cases of antifungal-resistant *C. albicans* due to extensive use of antifungal drugs in clinical settings [19,20]. Thus, finding alternative anti-candidal agents is of urgent importance.

One alternative of anti-candidal compounds is provided by plants. Some plant species produce secondary metabolites with anti-candidal activities, such as terpenes, terpenoids, and aromatic compounds [21]. A study reported that terpenes could inhibit cell growth, initiate apoptosis, and induce cell cycle arrest of yeast-like fungi, including *C. albicans* [22]. Terpenoids, a modified class of terpenes, can disrupt cell membrane integrity of *C. albicans* [23]. Mean-

while, eugenol, an aromatic compound, can kill *C. albicans* cell by disrupting its membrane morphology and function, causing oxidative stresses [24].

As a tropical country, Indonesia has a variety of medicinal plants which are traditionally used for treating *C. albicans* infection [25]. Plants from the Zingiberaceae family, such as *Alpinia galanga*, *Curcuma longa*, and *Curcuma xanthorrhiza*, were used for treating candidiasis [26–28]. Those plants produce anti-candidal secondary metabolites such as terpenoids (i.e., xanthorrhizol and curcumenol) and aromatic compounds (i.e., flavonoids and curcumin) [29, 30]. Another example is the fruit of clove *Syzygium aromaticum* L. (Myrtaceae), a native Indonesian plant whose main bioactive compound is anti-candidal eugenol [31].

Bioactive compounds from medicinal plants must be extracted first to be utilized as anti-candidal. The solvent is critical in the extraction process [32]. Methanol is an effective solvent for extracting anti-candidal compounds such as terpenoids, flavonoids, and polyphenol [33,34]. Besides that, the maceration method can prevent the damage of samples' bioactive components, especially the thermolabile ones [35]. This study aims to investigate the anti-candida (i.e., *C. albicans* strain ATCC 10231) activities of methanolic extracts of 11 medicinal plants from Indonesia. The result of this study is essential for developing anti-candidal food supplements and drugs.

2. Materials and Methods

2.1 Methanolic Extract Preparation

This study used 11 plant species from three families, i.e., Myrtaceae, Poaceae, and Zingiberaceae (Table 1, Ref. [31,36–44]). Plant materials were collected from Lawu Mountain, Jogorogo Village, Ngawi, East Java, Indonesia, at 7°3'31" S latitude and 112°32'0" E longitude (357 meters above sea level). Methanolic extract preparation was conducted based on a method developed by Wardana *et al*. [45]. One (1) kg of each plant powder was macerated with 3 L methanol for 24 hours at room temperature. The maceration process was repeated three times, after which the filtrate was passed through a Buchner funnel to obtain methanol extract. Each plant extract was then concentrated on a rotary vacuum evaporator to obtain a thick methanol extract. The thick methanol extract was then diluted in Dimethyl sulfoxide (DMSO) to obtain extract concentration of 500, 250, 125, 100, 50, and 25 mg/mL for anti-candida assay.

2.2 Anti-Candida Activities of the Plant Methanolic Extracts

The anti-candida activity of the 11 plants' methanolic extract was tested using a modified version of document M27-A3 published by the Clinical and Laboratory Standards Institute (CLSI) [46–48]. All assays were done in duplicate.

2.2.1 Inoculum Preparation of *C. albicans*

C. albicans ATCC 10231 used in this study was obtained from the Microbiology Laboratory, Department of Biology, Universitas Airlangga, Indonesia. One colony of *C. albicans* ATCC 10231 was inoculated in a Potato Dextrose Broth (PDB) and grown overnight at 37 °C (150 rpm). 60 μL of overnight culture was transferred into 3 mL PDB and grown at 37 °C (150 rpm) until their Optical Density (OD) at 600 nm was equal to 0.5 McFarland standard.

2.2.2 Disc Diffusion Assay

C. albicans culture from the previous step (100 μL) was spread onto Potato Dextrose Agar (PDA) using a sterile cotton swab. The PDA plates were then incubated at 37 °C for 48 hours with filter discs (6 mm) saturated with different dilutions of the methanolic extracts (25, 50, 100, 125, 250, 500 mg/mL). The solvents DMSO and nystatin (100 unit/disc) were used to dilute extract and acted as the negative and positive controls, respectively. The diameter of the clear area around the disc (i.e., inhibition zone) was measured using a vernier caliper, recorded, and considered an indication of antifungal activity of the plant methanolic extracts.

2.2.3 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the Plant Methanolic Extracts

Different concentrations of the methanolic extracts (1.85–250 mg/mL) were added to PDB media inoculated with 105 CFU/mL *C. albicans* in a 96-well microplate. The OD at 600 nm was measured using a microplate reader before and after incubation at 37 °C for 48 hours. The minimum inhibitory concentration (MIC) or the lowest concentration of the plant methanolic extract that prevents *C. albicans*' growth is observed when there is no increase in OD600 value after 48 hours of incubation. Meanwhile, the minimum fungicidal concentration (MFC) was determined by spreading 100 μL of *C. albicans* culture treated by plant methanolic extract at MIC concentration and two concentrations above it onto PDA plates. MFC is the lowest concentration that inhibits any *C. albicans* growth in the PDA plates.

3. Results

3.1 Plant Extraction Yield

Methanol extraction of 1 kg dry powdered plant materials yielded concentrated extract ranging from 72.1 to 183.5 gr (Table 2). The highest yield of plant extract was obtained from *C. longa,* while *C. citratus* gave the lowest result.

3.2 Anti-Candida Activities of the Plant Methanolic Extracts

Using a disc diffusion assay, 11 plant species commonly used in Indonesian traditional medicine were evaluated for their antifungal activity against *C. albicans*. The

Table 1. Data of plant species used in this study.

Family/species	Local name	Common name	Part used	Reported bioactive compounds	References
Myrtaceae					
Syzygium aromaticum	Cengkeh	Clove	Fruit	Eugenol, β-Caryophyllene, vanillin, crategolic acid/maslinic acid, kaempferol, rhamnetin, eugenitin, eugenin, ellagic acid, gallic acid, biflorin, myricetin, campesterol, stigmasterol. oleanolic acid, bicornin, quercetin, carvacrol	[31]
Poaceae					
Cymbopogon citratus	Serai/Sereh	Lemon grass	Bulb	Geraniol, geranial, geranyl acetate, myrcene, neral	[36]
Zingiberaceae					
Boesenbergia rotunda	Temu kunci	Fingerroot	Rhizome	Kaempferol, quercetin, hesperidin, caffeic acid, naringin, chlorogenic acid, p-coumaric acid, luteolin, diosmin	[37]
Curcuma aeruginosa	Temu hitam	-	Rhizome	Camphor, germacrone, borneol, 1,8-cineole, curzerene, germacrene a, germacrene b, camphene, limonene	[38]
Curcuma longa	Kunyit	Turmeric	Rhizome	Digalloyl-hexoside, caffeic acid hexoside, curdione, coumaric acid, caffeic acid, sinapic acid, qurecetin-3-d-galactoside, casuarinin, bisdemethoxycurcumin, curcuminol, demethoxycurcumin, isorhamnetin, valoneic acid bilactone, curcumin, curcumin-O-glucuronide	[39]
Curcuma heyneana	Temu giring	-	Rhizome	Curcumanolide A, oxycurcumenol, curcumenone, labda-8(17),12-diene-15,16-dial, isocurcumenol, curcumenol, germacrone	[40]
Curcuma xanthorrhiza	Temulawak	Javanese turmeric	Rhizome	Xanthorrhizol, α-terpinolene, *p*-cymen-7-ol, camphene, curcumin, α-pinene, camphor, *p*-cymene, germacrone, 1,8-cineole	[38]
Curcuma zedoaria	Kunyit putih	White turmeric	Rhizome	Curcumin, ethyl paramethoxycinnamate, β-turmerone, β-eudesmol, zingiberene, dihydrocurcumin, furanodiene, α-phellandrene, 1,8 cineole, β-elemene, germacrone	[41]
Zingiber montanum	Bangle	-	Rhizome	cis-3-(3',4'-dimethoxyphenyl)-4-[(E)-3''',4'''-dimethoxystyryl]cyclohex1-ene, (E)-4-(3',4'-dimethoxyphenyl) but-3-en-1-ol, 3,4-dimethoxybenzoic acid, 8-(13,14-dimethoxyphenyl)-2-methoxynaphto-1,4-quinone, and β-sitosterol	[42]
Zingiber officinale var. *rubrum*	Jahe merah	Red ginger	Rhizome	6-gingerol, 8- gingerol, 6-shogaol, 8-shogaol, quercetin, catechin, rutin, gallic acid, epicatechin, kaempferol, cinnamic acid, tannic acid, syringic acid, ferulic acid, geraniol, 1.8- cineole, linalool, farnesol	[43]
Zingiber officinale Rosc	Jahe putih	White ginger	Rhizome	Eudesmol, γ-terpinene, α-curcumene, zingiberene, alloaromadendrene, α-pinene, δ-cadinene, elemol, farnesal, (E)-β-farnesene, neril acetate, β-myrcene	[44]

results revealed that all plant extracts could inhibit *C. albicans* growth (Table 3). Two species, i.e., *Z. officinale* var. *rubrum* and *C. longa* showed a more extensive inhibition zone than nystatin at concentrations as low as 25 mg/mL (Table 3 and Fig. 1). The concentration of MIC and MBC from 11 plant species ranged from 3.91 to 15.62 mg/mL (Table 4). Methanolic extract of four species (i.e., *C. citratus*, *C. aeruginosa*, *C. xanthorrhiza*, and *Z. officinale* var. *rubrum*) had the lowest MIC and MBC value at 3.9 mg/mL (Table 4).

Table 2. Yield percentage of plant methanolic extract.

Plant species	pH	% Yield
Syzygium aromaticum	5.7	15.77
Cymbopogon citratus	5.5	7.21
Boesenbergia rotunda	5.9	16.28
Curcuma aeruginosa	6.2	15.03
C. longa	6.1	18.35
C. heyneana	6.1	15.15
C. xantorrhiza	6.2	17.87
C. zedoaria	6.0	13.52
Zingiber montanum	5.8	10.06
Z. officinale var. *rubrum*	5.9	10.80
Z. officinale Rosc	5.9	9.42

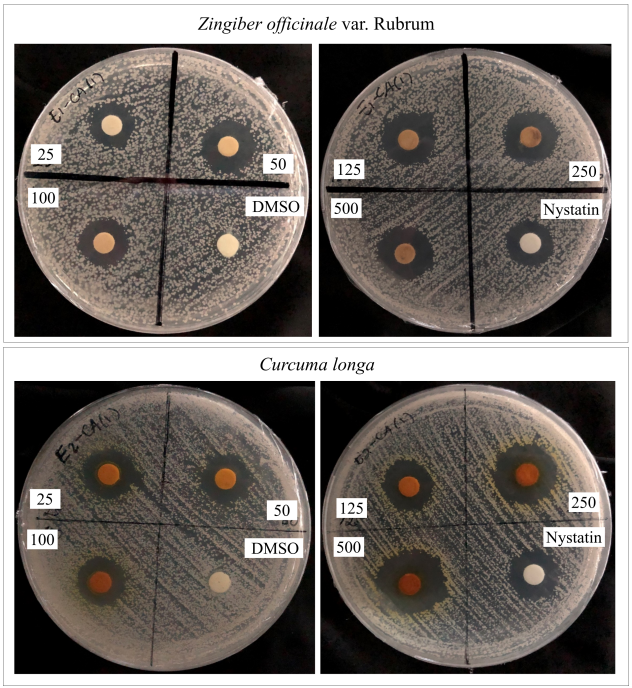

Fig. 1. Growth inhibition of *C. albicans* by *Z. officinale* var *rubrum* and *C. longa*. The methanol plant extract concentration used were 25, 50, 100, 125, 250, and 500 mg/mL. DMSO and nystatin (100 Units/disc) were used as the negative and positive control, respectively.

4. Discussion

In many Southeast Asian countries, medicinal plants are commonly used for antifungal activities. Among others, three plant species, i.e., *S. aromaticum*, *C. citratus*, *C. xanthorrhiza*, are widely used for treating candidiasis in Indonesia, Malaysia, and Thailand [26,49,50]. Extracting their organic solvent is one of the approaches to improve their anti-candida activities by bringing out related bioactive compounds [51,52].

Clove fruit (*S. aromaticum*) methanolic extract contains anti-candidal compounds such as flavonoids, polyphenols, and terpenoids [53,54]. However, there are limited re-

Table 3. The results of disc diffusion assay of plant methanolic extracts against *C. albicans*.

Plant species	Concentration (mg/mL)	Inhibition Zone (mm)
Syzygium aromaticum	25	7.25 ± 0.64
	50	7.60 ± 0.28
	100	8.40 ± 0.07
	125	8.18 ± 1.17
	250	11.08 ± 0.68
	500	11.10 ± 0.78
Cymbopogon citratus	25	7.08 ± 0.59
	50	6.95 ± 0.28
	100	6.95 ± 0.07
	125	6.73 ± 0.04
	250	7.03 ± 0.25
	500	6.68 ± 0.11
Boesenbergia rotunda	25	7.98 ± 0.53
	50	8.54 ± 0.62
	100	7.95 ± 0.00
	125	10.47 ± 0.05
	250	11.73 ± 1.80
	500	15.15 ± 0.28
Curcuma aeruginosa	25	6.70 ± 0.28
	50	7.30 ± 0.28
	100	7.53 ± 0.32
	125	7.25 ± 0.07
	250	7.58 ± 0.32
	500	7.30 ± 0.07
C. longa	25	14.30 ± 1.41
	50	14.63 ± 0.32
	100	13.95 ± 0.35
	125	14.68 ± 0.18
	250	17.13 ± 2.79
	500	19.60 ± 2.33
C. heyneana	25	6.75 ± 0.07
	50	7.63 ± 1.03
	100	7.03 ± 0.04
	125	6.63 ± 0.18
	250	7.53 ± 0.25
	500	8.53 ± 2.23
C. xantorrhiza	25	9.53 ± 4.84
	50	9.15 ± 3.32
	100	8.33 ± 3.01
	125	9.15 ± 1.41
	250	10.00 ± 0.28
	500	12.73 ± 5.06
C. zedoaria	25	6.63 ± 0.46
	50	6.38 ± 0.04
	100	6.73 ± 0.74
	125	7.55 ± 1.41
	250	6.80 ± 0.21
	500	7.05 ± 0.42

Table 3. Continued.

Plant species	Concentration (mg/mL)	Inhibition Zone (mm)
Zingiber montanum	25	6.15 ± 0.07
	50	6.34 ± 0.02
	100	6.19 ± 0.09
	125	6.10 ± 0.00
	250	6.13 ± 0.04
	500	6.83 ± 0.53
Z. officinale var. *rubrum*	25	12.48 ± 0.60
	50	14.18 ± 2.09
	100	13.68 ± 0.39
	125	14.20 ± 0.21
	250	16.60 ± 0.14
	500	16.48 ± 0.11
Z. officinale Rosc	25	6.47 ± 0.40
	50	6.73 ± 0.25
	100	7.45 ± 0.71
	125	7.40 ± 0.64
	250	6.83 ± 0.25
	500	6.63 ± 0.11
Nystatin	100 unit/disc	12.20 ± 0.11
DMSO	-	0 ± 0

Table 4. The MIC and MFC values of eleven plant species' methanolic extracts against *C. albicans*.

Plant species	MIC (mg/mL)	MFC (mg/mL)
Syzygium aromaticum	7.81	7.81
Cymbopogon citratus	3.91	3.91
Boesenbergia rotunda	15.62	15.62
Curcuma aeruginosa	3.91	3.91
Curcuma longa	7.81	7.81
Curcuma heyneana	7.81	7.81
Curcuma xantorrhiza	3.91	3.91
Curcuma zedoaria	15.62	15.62
Zingiber montanum	7.81	7.81
Zingiber officinale var. *rubrum*	3.91	3.91
Zingiber officinale Rosc	7.81	7.81

ports on the antifungal activity of clove fruit methanolic extract. To date, only one study by Vizhi *et al*. [54] evaluated the antifungal activity of the undiluted methanolic extract against *C. albicans via* disc diffusion assay. The reported inhibition activity was weaker than the results of the current study, where a much lower concentration of clove fruit methanolic extract was used. Furthermore, to our knowledge, our results are the first to report the MIC and MFC of clove fruit methanolic extract against *C. albicans*. When compared to extracts from different solvents (methanolic, diethyl ether, ethyl acetate, and n-hexane) of other parts of the plant (e.g., buds), our results are still superior in terms of growth inhibition observed in disc diffusion assay [55].

Methanolic extract of *C. citratus* bulb contained anti-candidal monoterpenes such as citral and geraniol. Citral can block the cell membrane synthesis and inhibit the cellular respiration of *C. albicans* [56]. Meanwhile, geraniol disrupts *C. albicans* membrane integrity and function by inhibiting ergosterol biosynthesis and plasma membrane H^+-ATPase [57]. Previous studies reported various effects of *C. citratus* methanolic extract on *C. albicans* growth. Aina *et al*. [58] and Ewansiha *et al*. [59] showed no inhibition of *C. albicans* growth, whereas a study by Zulfa *et al*. [60] showed lower MIC and MFC values than this study.

Two other species, i.e., *C. longa* and *Z. officinale* Rosc, are extensively explored for their anti-candidal activities. The main anti-candidal compound of these plants are polyphenol curcumin and gingerol, respectively, which damage *C. albicans* cell wall and plasma membrane [61, 62]. Previous studies [63,64] reported a higher MIC and MFC of *C. longa* methanolic extract than ours. Meanwhile, MIC and MFC of *Z. officinale* Rosc from our study are higher than previously reported results [65,66].

Up to our knowledge, there have not been any previous reports on MIC and MFC values of *B. rotunda*, *C. heyneana*, *C. zedoaria*, and *Z. montanum* methanolic extract. However, previous studies using the ethanolic extract from *B. rotunda* and *C. zedoaria* showed lower MIC and MFC values from the ethanolic extract of *B. rotunda* (MIC: 2.5 mg/mL, MFC: 5 mg/mL) and *C. zedoaria* (MIC90: 7.8 mg/mL) as compared to our results [67–70]. On the other hand, a previous study usingan essential oil extracted from hydrodistillation of *Z. montanum* also showed higher MIC and MFC values than our results [71]. Meanwhile, the only study in anti-candidal activity of *C. heyneana* [72] reported an inhibition zone in disc diffusion assay comparable to our result.

Among all methanolic extracts from Zingiberaceae plants used in this study, three samples (i.e., *C. aeruginosa*, *C. xantorrhiza,* and *Zingiber officinale* var. *rubrum*) showed the lowest MIC and MBC. The rhizomes of *C. aeruginosa* and *C. xantorrhiza* mainly contain anti-candidal monoterpene compounds camphor and terpinolene, respectively [38]. Camphor can downregulate genes related to biofilm formation, thus reducing the virulence of *C. albicans* [73]. Terpinolene works by disrupting *C. albicans* membrane integrity [74]. Another bioactive compound from *C. xantorrhiza* is xanthorhizzol, a bisabolane-type sesquiterpenoid that can disrupt *C. albicans* cell membrane [75]. Meanwhile, the main bioactive compounds of *Z. officinale* var. *rubrum* with antifungal activity are geraniol, 1.8-cineole, linalool, and farnesol which modify the permeability of *C. albicans* cell wall and sesquiterpenes ar-curcumene, which disrupts the cell membrane structure [27,76].

Philip *et al*. [77] showed no inhibition zone in the disc diffusion assay of *C. aeruginosa* methanolic extract, even at a high concentration of 500 mg/mL. Another study by Rukayadi *et al*. [78] showed a higher MIC and MFC values

of methanolic extract of *C. aeruginosa,* which is around 7 times and 13 times than our study, respectively. In the case of *C. xantorrhiza*, Novianti *et al*. [79] showed a higher inhibition zone (12.5 mm) than our result using 20 mg/mL methanolic extract. No studies reported MIC and MFC of methanolic *C. xantorrhiza.* However, two studies [80,81] reported a higher MIC and MFC of *C. xantorrhiza* from ethanolic extract than ours. For a similar reason, only one study [82] that used an ethanolic extract of *Z. officinale* var. *rubrum* showed a comparable MIC and MFC to our results.

Even though the MIC values from all 11 methanolic plant extracts are sometimes lower than in similar studies, they still showed antifungal activity against *Candida albicans*. The MIC values of this study can be considered in the moderate to low range of anti-candida activity compared to other similar studies [83–86]. The differences in anti-candida activity from previous studies might be influenced by several factors, such as the soil and climate where the plant materials were sampled [87–89]. Further investigation of the chemical constituents of the extracts and the cytotoxicity test against mammalian cells and test animals are needed to further develop plant extracts as the components of anti-candida drugs and health supplements.

5. Conclusions

Our results confirm the antifungal activities of methanolic extracts of 11 tropical medicinal plants from Indonesia against *Candida albicans* ATCC 10231. Among them, the methanolic extracts of *C. citratus*, *C. xanthorrhiza*, *C. aeruginosa*, and *Z. officinale* var. *rubrum* had the lowest MIC and MFC value, whereas in relatively low concentration, *Z. officinale* var. *rubrum* and *C. longa* showed antifungal activity comparable to nystatin. This study provides the first report on MIC and MFC values of *S. aromaticum* fruit, *C. xantorrhiza,* and *Z. officinale* var. *rubrum* methanolic extract. However, these results need to be interpreted cautiously, as this study only tested one strain of *C. albicans*. Different *C. albicans* strains can give different results due to their difference in sensitivity toward anti-candida plant extract. Therefore, further study using other *C. albicans* strains, including the ones obtained from patients (clinical strain), will be necessary to offer a bigger picture of anti-candida activity of plant extracts on *C. albicans*.

Author Contributions

AG and YSWM conceptualized the research study. AG and APW designed the research methodology. AG, APW, AYS, MRAW, NHW, VRH and NIDD performed the research and analyzed the data. AG, APW, AYS, MRAW, NHW, VRH and NIDD wrote the manuscript. AG, YSWM and ANK reviewed and contributed to editorial changes in the manuscript. AG, YSWM, NSA and ANK supervised the research. YSWM acquired the funding for the research. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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