Antifungal activity of aqueous and corn steep liquor extract of *Ficus exasperata*, *Anonna muricata* and *Azadiractha indica*

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Received: April 3, 2020
Accepted: May 19, 2020
Published on-line: September 14, 2020
Published: December 25, 2020

This study investigated the activity of aqueous and corn steep liquor (CSL) extracts of *Ficus exaperasta*, *Annona muricata* and *Azadirachta indica* against *Candida* spp. isolated from high vaginal swab samples. Phytochemical screening of the plants was done using standard methods, the antifungal activity of the plant's extracts and standard drugs were tested against isolates of *Candida* spp. using the agar well diffusion method; the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were also determined using microdilution standardized techniques. Phytochemical screening of the aqueous and CSL extracts of the plants revealed the presence of tannin, saponin, flavonoids, and terpenoids. Among the five *Candida* strain isolates, the zone of inhibition produced by the plant extracts against *C. albicans* shows a range of 0-18.3 mm; *C. krusei* (strain A): 5.1-24.5 mm; *C. krusei* (strain B): 0-18.0 mm; *C. kefyr* (strain A): 6.1-27.5 mm; and *C. kefyr* (strain B): 0-22.0 mm. The CSL extract had higher inhibitory action compared with aqueous extract, also *F. exasperata* and *A. muricata* gave better antifungal activity against the tested *Candida* strains. The MIC of the aqueous and CSL extracts of the *F. exasperata* ranged between 6.25-12.50 mg/mL; *A. muricata*: 3.125-12.500 mg/mL, while the aqueous and CSL extracts of *A. indica* was found to have no activity at all the tested concentrations against *C. albicans*, *C. krusei* (strain A) and *C. krusei* (strain B), similar observation for the MFC. This study proved the antifungal efficacy of aqueous and CSL extracts of *F. exasperata*, *A. muricata*, and *A. indica* against isolates of *Candida* species which are usually implicated in candidiasis.

Key words: *Candida species; Ficus exasperata; Annona muricata; Azadirachta indica; Corn Steep Liquor; Antifungal activity*

http://dx.doi.org/10.5937/leksir2040005A

1. INTRODUCTION

Fungal infections continue to be a source of concern for public health as it is known to affect many people worldwide. In recent times, infections caused by fungi are considered to be the fourth foremost causes of infections spread by blood where the prominent fungi concerned are those of *Candida* species (Pierce, 2005; Tsai et al., 2013). *Candida* spp. is one of the common fungal infection plaguing humans. These yeast species are characterized by a thin cell wall, ovoid cells that are typically 3-5 µm in diameter, often forming the normal flora of skin, mouth, vagina, and intestine. They are also known to be opportunistic pathogens that infect people with low immunity (Calderone and Fonzi, 2001; Rahman et al., 2011). *Candida* remains the major fungal pathogen responsible for the majority of human infections ranging from localized superficial to systemic candidiasis. It accounts for over 50% of all cases in the world, while an increase in the prevalence of yeast infections caused by *Candida* species other than *C. albicans* such as *C. glabrata*, *C. krusei*, *C. tropicalis*, and *C. parapsilosis* has been reported in different parts of the world (Pfaller and Diekema, 2007; Pinto et al., 2009). Thus, the quality of life could be affected by a fungal infection which is usually treated using antifungal medications. Some of these medications apart from being costly, have unpleasant side effects and may cause the resistance of the organisms to the antifungal agent. Consequently, there is a constant search for alternative antifungal treatments some of which are obtained from medicinal plants.

Medicinal plants form the backbone of traditional/alternative system of medicine and they have been the subject of research interest for pharmacological studies in the last couple of decades. This is as a result of the acceptance of the value of

medicinal plants as a possible source of novel compounds of therapeutic importance (Prusti et al., 2008). Among plants of medicinal importance, *Ficus exasperata*, *Annona muricata*, and *Azadirachta indica* are used for various therapeutic purposes.

Ficus exasperata Vahl. belongs to the family Moraceae and it is locally known as "sandpaper tree" and "Ewe ipin" in the Yoruba language of Western Nigeria. Different parts of the plant are locally used for treating various infectious diseases such as eye-sores, ringworm, stomach pains and leprosy, etc. (Bafor and Igbinuwen, 2009). Some pharmacological activities like anti-hypertensive, antioxidant, anti-inflammatory, anti-ulcer, anti-lipidic, anti-bacterial, and anti-fungal have been documented for *F. exasperata* (Sonibare et al., 2006).

Annona muricata L. belongs to the family Annonaceae, it is locally called soursop due to the slightly acidic taste of the fruit when ripe (Hutchinson and Dalziel, 2000). Different parts of the plants have been previously reported to possess bioactivity. For instance, its leaves and stem are considered to show cytotoxicity against cancer cells as a result of a compound called acetogenin activity which is not toxic to normal cells (Oberlies et al., 1995). Acetogenins and other phytocompounds present in the plant have collectively exhibited antitumor, parasiticidal, pesticidal, and antimicrobial activities (McLaughlin, 2008).

Azadirachta indica A. Juss. commonly-known as neem belongs to the family Meliaceae. It is indigenous to India and established in many of the tropical and subtropical countries including Nigeria. The plant has been reported to possess many pharmacological activities such as antioxidant and antifungal among others (Choudhary et al., 2017; Itelima et al., 2016; Mondall et al., 2009; Nahak and Sahu, 2010).

The bioactive substances in these plants are usually extracted with a wide range of solvents for various ethnobotanical applications. The literature is also well supported with information on the antimicrobial properties of various extracts of *F. exasperata* (Adebayo et al., 2009; Ajayi et al., 2012; Takon, 2013), *A. muricata* (Vijayameena et al., 2013) and *A. indica* (Mohammed and Omer, 2017). However, none of these studies has reported the activity of corn steep liquor (CSL) extracts against fungal species. The CSL which is a by-product of the wet corn milling process (Abdus-Salaam et al., 2014), and water are commonly used to prepare herbal decoctions which are utilized in the treatment and management of various disorders including fungal infection. In this study, we present findings on the antifungal activity of aqueous and corn steep extracts of *F. exasperata*, *A. muricata*, and *A. indica* against vaginal isolates of *Candida* species.

2. MATERIALS AND METHODS

2.1. Plant materials and extraction procedures

Leaves samples from *F. exasperata*, *A. muricata* and *A. indica* were collected in the early hours of the day from the arboretum of the Department of Forestry and Wildlife Management, Federal University of Agriculture, Abeokuta, Nigeria. The plant specimens were identified at the Forest Herbarium Ibadan. The leaves samples were air-dried for two weeks on a cabinet dryer and then milled to powdered form. Freshly made corn steep liquor (CSL), a byproduct of the corn wet milling process, was obtained from the local makers of pap, the main product of the wet milling process. Aqueous and CSL extracts were of the three plants were prepared according to the method previously used by Pai et al. (2010) with modifications. Sterile distilled water and CSL (both 500 mL) were used to extract 50 g of the powdered plant samples by boiling for 30 minutes on a hot plate (100 °C), allowed to cool and filtered using a sterile filter paper. The concentration of the filtrates was reduced to a semi-solid mass on a water bath to get the crude extracts of the three plant samples which were then stored at 4 °C in preparation for the phytochemical screening and fungal susceptibility testing of the extracts described in the subsequent sections.

2.2. Collection and isolation of *Candida* strains

High vaginal swabs from suspected cases of *Candida* infection were obtained from the Federal Medical Centre, Abeokuta. The obtained swabs containing *Candida* spp. were cultivated on potato dextrose agar (PDA) and *Candida* strains identified using standard microbiological procedures (Shah et al., 2012; Galle and Gianinni, 2004). In brief, macroscopic examination of the cultures considered rate of growth, colonial morphology, pigment production, texture, and colour of surface or reverse, while microscopic examination was done using the germ tube test.

2.3. Phytochemical analysis

Phytochemical screening of the plant extracts was carried out using standard methods for determination of tannins, saponins, anthraquinones, flavonoids, and terpenoids' presence. In brief, 2 g of the crude extract was re-dissolved in 10 mL of distilled water and CSL, then the presence of tannin was detected using the ferric chloride test, saponin's presence was detected using the Frothing test, anthraquinone was determined using the Bontrager's test, flavonoid was detected using the lead acetate test, while terpenoids' screening was done using the Salkowski's test (Harborne, 1973; Obasi et al., 2010; Roopashree et al., 2008).

2.4. Sensitivity testing of aqueous and corn steep liquor plant extracts against *Candida* isolates

Agar well diffusion method was used to test the sensitivity of the *Candida* isolates to the aqueous and CSL leaves extracts. This was done according to the method described by Kareem et al. (2010). Sterile cotton-wool swab was dipped into prepared inoculum and spread all over PDA plate. After each swabbing, the swab was passed around the edges of the agar surface medium and left to dry for few minutes at room temperature with the lid closed. Then, by using a sterile cork borer, four wells of 4 mm in diameter representing the four concentrations (100, 75, 50 and 25 mg/mL) of the sample extracts were made in the inoculated plate and each well labeled for each concentration. The three plants' crude extracts were each reconstituted with sterile distilled water and CSL to a stock concentration of 200 mg/mL after which four working concentrations of 100, 75, 50 and 25 mg/mL of aqueous and CSL of extracts of each plant were prepared from the stock then, 50 µL of each concentration was introduced into the respective wells using a micropipette in the inoculated plates. The plates were left for half an hour with the lid closed. The plates were incubated at 28 °C for 24 h and then observed for the zone of inhibition which is characterized by the clear area around the well. The experiment was done in triplicates and the zone of growth inhibition was measured with a transparent ruler and expressed in millimeters.

2.5. Determination of the minimum inhibitory concentration of plant extracts on *Candida* isolates

The modified method of Malwal and Sarin (2011) was employed for the determination of the minimum inhibitory concentration (MIC) of extract using the broth dilution method. For the broth dilution, the growth concentration was adjusted to 10^5 organisms/mL by using 0.5 mL McFarland turbidity

Table 1. Results of phytochemical screening of aqueous and CSL extracts of the test plants

	F. exasperata[a]		*A. muricata*		*A. indica*	
Phytochemical	Aqueous	CSL	Aqueous	CSL	Aqueous	CSL
Tannin	+	+	+	+	+	+
Saponin	+	+	+	+	+	+
Anthraquinone	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+

[a] Signs '+' and '-' denote presence and absence, respectively.

standard. Aqueous and CSL solution of 25 % was prepared from the leaves extracts as the stock solution. Potato dextrose broth (5 mL) was added in MIC tubes. One milliliter of plant extracts stock was added to each tube containing the broth at various concentrations (25, 12.5, 6.25 and 3.125 %). The tubes were incubated at 28 °C for 48 hours and observed for visible growth after shaking the tubes gently to mix. The experiment was done in duplicate. The MIC was taken as the lowest concentration of the extracts at which there is no turbidity after incubation. The negative control containing only broth appeared clear.

2.6. Determination of the minimum fungicidal concentration (MFC) of plant extracts on *Candida* isolates

This is usually carried out after MIC test, to determine the minimum concentration of the test antifungal agent that will kill the microorganism. The contents of the MIC wells were inoculated on sterile PDA separately. The plates were labeled indicating the well from which the isolate was plated and incubated at 28 °C for 48 hours for growth. Plates that showed growth of organisms were checked to determine the wells from which they were inoculated. The lowest concentration of the wells from which the plates were inoculated that did not show any growth was considered as the MFC of the organism (Levinson, 2016).

2.7. Antifungal susceptibility pattern

Antibiogram was performed using the method of Clinical and Laboratory Standards Institute (Kanthasamy et al., 1989). An inoculum was prepared and its turbidity was adjusted visually with the transmittance to that produced by a 0.5 McFarland standard. After obtaining the 0.5 McFarland standards for isolated species of *Candida* they were swabbed uniformly on the surface of Muller Hinton agar and allowed to dry under sterile conditions. After drying, antifungal discs of three commercial antibiotics namely Amphotericin B, Fluconazole and Ketoconazole were placed onto the surface of inoculated plates of *Candida*. The plates were incubated at 35 °C for 24 hours and after incubation zones of growth inhibition were measured, results were recorded as susceptible or resistant.

3. RESULTS

In other to assess the antifungal effects of CSL and aqueous extracts of three medicinal plants on clinical isolates of *Candida* spp., high vaginal swab samples obtained from suspected cases of candidiasis were used to isolate and test the potential effect of the tested extracts against the isolated organisms adopting the agar well diffusion method. Results from the high vaginal swab samples revealed that three *Candida* species were identified. One strain of *C. albicans* has been identified, while *C. kefyr* and *C. krusei* had two strains each. The morphological analysis of the three species showed that isolates had cream colour, spherical shape and the surface margin entire. *C. albicans* had a flat elevation while the strains of *C. kefyr* and *C. krusei* had a raised elevation. The biochemical analysis showed that the three species were urea negative. *C. kefyr* and *C. krusei* were also germ tube negative, while *C. albicans* was germ tube positive. The results of the phytochemical screening of the aqueous and CSL extracts of the species *F. exasperata*, *A. muricata* and *A. indica* are presented in Table 1. The results show the presence of all observed phytochemical compounds except anthraquinone.

The inhibitory activity of the aqueous and CSL extracts of tested medicinal plants against the isolated strains of *Candida* spp. is represented in Table 2. The mean zone of inhibition produced by the plant extracts against *C. albicans* show that for *F. exasperata* it ranged from 0-16.8 mm, for *A. muricata* ranged from 9.1-18.3 mm while for *A. indica* it ranged from 0-10.4 mm; the mean zone of inhibition observed in *C. krusei* (Strain A) indicate that for *F. exasperata* it ranged from 9.8-24.5 mm, for *A. muricata* it ranged from 9.4-23.4 mm while for *A. indica* it ranged from 5.3-14.4 mm; the mean zones of inhibition produced against *C. krusei* (Strain B) revealed that for *F. exasperata* it ranged from 0-18.0 mm, for *A. muricata* it ranged from 8.2-17.5 mm compared while for *A. indica* it ranged from 0-11.8 mm. Also, the mean inhibition zones produced against *C. kefyr* (Strain A) revealed that for *F. exasperata* it ranged from 10.3-25.1 mm, for *A. muricata* it ranged from 18.4-27.5 mm while for *A. indica* it ranged from 6.1-16.3 mm; against against *C. kefyr* (Strain B), the mean zones of inhibition revealed that for *F. exasperata* it ranged from 0-19.1 mm, for *A. muricata* it ranged from 2.1-22.0 mm while for *A. indica* it ranged from 0-16.4 mm. Both aqueous and CSL extracts of *A. muricata* had higher inhibitory activity compared with the other plants against all the *Candida* spp. while the least inhibitory effect was observed in *A. indica*.

The results of the antifungal sensitivity testing of the *Candida* strains against three commercial antibiotics are also shown in Table 2. Amphotericin B had the most sticking antifungal effect against the five strains of *Candida* spp. with the growth inhibition zone ranging from 15.0-21.0 mm while fluconazole had inhibitions on only the two strains of *C. kefyr*, while all the *Candida* strains demonstrated significant susceptibility to Ketonidazole except *C. albicans*. The result also suggests that the CSL extracts of *A. muricata* at the concentration range of 50-100 mg/mL showed better antifungal activity against the *C. kefyr* (Strain A) when compared with the standard antifungal drugs.

The results of the Minimum Inhibitory Concentrations (MICs) and Minimum Fungicidal Concentration (MFC) of the plant extracts against the tested *Candida* strains are presented in Table 3. The MICs of the aqueous and CSL extracts of the three medicinal plants were obtained by the broth dilution method where the MICs were determined as the lowest concentration of aqueous and CSL extracts that gave sterile culture after incubation. The MIC of the aqueous and CSL extracts of the

Table 2. Mean inhibition zone (mm) of *Candida* species after application of aqueous and CSL extracts of *F. exaperasta*, *A. muricata*, and *A. indic*a with commercial antibiotics as a control

	Extract	Conc.	*C. albicans*	*C. krusei*		*C. kefyr*	
Species		[mg/mL]		Strain A	Strain B	Strain A	Strain B
F. exasperata	Aqueous	25	-	9.8±0.9	6.2±0.6	17.2±1.7	-
	CSL	25	6.0±0.2	14.1±0.8	-	10.3±0.6	14.1±1.4
	Aqueous	50	3.3±0.6	11.7±1.4	9.2±1.3	19.4±1.2	10.0±1.0
	CSL	50	10.6±1.3	18.2±1.9	12.5±1.0	13.1±0.5	17.2±2.1
	Aqueous	75	10.3±0.8	16.3±1.2	11.9±0.8	24.3±2.0	14.4±1.4
	CSL	75	14.2±1.7	20.6±1.3	18.0±1.4	18.3±0.8	19.1±1.3
	Aqueous	100	13.1±0.9	21.3±1.7	15.1±1.2	25.1±1.9	15.7±0.8
	CSL	100	16.8±1.4	24.5±2.3	11.8±1.3	23.0±1.6	17.6±1.5
A. muricata	Aqueous	25	9.1±1.1	12.1±0.8	8.2±0.7	19.2±2.2	2.1±0.5
	CSL	25	11.5±0.6	9.4±0.8	12.2±0.7	18.4±1.4	11.4±1.1
	Aqueous	50	12.4±1.2	16.2±1.2	8.2±1.4	22.0±1.7	5.0±0.5
	CSL	50	14.1±0.5	17.3±1.2	13.5±1.5	23.4±2.1	18.0±1.2
	Aqueous	75	15.3±0.9	22.2±1.7	14.1±1.2	23.8±1.4	12.1±1.8
	CSL	75	16.2±1.4	18.1±0.8	14.3±0.7	24.1±1.6	17.2±1.6
	Aqueous	100	17.7±1.5	22.7±1.6	17.0±0.8	25.5±1.8	14.3±1.2
	CSL	100	18.3±1.8	23.4±1.8	17.5±1.2	27.5±0.6	22.0±1.7
A. indica	Aqueous	25	-	5.3±0.6	-	6.1±0.4	-
	CSL	25	-	5.9±0.3	2.0±0.6	7.2±0.8	-
	Aqueous	50	-	10.0±1.5	-	10.3±1.6	4.2±0.4
	CSL	50	-	6.8±0.4	6.4±1.4	9.1±0.4	8.1±0.8
	Aqueous	75	-	11.2±0.8	4.2±0.8	11.7±0.8	7.8±1.2
	CSL	75	7.5±0.7	11.8±1.3	11.4±1.4	11.4±1.1	10.3±1.4
	Aqueous	100	-	13.3±1.2	9.0±0.9	14.2±1.2	12.2±0.9
	CSL	100	10.4±1.6	14.4±0.7	11.8±1.6	16.3±0.8	16.4±1.3
Antibiotics		[µg/mL]					
Fluconazole		10	-	-	-	20.3±0.7	15.2±1.1
Ketonidazole		10	-	17.2±0.1	19.1±0.2	22.0±1.5	18.2±0.8
Amphotericin B		10	21.0±0.2	20.3±0.1	15.0±0.5	19.4±1.8	18.1±1.2

Table 3. Minimum inhibitory concentration (MIC) (mg/mL) and minimum fungicidal concentrations (MFC) (mg/mL) of aqueous and CSL extracts of test plants against isolates of *Candida* species

Test	Species	Strain	*F. exasperata*		*A. muricata*		*A. indica*	
			Aqueous	CSL	Aqueous	CSL	Aqueous	CSL
MIC	*C. albicans*	-	-	12.5	12.5	12.5	-	-
	C. krusei	Strain A	12.5	6.25	6.25	6.25	-	-
		Strain B	12.5	6.25	6.25	6.25	-	-
	C. kefyr	Strain A	12.5	6.25	6.25	3.125	25	12.5
		Strain B	6.25	6.25	6.25	3.125	25	12.5
MFC	*C. albicans*	-	-	25	25	25	-	-
	C. krusei	Strain A	25	12.5	12.5	12.5	-	-
		Strain B	25	12.5	12.5	12.5	-	-
	C. kefyr	Strain A	25	12.5	12.5	6.25	-	25
		Strain B	12.5	12.5	12.5	6.25	-	25

F. exasperata ranged between 6.25-12.5 mg/mL, though the aqueous extract was found to have no activity against cultures of *C. albicans*, The MIC for CSL and aqueous extracts of *A. muricata* ranged from 3.125-12.5 mg/mL, while the aqueous and CSL extracts of *A. indica* were found to have no activity at all the tested concentrations against *C. albicans*, *C. krusei* (Strain A) and *C. krusei* (Strain B). The MFC of both aqueous and CSL extracts was determined as the lowest concentration of the plant extracts that did not permit any visible growth of the inoculated test organism in broth culture. The aqueous extract of *F. exasperata* recorded no inhibition against *C. albicans* at the tested concentration range while the MFC of the aqueous and CSL extract against the other strains ranged from 12.5-25 mg/mL. The aqueous extract of *A. indica* gave no inhibition at the tested concentration range.

4. DISCUSSION

Many communities in the developing world continue to rely on the use of herbs for primary health care purposes. In the traditional medicinal applications, water and CSL are two of the commonly used solvent to prepare herbal decoction used to treat various ailments including fungal infection. Data available from the literature indicates that little or no information is available on the antifungal activity of the CSL extracts of the studied plants. Thus, in this study, the antifungal activity of the aqueous and CSL extracts of *F. exasperata*, *A. muricata*, *A. indica* were tested against *Candida* species isolated from high vaginal swab (HVS) samples. The presence of *Candida* in the HVS was ascertained, the genus *Candida* often forms the normal flora of the vagina, where some species may become opportunistic pathogens, if the defence mechanism of the host is weak (Bader et al., 2003). These *Candida* species in the vaginal flora are known to be responsible for candidiasis under low immunity. Three species of *Candida* (*C. albicans*, *C. kefyr* and *C. krusei*) were isolated from high vaginal swab samples, this agrees with previously reported studies in many countries that demonstrated that some of the prevalent yeast isolated from HVS were *C. albicans*, *C. krusei* and *C. kefyr* (Asticcioli et al., 2009; Ellabib and ElJariny, 2001; Hamza et al., 2008; Mohanty et al., 2007). Candidiasis has often been treated in hospitals with antibiotics. However, some reports have been published on the increasing development of resistance of microorganisms to antibiotics (Levy, 2002). Furthermore, studies have also shown that antibiotics usage for different human ailments including prophylactic and preventive purposes as well as the general well-being of the body have contributed to the increasing rise in resistance to antibiotics (Kothavade et al., 2010). Thus, it is not surprising that *Candida* species isolated from this study showed varying reactions to the commercially available antibiotics. *C. albicans* was found to be susceptible to Amphotericin B with the zone of inhibition of 21 mm while it was resistant to Fluconazole and Ketonidazole. The results of this study are consistent with a previous study which also suggest susceptibility pattern of *Candida* species to a diverse range of antibiotic (Hamza et al., 2008). The result from the antifungal testing of the aqueous and CSL extract of the studied plants suggests that both *F. exasperata* and *A. muricata* had a possible antifungal effect against the isolated *Candida* strains at higher concentration showing a partial dose-dependent trend, the CSL extract was found to particularly elicit greater antifungal effect that the aqueous extracts. It seems possible that this may be due to the presence of lactic acid bacteria and other bioactive components in the CSL which may have contributed to the increased extractive ability of the bioactive substances present in the medicinal plants. Furthermore, it is noteworthy that CSL on its own has been demonstrated to possess antibacterial activity (Abdus-Salaam et al., 2014); other solvent extracts of *F. exasperata* root bark have also been reported to possess significant activity against *Candida* species (Lawal et al., 2012), this corresponds with the findings in this study for both the aqueous and CSL leave extracts of *F. exasperata*. At lower concentration, the aqueous extract of *A. indica* was found to have little or no activity against *C. albicans*, *C. kefyr* (Strain B), and *C. krusei* (Strain B), while a dose-dependent inhibition was observed against *C. albicans* by the CSL extract of *A. muricata*. This trend is in agreement with the observations reported by Olaiya et al. (2016) where CSL extracts of *Citrullus colocynthis*, *Curculigo pilosa* and *Gladiolus psittacinus* demonstrated significant antibacterial effect against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonae* and *Salmonella typhi*. However, *F. exasperata* extract was reported to have no activity against *C. albicans* in the study by Adebayo et al. (2009) which is contrary to the findings in this study; this may be a reflection of the effect of the different solvent type used in the study. Various solvent extracts of *A. muricata* have been shown to exhibit antibacterial activity (Vijayameena et al., 2013). The findings in this study also matched those observed in the study by Pai et al. (2010) where an aqueous extract of *A. muricata* was reported to be highly effective against *Candida* species; it is also noteworthy that both aqueous and CSL extracts of *A. muricata* showed better activity against *C. kefyr* (Strain A) than all the conventional antibiotics. The minimal antifungal activity of *A. indica* extracts observed in this study mirrors those reported by Mahmoud et al. (2011) where little activity was found against *C. albicans*, though, in another study, tissue conditional mixed with *A. indica* extracts at two concentrations was reported to exhibit considerable potential effectiveness against *C. albicans* (Barua et al., 2017). The MIC values revealed that the aqueous and CSL leave extracts of the *F. exasperata* inhibited the growth of four of the tested pathogens within range of 6.25-12.5 mg/mL. The MIC for the aqueous and CSL extract of *A. muricata* ranged between 6.25-12.5 mg/mL and 3.125-12.5 mg/mL respectively, this shows the potential of the plant extract to inhibit the growth of the human fungal pathogens, however, only two of the *Candida* species gave a MIC value for *A. indica* extracts with the CSL extract inhibiting both strains of *C. kefyr* at a concentration of 12.25 mg/mL and no inhibition observed at the tested concentration ranged against the other *Candida* strains. In the same manner, the fungicidal effect of the tested extracts shows that only the aqueous extract of *F. exasperata* did not demonstrate a fungicidal effect against *C. albicans* at the tested concentration range. The MFC of the plant extracts tends towards the mid concentration for most of the plant extracts that showed activity with CSL extract of *A. indica* only demonstrating fungicidal action at 25 mg/mL against the two strains of *C. kefyr*. This observation justifies the use of this plant as an antimicrobial agent in traditional medicine. The antifungal activity shown by the extracts of the studied plants may be attributed to the presence of the secondary metabolites that were detected to be present in both the aqueous and CSL extracts of the *F. exasperata*, *A. muricata* and *A. indica*, this observation also supports the findings in other similar studies on different extracts of the studied plants (Iyanda-Joel et al., 2019; Nimenibo-uadia, 2017; Saleh Al-Hashemi and Hossain, 2016; Vijayameena et al., 2013). Some researchers have demonstrated the antifungal effects of various forms of tannin tested against a wide range of fungi including *Candida* species (Morey et al., 2016; Latté and Kolodziej, 2000), similarly, saponin rich extracts isolated from plants have also been reported to have good activity against *Candida* species (Coleman et al., 2010; Tsuzuki et al., 2007; Yang et al., 2006; 2018); likewise, the bioactivity of flavonoids and polyphenolics against *Candida* species have been documented (Patuwo et al., 2014; Salazar-Aranda et al., 2015). These evidence suggest that the antifungal activity elicited by the CSL and aqueous extracts of the plants may be due to the pres-

ence of these phytochemicals whether working alone or in synergy which could therefore, justify their folkloric use in alternative medicine. However, further studies are needed to isolate specific phytochemical compounds present in the CSL and aqueous extracts of the plants which may be responsible for the antifungal activity.

CONCLUSION

This study has shown the antifungal efficacy of aqueous and CSL extracts of *F. exasperata*, *A. muricata*, and *A. indica* against isolates of *Candida* species which are usually implicated in candidiasis, thereby providing additional evidence on the antimicrobial activity of CSL extracts of the plants; this has been attributed to its phytochemical extractive ability which is known to elicit antifungal activities.

ACKNOWLEDGMENTS

The authors are grateful to the microbiology section of the Federal Medical Centre, Abeokuta for providing the high vaginal swab samples and also to Mrs. Rafiu B. O of BMRC, FRIN for technical assistance during the laboratory analysis and the Forest Herbarium Ibadan for identifying the plant samples.

REFERENCES

Abdus-Salaam, R. B., Adepoju, P. A., Olaleye, O. N. and Adeoye, I. A. (2014). Studies on the antimicrobial effect of corn steep liquor on some diarrhoea causing organisms, *African Journal of Biotechnology* **13**(2): 332–335.

Adebayo, E., Ishola, O., Taiwo, O., Majolagbe, O. and Adekeye, B. (2009). Evaluations of the methanol extract of *Ficus exasperata* stem bark, leaf and root for phytochemical analysis and antimicrobial activities, *African Journal of Plant Science* **3**(12): 283–287.

Ajayi, O. B., Oluyege, J. O., Olalemi, O. M. and Ilesanmi, T. M. (2012). Nutritional composition, phytochemical screening and antimicrobial properties of the leaf of *Ficus exasperata* (Vahl), *Asian Journal of Biological and Life Sciences* **1**(3): 242–246.

Asticcioli, S., L., S., Daturi, R., Matti, C., Nucleo, E., Zara, F. and Pagani, L. (2009). Trends in frequency and *in vitro* antifungal susceptibility patterns of *Candida* isolates from women attending the STD outpatients clinic of a tertiary care hospital in Northern Italy during the years 2002-2007., *The new Microbiologica* **32**(2): 199–204.

Bader, T., Bodendorfer, B., Schröppel, K. and Morschhäuser, J. (2003). Calcineurin is essential for virulence in *Candida albicans*, *Infection and Immunity* **71**(9): 5344–5354.

Bafor, E. and Igbinuwen, O. (2009). Acute toxicity studies of the leaf extract of *Ficus exasperata* on haematological parameters, body weight and body temperature, *Journal of Ethnopharmacology* **123**(2): 302–307.

Barua, D., Basavanna, J. and Varghese, R. (2017). Efficacy of neem extract and three antimicrobial agents incorporated into tissue conditioner in inhibiting the growth of *C. albicans* and *S. mutans*, *Journal of clinical and diagnostic research* **11**(5): ZC97–ZC101.

Calderone, R. A. and Fonzi, W. A. (2001). Virulence factors of *Candida albicans*, *Trends in Microbiology* **9**(7): 327–335.

Choudhary, S., Dwivedi, A. K. and Singh, P. (2017). Antifungal activities of neem leaf extracts on the growth of *Macrophomina phaseolina in vitro* and its chemical characterization, *IOSR Journal of Environmental Science, Toxicology and Food Technology* **11**(4): 44–48.

Coleman, J. J., Okoli, I., Tegos, G. P., Holson, E. B., Wagner, F. F., Hamblin, M. R. and Mylonakis, E. (2010). Characterization of plant-derived saponin natural products against *Candida albicans*, *ACS Chemical Biology* **5**(3): 321–332.

Ellabib, M. S. and ElJariny, I. A. (2001). *In vitro* activity of 6 antifungal agents on *Candida* species isolated as causative agents from vaginal and other clinical specimens, *Saudi Journal of Medicine* **22**(10): 860–863.

Galle, L. C. and Gianinni, M. J. S. M. (2004). Prevalência e susceptibilidade de leveduras vaginais, *Jornal Brasileiro de Patologia e Medicina Laboratorial* **40**(4): 229–236.

Hamza, O. J., Matee, M. I., Moshi, M. J., Simon, E. N., Mugusi, F., Mikx, F. H., Helderman, W., Rijs, A. J., van der Ven, A. J. and Verweij, P. E. (2008). Species distribution and *in vitro* antifungal susceptibility of oral yeast isolates from Tanzanian HIV-infected patients with primary and recurrent oropharyngeal candidiasis, *BMC Microbiology* **8**(1): 135.

Harborne, J. B. (1973). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*, Springer Netherlands.

Hutchinson, J. and Dalziel, J. (2000). *Flora of West Tropical Africa Volume 1 Part 2*, Royal Botanic Gardens, Kew.

Itelima, J., Nwokedi, V., Ogbonna, A. and Nyam, M. (2016). Phytochemical screening and antimicrobial activity evaluation of aqueous and ethanolic extracts of the leaf of *Azadirachta indica* Juss (neem) on some microorganisms, *World Journal of Microbiology* **3**(1): 56–60.

Iyanda-Joel, W. O., Omonigbehin, E. A., Iweala, E. E. J. and Chinedu, S. N. (2019). Antibacterial studies on fruit-skin and leaf extracts of *Annona muricata* in Ota, Nigeria, *IOP Conference Series: Earth and Environmental Science* **331**: 012029.

Kanthasamy, A., Subramanian, S. and Govindasamy, S. (1989). Bactericidal and fungicidal effects of *Prosopis juliflora* alkaloidal fraction, *Indian Drugs* **26**: 390–394.

Kareem, K., Kareem, S., Adeyemo, O. and Egberongbe, R. (2010). *In vitro* antimicrobial properties of *Bridelia ferruginea* on some clinical isolates, *Agriculture and Biology Journal of North America* **1**(3): 416–420.

Kothavade, R. J., Kura, M. M., Valand, A. G. and Panthaki, M. H. (2010). *Candida tropicalis*: its prevalence, pathogenicity and increasing resistance to fluconazole, *Journal of Medical Microbiology* **59**(8): 873–880.

Latté, K. P. and Kolodziej, H. (2000). Antifungal effects of hydrolysable tannins and related compounds on dermatophytes, mould fungi and yeasts, *Zeitschrift für Naturforschung C* **55**(5-6): 467–472.

Lawal, I., Borokini, T., Oyeleye, A., Williams, O. and Olayemi, J. (2012). Evaluation of extract of *Ficus exasperata* Vahl. root bark for antimicrobial activities against some strains of clinical isolates of bacterial and fungi, *International Journal of Modern Botany* **2**(1): 6–12.

Levinson, W. E. (2016). *Review of Medical Microbiology and Immunology 14E*, McGraw-Hill Education. Google-Books-ID: TPnnCwAAQBAJ.

Levy, S. B. (2002). Factors impacting on the problem of antibiotic resistance, *Journal of Antimicrobial Chemotherapy* **49**(1): 25–30.

Mahmoud, D., Hassanein, N., Youssef, K. and Abou Zeid, M. (2011). Antifungal activity of different neem leaf extracts and the nimonol against some important human pathogens, *Brazilian Journal of Microbiology* **42**(3): 1007–1016.

Malwal, M. and Sarin, R. (2011). Antimicrobial efficacy of *Murraya koenigii* (Linn.) Spreng. root extracts, *Indian Journal of Natural Products and Resources* **2**(1): 48–51.

McLaughlin, J. L. (2008). Paw paw and cancer: Annonaceous acetogenins from discovery to commercial products, *Journal of Natural Products* **71**(7): 1311–1321.

Mohammed, H. A. and Omer, A. F. A. (2017). Antibacterial activity of *Azadirachta indica* (neem) leaf extract against bacterial pathogens in Sudan, *Ameri-can Journal of Research and Communication* **3**(5): 246–251.

Mohanty, S., Xess, I., Hasan, F., Kapil, A., Mittal, S. and Tolosa, J. E. (2007). Prevalence & susceptibility to fluconazole of *Candida* species causing vulvovaginitis, *The Indian Journal of Medical Research* **126**(3): 216–219.

Mondall, N., Mojumdar, A., Chatterje, S., Banerjee, A., Datta, J. and Gupta, S. (2009). Antifungal activities and chemical characterization of neem leaf extracts on the growth of some selected fungal species *in vitro* culture medium, *Journal of Applied Science and Environmental Management* **13**(1): 49–53.

Morey, A. T., de Souza, F. C., Santos, J. P., Pereira, C. A., Cardoso, J. D., de Almeida, R. S., Costa, M. A., de Mello, J. C., Nakamura, C. V., Pinge-Filho, P., Yamauchi, L. M. and Yamada-Ogatta, S. F. (2016). Antifungal activity of condensed tannins from *Stryphnodendron adstringens*: effect on *Candida tropicalis* growth and adhesion properties, *Current Pharmaceutical Biotechnology* **17**(4): 365–375.

Nahak, G. and Sahu, R. K. (2010). *In vitro* antioxidative acitivity of *Azadirachta indica* and *Melia azedarach* leaves by DPPH scavenging assay, *Journal of American Science* **6**(6): 123–128.

Nimenibo-uadia, R. (2017). Chemical composition and phytochemical screening of *Ficus exasperata* (Vahl) leaf, *Nigerian Journal of Pharmaceutical and Applied Science Research* **6**(1): 12–18.

Obasi, N., Egbuonu, A., Ukoha, P. and Ejikeme, P. (2010). Comparative phytochemical and antimicrobial screening of some solvent extracts of *Samanea saman* pods, *African Journal of Pure and Applied Chemistry* **4**(9): 206–212.

Oberlies, N. H., Jones, J. L., Corbett, T. H., Fotopoulos, S. S. and McLaughlin, J. L. (1995). Tumor cell growth inhibition by several Annonaceous acetogenins in an *in vitro* disk diffusion assay, *Cancer Letters* **96**(1): 55–62.

Olaiya, C., Idowu, P. and Karigidi, K. (2016). Antioxidative and antimicrobial activities of corn steep liquor anti-diabetic herb extracts, *Annals. Food Science and Technology* **17**(2): 272–279.

Pai, M. B. H., Prashant, G. M., Murlikrishna, K. S., Shivakumar, K. M. and Chandu, G. N. (2010). Antifungal efficacy of *Punica granatum*, *Acacia nilotica*, *Cuminum cyminum* and *Foeniculum vulgare* on *Candida albicans*: an *in vitro* study, *Indian Journal of Dental Research: Official Publication of Indian Society for Dental Research* **21**(3): 334–336.

Patuwo, C., Pasetto, S., Youngr, K. and Murata, M. (2014). Antifungal activity of flavonoids against *Candida albicans*, *43rd Annual Meeting & Exhibition of the AADR*.

Pfaller, M. A. and Diekema, D. J. (2007). Epidemiology of invasive candidiasis: a persistent public health problem, *Clinical Microbiology Reviews* **20**(1): 133–163.

Pierce, G. E. (2005). *Pseudomonas aeruginosa*, *Candida albicans*, and device-related nosocomial infections: implications, trends, and potential approaches for control, *Journal of Industrial Microbiology & Biotechnology* **32**(7): 309–318.

Pinto, E., Vale-Silva, L., Cavaleiro, C. and Salgueiro, L. (2009). Antifungal activity of the clove essential oil from *Syzygium aromaticum* on *Candida*, *Aspergillus* and dermatophyte species, *Journal of Medical Microbiology* **58**(11): 1454–1462.

Prusti, A., Mishea, S., Sahoo, S. and Mishea, S. (2008). Antibacterial activity of some indian medicinal plants, *Ethanobotanical leaflets* **12**: 227–228.

Rahman, A., Samoylenko, V., Jacob, M., Sahu, R., Jain, S., Khan, S., Tekwani, B. and Muhammad, I. (2011). Antiparasitic and antimicrobial indolizidines from the leaves of *Prosopis glandulosa* var. *glandulosa*, *Planta Medica* **77**(14): 1639–1643.

Roopashree, T. S., Dang, R., Rani, R. S. and Narendra, C. (2008). Antibacterial activity of antipsoriatic herbs: *Cassia tora*, *Momordica charantia* and *Calendula officinalis*, *International Journal of Applied Research in Natural Products* **1**(2): 20–28.

Salazar-Aranda, R., Granados-Guzmán, G., Pérez-Meseguer, J., González, G. and de Torres, N. (2015). Activity of polyphenolic compounds against *Candida glabrata*, *Molecules* **20**(10): 17903–17912.

Saleh Al-Hashemi, Z. S. and Hossain, M. A. (2016). Biological activities of different neem leaf crude extracts used locally in Ayurvedic medicine, *Pacific Science Review A: Natural Science and Engineering* **18**(2): 128–131.

Shah, S., Nasreen, S. and Sheikh, P. (2012). Cultural and morphological characterization of *Trichoderma* spp. associated with green mold disease of *Pleurotus* spp. in Kashmir, *Research Journal of Microbiology* **7**(2): 139–144.

Sonibare, M. A., Ogunwande, I. A., Walker, T. M., Setzer, W. N., Soladoye, M. O. and Essien, E. (2006). Volatile constituents of *Ficus exasperata* leaves, *Natural Product Communications* **1**(9): 1934578X0600100.

Takon, I. (2013). *In-vitro* evaluation of antibacterial activity of ethanolic and aqueous extracts of *Ficus exasperata* vahl. (Moraceae) leaves, *Journal of Microbiology and Biotechnology Research* **3**(4): 7–12.

Tsai, P.-W., Chen, Y.-T., Hsu, P.-C. and Lan, C.-Y. (2013). Study of *Candida albicans* and its interactions with the host: A mini review, *BioMedicine* **3**(1): 51–64.

Tsuzuki, J. K., Svidzinski, T. I., Shinobu, C. S., Silva, L. F., Rodrigues-Filho, E., Cortez, D. A. and Ferreira, I. C. (2007). Antifungal activity of the extracts and saponins from *Sapindus saponaria* L., *Anais da Academia Brasileira de Ciências* **79**(4): 577–583.

Vijayameena, C., Subhashini, G., Loganayagi, M. and Ramesh, B. (2013). Phytochemical screening and assessment of antibacterial activity for the bioactive compounds in *Annona muricata*, *International Journal of Current Microbiology and Applied Sciences* **2**(1): 1–8.

Yang, C.-R., Zhang, Y., Jacob, M. R., Khan, S. I., Zhang, Y.-J. and Li, X.-C. (2006). Antifungal activity of C-27 steroidal saponins, *Antimicrobial Agents and Chemotherapy* **50**(5): 1710–1714.

Yang, L., Liu, X., Zhuang, X., Feng, X., Zhong, L. and Ma, T. (2018). Antifungal effects of saponin extract from rhizomes of *Dioscorea panthaica* Prain et Burk against *Candida albicans*, *Evidence-Based Complementary and Alternative Medicine* **2018**: Article ID 6095307.