SUSCEPTIBILITY OF DRUG RESISTANT CANDIDA ISOLATES TO AQUEOUS LEAF EXTRACTS OF *Phyllanthus amarus*, *Senna alata* AND *Nymphaea lotus*

ISSN: 2141 – 3290
www.wojast.com

AKINJOGUNLA, O.J., ASAMUDO, N.U. AND DIVINE-ANTHONY, O.
Department of Microbiology, University of Uyo, Uyo, Akwa Ibom State.
papajyde2000@yahoo.com / 08064069404

ABSTRACT

The anticandidal efficacy of aqueous leaf extracts of *Phyllanthus amarus* (ALEPA), *Senna alata* (ALESA) and *Nymphaea lotus* (ALENL) on Fluconazole (FLU), Ketoconazole (KET) and Itraconazole (ITR) resistant strain of *Candida* was determined using disc diffusion technique. The percentage occurrence of *Candida* species was *C. albicans* (43.9 %), *C. glabrata* (24.5 %), *C. tropicalis* (12.2 %), *C. krusei* (7.1 %), *C. parapsilosis* (5.1 %) and *C. dubliniensis* 7(.1 %). The percentage yields of the ALEPA, ALESA and ALENL ranged between was 2.9 % and 3.7 %. The seven bio-active components common to ALEPA, ALESA and ALENL were alkaloids, flavonoids, saponins, tannins, anthraquinones, reducing sugar and terpenes. ≥ 24.5% and ≥ 27.6 % *Candida* isolates were KET resistant (KET^r) and FLU resistant (FLU^r), respectively. Varied levels of resistance to ITR were observed with *C. albicans, C. krusei* and *C. dubliniensis* showing 32.6 %, 14.3 % and 28.6 % resistance to ITR, respectively. All the *Candid*a isolates were sensitive to ALEPA at 80 $mgml^{-1}$ with inhibitory zones (ZIDs) ranging from 9.4 ± 0.2 mm to 20.2 ± 1.5 mm. ≥ 80 % *Candida* isolates were sensitive to growth inhibitory effects of ALESA at 40 $mgml^{-1}$ and 80 $mgml^{-1}$. FLU^r *C. tropicalis* (CT78) and KET^r *C. albicans* (CA81) were highly sensitive to ALENL at 80 $mgml^{-1}$ with ZIDs ranging between 18.8 ± 1.0 mm and 20.8 ± 1.2 mm. The regression values of the plants and ZIDs as exhibited by the *Candida* isolates ranged from 0.614 to 1.0. Consequently, these plants extracts would be therapeutically effective for the treatment of infections caused by *Candida* spp.

INTRODUCTION

Candida spp are the most common yeasts that infect humans (Talaro and Talaro, 1996). Although, these dimorphic fungi are normal microbiota of an individual's mucosal oral cavity, gastrointestinal tract and vagina, they are capable of causing superficial and life-threatening systemic infections in immune-compromised and immune-suppressed situations owing to their great adaptability to different host niches (Deepa *et al*., 2015). *Candida albicans* and non-albicans candida (NAC) such as *C. glabrata, C. krusei, C. tropicalis* and *C. parapsilosis* cause invasive fungal infections in human with increasing medical and economic importance due to high mortality rates, increased costs of care and duration of hospitalization (Pfaller and Diekema, 2007). The most frequently prescribed antifungal drugs for vaginal candidiasis are Clotrimazole and Fluconazole (Meis *et al*., 2000). The mechanisms of antifungal resistance are classified as either primary or secondary and are related to intrinsic or acquired characteristics of the fungal pathogens, involving either interference with the antifungal mechanism of the respective drug or a decrease in target drug levels (Talaro and Talaro, 1996).

Traditional medicine using plant extracts provides health coverage for many and exploitation of plants as traditional remedies occupy a fundamental place in developing countries (Planta and Gundersen, 2000; Akinjogunla *et al.*, 2011). *Phyllanthus amarus* (Schum and Thom), commonly called "carry me seed", or "gulf leaf flower", "hurricane weed", "windbreaker" belongs to the family Euphorbiaceae and is therapeutically effective in the treatment of jaundice, diarrhoea, skin

diseases, diabetes, hypertension and gastrointestinal related clinical problems (Oluwafemi and Debiri, 2008; Akinjogunla *et al*., 2012)). *Senna alata* Linn (Fabaceae) also known as ringworm bush, is a deciduous, an ornamental shrub, that grows well in forest areas of West Africa especially in Nigeria. The plant contains hydroxyanthraquinones, glycosides, chrysophanic acid, kampferin and sannoxide A and B (Abo *et al*., 1998) and has been reported to be efficacious for treating haemorrhoids, convulsion, gonorrhoea, constipation, inguinal hernia, heart failure, syphilis and diabetes (Ogunti and Elujoba, 1993). *Nymphaea lotus* Linn (Nymphaeaceae) is a perennial, herbaceous, aquatic plant localised in Central and Southern Europe, Asia, Middle East, North Africa, West Africa especially Nigeria (Abu-Zaida *et al*., 2008; Akinjogunla *et al*., 2009). *N. lotus* is therapeutically useful for treating dyspepsia, enteritis, diarrhoea, urinary problem, fever, insomnia and diabetes. The spread of multidrug-resistant fungi such as *Candida* spp and the reduced number of drugs available for treatment of infections caused by these fungi has necessitated the search for antifungals that could kill fungi or inhibit their growth.

This study was aimed at determining the efficacies of aqueous leaf extracts of *Phyllanthus amarus*, *Senna alata* and *Nymphaea lotus* on Fluconazole, Ketoconazole and Itraconazole resistant *Candida* Isolates from the high vaginal swabs.

MATERIALS AND METHODS

Collection of Samples

One hundred and twenty high vaginal swab (HVS) samples from women attending hospitals in Uyo, Akwa Ibom State, were aseptically collected using sterile swab sticks. The samples were immediately transported to the Microbiology Laboratory for mycological analysis.

Mycology of High Vaginal Swab (HVS) Samples

Each swab sample was aseptically inoculated on plates of Sabouraud Dextrose Agar (SDA) supplemented with streptomycin and aerobically incubated at 35 ^{o}C for 48 hrs. After incubation, the plates were observed macroscopically for yeasts growth (colonies with creamy, mucoid and musty appearance) and yeast colonies were sub-cultured onto freshly prepared plates of SDA and aerobically incubated at 35 ^{o}C for 48 hr. After incubation, the pure isolates were maintained on SDA slant at 4 ^{o}C. The pure isolates were further subcultured onto plates of CHROMagar Candida, aerobically incubated for 48 hrs at 35 ^{o}C and their colonial morphology and pigmentations on CHROMagar Candida were used for identification. Further identification of *Candida* species using Gram staining, germ tube production, chlamydospores production, sugar fermentation and assimilation tests were also carried out (Chander, 2009).

Susceptibility Testing of *Candida* isolates to Antifungal Drugs

In vitro susceptibility of *Candida* isolates to Itraconazole (ITR, 10 µg), Fluconazole (FLU, 25 µg) and Ketoconazole (KET, 10 µg) was carried out using disk diffusion technique. Ten microlitres of the inoculum suspension adjusted to turbidity of 0.5 McFarland standards was plated on Mueller Hilton Agar (MHA) supplemented with 2 % glucose and 0.5 g/ml methylene blue. The MHA plates were allowed to dry for 15 mins before the impregnated antifungal disc drugs were aseptically placed on the surface and aerobically incubated for 24 hrs at 35 °C. Inhibitory zones after incubation were measured in millilitres and categorized as sensitive (S), dose dependent susceptible (DDS) and resistant (R) according to CLSL (2000) and Pfaller *et al*. (2006).

Sources of Medicinal Plants

The leaves of *P. amarus*, *S. alata* and *N. lotus* were obtained from the forest in Uyo, Akwa Ibom State. The samples were authenticated in Department of Botany and Ecological Studies and later transferred to Pharmacognosy and Natural Medicine Laboratory, University of Uyo for processing. The leaves of *P. amarus*, *S. alata* and *N. lotus* were separately washed with distilled water to remove extraneous matters, air-dried at room temperature for one month, and pulverized using mortar and pestle into fine powder. The aqueous leaf extract of *P. amarus* was prepared by

soaking 2 kg of the powdered leaves into 1 litre of distilled water for 72 hrs with constant shaking at room temperature. It was then filtered using Whatman No 1 filter paper. The filtrate was evaporated to dryness with steam on water bath (45 °C), and the dried extract was weighed and stored in a refrigerator at 5ºC in screw capped bottle until required for use. The same procedure was repeated for powdered leaves of *S. alata* and *N. lotus*. The graded concentrations of the extracts were prepared using 100 ml of Dimethyl sulphoxide and shaken vigorously to obtain a homogenous mixture.

Percentage Yields of Aqueous Leaf Extracts of *P. amarus, S. alata* and *N. lotus*

The percentage yield of aqueous leaf extracts of *P. amarus, S. alata* and *N. lotus* was calculated as follows:

$$\frac{\text{Weight of the Initial Dried Sample}}{\text{Weight of the Extract}} \times 100$$

Phytochemical Screening of Plant Leaves

The phytochemical constituents of the aqueous leaf extracts of *P. amarus* (ALEPA), aqueous leaf extracts of *S. alata* (ALESA) and aqueous leaf extracts of *N. lotus* (ALENL) were analyzed using the methods described by Sofowora (1993); Trease and Evans (1996).

Susceptibility of Fluconazole, Itraconazole and Ketoconazole Resistant *Candida* Isolates to ALEPA, ALESA and ALENL

The anticandidal efficacies of ALEPA, ALESA and ALENL on Fluconazole, Itraconazole and Ketoconazole resistant *Candida* isolates were determined using disc diffusion method (CLSI, 2009). Sterile filter paper discs (6 mm diameter) impregnated with ALEPA, ALESA and ALENL solution of graded concentrations (20 mg/ml, 40 mg/ml and 80 mg/ml) were aseptically placed onto MHA agar plates which had previously been inoculated with 0.1 ml of standardized inoculum (*Candida* isolates) suspension using a sterilized forceps. The plates were aerobically incubated overnight at 35 ºC. Assays were performed in triplicate and the diameters of the inhibitory zones were measured in millimeters.

Statistical Analysis

The relationship between the different concentrations of ALEPA, ALESA, ALENL and the overall anticandidal activity, assessed as diameters of inhibitory zones with regard to the *Candida* isolates was determined by linear regression analysis

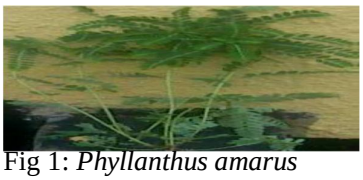
Fig 1: *Phyllanthus amarus*


Fig 2: *Senna alata*


Fig 3: *Nymphaea lotus*

RESULTS

Diverse Species of Candida Isolated from High Vaginal Swabs

Six *Candida* species comprising *C. albicans, C. glabrata, C. krusei, C. parapsilosis, C. tropicalis* and *C. dubliniensis* were isolated from the HVS. The distribution of *Candida* species was as follows: *C. albicans* 43.9 % (n=43), *C. glabrata* 24.5 % (n=24), *C. tropicalis* 12.2 % (n=12), *C. krusei* 7.1 % (n=7), *C. parapsilosis* 5.1 % (5) and *C. dubliniensis* 7.1 % (7) Table 1).

Extract Yield and Phytochemistry of Test Plants

The percentage yield of the ALEPA, ALESA and ALENL was 3.7 %, 2.9 % and 3.2 %, respectively (Table 2). The phytochemical analysis of ALEPA, ALESA and ALENL showed varied bio-active constituents (Table 3).

Table 1: The Occurrence of *Candida* Isolates in High Vaginal Swabs

Isolates	No of Occurrence	% of Occurrence
C. albicans	43	43.9
C. glabrata	24	24.5
C. krusei	7	7.1
C. parapsilosis	5	5.1
C. tropicalis	12	12.2
C. dubliniensis	7	7.1
Total	98	100

Table 2: Percentage Yields of Aqueous Leaf Extracts of *P. amarus, S. alata* and *N. lotus*

Plants	Weight of the Initial Dried Sample (g)	Weight of the Extract (g)	Percentage Yield
P. amarus	560	20.5	3.7
S. alata	615	18.2	2.9
N. lotus	582	18.9	3.2

Table 3: Phytochemical Constituents of Aqueous Leaf Extracts of *P. amarus, S alata* and *N. lotus*

Plant Extracts	Bio-Active Constituents	Occurrence
P. amarus	Alkaloids	+++
	Flavonoids	+++
	Saponins	+
	Tannins	++
	Cardiac Glycoside	+
	Anthraquinones	+
	Reducing Sugar	+
	Phlobatanins	ND
	Phenolics	+
	Terpenes	+
S. alata	Alkaloids	++
	Flavonoids	+
	Saponins	++
	Tannins	+++
	Cardiac Glycoside	ND
	Anthraquinones	+
	Reducing Sugar	+
	Phlobatanins	ND
	Phenolics	+
	Terpenes	+
N. lotus	Alkaloids	+++
	Flavonoids	++
	Saponins	++
	Tannins	+++
	Cardiac Glycoside	+
	Anthraquinones	+
	Reducing Sugar	+
	Phlobatanins	ND
	Phenolics	ND
	Terpenes	+

Keys: Present in very high concentration; ++: Present in moderately high concentration; +: Present in low concentration; ND: Not detected.

The ALEPA had alkaloids and flavonoids in a very high concentration (+++), tannins was in a moderately high concentration, while the concentrations of saponins, cardiac glycoside, phenolics, reducing sugar, anthraquinones and terpenes were low. The phytochemical constituent of ALESA were alkaloids (++), flavonoids (+), saponins (++), tannins (+++), anthraquinones (+), reducing sugar (+), phenolics (+), and terpenes. The eight bio-active constituents detected in the ALENL were alkaloids (+++), flavonoids (++), saponins (++), tannins (+++), cardiac glycoside (+), anthraquinones (+), reducing sugar (+) and terpenes (+) (Table 3).

Susceptibility of Candida Isolates to Plant Extracts

The results showed that 62 (63.3 %) *Candida* isolates were sensitive to FLU, 12 (12.2 %) isolates were dose dependent susceptible (DDS), while 24 (24.5 %) isolates were resistant to FLU. Of the 24 FLU resistant (FLU^r) *Candida* isolates, 10 isolates were *C. albicans, C. glabrata* (n=6), *C. krusei* (n=2), *C. parapsilosis* (n=1), *C. tropicalis* (n=3) and *C. dubliniensis* (n=2). $\geq$ 27.6 % *Candida* isolates were KET resistant (KET^r), 14.3 % isolates were DDS, while 58.2 % isolates were KET sensitive. Varied percentages of resistance of *Candida* isolates to ITR were observed with *C. albicans, C. glabrata, C. krusei* and *C. dubliniensis* showing 32.6 %, 20.4 %, 14.3 % and 28.6 % resistance to ITR, respectively (Table 4).

The FLU^r *C. albicans* (CA01), KET^r *C. albicans* (CA24), ITR^r *C. glabrata* (CG04), KET^r *C. parapsilosis* (CP06), FLU^r *C. parapsilosis* (CP22) and FLU^r *C. dubliniensis* (CD69) were resistant to ALEPA at 20 $mgml^{-1}$, while all the *Candid*a isolates were sensitive to growth inhibitory effect of ALEPA at 80 $mgml^{-1}$ with inhibitory zones (ZIDs) ranging from 9.4 ± 0.2 mm to 20.2 ± 1.5 mm (Table 5). The ALESA showed anti-candidal activity at different concentrations against FLU^r *Candida* isolates (n=6), KET^r *Candida* isolates (n=6) and ITR^r *Candida* isolates (n=3) (Table 5). KET^r *C. albicans* (CA24) and FLU^r *C. parapsilosis* (CP22) were resistant to ALESA at 20 $mgml^{-1}$, 40 $mgml^{-1}$ and 80$mgml^{-1}$. $\geq$ 80 % *Candida* isolates were sensitive to ALESA at 40 $mgml^{-1}$ and 80 $mgml^{-1}$. Of the 15 *Candida* isolates tested, 11 (73.3%), 14 (93.3%) and 15 (100%) *Candida* isolates were sensitive to ALENL at 20 $mgml^{-1}$, 40 $mgml^{-1}$ and 80 $mgml^{-1}$, respectively, having ZIDs ranging from 10.3 ± 0.1 mm to 20.8 ± 1.2 mm. FLU^r *C. tropicalis* (CT78), KET^r *C. albicans* CA81), KET^r *C. glabrata* (CG54) and FLU^r *C. glabrata* (CG15) were highly sensitive to ALENL at 80 $mgml^{-1}$ with ZIDs ranging between 18.8 ± 1.0 mm and 20.8 ± 1.2 mm (Table 5).

The regression values of ALEPA, ALESA, ALENL and ZIDs as exhibited by the *Candida* isolates ranged from 0.6139 to 0.9423; 0.6572 to 1.0 and 0.6477 to 0.9926, respectively (Table 6). The relationship between concentrations of ALEPA and ZIDs as exhibited by *C. albicans* (CA24), *C. glabrata* (CG04), *C. krusei* (CK08) and *C. tropicalis* (CT78) are shown in Figs 4-7. The relationship between concentrations of ALESA and ZIDs as exhibited by *C. albicans* (CA39), *C. glabrata* (CG04, CG54) and *C. krusei* (CK20) are shown in Figs 8-9.

DISCUSSION

Yeasts *Candida* infection is a common problem that causes significant morbidity and mortality (Asticcioli *et al*., 2009). Our findings showed *C. albicans* as the most frequently isolated yeast from HVS, followed by *C. glabrata* and this is in line with the findings of Asticcioli *et al.* (2009) who reported *C. albicans* and *C. glabrata* as the most common yeasts from HVS. In this study, non-*Candida albicans* were isolated from HVS and this substantiates the reports of Pfaller and Diekema (2007) on the increase in the prevalence of infections caused by non-*Candida albicans.* Fluconazole and itraconazole resistant isolates were obtained and this corroborates the previous reports of Galle and Gianinni (2004) whose studies conducted in Brazil reported 11.8 % fluconazole resistant *Candida* isolates. In Slovakia, Sojakova *et al*. (2004) also reported 13% fluconazole resistant and 18.5% itraconazole resistant *Candida* isolates.

Table 4: Susceptibility Profile of *Candida* Isolates from High Vagina Swabs to Fluconazole, Ketoconazole and Itraconazole

		Fluconazole			Ketoconazole			Itraconazole		
	No of	S	DDS	R	S	DDS	R	S	DDS	R
Isolates	Isolates	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
C. albicans	43	27 (62.8)	6 (13.9)	10 (23.3)	27 (62.8)	3 (7.0)	13 (30.2)	29 (67.4)	0 (0.0)	14 (32.6)
C. glabrata	24	15 (62.5)	3 (12.5)	6 (25.0)	15 (62.5)	5 (20.8)	4 (16.7)	17 (70.8)	2 (8.3)	5 (20.8)
C. krusei	7	5 (71.4)	0 (0.0)	2 (28.6)	3 (42.9)	1 (14.3)	3 (42.9)	5 (71.4)	1 (14.3)	1 (14.3)
C. parapsilosis	5	3 (60.0)	1 (20.0)	1 (10.0)	3 (60.0)	0 (0.0)	2 (40.0)	5(100)	0 (0.0)	0 (0.0)
C. tropicalis	12	9 (75.0)	0 (0.0)	3 (25.0)	8 (66.7)	2 (16.7)	2 (16.7)	10 (83.3)	2 (16.7)	0 (0.0)
C. dubliniensis	7	3 (42.9)	2(28.6)	2 (28.6)	1 (14.3)	3(42.9)	3 (42.9)	4 (57.1)	1(14.3)	2 (28.6)
Total	98	62 (63.3)	12 (12.2)	24 (24.5)	57 (58.2)	14 (14.3)	27 (27.6)	70 (71.4)	6 (6.1)	22 (22.4)

Keys: S: Sensitive; DDS: Dose Dependent Susceptible; R: Resistant; Itraconazole (S: $\geq$ 16, DDS: 10-15, R $\leq$ 9), Fluconazole (S: $\geq$19, DDS: 15- 18, R $\leq$ 14) and Ketoconazole (S: $\geq$ 30, DDS: 23-29, R $\leq$ 22).

Table 5: Susceptibility of Fluconazole, Itraconazole and Ketoconazole Resistant *Candida* Isolates to Aqueous Leaf Extracts of *P. amarus, S alata* and *N. lotus*

Isolates	Code of Isolates	Antifungal Resistance Profile	*P. amarus* Inhibitory Zones (mm ± SD)			*S. alata* Inhibitory Zones (mm ± SD)			*N. lotus* Inhibitory Zones (mm ± SD)		
			20 mg/ml	40 mg/ml	80 mg/ml	20 mg/ml	40 mg/ml	80 mg/ml	20 mg/ml	40 mg/ml	80mg/ml
C. albicans	CA01	FLU-resistant	NZ	12.1±0.2	13.9±0.5	NZ	13.0±0.5	14.4±0.3	NZ	13.7±0.2	15.0±0.5
C. albicans	CA24	KET-resistant	NZ	NZ	9.4±0.2	NZ	NZ	NZ	NZ	9.0±0.1	11.5±0.2
C. albicans	CA39	ITR-resistant	9.7±0.1	14.3±0.5	17.1±0.8	9.0±0.1	12.6±0.1	15.9±0.5	10.3±0.1	14.7±0.5	17.9±0.8
C. albicans	CA81	KET-resistant	16.2±1.0	18.0±1.2	19.5±1.0	13.4±0.3	14.3±0.5	15.0±0.5	13.8±0.3	16.1±1.0	19.0±1.0
C. glabrata	CG04	ITR-resistant	NZ	11.7±0.2	12.5±0.2	NZ	12.0±0.2	14.8±0.2	NZ	12.4±0.1	15.1±0.2
C. glabrata	CG15	FLU-resistant	15.5±0.5	18.1±1.0	20.2±1.5	13.0±0.2	16.6±0.8	17.3±1.0	13.4±0.3	16.9±1.0	18.8±1.0
C. glabrata	CG54	KET-resistant	14.1±0.5	16.3±0.5	17.8±1.0	13.3±0.2	14.0±0.3	15.4±0.6	14.5±0.5	17.0±1.0	20.6±1.5
C. krusei	CK08	FLU-resistant	10.3±0.1	14.0±0.5	15.2±0.3	NZ	12.5±0.1	15.0±0.6	12.0±0.2	15.4±0.5	16.7±0.5
C. krusei	CK20	KET-resistant	10.8±0.1	14.6±0.3	16.9±0.5	11.0±0.1	15.3±0.5	17.2±1.0	11.5±0.1	15.7±0.3	17.0±0.5
C. parapsilosis	CP06	KET-resistant	NZ	11.7±0.1	12.3±0.2	NZ	10.4±0.1	11.9±0.2	NZ	11.1±0.1	13.4±0.2
C. parapsilosis	CP22	FLU-resistant	NZ	NZ	10.5±0.1	NZ	NZ	NZ	NZ	NZ	9.7±0.1
C. tropicalis	CT78	FLU-resistant	14.6±0.3	17.2±0.7	19.3±0.5	13.0±0.2	15.7±0.5	18.1±1.0	14.8±0.5	17.7±1.1	20.8±1.2
C. tropicalis	CT97	KET-resistant	15.2±1.0	17.9±1.1	19.8±1.5	13.7±0.5	16.2±1.0	19.0±1.1	13.2±0.3	15.3±0.5	17.2±1.0
C. dubliniensis	CD82	ITR-resistant	12.3±0.2	15.0±0.5	16.4±0.5	12.9±0.2	15.7±0.5	17.2±0.5	11.3±0.2	15.0±0.5	16.7±0.5
C. dubliniensis	CD69	FLU-resistant	NZ	11.1±0.2	13.0±0.2	NZ	NZ	10.4±0.2	11.0±0.2	13.4±0.2	16.0±0.2

Keys: NZ: No Inhibitory Zone; mm: mean; SD: Standard Deviation; Each inhibitory zone included 6 mm diameter of the disc. Each value represents the mean of three replicates and standard deviation. FLU: Fluconazole; ITR: Itraconazole; KET: Ketoconazole.

Table 6: Regression Coefficient between Different Concentrations of *P. amarus*, *S. alata*, *N. lotus* and Inhibitory Zones Diameters Exhibited by *Candida* Isolates

Isolates	Code of Isolates	Regression (R^2)		
		P. amarus	*S. alata*	*N. lotus*
C. albicans	CA01	0.6864	0.6572	0.6477
C. albicans	CA24	0.8929	N/A	0.7655
C. albicans	CA39	0.8952	0.9544	0.9231
C. albicans	CA81	0.9423	0.9328	0.9687
C. glabrata	CG04	0.6274	0.7403	0.7310
C. glabrata	CG15	0.9381	0.7165	0.8117
C. glabrata	CG54	0.9133	1.0000	0.9926
C. krusei	CK08	0.7895	0.7200	0.8166
C. krusei	CK20	0.8943	0.8411	0.7821
C. parapsilosis	CP06	0.6139	0.6833	0.7245
C. parapsilosis	CP24	0.8929	N/A	0.8929
C. tropicalis	CT78	0.9381	0.9506	0.9711
C. tropicalis	CT97	0.9181	0.9754	0.9528
C. dubliniensis	CD82	0.8684	0.8740	0.8478
C. dubliniensis	CD69	0.7015	N/A	0.9724

In our study, the ALEPA contained alkaloids, flavonoids, tannins, saponins, cardiac glycoside, phenolics, reducing sugar, anthraquinones and terpenes. The detection of saponins, flavonoids, anthraquinones, terpenes and alkaloids in the ALEPA in this study is in dissimilarity with the reports of Senjobi *et al.* (2017), while detection of alkaloids, tannin and phenolics in the ALEPA is in consonant with the reports Erute and Egboduku, (2013). The antimicrobial activities of plants might be due to the presence of flavonoids and phenolics that inhibit cell membrane functions (Cushnie and Lamb, 2005; Rahman and Moon, 2007). Anticandidal activities of ALEPA on *C. albicans* and *C. krusei* were observed and this correlates the results of Erute and Egboduku, (2013) and Senjobi *et al.* (2017) who reported the antifungal activity of *P. amarus* on *Candida* spp. Varied concentrations of alkaloids, flavonoids, saponins, tannins, anthraquinones, reducing sugar, phenolics and terpenes were detected in ALESA and this substantiates the results of Owoyale *et al.* (2005) and Makinde *et al*. (2007). The anti-candidal potency of ALESA on *Candida* isolates in this study is in consonant with the results of Wuthi-udomlert *et al*. (2003) who reported the antimicrobial activities of aqueous extracts of *S. alata* on *Candida* spp. The anti-candidal activities this plant might be attributed to the presence of high concentration of tannins. Tannins have been reported to be toxic to filamentous fungi and yeasts (Scalbert, 1991). The secondary metabolites in ALENL were alkaloids, flavonoids, saponins, reducing sugar, tannins, anthraquinones, phenolics and terpenes and our results substantiate the reports of Akinjogunla *et al.* (2009). The anti-candidal activities of these plants may be due to the secondary metabolites that inhibit *Candida* through various mechanisms such as inhibition of ergosterol biosynthesis, disruption of cell membrane by protein precipitation and interference with fungal cell wall adhesion (Onyewu *et al*., 2003; Cavalcanti *et al*., 2011).

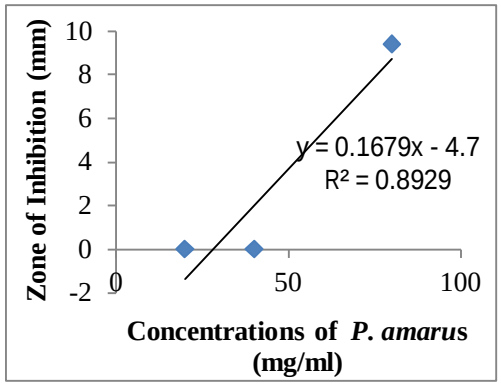

Fig 4: Relationship between Conc. of ALEPA and Inhibitory Zones as exhibited by CA24

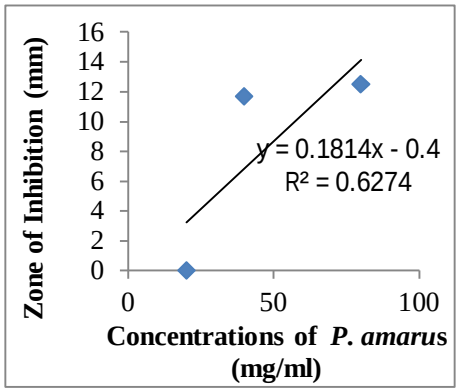

Fig 5: Relationship between Conc. of ALEPA and Inhibitory Zones as exhibited by CG04

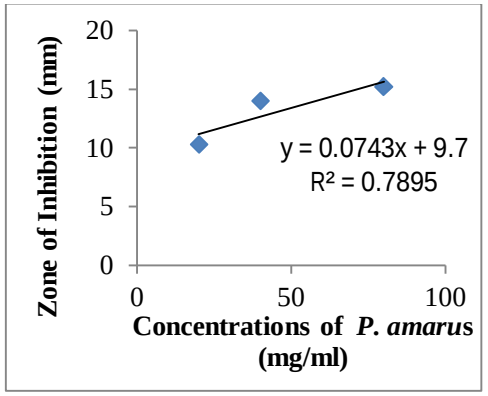

Fig 6: Relationship between Conc. of ALEPA and Inhibitory Zones as exhibited by CK08

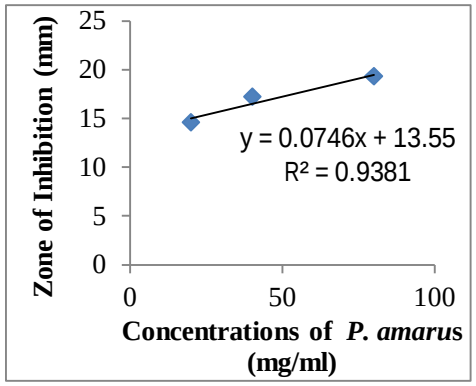

Fig 7: Relationship between Conc. of ALEPA and Inhibitory Zones as exhibited by CT78

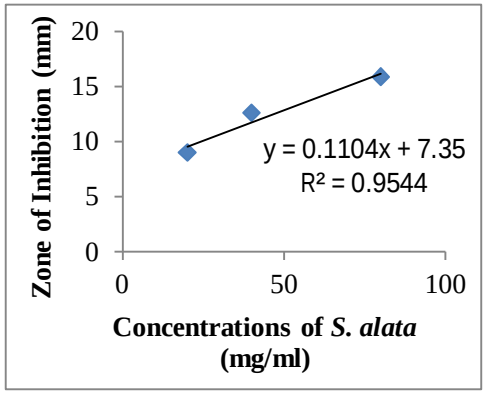

Fig 8: Relationship between Conc. of ALESA and Inhibitory Zones as exhibited by CA39

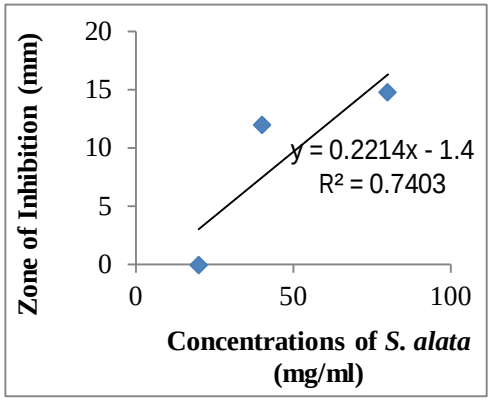

Fig 9: Relationship between Conc. of ALESA and Inhibitory Zones as exhibited by CG04

CONCLUSION AND RECOMMENDATION

Treatment of *Candida* infections by existing drugs faces a major hurdle owing to drug resistance, consequently, aqueous leaf extracts of *P. amarus, S. alata* and *N. lotus* would be therapeutically effective for the treatment of infections caused by *Candida* spp due to their secondary metabolites content.

REFERENCES

Abo, K.A., Adediwura, A.A. and Ibikunle, A.J. (1998). *First International Workshop on Herbal Medicinal Products*, University of Ibadan, Ibadan, Nigeria, 22-24.

Abu-Zaida, M.E., Mashaly, I.A., AbdEl-Monem, M and Torky, M. (2008). Economic potential of some aquatic plants growing in North –East, Nile-Delta. *Egypt Journal of Applied Sciences*, 8(1):1395-1405.

Akinjogunla, O. J, Adegoke, A.A., Udokang, I.P. and Adebayo-Tayo, B.C. (2009). Antimicrobial potential of *N. lotus* against wound pathogens. *J. Med. Plants Res.* 3(3): 138-141.

Akinjogunla, O.J., Bala, D.N. and Sylvester, M.A (2012). *In vitro* antibacterial efficacy of crude ethanolic leaf extract and alkaloidal fractions of *Phyllanthus amarus* on uro-pathogens. *Scientific Journal of Biological Sciences*, 1(4): 91-100.

Akinjogunla, O.J., Etok, C.A. and Oshoma, C.E. (2011). Preliminary phytochemistry and *in vitro* antibacterial efficacy of hydro-ethanolic leaf extracts of P. guajava on common urinary tract bacterial pathogens. *Bioresearch Bulletin*, 5:3()29-336.

Asticcioli, S., Sacco, L., 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 out-patients clinic of a tertiary care hospital in Northern Italy during the years 2002-2007. *New Microbiology*, 32: 199-204

Bharatiya, V.B. (1992). *Selected Medicinal Plants of India*. Bombany Tafa Press, pp235-237.

Cavalcanti, Y.W., Almeida, L. F. D. and Padilha, W.W.N. (2011). Atividade antifungica de Tres Oleos Essenciasis Sobre Cepas de *Candida. Revista Odontologica Brasileira Central*, 20(52) 68-73.

Chander, J. (2009). *A Text Book of Medical Mycology*, Candidiasis. (3rd edn), Mehta Publishers, New Delhi, pp 266-290.

Clinical Laboratory Standards Institute (2009). Method for antifungal disk diffusion Susceptibility testing of Yeasts; Approved Guideline-second edition. *CLSI Document M44-A2*. Clinical Laboratory Standard Institute, Wayne: Pennsylvania.

Cushnie, T.P. and Lamb, A.J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents*, 26(5): 343-356.

Deepa, K., Jeevitha, T. and Michael, A. (2015). In vitro evaluation of virulence factors of *Candida* spp from oral cavity. *Journal of Microbiology and Antimicrobials*, 7 (3): 28-35.

Erute, M.A. and Egboduku, O.W. (2013). Screening of some Nigerian medicinal plants for anti-candidal activity. *American Journal of Drug Discovery and Development*, 3: 60-71.

Galle, L.C. and Gianinni, M.J.S (2004). Prevalência e susceptibilidade de leveduras vaginais. *JBPMI*, 40: 229-236.

Makinde, A.A., Igou, J.J.G., TA'Ama, L., Shaibu, S.J. and Garba, A. (2007). Antimicrobial activity of *Cassia alata. African Journal of Biotechnology*, 6 1509-1510.

Meis, J., Petrou, M., Bille, J., Ellis, D. and Gibbs, D. (2000). A global evaluation of the susceptibility of *Candida* species to fluconazole by disk diffusion. *Ugeskr Laeger.* 162:1907-1908.

Ogunti, E.O. and Elujoba, A.A. (1993). Laxative activity of *C. alata, Fitoterapia*, 64(5): 437-39.

Oluwafemi, F and Debiri, F. (2008). Antimicrobial effect of *Phyllanthus amarus* and *Parquetina nigrescens* on *Salmonella typhi. African Journal of Bio Res*. 11: 215 – 219.

Onyewu, C., Blankenship, J.R., Del-Poeta, M., and Heitman, J. (2003). Ergosterol biosynthesis inhibitors become fungicidal when combined with calcineurin inhibitors against *Candida albicans, C. glabrata* and *C. krusei. Antimicrobial Agents and Chemothepy*, 47(3) 956-964.

Owoyale, J.A., Olatunji, G.A. and Oguntoye, S.O. (2005). Antifungal and antibacterial activities of extracts of *S. alata* leaves. *Journal of Applied Science and Environmental Management*, 9(3): 105-107

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

Pfaller, M.A, Diekema, D.J. and Sheehan, D.J. (2006). Interpretative Breakpoints for fluconazole and *Candida* revisited. A blueprint for the future of antifungal susceptibility testing. *Clinical Microbiology and Review*, 19: 435-444.

Planta, M. and Gundersen, B. (2000). Prevalence of the use of herbal products in a low income population. *Family Medicine*. 32: 252-257.

Rahman, M.A.A. and Moon, S.S. (2007). Antioxidant polyphenol glycosides from the plant *Draba nemorosa*. *Bull. Korean Chem. Soc.* 28(5): 827-831.

Scalbert, A. (1991). Antimicrobial properties of tannins. *Phytochemistry*, 30: 3875-3883.

Senjobi, C.T., Ettu, A.O., and Otujo, C.O. (2017). Antibacterial and antifungal activities of leaf extracts of *Phyllanthus amarus* (Euphorbiaceae). *Journal of Pharmacognosy and Phytotherapy*, 9(1): 6-10.

Sofowora, A. (1993). *Medicinal plants and Traditional medicine in Afric*a. Spectrum Books Ltd, Ibadan, Nigeria, 289.

Sojakova, M., Liptajova, D., Borovsky, M. and Subik, J. (2004) Fluconazole and itraconazole susceptibility of vaginal yeast isolates from Slovakia. *Mycopathologia*, 157(2):163–169.

Talaro, A. and Talaro, K. (1996). *Foundations in Microbiology*, (2nd edn).Wm. C. Brown Publisher, USA. pp 673-701.

Trease, G. E. and Evans, W. C. (1996*). A Textbook of Pharmacognosy*. (14th Edn), Bailliere Tindall Ltd., London, pp 60-75.

Wuthi-udomlert, M., Prathanturarug, S., Soonthomchareonnon, N. (2005). Antifungal activity of *Senna alata* extracts using different methods of extraction. *ISH Acta Hortic*, 597: 205-208.