Available online: www.notulaebiologicae.ro
Print ISSN 2067-3205; Electronic 2067-3264
Not Sci Biol, 2019, 11(3):376-382. DOI: 10.15835/nsb11310443
Original Article

In vitro Control of Oral Thrush Causal Organisms Using Medicinal Plants Extracts

Abosede M. EBABHI[1]*, Adedotun A. ADEKUNLE[2], Yoma I. OGHENERHABOKE[2]

[1]*University of Lagos, Distance Learning Institute, Akoka, Lagos, Nigeria; aebabhi@unilag.edu.ng (*corresponding author)*
[2]*University of Lagos, Faculty of Science, Department of Botany, Akoka, Lagos, Nigeria; aaded63@yahoo.com; yomaissac74@gmail.com*

Abstract

Oral hygiene is important to the generality of the human healthcare system. For this, the antifungal activities of the aqueous and ethanol extracts of four medicinal plants of *Jatropha curcas* (stem), *Eucaluptus golbulus* (leaves), *Vernonia amygdalina* (stem) and *Zanthoxylum zanthoxyloides* (root) were carried out *in vitro* against three species of *Candida* associated with oral thrush namely *C. albicans, C. glabrata, C. tropicalis* using the disc diffusion agar assay. The zones of inhibition varied with the test organisms as well as the extracts. The ethanolic extract of *Jatropha curcas* showed the highest zone of inhibition of 10.88 ± 0.22 mm against *C. albicans* while the least zone of inhibition (6.13 ± 0.13 mm) was exhibited by the ethanol extract of *Z. zanthoxyloides* on *C. glabrata*. The preliminary phytochemical screening showed the presence of tannin, saponin, alkaloids, flavonoids and reducing sugar in all plant samples. This study can be further used as a foundation for the screening of phytochemical constituents by pharmaceuticals for the control and eradication of oral thrush.

Keywords: *Candida* spp.; medicinal plants; mouth swab; oral thrush; phytochemicals

Introduction

Thrush is a fungal infection (mycosis) of any species from the genus *Candida*. This is commonly referred to as yeast infection and technically as candidiasis, moniliasis and oidiomycosis (William *et al*., 2006). Candidiasis encompasses infection that range from superficial, such as oral thrush and vaginitis, to systemic and potentially life-threatening disease. It is often confined to severely immune-compromised persons such as cancer, transplant and AIDS patients, as well as nontrauma emergency surgery patients (Koukpupetis *et al*., 2010). *Candida abicans* is the most common agent of thrush in humans (Walsh and Dixon, 1996).

Medicinal plants have been part of human medicine since the dawn of civilization. These plants have made backbones of traditional medicinal systems in Nigeria (Akerele, 1988) and other parts of the world like India (Nayak *et al*., 2011). The use of plant extracts to treat microbial infections is also reported in ancient Ayurvedic compendium 'Charack Samhita' and 'Sushrat Samhita' (Kelmanson *et al*., 2000). The search for new therapeutic treatments for various disease conditions is expanding due to increased prevalence of drug resistant microorganisms (Fidel *et al*., 1999; Samuel *et al*., 2014). In many developing countries, plants have been looked at as a very promising source of new compounds for drug discovery and development (Kong *et al*., 2003). Plant extracts and their products are clinically safer than antibiotics (Srinivasan *et al*., 2001). The aim of this study is to control oral Candidiasis in vitro using medicinal plants which will serve as a reference point for further research on controlling oral infections.

Received: 13 Feb 2019. Received in revised form: 27 Aug 2019. Accepted: 13 Sep 2019. Published online: 30 Sep 2019.

Materials and Methods

Isolation and identification of microorganisms

Mouth swab was collected from postgraduate students at the hall of residence in a tertiary institution in Southwest, Nigeria by using sterile swab sticks to scrap the surface of the tongues before early morning wash of the mouth as described by Ochei and Kolhatkar (2000). The swab sticks were aseptically placed in separate seal up plastic bags for transport to the lab. A total of 42 students were routinely sampled for three weeks on a weekly basis. All samples were routinely grown on Sabouraud dextrose agar (SDA) (Oxoid, Basingstoke, England) for 72-96 hours at 30 °C. Growth medium was prepared in accordance to manufacturer's specification. Pure cultures of isolated organisms were certified as fungi using their cultural, morphological characteristics as well as comparing them with confirmed representatives of different species in relevant texts like Ellis *et al*. (2007). Further biochemical tests were performed by Gram staining according to the method described by Beveridge (2001). Sugar fermentation test was carried out using a modified method of Olutiola *et al*. (2000). Urea hydrolysis test and sensitivity to chloramphenicol were carried out using the methods of Seeliger (1956) and Kirby *et al*. (1996) respectively. Photomicrographs of the isolates were taken with Motic Camera 2000.

Collection, identification and preparation of plant extracts

Plant parts of *Jatropha curcas* (stem), *Vernonia amygdalina* (stem) and *Eucalyptus globules* (leaves) were obtained from the Botanical and Zoological garden of the University of Lagos. The roots of *Zanthoxylum zanthoxyloides* were purchased from Oyingbo herbal market, Mainland Local Government Area of Lagos state. The plants were all identified at the Lagos University herbarium, University of Lagos. The plant parts were cut into bits of 2.0 cm and shade dried for about two weeks. The plant materials were all ground into fine powder using commercial blender. Two hundred grams of each sample was soaked in conical flasks containing different solvent of aqueous and ethanol at 1:2 w/v and observed on a shaker for 72 hours. The extracts were filtered through a Whatman No 1 filter papers and Muslin cloth severally into separate beakers and concentrated to dryness with the aid of a rotary evaporator. The stocks were kept at 4 °C in a refrigerator until further use (Silva *et al*., 2014).

Qualitative preliminary phytochemical screening of the plant samples was carried out using standard procedures as described by Edeoga *et al*. (2005), Sofowora (2006), Trease and Evans (2009) and Ashafa and Umebese (2012). The phytochemicals analyzed include tannins, phenols, saponins, alkaloids, flavonoids, steroids, cardiac glycosides and reducing sugars. The extracts were later subjected to quantitative analysis based on the methods of Edeoga *et al*. (2005) and Prohp and Onoagbe (2012).

Test for purity of the organic extracts

Each extract obtained was tested to ensure its purity via dropping on a sterile plate containing potato dextrose agar and well spread on the agar. The plates were incubated at 27 °C based on the method described by Okigbo *et al*. (2009). The plates were examined for possible growth of contaminants, the absence of which confirmed the purity of the test extracts.

Antifungal assay of the plant extracts

The antifungal assay was carried out using the disc diffusion agar method of Adekunle and Ikumapayi (2006) and Valle *et al*. (2016). The disc preparation involved the use of filter papers (Whatman No. 1 filter paper) perforated into discs, placed in aluminium foil, wrapped and sterilized in an autoclave. The discs were dried in an oven at 60 °C for 30 minutes. Each plant extract was reconstituted with 1 ml of distilled water respectively at concentrations of 100 mg/ml and 200 mg/ml, the discs were later soaked in the prepared extracts for 24 hrs prior to use. Spore suspension of 10^5-10^7 cells/ml, counted with haemocytometer was made (Lee *et al*., 1981). A micropipette was used to introduce 0.1 ml of the spore suspension on sterile plates of Saboraud dextrose agar (SDA) before spreading with a glass rod under sterile conditions to ensure even distribution of the spores. These were then dried at room temperature for 15 mins prior to the application of the discs. The discs (4) which had been soaked in plant extract were placed at the four edges of the surface of the agar plate with the aid of a sterilized forceps. These were done in triplicate. The inoculated plates were later placed in an incubator at 28 °C and observed after 48 hours. The extent of inhibition was determined by measuring the diameter of the inhibition zone using a transparent metre rule (Booths *et al*., 1971). The mean zone of inhibition of the three replicated tests of the plants extracts on the test organisms were expressed in millimetres. The discs were also impregnated in equivalent volume of distilled water as negative control and griseofulvin (anti-fungal drug) served as the positive control measure.

Statistical analysis

The data were expressed as mean ± S.E.M. and were statistically analysed using one-way analysis of variance (ANOVA). Means were separated by the Duncan new multiple range test. Values were considered significant at $p<0.05$ (Kim, 2015).

Results

Isolation and identification of microorganisms

The results on the study of biological control of oral candidiasis using medicinal plants revealed that three fungi isolates were obtained in the course of this study. The isolated fungi were identified as *Candida albicans*, *Candida glabrata* and *Candida tropicalis*. All isolated organisms showed resistance to chloramphenicol which was indicated by the growth in the presence of the discs. The antibiotic is usually added to fungi growth media to inhibit the growth of Gram positive and Gram negative bacteria. In the biochemical test, the *Candida spp* were able to break the urea at 72 hour of incubation which was indicated by a colour change from pale pinkish-red to pale orange colour. The set up experiment was observed for 4 days. The lack of

colour change is an indication of a negative result. The isolated organisms were confirmed as yeast via the fermentation analysis. Result is has depicted in Table 1.

Qualitative and quantitative analysis of plant extracts

The voucher number of the authenticated plants are LUH 6992 (*Jatropha curcas)*, LUH 7045 (*Vernonia amygdalina)*. LUH 4689 (*Eucalyptus globules)* and LUH 6317 (*Zanthoxylum zanthoxyloides)*. The qualitative analysis shows that tannin, saponin, alkaloid and reducing sugar are present in all plant extracts. Phenol was absent in *Jatropha curcas*. Steroid and cardiac glycoside were equally absent in *Eucalyptus globules* while in *Vernonia amygdalina,* steroid was the only phytochemical absent. Result is as presented in Table 2.

For the quantitative analysis alkaloids had the highest quantity of 33.64 mg/g while steroids had the least quantity of 13.21 mg/g in *J. curcas*. The ethanolic extract of *Eucalyptus globulus* showed that steroids and cardiac glycosides were absent. Like in *Jatropha curcas,* alkaloids had the highest quantity of 41.98 mg/g while saponin had the least quantity of 19.38 mg/g in *E. globulus*. The ethanolic extract of *Vernonia amygdalina* revealed that alkaloid recorded the highest quantity of 46.37 mg/g while cardiac glycoside had the least quantity of 15.32 mg/g. *Zanthoxylum zanthoxyloides* ethanolic extract showed that alkaloid similarly recorded the highest quantity of 32.15 mg/g with steroids being the least of 11.61 mg/g as depicted in Table 3.

Table 1. Phenotypic characteristics of the isolates

Biochemical characteristics	Fungal isolates		
	C. albicans	*C. glabrata*	*C. tropicalis*
Cell morphology	Ovoid	Globose	Round
Gram reaction	+	+	+
Sucrose	-	-	V
Glucose	+	+	+
Starch	-	-	-
Lactose	+	-	+
Galactose	-	+	+
Maltose	-	+	+
Fructose	+	+	+
Dextrose	+	+	+
Urease	+	+	+
Resistance to chloramphenical	+	+	+

+ = Present - = Absent V = Variable

Table 2. Qualitative analysis of phytochemicals present in plant extracts

Phytochemical / Plan extract	Tannin	Phenol	Saponin	Alkaloid	Flavonoid	Steroid	Cardiac glycoside	Reducing sugar
J. curcas (E)	+	-	+	+	+	+	+	+
E. globulus (E)	+	+	+	+	+	-	-	+
V. amygdalina (E)	+	+	+	+	+	-	+	+
Z. zanthoxyloides (E)	+	+	+	+	+	+	+	+

+: Present, -: Absent E: Ethanol

Table 3. Quantitative analysis of phytochemicals in plant extracts

Phytochemicals (mg/ g)	Plant samples			
	J. carcas	*E. globulus*	*V. amygdalina*	*Z. zanthoxyloides*
Tannin	22.05	29.06	23.33	30.76
Phenol	0.00	19.38	15.69	21.66
Saponin	31.50	29.25	33.81	32.15
Alkaloid	33.64	41.98	46.37	39.04
Flavonoid	32.24	22.50	18.36	19.12
Steroid	13.21	0.00	0.00	11.61
Cardiac glycoside	13.99	0.00	15.32	14.26
Reducing sugar	15.77	23.76	20.09	20.56

Antifungal assay

The test for purity of the extracts showed no microbial growth after incubation. The results on Figs. 1 and 2 show the zones of inhibition of the different plant extracts on the fungal isolates. The analysis of variance revealed that the rate of inhibition differed significantly ($p < 0.05$) among the extracts. The ethanol extracts of the plant samples showed higher level of inhibition on the test organisms as compared with the antifungal drug used (positive control). For the extracts at 200 mg/ml, the highest rate of inhibition was observed on *C. albicans* (10.88±0.22 mm) using the ethanol extract from *Jatropha curcas* while the least zone of inhibition was observed in *C. glabrata* at 6.13±0.13 mm from both extracts of *Zanthoxylum zanthoxyloides*. However, both aqueous and ethanolic extracts of *Jatropha curcas* showed a significant ($p< 0.05$) level of inhibition (9.88±0.32 mm; 10.38±0.24 mm respectively) on the growth of *C. tropicalis* while they had no significant inhibitory effects on the growth of *C. glabrata*. The results showed that the ethanolic extract of *Eucalyptus globulus* had a significant ($p< 0.05$) rate of inhibition (10.13±0.32 mm) than the aqueous extract (9.00±0.24 mm) on the growth of *C. albicans*. Both aqueous and ethanolic extracts produced significant level of inhibition (9.62±0.43 mm) on the growth of *C. tropicalis* while the inhibitory effect (6.25±0.13 mm) on the growth of *C. glabrata* was insignificant. However, at 100 mg/ml the ethanol extract of *E. globulus* showed significant level (9.50±0.20 mm) of inhibition on *C. tropicalis* while no significant difference was observed on the other extracts though they all showed fungistatic activities on all test isolates.

Discussion

Oral candidiasis (mouth thrush) is a yeast infection which develops inside the mouth and on the tongue of human. This condition is commonly found in children, the aged and the immunosuppressed (Umeh and Okolocha, 2005; Silva *et al*., 2012). Oral candidiasis is a *Candida* disorder that can be treated medically and traditionally using herbal remedies. This study examined the effects of both aqueous and ethanolic extracts of four medicinal plants on oral candidiasis causal organisms isolated from postgraduate students of a tertiary institution. A total number of 42 students were sampled. The identified isolates were *Candida albicans, Candida tropicalis* and *Candida glabrata. C. albicans* is a well-known causal agent of candidiasis in human (Calderone, 2002) while *C. glabrata* has been implicated as a prevalent pathogenic yeast by Rodrigues and Henriques (2017) in their study on oral mucositis.

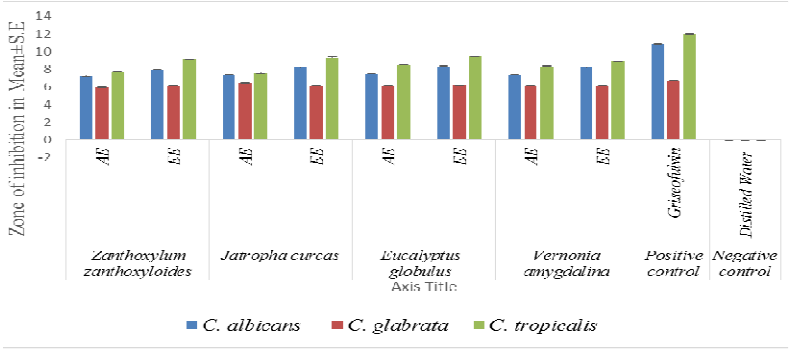

Fig. 1. The zone of inhibition induced by the medicinal plant extracts at 200 mg/ml on isolated *Candida* spp from oral thrush patients (AE=Aqueous extract, EE=Ethanol extract)

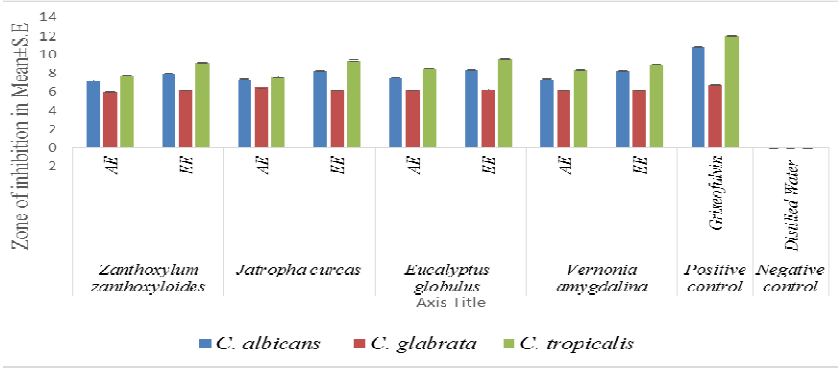

Fig. 2. The zone of inhibition induced by the medicinal plant extracts at 100 mg/ml on isolated *Candida* spp from oral thrush patients (AE=Aqueous extract, EE=Ethanol extract)

According to Fidel *et al*. (1999), *C. glabrata* is ranked the second or third causative agent of superficial (oral, esophageal, vaginal, or urinary) or systemic candida infections, which are often nosocomial. Its infections have been noted to have high mortality rate in compromised, at-risk hospitalized patients. The isolation of *Candida* spp in our studies also confirms with the work of Redding *et al*. (2002) who reported that a frequent combination of mixed species infection by *Candida* species is *C. glabrata* and *C. albicans*, which has been found in approximately 70% of patients with oral candidosis. Flevari *et al*. (2013) also stated that *Candida* species remain the most important cause of opportunistic infections worldwide, affecting predominantly patients over 65 years old, while they are considered to be the fourth most common cause of nosocomial bloodstream infections.

The issue of pathogenic organisms mutating against various synthetic compounds and known therapeutic drugs has led scientists to constantly be on the search for new and effective compounds which can eradicate the menace of diseases in the human race. The phytochemicals found in most plants have been shown to be most effective in the treatment of diseases and they provide an important source of all the world's pharmaceuticals. The results from the preliminary phytochemical screening in our present study showed that the sampled plants of *Jatropha curcas, Eucalyptus globulus, Vernonia amygdalina* and *Zanthoxylum zanthoxyloides* possess most of these bioactive compounds like tannin, cardiac glycosides, steroids, terpenoids, flavanoids, and alkaloids which were found in reasonable amount in the ethanol extracts of the plants' parts. Alkaloid, tannin and saponin were present in all samples. The presence of these compounds have been confirmed in *J. curcas* by Sharma *et al*. (2012); in *V. amygdalina* by Alara *et al*. (2018); in *E. globulus* by Takahashi *et al*. (2004) and Egwaikhide *et al*. (2009) while the phytochemical screening of *Z. zanthoxyloides* by Kosh-Komba *et al*. (2017) also revealed the presences of these bioactive compounds. The antifungal activities of all sampled plants can be linked to the presence of tannin. Tannin has been reported to inhibit the cell protein synthesis of microorganisms as it forms irreversible complexes with proline rich protein (Shimada, 2006).

The presence of phytochemical compounds in the plants extracts indicates that some of the phytochemical compounds might be responsible for the potential antifungal activities. The results of this study showed that the aqueous and ethanolic extracts of *Vernonia amygdalina* had insignificant inhibition on the growth of *C. glabrata* but had significant inhibition on the growth of *Candida albicans* at 200 mg/ml. In the same vain, Akinpelu (1999) had also stated that the methanolic extract at 60% had no effect on *C. albicans*. The aqueous and ethanolic extracts of *Z. zanthoxyloides* exhibited a significant inhibition on the growth of *C. tropicalis* while the inhibition on the growth of *Candida albicans* was considerable. This result is supported by Adebiyi *et al*. (2009) who observed in their study that the use of ethanolic extracts of the root of *Zanthoxylum* sp gave a considerable inhibition on the growth of *Candida* sp. Thus, reducing the level of oral infection in some selected natives who use this root as chewing-stick in the western part of Nigeria. In Central African Republic, Kosh-Komba *et al*. (2017) also showed that the water-alcohol (50/50, alcohol 95°) extract of Z. zanthoxyloides root is very effective against *C. albicans* with high concentration (4 mg/disc). The results of our study illustrated that the ethanolic extracts of *Jatropha curcas* had significant inhibitory effects on the growth of *C. albicans* and *C. tropicalis* than the aqueous extract. This result is supported by Agbogidi *et al*. (2013) who reported that the sap from the stem of *Jatropha curcas* inhibited the growth of microbes on the tongues of selected people and it gave relief to toothaches. This result is also in accordance with the work of Osemene *et al*. (2013) that showed the antimicrobial properties of *Jatropha curcas* on some oral disorders. However, the aqueous and ethanolic extracts of *J. curcas* had no significant inhibition on the growth of *C. glabrata*. Ethanolic extracts of *Eucalyptus globulus* showed significant inhibition on the growth of *C. albicans* in comparison to the aqueous extract. The work of Hardel and Sahoo (2011) lay credence to this as they revealed that a 50% ethanolic extract of *E. globulus* leaves had antifungal activity against oral pathogenic microorganisms with minimum inhibition concentration (MIC) values ranging from 0.20-6.25 mg/ml.

The antifungal activities of these plants parts can be attributed to the presence of some bioactive components in them. Many authors for instance have suggested that the presence of saponins confer many plants with antifungal activities. Saponins are surface active agents which interfere with or alter the permeability of the cell wall of microorganisms. Sule *et al*. (2010) have reported saponins as a major antifungal secondary metabolite. It is also believed that the interaction with steroids of the fungal membrane is the mechanism of antifungal activity of saponins as reported by Arif *et al*. (2009) and Damke *et al*. (2011). The results of this study showed that the leave, stem and root extracts of *E. globulus, J. curcas* and *Z. zanthoxyloides* respectively had significant antifungal activities on *C. albicans* and *C. tropicalis* as compared to the antifungal drug used as control. These extracts had no significant effects on the growth of *C. glabrata* a causal agent of oesophageal thrush. However, the ethanolic extract of *V. amygdalina* stem showed a significant fungistatic effect on the growth of *C. glabrata. C. glabrata* has been severally reported (Krogh-Madsen *et al*., 2006; Lass-Flörl, 2009; Rodrigues and Henriques, 2017) to be resistant to many antifungal drugs especially the azoles containing drugs.

Conclusions

The issue of oral thrush and mouth odour has become alarming in our environment especially among the youths. Many people find it difficult to seek medical assistance as a result of economic status and possible public embarrassment. The use of various types of plants such as *Jatropha curcas, Eucalyptus globulus, Vernonia amygdalina* and *Zanthoxylum zanthoxyloides* as chewing-stick to clean the mouth is a common practice. This study therefore established the efficacy of some medicinal plants in eradicating the causal organisms of oral thrush.

These plants can be exploited for their potential in mouthwash and in economically produced pastries for the treatment of oral thrush as they are eco-friendly and economically available.

Conflict of Interest

The authors declare that there are no conflicts of interest related to this article.

References

Alara OR, Nour AH, Olalere OA (2018). Ethanolic extraction of bioactive compounds from *Vernonia amygdalina* leaf using response surface methodology as an optimization tool. Journal of Food Measurement and Characterization 12(2):1107-1122.

Arif T, Bhosale JD, Kumar N, Mandal TK, Bendre RS, Lavekar GS (2009). Natural products - antifungal agents derived from plant. Journal of Asian Natural Product Research 11(7):621-638.

Adebiyi AO, Koekemuoer T, Adebiyi AP, Smith N, Baxter E, Naude RJ, Van de Vanter M (2009). Antimicrobial and antioxidant activities of crude extracts of two Nigerian chewing sticks. Pharmaceutical Biology 47(4):320-327.

Adekunle AA, Ikumapayi AM (2006) Antifungal property and phytochemical screening of the crude extracts of *Funtumia elastica* and *Mallotus oppositifolus*. West Indian Medical Journal 55 (4): 219 -223.

Agbogidi OM, Akparaobi SO, Eruoto PG (2013). Health and environmental benefits of *Jatropha curcas* Linn. Unique Research Journal of Agricultural Sciences 1(5):76-79.

Akerele O (1988). Medicinal plants and primary health care: An agenda for action. Fitoterapia 59(5):355-363.

Akinpelu DA (1999). Antimicrobial activity of *Vernonia amygdalina* leaves. Fitoterapia 70(4):432-434.

Ashafa AOT, Umebese C (2012). Phytochemical screening, antibacterial and antifungal activity of *Garuleum woodii* Schinz. root extracts against human pathogenic microbes. Journal of Medical Plants Research 6(42):5513-5518.

Beveridge TJ (2001). Use of Gram stain in microbiology. Biotechnology and Histochemistry 76(3):111-118.

Calderone RA (2002). Introduction and historical perspectives *Candida* and *Candidiasis*. ASM Press, Washington, DC, pp 15-25.

Damke E, Tsuzuki JK, Cortez DAG, Ferreira ICP, Bertoni TA, Batista MR (2011). *In vivo* activity of *Sapindus saponaria* against azole-susceptible and resistant human vaginal *Candida* species. BMC Complementary and Alternative Medicine 11(1):35.

Edeoga HO, Okwu DE, Mbaebie BO (2005). Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology 4(7):685-688.

Egwaikhide PA, Okeniyi SO, Gimba CE (2009). Screening for anti-microbial activity and phytochemical constituents of some Nigerian medicinal plants. Journal of Medicinal Plant Research 3(12):1088-1091.

Ellis D, Davis S, Alexiou H, Handke R, Bartley R (2007). Description of medical fungi. Mycological Unit, Women's and Children's Hospital, North Adelaide, pp 198.

Fidel PL, Vazquez JA, Sobel JD (1999). *Candida glabrata*: review of epidemiology, pathogenesis, and clinical disease with comparison to *C. albicans*. Clinical Microbiology Reviews 12(1):80-96.

Flevari A, Theodorakopoulou M, Velegraki A, Armaganidis A, Dimopoulos G (2013). Treatment of invasive candidiasis in the elderly: a review. Clinical Interventions in Aging 8:1199-1208

Hardel DK, Sahoo L (2011). A review of phytochemical and pharmacological properties of *Eucalyptus globulus:* a multipurpose tree. International Journal of Research in Ayurveda and Pharmacy 2(5):1527-1530.

Kelmanson JE, Jager AK, van Staden J (2000). Zulu medicinal plants with antibacterial activities. Journal of Ethnopharmacology 69(3):241-246.

Kim Y (2015). Statistical notes for clinical researchers: post-hoc multiple comparisons. The Korean Academy of Conservative Dentistry, http://dx.doi.org/10.5395/rde.2015.40.2.172, accessed 4th January 2017.

Kirby WWM, Bauer AW, Sherris JC (1966). Antibiotic susceptibility testing by standardized single disk method. American Journal of Clinical Pathology 45(4):493-496.

Koukpupetis T, Manolakaki D, Velhamos G (2010). Candida infection and colonization among nontrauma emergency surgery patients. Virulence 1(5):359-366.

Kong J, Goh NK, Chia TF (2003). Recent advances in traditional plant drugs and orchids. Acta Pharmacologica Sinica 24(1):7-21.

Krogh-Madsen M, Arendrup MC, Heslet L, Knudsen JD (2006). Amphotericin B and caspofungin resistance in *Candida glabrata* isolates recovered from a critically ill patient. Clinical Infectious Disease 42(7):938-944.

Kosh-Komba E, Aba Toumnou L, Zinga I, Touckia I, Nembara PU, Mukeina G, … Syssa-Magale JL (2017). Phytochemical screening, antifungal and antibacterial effect of *Zanthoxylum zanthoxyloides* and *Zanthoxylum macrophylum* used in traditional medicine in Yamboro (Central African Republic). European Journal of Medicinal Plants 19(3):1-11.

Lass-Flörl C (2009). The changing face of epidemiology of invasive fungal disease in Europe. Mycoses 52(3):197-205.

Lee SS, Robinson FM, Wong HY (1981). Rapid determination of yeast viability. Biotechnology and Bioengineering Symposia 11:641-649.

Nayak A, Nayak RN, Soumya B, Bhat K, Kudalkar M (2011). Evaluation of antibacterial and anticandidal efficacy of aqueous and alcoholic extracts of neem (*Azadirachta indica*) and *in vitro* study. International Journal of Research in Ayurveda and Pharmacy 2:230-235.

Ochei J, Kolhatkar A (2000). Medical laboratory science. Theory and practice. Tata Mcgraw-Hill Publishing Company Limited: New Delhi. 2nd Edition, pp 331-349.

Ogundare AO, Adetuyi FC (2007). Growth studies on microbial population of bread baked with wheat flour from South Western Nigeria. Journal of Food, Agriculture and Environment 34:85-87.

Okigbo RN, Anuagasi CL, Amadi JE, Ukpadi UJ (2009). Potential inhibitory effects of some African tuberous plant extracts on *Escherichia coli, Staphylococcus aureus* and *Candida albicans*. International Journal of Integrated Biology 6(2):91-98.

Olutiola PO, Famurewa O, Sonntag HG (2000). Introduction to general microbiology: a practical approach. Bolaby Publication, Lagos, pp 267.

Osemene KP, Ilori MO, Elujoba AA (2013). Examining the nature of herbal research and development in Nigeria. International Journal of

Biology, Pharmacy and Applied Sciences 1(2):133-142.

Redding SW, Kirkpatrick WR, Coco BJ, Sadkowski L, Fothergill AW, Rinaldi MG, … Patterson TF (2002). *Candida glabrata* oropharyngeal candidiasis in patients receiving radiation treatment for head and neck cancer. Journal of Clinical Microbiology 40(5):1879-1881.

Rodrigues CF, Henriques M (2017). Oral mucositis caused by *Candida glabrata* biofilms: failure of the concomitant use of fluconazole and ascorbic acid. Therapeutic Advances in Infectious Diseases 4(1):10-17.

Samuel TO, Adekunle AA, Ogundipe OT (2014). *In-vitro* activities of eight antifungal drugs against some clinical isolated fungi. University of Lagos Journal of Basic Medical Sciences 2(2):47-51.

Seeliger HPR (1956). Use of a urease test for the screening and identification of Cryptococci. Journal of Bacteriology 72(2):127-131.

Sharma AK, Gangwar M, Tilak R, Nath G, Sinha AS, Tripathi YB, Kumar D (2012). Comparative *in vitro* antimicrobial and phytochemical evaluation of methanolic extract of root, stem and leaf of *Jatropha curcas* Linn. Pharmacognocy Journal 4(30):34-40.

Shimada T (2006). Salivary proteins as a defense against dietary tannins. Journal of Chemical Ecology 32(6):1149-1163.

Silva S, Negri M, Henriques M, Oliveira R, Williams DW, Azeredo J (2012). *Candida glabrata, Candida parapsilosis* and *Candida tropicalis*: biology, epidemiology, pathogenicity and antifungal resistance. FEMS Microbiology Reviews 36(2):288-305.

Silva E, Martins S, Alves E (2014). Essential oils from the control of bacteria speck in tomato crop. African Journal of Agricultural Research 9(34):2624-2629.

Sofowora A (2006). Medicinal plants and traditional medicine in Africa. Ibadan, Spectrum Books Limited, pp 285.

Srinivasan D, Thirumurugen N, Hansi T, Persumalasamy PL (2001) Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. Journal of Ethnopharmacology 74(3):217-220.

Sule WF, Okonko IO, Joeseph TA, Ojezele MO, Nwanze JC, Alli JA, … Ojezele OJ (2010). *In vitro* antifungal activity of *Senna alata* Linn. crude leaf extract. Research Journal of Biological Sciences 5(3):275-284.

Takahashi T, Kokubo R, Sakaino M (2004). Antimicrobial activities of *Eucalyptus* leaf extracts and flavonoids from *Eucalyptus maculata*. Letters in Applied Microbiology 39(1):60-64.

Trease GE, Evans WC (2009). Pharmacognosy (16th ed). Edinburgh. Saunders Elsevier.

Umeh CN, Okolocha MB (2005). The occurrence of oral thrush yeasts among children in Onitsha urban, Anambra state, Nigeria. Journal of Medical Sciences 5(3):177-180.

Valle D, Cabrera E, Puzon J, Rivera W (2016). Antimicrobial activities of methanol, ethanol and supercritical CO_2 extracts of Philippine *Piper betle* L. on clinical isolates of Gram Positive and Gram Negative bacteria with transferable multiple drug resistance. Plos One 11(1):e0146349.

Walsh TJ, Dixon DM (1996). Deep mycoses. Barons Medical Microbiology (4th ed) pp 204-207.

William D, Timothy G (2006). Andrew's diseases of the skin. Clinical Dermatology - Expert Consult, Online and Print. Elsevier Health Sciences.