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Full Length Research Paper

Prevalence of Vulvovaginal Candidiasis amongst pregnant women in Maroua (Cameroon) and the sensitivity of *Candida albicans* to extracts of six locally used antifungal plants

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Abstract

This investigation was carried out at two referral hospitals of Maroua (Far-North, Cameroon), involving 211 (112 pregnant and 99 non pregnant) women of age interval 16-45, to estimate the prevalence of vulvovaginal candidiasis (VVC) amongst pregnant women and to determine the sensitivity of *Candida albicans* culture to extracts of six locally used antifungal plants. Prevalence and various potential risk factors for VVC were assessed during a survey. Women provided vaginal swabs. Isolation of *Candida albicans* from swabs was done using Sabouraud Chloramphenicol Agar (SCA) (manufactured by Bio-Rad). The sensitivity of *Candida albicans* cultures was determined on SCA and Muller Hinton agar to the six plants extracts. The results indicated that the prevalence of VVC was higher amongst pregnant (55.4%) than non-pregnant women (35.4%). Associated risk factors such as douching habits, use of antibiotics and poor toilet facilities and sanitary conditions favor this prevalence. *Candida albicans* does not appear to be transmitted sexually, and episodes of VVC do not appear to be related to the number of sexual partners. Amongst the plants studied *Allium sativum*, *Sena alata* and *Ocimum gratissimum* had the greatest inhibitory activity against *Candida albicans*. Because of their antifungal effects, clinical trial should be performed to determine the usefulness of the extracts of these plants for the treatment of VVC.

Keywords: Vulvovaginal Candidiasis, Prevalence, Pregnant women, Antifungal activity, *Candida albicans*

INTRODUCTION

Vulvovaginal candidiasis (VVC) is an infection most commonly due to the fungus *Candida albicans* (Nviriesy, 2008). An estimated 70 to 75 % of healthy adult women have at least one episode of VVC during their lifetimes (Sobel *et al.,* 1998). Prevalence rates are higher in women treated with broad spectrum antibiotics (Singh, 2003), pregnant women, diabetic women (De Leon *et al*., 2002; Donders, 2002) and women with HIV/AIDS (Duerr *et al*., 2003; Reed *et al.,* 2003). Akah *et al.* (2010), in Enugu State (Nigeria) found that pregnant women had uncomplicated VVC. Another survey, at the Port Moresby General Hospital, New Guinea, indicated that, of the 206 pregnant women examined, 23% were found to have *Candida albicans* infection (Klufio *et al*., 1995). A study on HIV/AIDS patients attending the Nylon Health District Hospital in Douala (Cameroon), showed that out of 304 HIV-positive individuals, a total of 204 (67.1%) had more than one predisposing condition to candidiasis, with those on antibiotic therapy having the highest prevalence (63.7%) followed by pregnant women (7.3%) (Njunda *et al*., 2011).

Classic medical treatment of VVC lies on the use of antifungal drugs such as Nystatin, Amphotericin B and Imidazoles. However, the more current and most effective antifungal drugs are very expensive and out of reach for many Africans, majority of who reside in the

rural areas. These antifungal drugs may also be associated with some serious side effects (Barret, 2002). Research has also shown an increasing resistance of fungi to imidazole derivatives. Thus, the prescription of antifungal drugs should be hardly based on proper diagnosis and antifungal sensitivity tests (Yongabi *et al*., 2009).

The use of medical plants, which has fewer side effects and is economically cheaper, has been recently taken into consideration (WHO, 2002; Ajose, 2007; Lee *et al*., 2007). Singh *et al.,* (1995) identified "cinnamic aldehyde" in cinnamon as antifungal material. Another study carried out on the effect of 45 Indian plants on human resistant pathogens to different drugs revealed that 24 plants showed antifungal activity against *Candida sp* (Ahmad and Beg, 2001). In Bamenda (North-West, Cameroon), Yongabi *et al.* (2009) found that methanol extracts of *Mangifera indica* (mango) seeds, *Aspilia africana* (African iodine) leaves, *Ageratum conyzoides* (goat weed/king grass) leaves, *Allium sativum* (garlic) cloves, *Vernonia amygdalina* (bitter leaves), *Khaya senegalensis* (drywood mahogany) seeds, *Moringa oleifera* (drum stick/horseradish) seeds and *Persea americana* (avocado) seeds exhibited appreciable growth inhibition of *Candida sp.*

Despite the progress made to determine the prevalence rate of VVC in other regions of Cameroon (Enow *et al*., 2008; Njunda *et al*., 2011) little research has been focused on the Far-North region. Far-North region has a rich flora of plants whose products are being used against fungal infections by the local population, but the antifungal effect of these plants is not yet scientifically proven. Determining the antifungal properties of these plants would thus enable their safe use in traditional medicine for the treatment of *Candida* and other fungal infections.

The objective of this study was to estimate the prevalence of VVC amongst pregnant women in Maroua (Far-North, Cameroon) and determine the sensitivity of *Candida albicans* culture to six extracts of locally used antifungal plants.

MATERIALS AND METHODS

Study site

The study was conducted at Maroua Regional Hospital and the Social Insurance Fund Hospital (10°35'85"N; 14°14'19"E) in Cameroon. These are two referral hospitals for three districts (Maroua 1, 2 and 3) of Maroua town.

The population of Maroua is estimated at 272,000 inhabitants in January 2010, which is 10.6% of the Far-north region and 1.7% of the population of Cameroon (Anonymous, 2010). The inhabitants constitute 51% Moslems, 47% Christians and 2% neither Moslem nor Christians. The natives of this town are the Giziga and Bi-marvas who are organized into a community (lamida) controlled by a lamido. Some ethnic groups like the Tupuri and Mundang are well represented. Artisanal activities are well developed (Noumi and Kolaipla, 2011).

The climate is tropical and of the Sudano-Sahelian type. The averages of rainfalls and temperatures are 830 mm and 25.92°C respectively. The humid season lasts for five months (May–September). The minimal temperatures are observed from December to January, periods corresponding to the continental influence. The maximum temperature occurs in April and corresponds to the end of the dry season. The set of rivers in the locality are characterized by non-permanent outflows (Seignobos and Iyebi-Mandjek, 2000).

Vegetation is a spiny steppe of the sahelian sector. Amongst the woody plants, *Acacia hockii* is very extensively dominant. One also notes: *Anogeissus leiocarpus, Combretum glutinosum, Boswellia dalzielii, Accacaia senegalensis, Sterculia setigera* and *Balanites aegyptiaca* (Letouzey, 1985 cited by Noumi and Kolaipla, 2011).

Ethical Consent

A research authorization was obtained from the University of Maroua. An ethical clearance was also obtained from the Regional Delegation of Health. Approvals were also granted by the authorities of the Social Insurance Fund (SIF) and Maroua Regional hospitals where this study was carried out. Participation was voluntary and informed consent was obtained from the participants.

Assessment of the prevalence of VVC

This study was conducted over a period of three months (February 2012 to April 2012) involving 211 women (112 pregnant women and 99 non pregnant women) of age interval 16-45 presenting to the Maroua Regional Hospital and the Social Insurance Fund (SIF) Hospital for ante-natal visit and routine high vaginal swab test. Information on the level of education, occupation and age of the women was obtained through a data form to determine the prevalence among these groups. Required laboratory test were therefore carried out to confirm the presence of vaginal *Candida sp.*

Administration of questionnaire

A questionnaire was administered in order to assess the risk factors that influence the prevalence rate of VVC in Maroua. The questionnaire was first pre-tested, and then administered to women of different age groups and social

classes, randomly selected in the locality of Maroua. Participation was voluntary after obtaining informed consent from the participants. After explanations on how to fill the questionnaire, the participants then proceeded to fill in the required information. Participants who understood neither French nor English were asked questions in the *Fulfulde* (local language) by a guide who in turn filled the questionnaires on their behalf. The questionnaire was first checked visually for completeness, obvious errors and inconsistencies.

Sampling technique

Those selected for the study were women resident in Maroua, presenting abnormal vaginal discharge and symptomatic diagnosis of VVC.

Preparation of patients

The patients were sensitized on the conditions to be respected prior to specimen collection which included the following:

- Patients were advised to avoid any form of treatment four weeks prior to swab collection to improve the chance of positive results.
- They should avoid douching and sexual intercourse 24 hours prior to sample collection.
- Patients were received between the hours of 8:00-11:00 am for sample collection.

Collection of specimens

According to the laboratory procedures of the WHO (2009), a sterile vaginal speculum (single use, manufactured by Changzhou Shaungma medical devices co. ltd.) was moistened in warm sterile water and inserted into the vagina to examine and appreciate the state of the cervix. A sterile cotton-wool swab stick (single usage, sterilized with ethylene oxide and manufactured by NAFDAC industries ltd) was then inserted 20-30 mm into the endocervical canal and gently rotated against the endocervical wall to pick a high vaginal swab. The swab stick was immediately replaced in its casing and labeled appropriately with the patient's information (first name, surname, date of birth, location, physician, telephone number, time and date). The patient's details were also filled in a request form.

Specimen Analysis

The specimens collected were then analyzed using standard laboratory procedures laid down by the WHO (2009).

Macroscopic examination of specimen

After collection of the vaginal smears, macroscopic examinations were done and this involved commenting on; the state of the cervix (normal or inflamed), the amount of discharge (abundant, less abundant or normal), the color of the discharge (yellowish or whitish), the aspect (paste like, slimy) and the odor (odorless) of discharge. The pH of the vaginal secretions was determined by narrow-range pH paper.

Microscopic examination of specimen

Wet preparation

One sample of vaginal swab in 1 to 2 drops of 0.9% normal saline solution was diluted on slide and a second sample was diluted in 10% potassium hydroxide (KOH) solution on another slide (ground edges, manufactured by Medifield Equipment and Scientific limited, England). Cover slips were placed on each slide and then examined under a light microscope (Humascope). The yeast or pseudohyphae of *Candida* species were more easily identified in the KOH specimen. Yeast cells were also seen in the normal saline preparation of positive samples. The cells were immobile and budding cells could be appreciated although the pseudohyphae formed were not easily detectable.

Dry preparation

In accordance with the gram staining procedures of the WHO laboratory manual, exudates of the specimens collected were spread on another slide to give a smear which was gram stained.

Culture and identification of *Candida albicans*

The media used for this analysis was the Sabouraud Chloramphenicol Agar (manufactured by Bio-Rad), a selective media for fungal growth. *Candida albicans* was cultured on SCA following the standard laboratory procedures laid out by WHO (2009). The plate was incubated at 37 ^{o}C for 48 hours. A germ tube test was carried out to confirm the *Candida albicans* species.

Determination of sensitivity of *Candida albicans* culture to plant extracts

Sources of plant materials

An investigation form was used to get inquires on plants frequently used to treat fungal infections by natives in the

localities of Maroua. The plants and the parts used were then selected based on their medicinal traditional usage by the natives of Maroua and other regions in Cameroon, to manage and treat several skin diseases such as diaper rash in babies and vaginal itches in women. The plant specimens were then washed and dried at room temperature for a week after which they were pulverized individually in a mortar using a pestle and stored in air tight clinical plastic bags. The different plants selected for this study and the various parts of the plants used are presented in Table 1.

Extraction procedure

Fifty grams (50 g) of the dried plant material was added to 250 ml of methanol (1:5 [w/v]) in 500 ml beakers (Pyrex) and allowed to extract for 72 h (Irobi and Daramola, 1993). The solutions were filtered using filter paper and the filtrate solvent was evaporated under vacuum at 100^{o}C using a rotary evaporator. The resulting extracts were stored in sterile screw-capped bottles and kept at room temperature.

Determination of diameters of growth inhibition

The agar diffusion method, according to Collins *et al*. (1995) was used. 0.2 g of the plant extracts were reconstituted in 2.5 ml of methanol. One colony of the conserved yeast (*Candida albicans*) culture was dissolved in 2 ml of distilled water in a test tube and inoculated in MHA and SCA plates. Wells of 6 mm in diameter were made in the agar with a sterile stainless steel cork borer and 0.05ml of the extracts was added to each well, controls comprised extraction solvent methanol (Negative control) and 200 mg of Nystatin standard disk (Positive control). The agar plates were incubated at 37 ^{o}C and 40 ^{o}C for 48 h. The different zones of inhibitions around each plant extract and the controls were recorded. These zones of inhibition around the wells containing the extract were taken to indicate the antifungal activity of the plant extract against the test organism.

Determination of Minimum inhibitory concentration (MIC)

Five test tubes were labeled with different weights (0.005g, 0.01g, 0.05, 0.10, and 0.15g) for each plant extract, giving a total of 35 test tubes for the six plant extracts. Different weights of the plant extracts were measured using a sensitive balance (0.005g, 0.01g, 0.05, 0.10, and 0.15g) and placed in the appropriately labeled test tubes. 2.5 ml of methanol was then added into each tube to produce different final concentrations. Negative control test tubes containing only methanol were also prepared.

The estimation of MIC of the crude extracts was carried out using the Broth Dilution Technique by Akinpelu and Kolawole (2004). Two aliquots (2 ml) of different concentrations of the five-fold dilutions of each crude extract prepared were added to 18 ml of pre-sterilized molten Sabouraud in small test tubes (already labeled), and mixed allowed to set. The surface of the medium was allowed to dry under laminar flow before inoculating with 48 h *Candida albicans* cultures. The test tubes were later incubated at 37° C for up to 48 h after which they were examined for the presence or absence of growth (Adesokan *et al.,* 2007). The MIC was taken as the lowest concentration that prevented the growth of the test microorganism.

Data Analysis

The data collected from the sample analysis of VVC as well as that from the questionnaire to assess the prevalence of VVC and risk factors respectively was analyzed using the SPSS data base version 17.0. The association between two variables such as the state, occupation, age and result obtained (positive/negative) was established using the Chi square and Fisher's exact tests and results were considered statistically significant when the P-value was less than or equal to 0.05. Interpretation of antifungal sensitivity testing and MIC were done using the EUCAST (European Committee on Antimicrobial Susceptibilty Testing) version 1.3, 2010. The percentage yield of the crude extract was determined using the formula: Percentage yield = X l – X 2 / X1 x 100.0%. [Where, X l = weight of the dry powdered leaves before extraction (50.0g); X2 = weight of the dry chaff after extraction].

RESULTS

Occurrence of VVC amongst pregnant and non-pregnant women

The computed prevalence of VVC amongst pregnant and non-pregnant women showed that 70% of the pregnant women had clinical symptoms of VVC but 55.4% of them were microbiologically confirmed positive. The result indicated that the prevalence rate of VVC was higher amongst pregnant women (55.4%) than amongst non-pregnant women (35.4%). Some 14.6% (pregnant) and 29.6% (non-pregnant) women who were symptomatic (pruritus and leucorrhoea) had negative cultures.

Occurrence of VVC according to age group, education level and occupation amongst pregnant and non-pregnant women

The results in Table 2 show that the prevalence of VVC was higher in the age group of 25-31 (46.8%) amongst

Table 1. Plants and plant parts selected

Plant selected	Plant parts
Moringa oleifera	Leaves
Carica papaya	Leaves
Allium sativum	Cloves
Allium cepa	Bulb
Sena alata	Leaves
Ocimum gratissimum	Leaves

Table 2. Occurrence of VVC (%) according to age group, education level and occupation amongst pregnant and non-pregnant women

Categories		Results	
		Pregnant	Non Pregnant
Age	<18	4.8	5.7
	18-24	25.8	45.7
	25-31	46.8	25.7
	32-38	16.1	20.0
	≥39	6.5	2.9
Education Level	Illiterate	8.1	20.0
	Primary	50.0	40.0
	Secondary	33.9	25.7
	Higher	8.0	14.3
Occupation	Unemployed	66.1	74.3
	Employed	17.8	14.3
	Student	16.1	11.4

pregnant women and 18-24 (45.7%) amongst non-pregnant women. On the other hand, the prevalence was low amongst pregnant women of the age group <18 (4.8%) and non-pregnant women of the age group ≥ 39 (2.9%). Those who had received just primary education had the highest prevalence (50% and 40%) in both pregnant and non-pregnant women respectively whereas a low prevalence was observed amongst those who had attained a higher level of education in both pregnant (8.0%) and non-pregnant women (14.3%) (Table 2). The prevalence was higher amongst the unemployed pregnant women (66.1%) and non- pregnant women (74.3%) but low amongst students in both pregnant (16.1) and non-pregnant women (11.4%).

Occurrence of VVC, state of occurrence and the number of sexual partners amongst women

The occurrence of VVC amongst women and the state in which this infection occurred were assessed. We found that 54% of women have had at least one episode of VVC, 38% out of pregnancy, 14% during pregnancy and 4% in both states. A handful of women (77%) had just one sexual partner while 14% of them are not sexually involved; few of them (8% and 1%) had 2 and 3 sexual partners respectively.

Risk factors associated with VVC

Douching habits and agents used for

Inquiries on the douching habits and agents used for douching showed that 41% of women do a daily douching while 30%, 6% and 23% do a weekly, bimonthly and a monthly douching respectively. Also, most of the women (54%) used antiseptics for douching whereas 41% used just water for douching and 5% used other agents different from antiseptics and water.

Use of antibiotics and contraceptive pills by women

Information from the questionnaire showed that 72% of women frequently take antibiotic whereas 28% don't. On the other hand, a majority (78%) of the women do not use

Table 3. Percentage yield from methanolic extraction of each plant

Plants selected	Weight of dry powder(g) Before/After extraction		Weight of crude methanolic extract(g)	Percentage yield(%)
Moringa oleifera	50	46	4	8
Carica papaya	50	45	5	10
Allium sativum	50	48	2	4
Allium cepa	50	42	8	16
Sena alata	50	44	6	12
Ocimum gratissimum	50	46	4	8

Table 4. Diameters of inhibition of plant extracts against *Candida albicans*

Plant extract	Diameter of inhibition(mm)*					
	MHA		SCA			
	37.8 °C	Sensitivity	37.8 °C	Sensitivity	40.2 °C	Sensitivity
Moringa oleifera	8.0	Intermediate	8.5	Intermediate	6.0	Resistant
Carica papaya	7.0	Resistant	7.1	Resistant	6.0	Resistant
Allium sativum	7.5	Intermediate	13.0	Sensitive	11.0	Sensitive
Allium cepa	7.8	Intermediate	8.0	Intermediate	8.3	Intermediate
Senna alata	9.5	Sensitive	9.5	Sensitive	11.0	Sensitive
Ocimum graticimum	9.0	Sensitive	9.0	Intermediate	10.0	Sensitive
Nystatine	11.0	Sensitive	12.0	Sensitive	11.5	Sensitive
Methanol	6.0	Resistant	6.0	Resistant	6.0	Resistant

* <7.5 = Resistant, 7.5-8.9 = Intermediate, ≥9 = Sensitive

contraceptive pills, 15% of them use it occasionally while 7% use it monthly.

Toilets and underwear types commonly used by women

The two commonly used toilets systems by women of this region are the pit and water system toilets, of which 59% of these women use the pit toilet while 41% use the water system. Cotton underwears is that which is often used by the women (46%) of this region though some (16%) of them also use synthetic underwears while others (38%) use both the cotton and synthetic underwears.

Sensitivity of *Candida albicans* to plants extracts

Percentage yield from the methanolic extraction of plants

After methanolic extraction of plants the results of the percentage yield per plant showed that the highest yields were obtained from *Allium cepa* (16%) and the lowest from *Allium sativum* (Table 3).

Results of diameter of zones of growth inhibition

The diameter of the zones of inhibition of the different plant extracts on *Candida albicans* (Table 4) growing on SCA and MHA at different temperatures of incubation were obtained. Results showed that *Allium sativum* had the highest zone of inhibition (13.0mm and 11.0mm) to *Candida albicans* on SCA at 37.8 °C and 40.2 °C respectively, followed by *Senna alata* (9.5mm and 11.0mm) while *Carica papaya* leaves had the least zone of inhibition (7.1mm and 6.0mm). The sensitivity of *Candida albicans* to the various plant extracts was classified as being resistant, intermediate or sensitive according to EUCAST version 1.3 as indicated in the Table 4. The results obtained from sensitivity testing on MHA were slightly lower than those on SCA.

Minimum inhibitory concentration of plants extracts on *Candida albicans*

The minimum inhibitory concentration for each plant extract was determined after serial dilution of the different plant extracts to varying concentrations. The results of the minimum inhibitory concentration of each plant

Table 5. Minimum inhibitory concentration of plant extracts.

Plant extracts	Concerntration of plant extract used in mg/ml				
	0.01 mg/ml	0.02 mg/ml	0.03 mg/ml	0.04 mg/ml	0.05mg/ml
Moringa oleifera	G	G	NG	NG	NG
Carica papaya	G	G	G	G	NG
Allium sativum	G	NG	NG	NG	NG
Allium cepa	G	G	G	G	NG
Senna alata	G	NG	NG	NG	NG
Ocimum graticimum	G	NG	NG	NG	NG

NG = no growth, G = growth

extracts are presented in Table 5. These results show that *Allium sativum, Ocimum graticimum* and *Senna alata* had the lowest minimum concentration of inhibition at 0.02mg/ml while *Allium cepa* and *Carica papaya* had the highest (0.05mg/ml).

DISCUSSION

The study showed high prevalence rates (55.4%, $p<0.05$) of vulvovaginal candidiasis amongst pregnant women in Maroua indicating a significant relationship between VVC infections during pregnancy due to changes in the levels of female sex hormones, such as estrogen and progesterone (Dennerstein and Ellis, 2001; Tarry *et al*., 2005). The prevalence was observed amongst those of the age group 25-31 (46.8%), as well as among those with a primary level of education (50%), in spite the fact that there exist no relationship between age ($p>0.05$) and level of education ($p>0.05$), to VVC infection. Thrush most commonly affects women in their twenties and thirties. Thrush is less common in girls who have not yet started menstruating and women who have reached menopause (Pray, 2006). A high rate of VVC infection was also observed among the unemployed women (66.1 %, $p<0.05$), thus indicating a relationship between social status and VVC infection. The unemployed women are more exposed to this infection because of limited resources or finances to take adequate care of themselves.

About 70 % of tested women had clinical symptoms of VVC and as high as 55.4 % were microbiologically confirmed. Nikolov *et al*., (2006) reported 88.3 % prevalence by microscopy while Klufio *et al*., (1995) reported 57 % infection microbiologically. The high rates are in conformity with the fact that *Candida albicans* is both the most frequent colonizer and responsible for most cases of vulvovaginitis (Singh, 2003; Hainsworth, 2002; Watson *et al*., 2001). Out of 211 women (112 pregnant and 99 non pregnant) who attended antenatal clinic, 70% of the pregnant women and 55% of the non-pregnant women presented with symptoms of vaginal itching (pruritus vulvae) and discharge (leucorrhoea). A total of 55.4% and 35.4% of this numbers respectively had positive culture of *Candida spp.* (significant VVC). Some 14.6% (pregnant) and 29.6% (non-pregnant) women who were symptomatic (pruritus and leucorrhoea) had negative cultures. This illustrates that most patients with symptoms of VVC are actually confirmed to have VVC after clinical analyses though some few cases will have these symptoms but show negative results of VVC. Symptomatic VVC with negative culture may be indicating of other vagina infections.

After analyzes of questionnaires results showed that the most probable factor favoring the prevalence rate of VVC in the town of Maroua include; douching habits and agents, antibiotics and toilet systems. Frequent douching with antiseptics can alter the microbial flora of the vagina thus exposing it to *Candida* infection. Any type of antibiotics can increase the risk of developing thrush, but in order to develop the condition, the *Candida* fungus must already be present. Prevalence of VVC as a result of antibiotic use is also confirmed by Pirotta & Garland (2006) and Bluestein *et al*. (1991) who reported 28%-33% frequency of VVC with antibiotic use. Toilet systems such as the pit toilet may increase the risk of developing thrush because of their poor sanitary condition and maintenance.

Methanolic extraction of plants showed higher yields (16%) of *Allium cepa* indicating high degree of solubility of its contents to methanol, unlike *Allium sativum* (4%) which has a lower solubility.

Sensitivity testing was more appreciable on SCA than on MHA because SCA is the selective media for *Candida albicans* (Murray *et al.,* 2007). Sensitivity on SCA showed that *Allium sativum* (13.0 mm) has the greatest antifungal activity to *Candida albicans* than all the other plant extracts. Yin and Tsao (1999) reported garlic to have the greatest antifungal effect compared to other plants while Zohreh *et al*. (2011) confirmed the inhibitory effect of *Allium sativum* (18.64 mm) on *Candida albicans* due to the action of allicin a sulfur-containing compound found in garlic. *Sena alata* (9.5 mm) leaves on SCA at 37 ^{o}C showed strong antifungal properties against *Candida albicans* which is in consonance with the findings of Villasenor *et al*. (2002) reporting the effect of *Sena alata*

leaves against *Trichophyton mentagrophytes* due to their phytochemical contents which caused inhibitory effects on the mycelia growth of the fungi. *Occimum gratissimum* leaves cultured on the same culture media and temperature also exhibit antifungal effect against *Candida albicans* with a zone of inhibition of 9.0 mm. The antifungal effects of these plants when cultured on the same culture media (SCA) but with increased temperature (40.2 ^{o}C) showed an increase in their diameter of inhibition. This revealed that the antifungal properties of these plants increase with temperature (Doughari, 2006). This may be an indication that the bioactive compounds of the plants are heat stable and explains their extraction and ethno-botanical application process at high temperatures without losing their efficacy.

Results of the minimum inhibitory concentration revealed that *Candida albicans* is resistant to all plants extract at 0.01 mg/ml. The MIC for *Allium sativum*, *Sena alata* and *Ocimum gratissimum* is 0.02 mg/ml confirming their strong antifungal properties. MIC results of the crude bark extract of *Sena alata* for all the tested dermatophytes was at 5.0 mg/mL (Sule *et al*., 2011). The MIC of *Moringa oleifera* is at 0.03 mg/ml indicating its weak antifungal properties. The MIC of *Allium cepa* and *Carica papaya* are at 0.04 mg/ml, identifying it as a very weak antifungal plant against *Candida albicans*. At 0.05 mg/ml all the plants extract exhibit antifungal effect against *Candida albicans* implying that at higher concentrations all plants extract will show antifungal properties.

Comparing the results of antifungal sensitivity testing and that of the minimum inhibitory concentration, it is observed that there is a relationship between these results. *Allium sativum* which had the strong antifungal properties had the lowest MIC value whereas *Carica papaya* which had the lowest antifungal properties had the highest MIC value.

In accordance with the above analyses, *Allium sativum*, *Sena alata* and *Ocimum gratissimum* have proven to be the most probable antifungal plants. On the contrary, *Carica papaya* and *Allium cepa* present the lowest antifungal effects and are considered as very weak antifungal plants. In this light, the above plants proven to have antifungal effect could be used by the local population of Maroua to prevent VVC infection thereby reducing its prevalence in this region.

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