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IN-VITRO ANTIFUNGAL EFFECT OF *GARCINIA KOLA* AND GARLIC (*ALLIUMS SATIVUM*) ON VAGINAL ISOLATES OF *CANDIDA*

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ABSTRACT

Background/Objectives:

Within the last decade there has been an emergence of antifungal drug resistance. *Alliums sativum* and *Garcinia kola* seeds were tested for their anticandidal properties in comparison with fluconazole and miconazole.

Methods: High Vaginal swab samples from patients with vulvovaginal candidiasis were processed and identified to the species level by germ tube method, morphology on corn meal agar and sugar fermentation reactions. Methanol and aqueous extracts of *Garcinia kola* and *Alliums sativum*, as well as fluconazole and miconazole were tested in-vitro using the agar dilution method.

Results: One hundred and twenty six women with symptoms of vulvovaginal candidiasis were sampled and *Candida species* were isolated from 25 of them. *Candida spp.* identified were *C. albicans* (44%), *C. tropicalis* (28%), *C. glabrata* (16%) and *C. parapsilosis* (12%). All species except *C. glabrata* were inhibited by fluconazole and miconazole, all isolates of the same species having same minimum inhibitory concentrations (MICs). The highest MICs (25 mg/ml) with the alcoholic extracts were shown by *C. albicans* and *C. glabrata* and the lowest MICs (12.5 mg/ml) were shown by *C parapsilosis* and *C tropicalis*. All the isolates tested with *Garcinia kola* aqueous extract had a uniform MIC of 50 mg/ml, those tested with garlic aqueous extract had an MIC of 200 mg/ml. *C. albicans* and *C. glabrata* had MIC of 200 mg/ml of the alcoholic extract but *C. tropicalis* was inhibited at 25 mg/ml.

Conclusion: We found that *Garcinia kola* and *Alliums ativum* have activity against the vaginal *Candida* species isolated thus showing promise as alternative therapy for vaginal candidiasis.

Keywords: *Alliums ativum, Candida spp, Garcinia kola*, Minimum inhibitory concentrations

INTRODUCTION

Candida vaginosis is one of the most frequent infections of the female genital tract. At least 75% women suffer once in their life from one episode of a candida infection (1-3). Although *Candida albicans* is the pathogen identified in most patients with vulvovaginal candidiasis, other possible pathogens include *Candida tropicalis, Candida glabrata, Candida parapsilosis* amongst others, which are responsible for up to 33 percent of recurrent infections (4-6). *Candida tropicalis* and *glabrata* are the most important of the *non-C albicans* infections (7, 8). *Candida species* other than *albicans* have been found to cause yeast vaginitis (8). Relatively higher antifungal resistance rate of non-*C albicans species* may contribute to higher rates of recurrent infections.

Imidazole is the first-line treatment for *C. albicans* infections. *In vitro* studies have shown that imidazole antifungal agents such as miconazole and clotrimazole are not as effective against non-*C. albicans* fungi as against *C. albicans* fungi. *C. tropicalis* and *C. glabrata* are 10 times less sensitive to miconazole than is *C. albicans* (9, 10). The recognition of yeast speciation and the need for use of a broad-spectrum antifungal preparation that covers these organisms is now apparent (11-14). However many of the commonly used antifungal drugs are of limited use due to their toxicity and side effects which includes dangerous drug interactions, liver damage, and heart failure (15). Within the last decade there has been an emergence of antifungal drug resistance, which was uncommon in the past (11-14)

Recently in developing countries the antimicrobial effects of plant extracts have been reported and several attempts made to destroy bacteria and their spores by the application of these extracts (16 -21). In addition, plants extracts promote good human health and several plant extracts are effective against a number of human pathogens including *Candida albicans* (21, 22). Since many of these compounds are currently available as unregulated botanical preparations and their use by the public is increasing rapidly, clinicians need to consider the consequences of patients self-medicating with these preparations.

Medically important strains of fungi have been reported to have multiple drug resistance and this has led to development of more potent synthetic antifungal drugs (23-26). These new antifungal drugs are not readily available in our environment and when available is expensive thus making compliance an issue. The alcoholic extract of *Garcinia kola* has been reported to exhibit significant sensitivity and inhibitory activities against fungi and bacteria (27, 22). *Alliums sativum* has also been reported to have antifungal activity (28).

This work aimed to investigate the antifungal activity of *Garcinia kola* and *Alliums sativum* on various *species* of *Candida* isolated from the vagina with a view of possibly recommending their incorporation into formulations of efficacious drugs for the treatment of vaginal candidiasis in future.

MATERIALS AND METHODS

Study Design: Clinical isolates of *Candida* causing vulvovaginitis in women attending two separate centers of a community clinic in Lagos were exposed tofluconazole and micaconazole and some plants on trial. The study was conducted between May and September 2007 in the Department Medical Microbiology and Parasitology of the College of Medicine, Idi-Araba, Lagos. It was approved by the Research and Ethics Committee of the LUTH and informed consent was obtained from the study participants.

Study Population

High vaginal swab samples were collected from women attending the Lekki and Idi-Araba branches of a community healthcare facility for women and children in Lagos. Candidiasis was diagnosed in the women if they complained of vaginal discharge and pruritus, and *candida spp*. were seen on gram staining or culture of their vaginal discharge. Specimens were cultured on Sabouraud dextrose agar (Oxoid) incubated at 37^0C. Isolates were identified to the species level by germ tube method, morphology on cornmeal agar and sugar fermentation reactions. Identified isolates were stored on nutrient agar slant at room temperature for subsequent susceptibility testing.

Preparation of Drug Concentrations

In this study, isolates from patients were subjected to antimicrobial susceptibility testing according to the method recommended by Clinical Laboratory Standard Institute (CLSI, 2007). Antimicrobial agents used were as follows Fluconazole: (Merck Inc., West Point, PA, USA); and Miconazole (Rodhia Farma Ltd, Sao Paulo, SP, USA); Antifungal agents were reconstituted according to the manufacturers' instructions and serial two-fold dilutions (ranging from 0.06 µg/ml to 64 µg/ml) were prepared on the day of the test and added to Mueller Hinton Agar. Plates were inoculated with 105 cfu/ml of isolates. Control plates without antimicrobial agents were inoculated before and after each set of drug-containing plates. Plates were then incubated aerobically for 24 at 37^oC. A reference strain of *C. albicans* ATCC 25285 was included as control. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the antibiotic that yielded no bacterial growth.

Preparation of Plant Extracts

The Seeds of *Garcinia kola* were purchased from Mushin market (Lagos, Nigeria) and identified in the Pharmacognosy department of University of Lagos; Idi-Araba. The seeds were air dried at room temperature (29) grinded into powder form, the powdered plant was loaded into a soxhlex extractor and extracted using methanol and sterile water.

Cloves of Garlic *Alliums sativum* were purchased from same market and identified in the Pharmacognosy department of University of Lagos, Idi-Araba .The outer coat were removed, the cloves were air dried at room temperature then grounded into powder form. The powdered plant was loaded into a soxhlet extractor and extracted using methanol and water.

Agar Preparation for Plant Extract Sensitivity

The MIC was determined using the agar dilution method. Each of the plant extracts were incorporated into Mueller Hinton agar at different concentrations obtained by weighing the desired concentration of each of the plants into appropriate volume of Mueller Hinton agar.

Each of 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml concentrations was prepared for both the *Garcinia kola* aqueous and alcoholic extracts. Weighing 5g, 2.5g, 1.25g and 0.625g of each of the extract into a 100ml of Mueller Hinton agar (Oxoid, UK) and mixing vigorously to obtain a homogenous mixture achieved these concentrations.

For the Garlic (*alliums sativum*), each of 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml concentrations were prepared for both the aqueous and alcoholic extracts. Weighing 20g, 10g, 5g, 2.5g, 1.25g and 0.625g of each of the extract into a 100ml of Mueller Hinton agar and mixing vigorously achieved these concentrations.

Inoculum Preparation

Three to five isolated colonies of similar colony morphology were picked from positive plates and subcultured onto SDA. The plate was incubated and used for the initial inoculums preparation.

Using the tip of a sterile applicator stick, five isolated colonies of similar colony morphology at least 1mm in diameter were picked, and added to 5ml of sterile

0.85% NaCl, mixed for 15 to 20s. The suspension was adjusted to 0.5 McFarland standards.
Each of the prepared plates with the antimicrobial agents was inoculated with 0.1ml of the prepared inoculums. The plates were incubated at 37◦C for 24 to 48 hours and then observed thereafter, plates with growth were interpreted as positive while those without growth were said to be negative.
For each of the antifungal agents and plant extracts concentrations prepared, control plates were also incubated along without inoculums.

RESULTS

One hundred and twenty six vaginal swabs were collected from women attending both clinics and 25 (19.8%) of the women had vulvovaginal candidiasis. Four species of *C.* were isolated; *Candida albicans* 11 (44%) was the most commonly isolated followed by *C. tropicalis* 7 (28%), *C.andida glabrata* 4 (16%) and *C parapsilosis* 3 (12%).

All species except *C. glabrata* were inhibited by fluconazole and miconazole, with isolates of the same species having the same minimum inhibitory concentrations (MIC) (Table 1 and 2). The effect of methanol and aqueous extract of *Garcinia Kola* was also tested on all the isolates. The different *species* of *candida* were inhibited by various concentrations to the *Garcinia kola* extract. (Tables 3 and 4) . The aqueous extracts of the herbs were less active than the alcoholic extracts. There were also variations in the MICs of *Garcinia kola* for different species of *Candida.* The highest MICs with the alcoholic extracts were shown by *C. albicans* and *C. glabrata.* They were inhibited at a concentration of 25 mg/ml and the lowest MICs were shown by *C. parapsilosis* and *C. tropicalis* they were inhibited at 12.5 mg/ml (Tables 5 and 6). All the isolates tested with *G. kola* aqueous extract had a uniform MIC of 50 mg/ml.

The aqueous extract of Garlic was also less active than the alcoholic extract and the MICs varied for different species of *Candida*. *Candida albicans* and *C glabrata* which were inhibited only at a concentration of 200 mg/ml of the alcoholic extract but *C. tropicalis* was inhibited at 25 mg/ml. All the isolates tested with garlic aqueous extract had a uniform minimum inhibitory concentration of 200 mg/ml.

TABLE 1: SENSITIVITY OF *CANDIDA SPECIES* TO FLUCONAZOLE

Candida species	64 µg/ml	32 µg/ml	16 µg/ml	8 µg/ml
C. glabrata	+	+	+	+
C. tropicalis	-	-	+	+
C. albicans	-	-	+	+
C. parapsilosis	-	-	+	+

TABLE 2: SENSITIVITY OF *CANDIDA SPECIES* TO MICONAZOLE

Candida species	16ug/ml	8ug/ml	4ug/ml	2ug/ml
C. glabrata	-	+	+	+
C. tropicalis	-	-	+	+
C. albicans	-	-	+	+
C. parapsilosis	-	-	+	+

TABLE 3: SENSITIVITY OF *CANDIDA SPECIES* TO ALCOHOLIC EXTRACT OF *GARCINIA KOLA.*

Candida species	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml
C. albicans	-	-	+	+
C. tropicalis	-	-	-	+
C. glabrata	-	-	+	+
C. parapsilosis	-	-	-	+

TABLE 4: SENSITIVITY OF *CANDIDA SPECIES* TO AQUEOUS EXTRACT OF *GARCINIA KOLA.*

Candida species	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml
C. albicans	-	+	+	+
C. tropicalis	-	+	+	+
C. glabrata	-	+	+	+
C. parapsilosis	-	+	+	+

TABLE 5: SENSITIVITY OF *CANDIDA SPECIES* TO ALCOHOLIC EXTRACT OF *ALLIUMS SATIVUM*

Candida species	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml
C. albicans	-	+	+	+	+	+
C. tropicalis	-	-	-	-	+	+
C. glabrata	-	+	+	+	+	+
C. parapsilosis	-	-	+	+	+	+

TABLE 6: SENSITIVITY OF *CANDIDA SPECIES* TO AQUEOUS EXTRACT OF *ALLIUMS SATIVUM*.

Candida species	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml
C. albicans	-	+	+	+	+	+
C. tropicalis	-	+	+	+	+	+
C. glabrata	-	+	+	+	+	+
C. parapsilosis	-	+	+	+	+	+

KEYS FOR TABLES 1-6
+: Growth -: No growth

DISCUSSION

Most cases of vaginitis in the study was caused by *C. albicans,* which accounted for 44% of all isolates while the non- *C. albicans* accounted for 56%. This is consistent with previous findings (30,31).This study demonstrated the inhibitory effects of local herbs in comparison with known antifungal agents on vaginal Candidasis, Fluconazole and miconazole are azole anticandidal agents known to be highly active against Candida and so their inhibitory effects in this study is not surprising. The herbs investigated also showed inhibitory effects, but their MICs were high, compared to 2.5-7.5mg/dl reported by Akerele *et al* 2008 (27); implying that a high systemic concentration would be required for therapeutic effects, which implies possibility of systemic toxicity usually associated with a high therapeutic dose. There is a need to carry out a toxicity study. In addition we suggest that these agents can serve as good topical agents if the results are generated and the results of all studies are corroborated in a larger study or clinical trials.

Both herbs are easily and locally available. As shown by the higher MIC of Garlic, *Garcinia kola* may have better antifungal properties *in vitro* against vulvovaginal candida. *Garcinia Kola* seeds are rich in phytonutrients such as flavonoids, phenolic compounds, tannins, saponialkaloids. The phenolic compounds are antimicrobial agents. Phenolic compounds have been extensively used in disinfection (18). The antifungal activity of *Garcinia kola* has been attributed to the presence of hydroxybiflavonoids (22, 32) and that of A*lliums sativum* to Allicin, *S*- allylcysteine and saponins (33-35). It would be interesting and beneficial to determine the time kill effect of these substances to investigate effective use as possible disinfectants.

A comparison of the results of both aqueous and methanolic extracts shows that the methanolic extract is a better antifungal agent than the aqueous extract and this is similar to previous findings (27,28). Methanol is an organic solvent and will dissolve organic compounds better and as such will liberate the active ingredients required for antimicrobial activity (27, 28). It therefore possibly shows that the solvent used in extraction affects the degree of microbial activity. It is already known that alcohol has antibacterial effect. It will be worthwhile to investigate its effect on Candida before concluding that alcohol itself has anti candidal effect.

This study shows that the extracts of *Garcinia kola* and *Alliums sativum* possess anticandidal activity and provide preliminary evidence of the presence of one or more soluble constituents with antifungal properties. The antifungal properties can be investigated further by purifying and characterizing the active agents and by determining toxicological effect if any on normal vaginal micro flora. There is need for more work on these plants extracts to usher in a cheap and readily available antifungal agent.

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