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ANTIFUNGAL ACTIVITIES OF SOME MEDICINAL PLANTS ON THE GROWTH OF *CANDIDA ALBICANS*

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ABSTRACT

The aim of this study was to determine the *in vitro* effects of aqueous and ethanolic extract of four local plants: Alligator pepper (*Aframomum melegueta*), Ginger (*Zingiber officinale*), Cloves (*Syzygium aromaticum*) and Ashanti pepper (*Piper guineese*) against the growth of *Candida albicans.* The antifungal activity was carried out by agar well diffusion method. The ethanolic extracts of these plants inhibited the growth of *Candida albicans* (6.25, 12.5,25, 50,100,150,200 and 500mg/ml) while the aqueous extract had no inhibition on the growth of the organism at all concentrations. The minimum inhibitory concentration (MIC) of ethanolic extract of these plants ranged from 6. 25-12.5mg/ml. The phytochemical analysis of the ethanolic extract revealed the presence of flavonoids, terpenoids, alkaloids and saponins. This study shows that the ethanolic extracts of these plants can be used to treat fungal infections as alternative to conventional antibiotics.

KEYWORDS: Antifungal activity, phytochemical, plant extract, *Candida albicans* and MIC.

INTRODUCTION

Fungal infections due to *Candida* species are an important cause of morbidity and mortality especially in immunocompromised patients (Al-Hussaini and Al-Mohana, 2010). The use of

available treatment options for invasive mycoses is limited due to narrow spectrum of activity, drug resistance, toxicity and drug –interactions (Eggimann*et al*., 2003). In view of this, there is a need to develop more effective and less topic agents for the treatment of common as well as drug resistant fungal infections (Eggimann*et al*., 2003). Plants have been seen as a good source of antimicrobial agent for the treatment of common as well as drug resistant fungal infections (Al- hussani and Al-mohana. 2010). Plants have been seen as a good source of antimicrobial agent. Some of the active ingredient of the extracts of some plants have been isolated, tested and documented (Akinpelu and kolawole, 2004). The world Health Organization estimated that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population. The clinical efficacy of many existing antibiotics is being threatened by the multidrug-resistant pathogens (Amalu and Okechukwu, 2014). The increasing failure of chemotherapeutic and antibiotic resistance exhibited by pathogenic microbial agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Eyogmatig*et al*., 2007; Gull. 1992). Plants have been major source of medicine and plant secondary metabolites and have been attributed for most plants therapeutic activities (Akinpelu and kolawole, 2004; Akoachere*et al*., 2002). The aim of this study is to determine the *in vitro* effects of somelocal medical plants against the growth of *Candida albicans* and compare their results with the antifungal activityof standard anti-*Candida*drug (clotrimazole) in culture media.

MATERIALS AND METHODS

Collection of plant specimen

Alligator pepper (*Aframomummelegueta*), ginger (*Zingiberofficinale*) and Ashanti pepper uziza (*Piper guineense*) were purchased from Ogbete main market, Enugu State, Nigeria. The plants were identified and taxonomically authenticated at the herbarium of the Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology.

Collection of Test Microorganisms

The test organisms were collected from the clinical laboratory of Department of Microbiology, University of Nigeria, Nsukka.

Preparation of plant extracts

Seeds and flowers of Alligator pepper (*Aframomum melegueta*), cloves (*Syzygium aromaticum*), Ginger (*Zingiber officinale*), Ashanti pepper (*Piper guineense*) were dried at

room temperature, crushed and ground in a grinding machine to obtain a fine powder for each plant. All the grinded plants were weighed and pached in an air tight container.

Ethanolic Extraction

Twenty gram (20g) of each powdered plant sample were mixed with 100 ml of absolute ethanol in the 250 ml conical flask. The flask was covered with cotton wool. The mixture was kept for 72 hours at room temperature. During this period, the mixture was agitated at several intervals. After 72 hours, this was filtered first with sterile muslin cloth and later with a whattman filter paper to remove the coarse plant materials. The filtrates of each tested plant were evaporated to dryness using a rotary evaporator at 40^0c. The final dried samples were weighted and stored at 20^0c until use.

Aqueous Extraction

Twenty gram (20 g) of each powdered plant sample were mixed with 100 ml of sterile distilled water in 250 ml conical flask; corked with cotton wool. This mixture was kept for 72 hours at room temperature and frequently agitated. After 72 hours, this was filtered first with sterile muslin cloth and later with a whattman filter paper to remove the coarse plant materials. The filtrates of each tested plant were evaporated to dryness using a rotary evaporator at 40^0c. The final dried samples were weighted and stored at 20^0c until use.

Antifungal (standard)

Clotrimazole (Candistan solution, each 20 ml contains clotrimazole 0.2 g). The Arab Drug company, Cairo- A.R.E was used as the antifungal control at same concentrations in this study.

Antifungal Susceptibility Test

A serial dilution of each extract was prepared for studying the antifungal activities by using agar well diffusion method at different concentrations according to Al-Hussaini and Al-Mohana, (2010). Two gram (2 g) of each dry extract was diluted with 5 ml of 96% of ethanol to obtain stock solution at a concentration of 400 mg/ml. from this stock solution, various concentrations were made including 200 mg/ml (consist of 2 ml of 96% ethanol and 2ml of the stock solution at 400 mg/ml concentration), 150 mg/ml (prepared by adding 1ml of 96% ethanol to 3ml of the extract solution at a concentration of 200 mg/m1), 50 mg/ ml (prepared by adding 1ml of the extract solution at a concentration of 100 mg/ml and adding to 1ml of 96% ethanol), 25mg/ml (done by mixing 1ml of 96% ethanol with 1ml of the extract

solution at a concentration of 50mg/ml). The same serial dilution was carried out for the aqueous extract. *Candida isolates* were subcultured in nutrient broth media (oxoid) that was prepared by dissolving 1.3g of nutrient broth in 1.0ml of distilled water, shaking well and heated for several minutes using water bath to ensure complete dissolution, then sterilized for 15 minutes at 15 ip pressure in an autoclave. Several colonies of *Candida* were suspended with the aid of sterile cotton swab in sterile tube containing 10ml of nutrient broth. After mixing, the tube was incubated at 37^0c for 24 hr to produce a fungal suspension of moderate turbidity. Sabouraud dextrose ager (SDA) medium was prepared according to the manufacturer's instructions by dissolving 6.5g of the SDA in 100ml of distilled water, then shaked, heated and autoclaved. This medium was poured aseptically at 45^0c into sterilized petri plates. Afterwords, wells were dug in the plates with the aid of a sterilized cork borer of 6mm diameter. A sterile cotton swab was dipped into the fungal suspension and this was streaked all over the entire surface of the plate and the excess was removed. A total of 0.1ml of each concentration of each extract was poured into the wells and ethanol was used as a control. Antifungal drug was also used on each different plate. These experiments were allowed to stand for one hour for proper diffusion and then incubated at 37^0c for 24-46h. The diameter of zone of inhibition around the wells was measured in millimeters with help of transparent ruler. This was taken as an index of the degree of sensitivity of the test organisms to both ethanol and water extracts.

Determination of Minimum Inhibitory Concentration

Minimum inhibitory concentration was determined by agar well diffusion method as described by Ogata *et al*. (2000). The concentrations used in the antifungal susceptibility test were diluted further (2 fold-dilution) to get different concentrations (12.5mg/ml, 6.25mg/ ml, 3. 125mg/ml). The plates containing sabouraud dextrose agar (SDA) were spread with 0.1ml of the fungal inoculums and wells (6mm in diameter) were cut from agar plates using a sterilized cork borer and were filled with 0.1ml. The plates were incubated at 37^0c for 24-48h and the diameter of resultant zone of inhibition was measured. The least concentration of the extracts that show inhibitory effects on the test organism was taken as the minimum inhibitory concentration.

Qualitative Phytochemical Screening of The Extracts

Simple standard chemical tests were carried out for the qualitative phytochemical screening of the extracts. These tests were used to detect the presence of bioactive agents such as the

alkaloids, tannins saponins and terpenoids. The pyto- constituents were assayed for using standard methods described by Trease and Evans (1978). The phytochemical analysis was carried out in the Department of Applied Microbiology and Brewng, Enugu State University of Science and Technology, Enugu State (ESUT).

Statistical Analysis

Data obtain from each parameter was subjected to a statistical analysis of variance techniques to determine the level of significance in different parameters of the extracts.

RESULTS

The *in vitro* antifungal activities of four local plant extracts on *Candida albicans* were shown in Table1while the standard antifungal drug was shown in Table 2. The ethanolic extracts of four plants exhibited various antifungal activities against the strain of *Candida albicans* according to the type of plant and the used concentrations. Amongst the plants studied, the most active extract was that obtained from clove (*Syzygium aromaticum*) which gave zone of inhibition of about (12mm, 14mm, 20mm and 22mm), at a concentration of 50, 100,150,100,150,100 and 400mg/ml respectively. Other extracts gave considerably inhibition on the *Candida albicans*. Aqueous extract of these plants did not give any inhibition at all concentrations.

Table 1: Zone of Inhibition (mm) of Ethanol Extracts of the four plants on *Candida albicans.*

Type of plant	Extract concentration mg/ml				
	50	100	150	200	400
Clove (*Syzygium aromaticum*)	12	14	20	20	22
Alligator pepper *(Aframomum melegueta)*	11	13	15	19	20
Ashanti pepper *(Piper guineese)*	12	13	14	17	20

Culture media when used as positive control

Positive control	inhibition zone (mm)
Clotrimazole 30mg/ml	22mm

Table 3: shows the minimum inhibitory concentration of ethanol extracts of the four plants on *Candida albicans*. The result showed that for clove(*Syzygium aromaticum*) the least concentration that inhibited the growth of *Candida albicans* was 6.25mg/ml while Alligator

pepper (*Aframomummetegueta*), Ashanti pepper (*Piper guineenses*) and Ginger (*Zingiberofficinale*), the least concentration that inhibited the growth of *Candida albicans* was 12.5mg/ml respectively.

Table 3: Minimum inhibitory concentration of extracts on *Candida albicans.*

Types of plant	extract concentration (mg1ml)
	3.125 6.2512.5 25 50 100 150 200400
Clove (*Syzygiumaromaticum*)	04 8 10 11 13 17 18 20
Alligator pepper(*Aframomummetegueta*)	0 0 5 9 10 11 13 18 15
Ashanti peper(*Piper guineense*)	00 4 8 9 10 12 14 20
Ginger (*Zingiberofficinale*)	0 0 3 6 8 11 12 15 17

Table 4: Phytochemical result of the plant extracts.

Phytochemical	Alligator pepper (*Aframomum metegueta)*	clove *(Syzygium aromaticum)*	Ashanti pepper(piper *guineense)*	Ginger *(Zingiber officinale*)
Alkaloids	+	+	+	+
Flavonnoids	+	+	+	+
Saponins	-	+	-	-
Terpenoids	+	+	-	+

DISCUSSION

Candida species has become important opportunistic fungal pathogens that cause oral infections in immunocompetent and immunocompromised individuals. An increase in the side effects of synthetic drugs haveimproved the use of plant extract to be a better alternative to these hazardous chemicals. The presents study showed that the ethanolic extract of the plants tested had antifungal activity against the growth of *Candidaalbcians* while the aqueous extracts had no effects on the growth of *Candida albicans.* This is in agreement with the work of Anyanwu and Nwosu (2013) who reported that aqueous extracts of P*ipperguineense* leaves had no effect on C*andida albicans* in all concentrations except in 20mg/ml. This observed different between ethanol extracts and aqueous extracts may be due to insolubility of active compounds in water or the presence of inhibitors to the antifungal components (Okigbo and Ogbonnya, 2006). Amadioha and Obi (1999), Okigbo and Ajale (2005a) and Okigbo*et al*. (2005b) reported that inactivity of plant extracts may be due to age of plant, extracting solvents, method of extraction and time of harvesting of plant materials. The result

of this study showed that the ethanolic extracts of *Syzygiuma romaticum*. *Aframomum melegueta*, Piper guineense and *Zingiber officinale* inhibited the growth of *Candida albicans.* This suggests that the plant extracts are broad spectrum in activity. Higher antifungal activity of these extracts were observed on *Candida albicans* at high concentration. This is similar to the earlier result obtained by Oyagade*et al*. (1999), Akpulu(1994) and Oladunmoye (2007). Also Agarwal *et al*. (2010) reported that clove oil and ginger had effects on *Candida albicans*with inhibition zones of 13.8 mm and 16.0 mm respectively. They also reported that clove oil not only act as potent antifungal agents against *Candida albicans* but also perform better than fluconazole. The antifungal effects of these plant extracts are due to the phytochemical constituents present in them. *Syzygiumaromaticum*, *Aframomummelegueta*, *Piper guineense* and *Zingiberofficinale* are rich in phytonutrients such as flavonoids, phenolic compound, tannins, saponin, terpernoids and alkaloids. The biological function of flavonoids include protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumors (Okwu, 2004). This may be the reason for the use of the extracts of some of these plants in the treatment of intestinal problems in herbal medicine (Okwu, 2004). These plants have some quantity of saponin content. Some of the general characteristics of saponin includeformation of foam in aqueous solutions, haemolytic activity, cholesterol binding properties and bitterness (Okwu, 2004). Alkaloids ranked the most efficient therapeutically significant plant substance. Pure isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects. Tannins hasten the healing of wounds and inflamed mucous membrane (Okwu and Okwu, 2004). The presence of tannins in these plants strongly supports its use in treating wounds, burns and hemorrhoids in herbal medicine. The relative susceptibility of the organism tested should be due to the oil as well as the component's capability of inducing cell lysis (Ijeh*et al*., 2004). This study showed that minimum inhibitory concentration (MIC) of the ethanolic extracts ranged between 6.25 and 12.5 mg/ml. this is in agreement with Anyanwu and Nwosu (2013) who reported the MIC of ethanolic extract of *Piper guineense*to be between 5.0 and 10.0 mg/ml. Clotrimazole is an antifungal drug and it was used as a positive control in this study. The result showed that clotrimazole had an inhibitory effect on *Candida albicans* with inhibition zone of 22 mm. This study agrees with the work of Al-Mohana, (2010) who reported thatclotrimazolehas effect on *Candida albicans* with inhibition zone of 18.8mm. This is also in agreement with Agarwal *et al*. (2010) who reported that fluconazole the same azole drug completely inhibited the growth of this

organism. Clotrimazole belongs to azole antifungal agent and hence has direct effect on the fatty acids of cell membranes thereby leads to the depletion of ergosterol in the membrane.

CONCLUSION

From this present study, it was observed that ethanol extracts exhibited high inhibitory activity on the test organism. This is because of the ability of ethanol to extract more of the essential oils and secondary plant metabolites which are believed to exert antifungal activity on *Candida albicans.* This study therefore, justifies the use of the plant extracts as an antifungal agent.

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