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ANTIFUNGAL POTENCY OF POTASH COMPOUNDS AGAINST CANDIDA SPECIES ISOLATED FROM HIGH VAGINAL SWABS OF WOMEN ATTENDING A TEACHING HOSPITAL IN NIGERIA

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ABSTRACT
Antifungal research and development are unfortunately challenging, and no considerable advancements in antifungal therapies have been achieved recently. The aim of this research was to evaluate the antifungal potency of potash compounds (potash alum, trona and palm ash) against *Candida* species isolated from high vaginal swabs of women attending a Teaching hospital in Nigeria. Four hundred and fifty high vaginal swab samples were collected from patients suspected of having vulvo-vaginal candidiasis. These were inoculated on Sabouraud dextrose agar supplemented with 50µg/ml chloramphenicol and incubated aerobically at 25^{o}C for 24hours. The yeast isolates were identified based on their morphological, physiological, biochemical and molecular characteristics. Antimicrobial activity of the natural compounds on the *Candida* isolates was evaluated using the agar-well diffusion method. The Minimum inhibitory concentration (MIC) and Minimum fungicidal concentration (MFC) of the agents were determined using the broth dilution method. The yeast isolates included: *Candida albicans* (51%), *C. tropicalis* (28%), *C. glabrata* (15%) and *C. parapsilosis* (6%). Trona gave the highest inhibition zone diameter (IZD) of 31.00±1.41mm against *Candida glabrata*, potash alum, 30.00±1.41mm against *C. parasilopsis* and palm ash, 17.50±0.71mm against *C. glabrata* at 200mg/ml. Ketoconazole (at 200mg/ml) gave the IZD of 21.00±1.41mm against *C. albicans*, 21.50±0.71mm against *C. tropicalis*, 20.50±0.71mm against *C. glabrata* and 19.00±1.41mm against *C. parapsilosis*. The MIC and MFC of the test agents varied among the *Candida* isolates. For *C. albicans*, potash alum MIC was (50mg/ml), Trona (100mg/ml), Palm ash (100mg/ml) and Ketoconazole (50mg/ml). The study revealed that potash compounds are potent anticandidal agents and could serve as good alternatives to our conventional antifungal antibiotics.

KEYWORDS: Antifungal, Potash Compounds, Candida *species*, Inhibition zone diammeter, *Candidiasis.*

INTRODUCTION
The body normally hosts a variety of germs, including bacteria and fungi. Some of these are useful to the body, some produce no harm or benefit, and some can cause harmful infections. Some fungal infections are caused by fungi that often live on the hair, nails, and outer skin layers. They include yeast-like fungi such as *Candida*. It is commonly found as commensal yeast in the mucous membranes of humans and other warm-blooded animals.[1,2]

A weakened or undeveloped immune system or metabolic illnesses are significant predisposing factors of candidiasis. Most candidal infections result in minimal complications such as redness, itching, and discomfort, though complications may be severe or even fatal if left untreated in certain populations.[3,4]

Pharmaceutical industries primarily strive to deliver new drugs to the market through the complex activities of drug discovery and development. Drug discovery is a process which is intended to identify a small synthetic molecule or a large biomolecule for comprehensive evaluation as a potential drug. New drugs are continually required by the healthcare systems to address unmet medical needs across diverse therapeutic areas.[5]

Potash is the common name for various mined and manufactured salts that contain potassium in water – soluble form. The name derives from "pot ash", which refers to plant ashes soaked in water in a pot, the primary means of manufacturing the product before the industrial era. Potash refers to potassium compounds and potassium-bearing materials. Commonly available potash compounds include trona, potash alum and palm ash.[6]

Alum with the molecular formula, KAl(SO4)2.12H2O) is a colorless, odorless crystalline solid that turns white in air. It is a transparent salt-like substance that is used in cooking as well as for medicinal purposes. They are commonly found in Nigeria, India, Egypt, Nepal, Italy, Philippines and many parts of Asian countries. There are a variety of commercially available alums; soda alum, ammonium alum, chrome alum, selenate alum and aluminium sulphate. The potency of alum as an antimicrobial agent had been visibly demonstrated over the years through the myriads of its beneficial activities and relevance in a broad spectrum of human research and development.[7,8,9]

Trona (akanwu) is a type of lake salt that is dry and hydrated in nature. Akanwu salt is the second most popularly used salt in Nigeria. The medicinal use of trona for all sorts of ailments has been reported by few scholars. It is used in some concoction for curing cough and ameliorating toothache, stomach pains, and constipation.[10,11] Palm ash is a white, grey or black left over after burning of palm tree parts (such as *Elaeis guineensis*). Palm bunch ash (PBA), traditionally known as Ngu in south eastern Nigeria, is used in place of trona (Akanwu) as food additive and tenderizer.[11]

The increased risk of fungal diseases particularly in immunocompromised patients, limited repertoire of antifungal drugs, toxicity and the development of resistance to the available antifungal drugs, have increased the demand for the development of new and effective antifungal agents. These have resulted to intensified efforts on antifungal drug discovery to develop more readily available, clinically effective and safer antifungal agents.

The aim of this research was to evaluate the antifungal potency of potash compounds against *Candida* species isolated from high vaginal swabs of women attending a Teaching hospital in Nigeria.

MATERIALS AND METHODS

Study Area, Samples Collection and Processing

This study was carried out at the Laboratory Unit of Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

With the permission of the ethical committee of Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka and the assistance of the Medical Laboratory Scientist of the Hospital's Laboratory unit, four hundred and fifty swab samples were aseptically and properly collected from patients suspected of suffering from candidiasis (vulvo-vaginal candidiasis). Plates of Sabouraud dextrose agar media supplemented with chloramphenicol (50µg/ml) were inoculated and incubated aerobically at 25^{o}C for 24 hours.[12]

The potash compounds used for this study were potash alum (Tawas), Trona (akanwu or kanwa) and palm ash (ngu). These potash compounds were hygienically selected after purchase from the Eke-Awka market in Awka South Local Government Area of Anambra State, Nigeria. The samples were transferred into sterile containers, and transported to the laboratory for processing and analysis as described by Kamka-Evans.[13]

Obvious impurities were gently and carefully removed manually, from the samples after which known weights of the compounds were soaked in water at room temperature and placed in the shaker at 60rpm at 40^{o}C. The samples dissolved completely within two hours.

Identification of Yeast Isolates

The yeast isolates were identified based on their morphological, physiological, biochemical and molecular characteristics which included sugar fermentation test,[14] growth on cornmeal agar as described by,[14] germ tube test, [15] growth on Chromogenic *Candida* agar[16] and nucleic acid sequence analysis.[17, 18]

In vitro Evaluation of Antifungal Activity of the Solutions

This assay was done using the agar-well diffusion method as described by,[16,19] Stock solutions (200mg/ml) of the potash compounds were prepared by weighing out 2g each of the stones and dissolving in 10ml of sterile water in test tubes. A double fold serial dilution of the stock solution was performed to obtain 100mg/ml, 50mg/ml and 25mg/ml concentrations. Plates of Mueller Hinton Agar supplemented with 2% glucose were aseptically prepared. Using 9mm cork-borer, 9mm diameter wells were bored through the already gelled MHA medium. The agar plates were seeded with 2 x 10^{5}cfu/ml (equivalent to 0.5 McFarland Standard) of the *Candida* isolates. This was done by adding 0.1ml of the above McFarland value onto the surface of the plates, and gently spreading the inoculums onto the plate surface with sterile bent glass rod. Then, 0.5ml of the test solutions were added into the wells using sterile 1ml glass pipette. Positive and negative controls were prepared by adding 0.5ml of ketoconazole and distilled water respectively into some wells. The experimental set up was incubated at 25^{o}C for 24 hours. The experiments were performed in triplicate and the results reported as average of 3 experiments. Antifungal activity was determined by measuring the inhibition zone diameter (mm) produced after 24hrs of incubation. The inhibition zone diameter was reported as Mean±Standard deviation.

Determination of MIC and MFC using broth dilution method

From the stock concentration of 200mg/ml of the test agents, various concentrations of the potash compounds were made in Sabouraud dextrose broth by double fold serial dilution to obtain, 100mg/ml, 50 mg/ml, 25 mg/ml,

12.25mg/ml, 6.325 mg/ml, 3.125mg/ml and 1.5625 mg/ml. Each dilution in a test-tube was inoculated with 0.2 ml of the broth culture of test isolates diluted to 0.5 McFarland standards. All the tubes were incubated at 25°C for 24 hrs. The lowest concentration showing no visible growth was recorded as the minimum inhibitory concentration (MIC) for each organism. From each negative tube in MIC assay, 1 ml was transferred onto the surface of freshly prepared Sabouraud Dextrose Agar plates (without the test agents) using spread plate method and incubated at 25°C for 48 hrs. The lowest concentration showing no visible growth on SDA was recorded as minimum fungicidal concentration (MFC) for each organism.[20,21]

RESULTS AND DISCUSSION

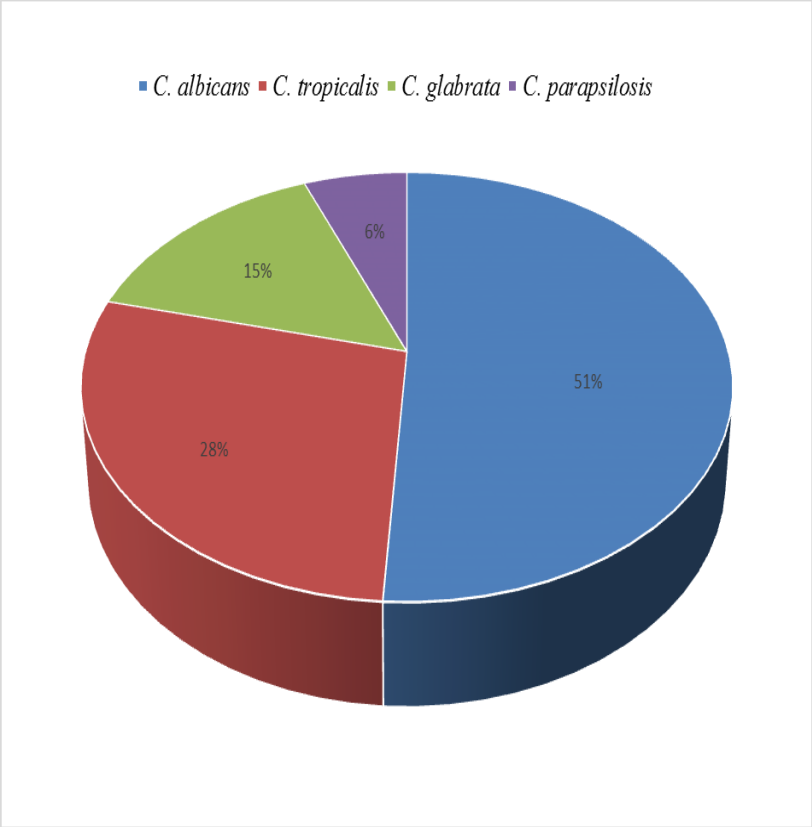

Figure 1: Frequency of the isolates from the HVS Samples.

Table 1: Inhibition zone diameter of potash compounds on *Candida albicans* using the agar- well diffusion Method.

Concentration (mg/ml)	Potash alum (mm)	Trona (mm)	Palm ash (mm)	Ketoconazole (mm)
12.5	-	-	-	-
25	13.50±0.71	12.00±1.41	-	-
50	19.50±0.71	18.00±1.41	10.00±0.00	14.50±0.71
100	27.50±0.71	24.50±0.71	12.50±0.71	19.00±1.41
200	29.00±1.41	29.00±1.41	16.50±0.71	21.00±1.41

Positive control for disc & well respectively = Ketoconazole. Negative control (distilled water) = 00mm.

Table 2: Inhibition zone diameter of potash compounds on *Candida tropicalis* using the agar-well diffusion Method.

Concentration (mg/ml)	Potash alum (mm)	Trona (mm)	Palm ash (mm)	Ketoconazole (mm)
12.5	-	-	-	-
25	12.00±1.41	-	-	-
50	16.00±1.41	12.00±1.41	-	14.00±1.41
100	20.00±0.00	17.50±0.71	11.50±0.71	16.50±2.12
200	26.00±0.00	29.00±1.41	16.00±1.41	21.50±0.71

Positive control for disc & well respectively = Ketoconazole. Negative control (distilled water) = 00mm.

Table 3: Inhibition zone diameter of potash compounds on *Candida glabrata* using the agar-well diffusion Method.

Concentration(mg/ml)	Potash alum (mm)	Trona (mm)	Palm ash (mm)	Ketoconazole (mm)
12.5	-	-	-	-
25	12.50±0.71	12.00±1.41	-	-
50	17.50±0.71	17.50±0.71	10.50±0.71	11.50±0.71
100	19.50±0.71	22.00±1.41	14.00±1.41	14.00±0.00
200	26.50±0.71	31.00±1.41	17.50±0.71	20.50±0.71

Positive control for disc & well respectively = Ketoconazole. Negative control (distilled water) = 00mm.

Table 4: Inhibition zone diameter of potash compounds on *Candida parapsilosis* using the agar-well diffusion Method.

Concentration (mg/ml)	Potash alum (mm)	Trona (mm)	Palm ash (mm)	Ketoconazole (mm)
12.5	-	-	-	-
25	11.00±0.00	11.50±0.71	-	-
50	16.00±2.83	16.50±0.71	10.50±0.71	12.00±1.41
100	22.00±1.41	23.00±1.41	14.00±0.00	14.50±0.71
200	30.00±1.41	30.00±1.41	16.50±0.71	19.00±1.41

Positive control for disc & well respectively = Ketoconazole. Negative control (distilled water) = 00mm.

Table 5: MIC Determination of the Potash Compounds against the isolates using Broth Dilution Method (mg/ml).

Potash Compounds	*C. albicans*	*C. tropicalis*	*C. glabrata*	*C. parapsilosis*
Potash Alum	50	25	12.50	12.50
Trona	100	50	6.25	6.25
Palm Ash	100	100	100	100
Ketoconazole	50	25	50	12.50

Table 6: MFC Determination of the Potash Compounds against the isolates (mg/ml).

Potash Compounds	*C. albicans*	*C. tropicalis*	*C. glabrata*	*C. parapsilosis*
Potash Alum	50	50	12.50	25
Trona	100	100	12.50	25
Palm Ash	100	200	100	100
Ketoconazole	50	25	50	25

DISCUSSION

This research work has investigated the antifungal potency of Potash Compounds against *Candida* species isolated from High Vaginal Swabs of Women attending a Teaching Hospital in Nigeria. The potash compounds analyzed were potash alum, trona and palm ash. Four hundred and fifty high vaginal swab samples were collected and analysed. One hundred and eighty-eight HVS (42%) were positive to *Candida* growth; *C. albicans* (51%), *C. tropicalis* (28%), *C. glabrata* (15%) and *Candida parapsilosis* (6%) as shown in figure 1. Watson *et al*.[22] also reported that *Candida albicans* is the most common type of fungus to cause yeast infections. Watson's work on yeast infections further revealed that the fungus, *C. albicans* is responsible for most vaginal yeast infections. Thus, *C. albicans* is the most aetiological agent of vaginal candidiasis (51%) as shown in the result which is in concordance with the findings by Watson *et al*.[22]

The research work also revealed that potash compounds are good antimicrobials, though with varying levels of potency *in vitro* against the isolates. Among the potash compounds analysed, potash alum (otherwise known as tawas) gave the highest inhibition zone diameter and best MIC/MFC values against *Candida albicans* while trona gave the best IZD against *C. tropicalis, C. glabrata* and *C. parapsilosis* as shown in Tables 1 - 4. The least antifungal activity was observed with palm ash. Ali *et al*.[7] pointed out that the potency of alum as an antimicrobial agent had been visibly demonstrated over the years through myriads of its beneficial activities and relevance in a broad spectrum of human research and development. According to Shalli *et al.*[23] alum has been used as a remedy for the treatment of vaginal discharge. Ntukidem *et al.*[24] reported that trona exhibited antimicrobial activity against pathogenic bacteria and fungi organisms including *Pseudomonas aeruginosa, Proteus spp, Candida albicans and C. pseudotropicalis.*

Brahmachari,[8] stated that notable advantages in the use of alum include cost effectiveness, availability, non-toxicity, reusability and ecofriendliness. Thus the antifungal effectiveness of the potash compounds against *Candida* isolates from High vaginal swab samples has been revealed through their IZD, MIC and MFC values as shown in Tables 1 - 6.

CONCLUSION

There is serious global concern about rising incidence of emerging fungal infections, toxicity and resistance to the available antifungal antibiotics, especially among immunocompromised individuals. The continuous search for more effective, less toxic and cheaper raw materials to feed the pharmaceutical industries is inevitable. The raw materials include natural compounds such as potash compounds. Thus, this study has shown that potash compounds such as potash alum, trona and palm ash could serve as good alternatives to the conventional antifungal antibiotics.

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