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Research Article

In Vitro Antifungal Efficacy of *Allium Cepa* And *Allium Sativum*: A Comparative Study with Commercial Drugs

Anazodo C. A.[1*], Abana C. C. [1], Agu K. C. [1], Victor-Aduloju A. T.[1], Okoli F. A.[1], Ifediegwu M. C.[1], Awari V. G.[2], Chidozie C. P.[3]

[1]Applied Microbiology and Brewing Department, Nnamdi Azikiwe University, PMB 5025, Awka, Nigeria

[2]Microbiology Department, Tansian University, Umunya, Nigeria

[3]Paediatrics Department, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria

Abstract:

The in vitro activities of water, methanol, acetone, ethanol, and chloroform extracts of garlic *(Allium sativum)* and Onions (Allium cepa) were investigated. For comparison, activities of commercially sold antifungals viz Nystatin, Fulcin, and *Cotrimoxazole* against *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Penicillium spp, Trichophyton schoeleinii, and Neurospora clasa* were also investigated. The largest zone of inhibition was exerted by the ethanol and water extracts of garlic (Allium sativum) against *Aspergillus niger, A. fumigatus, T. Schoeleilnii, and penicillium spp. The antifugal* activity of onions was less marked while its water extracts showed no activity. The lack of inhibition on *Neurospora clasa* was conspicuous for both the garlic *(Allium sativum)* and Onions (Allium cepa) extracts. The commercial drugs were significantly more active against the tested fungi (MIC range 1.56-50ug/ml). Fulcin showed more activity than the rest of the commercial drugs tested. The activities of fulcrum for all the fungal species far surpassed those of nystatin and were even superior to those of *Cotrimoxazole on Aspergillus niger and Aspergillus fumigatus. Nystatin* showed a higher MIC range of 12.5-50 µg/ml for the tested fungal species. The MFC of the drugs was mostly 2-3 times their MIC values. These results suggest that overall, the fungal species tested showed more sensitivity to the commercially sold antifungals than that of the extracts.

Keywords: *In-vitro*, Antifungal Efficacy, *Allium cepa*, *Allium sativum*, Drugs

Introduction

The use of medicinal herbs in the treatment of infections is an age-old practice. In Nigeria, several species are used by many ethnic groups for the treatment of various ailments ranging from minor infections to dysentery, skin diseases, asthma, malaria, typhoid fever, and a horde of others [31-38]. Bulbs of Garlic *(Allium sativum)* Alliaceae have been used as a species, food, and folk medicine since ancient times. It has been shown to possess insecticidal, antimicrobial, antiparasitic, and antitumor properties (26). In addition, garlic extract has been reported to show an in vitro growth inhibition effect against a large number of yeasts including candida spp and fungi such as *Cocidioides immitis* (3,25) also have a protective effect against in vivo fungal infections. The active component of garlic is thought to be a di-allyl thiosulfate (allicin), a sulfur-containing compound.

Antiprotozoal, antibacterial, and antifungal effects have been substantiated in vitro and found to be due specifically to allicin, methylallylthiosulfinate, and allylmethylthiosulfinate (24). Thiosulfinate compounds are released from garlic cloves after tissue disintegration caused by chewing, cutting, or pressing (28).

The in vitro and in vivo antifungal activity of garlic has been detected in serum after injection of garlic *(Allium sativum*) and in cerebrospinal fluid (CSF) after intravenous administration of a commercial garlic extract (3). Recently, commercial A sativum extracts have been administered intravenously with amphotericin B to treat cryptococcal meningitis. *A sativum* (garlic) compound has been demonstrated to possess potent anti-cryptococcal activity and act synergistically with amphotericin B under in vitro conditions to inhibit growth and kill *Cryptococcus neoformans.* Other plants, that as Zanthoxylum leprieurii and Zanthoxylum xanthoxyloides commonly used in Cameroon for the traditional treatment of certain infectious diseases: skin infections, gonococcies, urinary infections, dysentery (17).

Human infections, particularly those involving the skin and mucosal surfaces, constitute a serious problem, especially in tropical and subtropical developing countries, dermatophytes and *Candida spp*. being the most frequent pathogens. Although several antimycotic drugs are available at present, their use is limited by several factors, Such as low potency, poor solubility, emergence of resistant strains, and drug toxicity. Therefore, there is a distinct need for the discovery of new, safer, and more effective antifungal agents.

Plants used in traditional medicine usually constitute an important source of new biologically active compounds. Thus, to search for

new antifungal agents, an ethnobotanical survey was carried out in Paraguay. Extracts of Paraguay plants assayed were active against *Aspergillus niger* and only the dichloromethane extract of *Tabebuia avellanedae* showed active against *A. fumigatus* and *Candida albicans*. Garlic *(Allium sativum)* could be used as an effective antidermatophytic agent.

Further purification and extraction of the active principle of garlic would give a true antidermatophytic activity comparable to standard antifungal drugs (26). Onion and garlic contain many sulfur-containing active principles mainly in the form of cysteine derivatives, viz S-alkyl cysteine sulfoxides which decompose into a variety of thosulfinates and polysulfides by the action of an enzyme allinase on extraction. Decomposed products are volatile and present in the oils of Onion and garlic. They possess antidiabetic, antibiotic, hypocholesterolemic, fibrinolytic, and various other biological actions. In addition to free sulfoxides in alliums, some non-volatile sulfur-containing peptides and proteins possess various activities and thus make these vegetables an important source of therapeutic agents. As allyl and related sulfoxides are inhibiting thiol group enzymes, alliums are to be used only in limited quantities. Reports have shown that fresh garlic extract has a greater efficacy than garlic powder extract as indicated both by its effects on morphology and inhibition of growth (14). The water-soluble extracts of garlic bulbs, green garlic, and green onions showed an inhibitory effect against the growth of *A. niger, A flavus* (29).

With the increase in the incidence of systemic mycosis in recent years, there has been an increasing emphasis on the importance of antifungal chemotherapy. Since the introduction of fluconazole for clinical use, aspergillosis has replaced candidosis as the most important issue in the control of mycosis. Thus the development of broad-spectrum antifungal agents with a potent anti-Aspergillus activity and good safety is needed. Posaconazole is a new triazole antifungal agent synthesized by the Schering-Plough Research Institute.

The in vitro antifungal activities of posaconazole against clinical isolates of *Candida albicans*, other yeast-like fungi, and *Aspergillus fumigatus,* as well as against stock strains of a range of pathogenic fungi (13). In vitro and in vivo susceptibility studies activity against a wide spectrum of pathogenic fungi such as *Candida spp, Cryptococcus neoformans, Blastomyces dermatitides, Pneumocystis* carinil, *Aspergillus spp,* and several emerging fungal pathogens (6). The presence of toxigenic fungi and mycotoxins in foods and grains stored for a long time presents a potential hazard to human and animal health. Many investigators have used essential oils such as cinnamon, peppermint, basil, and thyme to protect maize kernels against *A flavus* infection, without affecting germination and corn growth (16). Another related plants, that is ginger (*Zingiber officinale*), and African oil bean (Pentaclethra macrophylla) belonging to the family Mimosaceae have also been reported to possess antibacterial activity (9). Also, extracts of Landolphia owerrience (Igbo: utu) family *Apocynaceae* have antibacterial activity (19).

Many new antifungal agents have emerged in clinical use during the last several years. These antifungal agents are mostly N-substituted imidazole or triazole derivatives. The Azole drugs possess a broad spectrum high intensity of activity and tolerable side effects in men. The inhibitory effects of azole compounds on *dermatophytes* and non-dermatophytes have been demonstrated in the results of in vitro activity of seven azole compounds against some clinical isolates of non-dermatophytic filamentous fungi and some dermatophytes (20). In vitro antifungal susceptibility tests may be useful but reliable methods need to be developed to detect the activity of antifungal agents that may predict clinical outcomes. Interpretative breakpoints have been proposed for some antifungals (fluconazole, Itraconazole, and flucytosine) based on a comparison of clinical outcomes with the in vitro results (5).

In recognition of information in the literature on the medicinal value of garlic *(Allium sativum)* and onions, the present investigation was carried out to ascertain if extracts of these plants have any in vitro antimicrobial properties together in comparison with commercially made antifungals.

Materials and Methods

The materials used for microbial work and media preparation are stated in the appendix.

Sample collection

Bulbs of *Allium sativum* (garlic) were purchased from the Eke-Awka market along with bulbs of *Allium cepa* (onions). The commercially sold antifungal (Nystatin batch no NT - 07). Fulcin lot N. LB03890 and cotrimoxazole (lot no- 20160001) were obtained from Chisom Pharmaceuticals Awka, Nigeria. These drugs were used to compare the in vitro antifungal activities of onions and garlic.

Characterization and Identification of Test Fungi

The pure cultures of the test fungi viz. *Penicillium spp, Neurospora clasa, Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, and Trichophyton schoeleinii* were obtained from Conig_Simonne Laboratories, Awka. Preliminary fungal characterization was done by studying the cultural characteristics and employing the slide culture wet mount technique as Described by Agu and Chidozie [39], for evaluating the fungal microscopic features concerning the Manual of Fungal Atlases according to [40-41]. The cultures were maintained on Sabouraud dextrose agar slants and preserved at 4-5℃.

Extraction procedure

The extraction was done according to the method of (9). Two (2) grams of each garlic (*Allium sativum)* and onions were crushed

using a pestle and mortar. Each sample was then macerated in 50mls of cold 95% ethanol in a bottle, as well as in 50mls of acetone, water, and chloroform. The homogenate was allowed to stand for 48 hours and then filtered using (Whatman No. 1) filter paper. The filtrate was then used for the experiment.

Preparation of commercial antifungal drugs

Varying solubilities of the three drugs necessitated the use of two different solvent systems, 50mg of Nystatin and fulcin each was dissolved in 5mls of absolute ethanol while 50mg of cotrimoxazole was dissolved in 5mls of sterile water to give a concentration of 10000µg/ml. This original concentration was diluted twofold to give a concentration of 5000µg/ml. Then Iml of this concentration (5000µg/ml) was added to 24mls of SDA broth giving the concentration of 200µg/ml. Then the 200µg/ml was serially diluted further byo-fold leading to the final drug concentration which ranged from 1.56 to 200µg/ml for each of the cotrimoxazole, Nystatin, and Fulcin.

Preparation of Inocula

The fungal species listed above were maintained on Sabouraud dextrose agar slants for 2-3 days, and easily produced hypha. A hyphal suspension was prepared by putting 2mls of sterile water in the agar slants maintained in six sterile bijou bottles for the six fungal strains tested.
Then, a wire loop was used to scrap out the organisms from the slants and the bottles shook gently to mix the organisms with the sterile water. These mixtures were poured out into six different sterile bijou bottles and 1ml of sterile water was added to each of the bijou bottles making the volume up to 3mls. After this, sterile glass beads were added to each of these bijou bottles containing the hyphal suspensions and shaken for 2 minutes to break the hypha.

Screening for antifungal activity of the extracted solvent.

The in vitro antimycotic activity of absolute ethanol, water, methanol, and chloroform extracts of each sample was evaluated by agar well diffusion assays (9). Loopfuls of the hyphal suspension of each of the six organisms were inoculated in the center of the Sabouraud dextrose agar medium in Petri dishes, after this, a sterile bent glass rod (spreader) was used for spreading the cell suspensions of the test organisms on the agar surface. Then wells of 9mm were cut in the agar plate using the sterile cork borer. The holes were then filled with 4 drops (about 0.2mls of each of the extract and carrier solvent controls were loaded in the wells (holes). The plates were incubated at 25°c for 48- 72 hours. The zone sizes were measured. The diameter of the inhibition zone around each well was recorded. A zone of 20mm (including the diameter of the cork) was taken as an indicator of active inhibition.

Agar dilution test procedure for commercial antifungal.

Each drug concentration in different test tubes was thoroughly mixed with 5mls of Sabouraud dextrose agar medium in test tubes, then, poured out in Petri dishes and allowed to solidify after which the test strains were inoculated from the prepared hyphal suspensions. The drug-free medium and medium supplemented with absolute ethanol were used as controls.

Minimum inhibitory concentration (MICs) and Minimum Fungicidal Concentration (MFC) on determination of the drugs

The drug-containing plates as well as drug-free plates were inoculated with 0.05ml of the inoculum and incubated at 25^0C until visible colonies appeared in the control plates in 48 - 96hrs. The MIC was the lowest of each drug concentration completely inhibiting growth. The MFC was determined as follows, the surface of the medium in all the plates showing no growth was touched with a sterile wire loop and streaked on drug-free SDA plates and incubated at 25°C until growth appeared in the control plate for 96hrs. The minimum fungicidal concentration (MFC) was interpreted as the lowest drug concentration with no macroscopically visible growth.

Results

The results of testing the antifungal activity of garlic (*Allium sativum)* and onions *(Allium cepa)* extracts by the agar well diffusion method are set out in Tables 2 and 3 respectively

TABLE 2: ANTIFUNGAL SPECTRUM OF GARLIC EXTRACT AGAR DIFFUSION ASSAY AGAINST SOME FUNGAL SPECIES

Test Organism	Inhibition zone diameter (mm) garlic extracts in various insolventS			
	Ethanol	**Acetone**	**Water**	**Chloroform**
Aspergillus niger	26	21	27	24
Aspergillus Fumigatus	27	25	21	23
Aspergillus Flavus	23	21	26	18
Trichophyton Schoeleinii	22	-	18	16

Penicillium spp	21	16	15	-
Neurospora clasa	-	-	-	-

No inhibition of growth

TABLE 3: ANTIFUNGAL SPECTRUM OF ONION EXTRACT AGAR DIFFUSION ASSAY AGAINST SOME FUNGAL SPECIES

Test Organism	**Inhibition zone diameter (mm) Onion extracts in various insolvent**			
	Ethanol	**Methanol**	**Water**	**Chloroform**
AsperGilles niger	20	18	-	-
Aspergillus Fumigatus	19	18	-	16
Aspergillus Flavus	24	22	-	20
Trichophyton Schoeleinii	25	15	-	21
Penicillium spp	13	-	-	18
Neurospora clasa	-	-	-	-

-‘ No inhibition of growth

All the organisms tested displayed varying sensitivities to the various drops of the extracts as evident from Table 2, the garlic extracts showed a significant antifungal activity for Aspergillus niger, *Aspergillus fumigatus*, and *Aspergillus flavus*, as seen by wide zones of inhibition. The activity was only slight for *Trichophyton schoelenii*, *Penicillium spp* except *Neurospora clasa* which showed no sensitivity to the extracts. The methanol, ethanol, and chloroform extracts of onion produced larger inhibition zones than its water extracts which exhibited no activity (Table 3): The ethanolic extracts of onion showed slight inhibitory activity for *Penicillium spp* and no activity for *Neurospora clasa.* The lack of activity on *Neurospora clasa* was conspicuous for both the garlic and onion extracts.

Commercial drug sensitivity tests *in vitro*

As noted before, these studies include experiments with three commercially sold antifungal agents. The results of the sensitivity of these antifungals against the test organisms are shown in Tables 4 and 5.

TABLE 4: MINIMUM INHIBITORY CONCENTRATION (MIC) OF 3 ANTIFUNGAL AGENTS

Fungi	**MICs (µglml) of various drugs**		
	Nystatin	**Fulcin**	**Cotrimoxazole**
Neuospora Clasa	12.5	1.56	-
Penicillium Spp	25	12.5	6.25
Trichophyton Schoeleinii	25	6.25	6.25
Aspergillus niger	50	3.13	-
Aspergilluis flavus	50	3.13	12.5
Aspergillus fumigatus	50	6.25	-

-; no inhibition

TABLE 5: MINIMUM FUNGICIDAL CONCENTRATION (MFCs) OF 3 ANTIFUNGAL AGENTS

Fungi	MFCs (µglml) of various drugs		
	Nystatin	Fulcin	Corimoxazole
Neuospora Clasa	50	12.5	-
Penicillium Spp	100	25	50
Trichophyton Schoeleinii	50	50	25
Aspergillus niger	100	25	-
Aspergilluis flavus	200	50	50
Aspergillus fumigatus	100	25	-

A comparison of the MIC data showed that Nystatin had the highest MICS and Fulcin, the lowest. Fulcin was the most active of all the antifungal agents tested with the MIC value ranging from 1.56 to 12.5µglml. Generally, *Trichophyton Penicillium spp* and *Aspergillus flavus* were more sensitive to the drugs than other fungal species tested.

Of the fungi tested, Neurospora class, *Aspergillus niger, and Aspergillus fumigatus* are known to have markedly no susceptibility to the cotrimoxazole, because, no inhibition of these species was observed up to the highest concentration of 200µg/ml. The MIC range of 1.56 to 12.5µglml was obtained for *Neurospora clasa* for both fulcin and Nystatin. Complete growth inhibition was achieved for three species, *Neusospora clasa, A niger, and A fumigatus* for both Nystatin and Fulcin at a concentration range of 3.13 to 50uglml Cotrimoxazole showed potent inhibitory activity against *Penicillium spp, Trichophyton schoeleinii* and *Aspergillus flavus* (MIC range, 6.25 to 12. 5µgiml). *Neurospora clasa* showed the highest susceptibility to Nystatin with a low MIC of 12. 5µglm. a similar level of antifungal activity against *Aspergillus flavus* was seen for cotrimoxazole. The MICS of the drugs were measured only on SDA medium. Of all the fungi tested, in Fulcin, *Neurospora clasa* was more sensitive to the drug at a MIC value of 1.56µglml than the rest of the organisms which are sensitive at MIC not less than 3.13µglml. The highest MIC values of 50uglml were recorded for nystatin against all the Aspergillus species tested. The minimum fungicidal concentration of these drugs was mostly 2-3 times their MIC values. The fungal species tested showed more sensitivity to the commercially sold antifungal than that of the extracts, in the fact that the commercially sold antifungal has fungicidal effects on the fungal species while the extracts have only the inhibitory effect.

Discussion and Conclusion

This study shows that ethanol and chloroform extracts of both garlic and onions possess antifungal activity against the tested pathogens (Tables 2 and 3). The *Aspergillus species (A. flavus, A. fumigatus, A. niger)* appeared to be quite susceptible to the antifungal effects of the extracts as shown by the large zones of inhibition (18-27mm). This observation is of particular interest since the local people in Nigeria use garlic against otomycosis, the type of ear infection caused by these microorganisms. The ethanolic, chloroform extracts of both garlic and onions also proved active against *Trichophyton schoeleini,* a fungus associated with the pathogenesis of *tinea capitis, tinea corporis,* and onychomycosis in man (26). The extracts were also active against *Penicillium spp*. This observation suggests that the antifungal principles in *Allium sativum* and *Allium cepa* have a broad spectrum of antifungal activity. The wide spectrum of activity of the ethanolic extracts of both garlic and onions over the water extracts is significant because the traditional administration of bulb-based herbal medicine is in the form of the former (in kai-kai, a locally distilled gin). Old herbal knowledge and methods are being replaced by modern ideas and techniques.

Synthetic drugs are made to replace natural remedies, denatured processed foods, and replacing natural foods. The reason is that man now goes for a fat life full of cases, demanding no effort and time (15). The result of the in vitro commercial antifungal drug presented here demonstrates that these compounds are inhibitory to fungal species tested. However, the *Aspergillus* species were inhibited by Nystatin at a higher MIC value (50uglml) than the fulcin (3.13-6.25µg/ml). Fulcin showed the highest activity against all the fungi tested while cotrimoxazole showed lower in vitro activity. The MIC values of these commercial drugs obtained in this study are similar to those obtained by other investigators against different species of fungi (5, 12). A good in vitro activity of fulcin against the fungi tested is suggestive of its excellent therapeutic value. The Welsh onion extracts have been shown to inhibit the atlatoxin-producing fungi (7) while the extracts of *Allium sativum* (garlic) have been shown to have an in vitro antifungal effect against the *Aspergillus spp* involved in otomycosis (21). The report of (3) showed the in vitro synergism of concentrated *Allium sativum* extract and amphotericin B against *Cyptococcus neoformans.* This study leads laboratory support for the treatment of cryptococcal infections with concentrated garlic extracts. People all over the world have used herbs to Cure and control different diseases that are peculiar to their sub-regions and Nigeria is no exception. The therapeutic values of Onion *(Allium cepa*) and garlic (*Allum sativum*) have been shown, that they possess antidiabetic, antibiotic, hypocholesterolemic, fibrinolytic, and various other biological actions (2) while the garlic (*Allium sativum)* is an anti-candida agent(14). The strong activity of the ethanolic extracts of *Landolphia Owerrience* has been shown against known etiologic agents of diseases traditionally treated with this plant root, of similar preparations which provides scientific justification for the use of the herb in ethnomedical practice in Nigeria (19).

A review of the data presented in this study reveals important information on the increase in the in vitro antifungal sensitivity of commercial antifungals against the extracts. The extracts are only fungistatic in action while the commercially sold antifungals are both fungistatic and fungicidal.

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