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Antidermatophytic activity of garlic (Allium Sativum) extract on dermatophytes isolated from children of Katsina-Ala Local Government Area, Benue State

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Abstract

Forty related species of fungi belonging to the dermatophytic genus, such as *Trichophyton, Microsporum,* and *Epidermophyton,* have been discovered by studies as possible etiological agents of dermatophytosis. The Antidermatophytic activity of methanolic extracts of garlic (*Allium sativum*) was investigated against isolates of dermatophytes obtained from fifty primary school children in Katsina-Ala Local Government Area of Benue State. Garlic extracts were obtained using the cold maceration method, and the isolates were obtained using standard microbiological methods. The isolated organisms were *Trichophyton, Microsporum,* and *Epidermophyton* species. The zones of inhibition obtained were recorded in mean ± standard deviation of duplicate values. *Trychophytum* sp exhibited a mean value of 15.50±0.71, *Epidermophytum* sp had 9.00±0.00, while *Microsporum* sp had 10.50±0.71, all at 50mg respectively. At 100mg, the mean zones of inhibition were 20.50±2.12 on *Trychophytum* sp, 15.50±0.71 on *Epidermophytum* sp, and 14.50±2.12 on *Microsporum* sp. Finally, the mean zones of inhibition at 200mg were 31.00±1.41 on *Trychophytum* sp, 18.00±0.00 on *Epidermophytum* sp, and 17.50±0.71 on *Microsporum* sp. The MIC was 50mg each. The findings of this study showed that the extracts of garlic had a marked significance in inhibiting the test organisms. As the findings of this study compared favourably with previous studies on antifungal activity of garlic, the plant might be a promising source of drugs for the treatment of dermatophytic infections.

Keywords: Antidermatophytic Activity, Benue State, Dermatophytes, Garlic, Katsina-Ala LGA

1. Introduction

Studies have identified a group of 40 closely related species of fungus in the dermatophytic genus, including Trichophyton, Microsporum, and Epidermophyton, as potential etiological agents of

dermatophytosis. It is generally recognized that dermatophytosis is a common ailment that affects children's health and well-being all around the world (Bhatia and Sharma, 2014). Humidity, excessive clouding, and poor hygiene create the ideal conditions for dermatophytes to grow and spread (Sharma et al., 2015). Dermatophytes are the most prevalent form of fungus that infects skin, hair, and nails (Seebacher, 2010). These illnesses can produce a variety of clinical signs, such as majocchi's granuloma, tinea capitis, tinea pedis, tinea corporis, and tinea cruris. Traditional antifungal drugs can be used to treat these dermatophytes; however, these infections may recur in the same or other places (Sudha et al., 2016).

2. Literature review

Almost all cultures and civilizations throughout human history have used herbal treatments to heal infections since plants have long been used to treat infectious disorders. This is still a common practice today, according to Rad and colleagues (2018). Much attention has been focused on the use of plant extracts to treat fungal infections because of the lack of efficacy, unfavorable side effects, and resistance associated with some of the existing treatments. The bulb known as garlic (Allium sativum) belongs to the lily family Liliaceae. It is found in every tropical country and is commonly called garlic. It's compounds with sulfur bases that are fragrant, enhancing its flavor and scent. Allium sativum has been shown to suppress a range of pathogenic bacteria, viruses, and fungi. Garlic's antibacterial qualities are attributed to allicin, a crucial component. Allicin is unstable and quickly decomposes to yield diallyl sulphide, diallyl disulphide, diallyl trisulphide, allyl ethyl trisulphide, dithiis, and ajoene according to Khorshed et al (Khorshed et al., 2016).

3. Research methodology

3.1. Sample collection

3.1.1. Plant sample collection.

The plant sample (garlic cloves) was obtained at North Bank Market, Makurdi metropolis, they was transported to the Microbiology research laboratory, Joseph Sarwuan Tarka University, Makurdi. The fresh garlic cloves were peeled and blended with a blender to obtain the paste from which the extract was made.

3.2. Aqueous Garlic Extract

Two hundred and fifty grams of crushed garlic paste was mixed with 500 ml of double standard distilled water in a glass container to obtain a homogenous mixture by stirring it occasionally for some time. The mixture was filtered and further centrifuged at 10,000 rpm for 20 min. The supernatant was filtered through a 0.2-mm pore size Wattman filter paper grade 1 to remove any impurity.

3.3. Ethanolic Garlic Extract

Two hundred and fifty grams of crushed garlic paste was mixed with 500 ml of ethyl alcohol in a glass container to obtain a homogenous mix by stirring it occasionally for some time at 35°C. The mixture was filtered and further centrifuged at 10,000 rpm for 20 min. The supernatant was filtered

through a 0.2-mm pore size Wattman filter paper grade 1 to remove any impurities. Thus, the obtained alcoholic extract was concentrated by heating to evaporate ethyl alcohol and to obtain the crude extract.

3.4. Swap Sample Collection

About 50 swap samples were randomly collected from affected areas on the skin of children from Katsina-Ala Local Government Area, Benue State. They were transported to the Research Laboratory of Joseph Sarwuan Tarkar University, Makurdi, and were analysed using standard microbiological procedures under aseptic conditions.

3.5. Preparation of Different Concentrations of Garlic Extract

Double standard dilution method by Molan (2001), was used to obtain five different concentrations of the crude aqueous garlic extract (AGE) and Ethanolic garlic extract (EGE), these will be 500 mg/mL, 250 mg/mL, 200mg/mL, 125 mg/mL, and 100mg/mL respectively. This was done by diluting appropriate milligrams of the extracts into corresponding volumes of solvents.

3.6. Media Preparation and Inoculation of samples.

All media to be used were prepared in accordance with the manufacturers' instructions.
They were sterilised at 121oC in an autoclave and at a pressure of 15 pounds for 15 minutes. They will then be poured into plates upon cooling to a temperature of about 42-45oC and the swap samples were inoculated using the pour plating method following a serial dilution.
Fungal Isolation and Identification
Identification of the fungal isolates was based on morphological characteristics, such as shape, pigmentation, and microscopic examination using lactophenol cotton blue stain.

3.7. Microscopic Examination of the Isolates

The fungal isolates were mounted in lactophenol cotton blue stain solution on slides with cover slips and microscopically examined for spores and vegetative bodies according to the method described by the authors (Barnett & Hunter, 2001). The isolates thus obtained were subjected to the aqueous and ethanolic garlic extract at different concentrations using agar well diffusion technique.

3.8. Agar Well Diffusion Procedure

3.8.1. Inoculum Preparations Using Macfaland Standard

The colonies were transferred from the plates to the broth medium with a sterilized straight nichrome wire. The turbidity was visually adjusted with the broth medium to equal that of a 0.5 MacFarland unit turbidity standard that was freshly prepared by reacting Barium chloride with sulfuric acid.

3.8.2. Inoculation of Agar Plate

After adjusting the inoculum to a 0.5 MacFarland unit turbidity standard, a sterile cotton swab was dipped into the inoculum and rotated against the wall of the tube above the liquid to remove excess inoculum. The entire surface of a potato dextrose agar plate was swabbed three times, rotating plates approximately 60° between streaking to ensure even distribution. The inoculated plates were allowed

to stand for at least 3 minutes but not longer than 15 minutes before the wells were punched in the agar plate. A hollow tube of 5 mm diameter was taken and heated. It was pressed on the inoculated agar plate and removed immediately after making a well in the plate. Likewise, five wells were made on each plate.

500 mg/ml, 250 mg/mL, 200mg/m, 125 mg/mL, and 100mg/mL of AGE and of EGE concentrations were added into the respective wells on each plate, and the plates were incubated within 15 min of compound application for 18-24 hours at 37°C. Plates with confluent or nearly confluent growth were read. The diameters of the inhibition zone were measured to the nearest whole millimeter by holding the calipers (Ali et al., 2017).

4. Data analysis

Data obtained was analyze using the Statistical Package for Social Sciences (SPSS) version 22. Descriptive statistics were used to determine the mean and the standard deviation of variables.

5. Results and discussions

Table 1: Phytochemical Analysis of Garlic Extract

Metabolites	Reactions
Phenolics	+
Flavonoids	+
Tannins	+
Saponins	+
Alkanoids	+
Terpenoids	-
Glycosides	-
Steroids	+

+= Positive

- Negative

Table 2: Macroscopic and Microscopic Characteristics of Dermatophytes

Macroscopic	Microscopic	Fungi isolates
White and creamy powdery surface, red pigment on reverse	Tear-shaped microconidia	*Trychophytum* sp
Yellowish powdery colonies	Club shaped microconidia in clusters	*Epidermophytum* sp
Powdery, buff coloured colonies	Abundant, thin walled microconidia with 4-6 septa	*Microsporum* sp

Values are ± standard deviation of duplicate value means on the same row with different superscripts differing significantly

Table 3: Zones of Inhibition after Sample Treatment

Treatment	*Trychophytum* sp	*Epidermophytum* sp	*Microsporum* sp
50mg	15.50±0.71	9.00±0.00	10.50±0.71
100mg	20.50±2.12	15.50±0.71	14.50±2.12
200mg	31.00±1.41	18.00±0.00	17.50±0.71
P value	0.004	0.00	0.03

Values are ± standard deviation of duplicate values means on the same row with different superscripts that differ significantly.

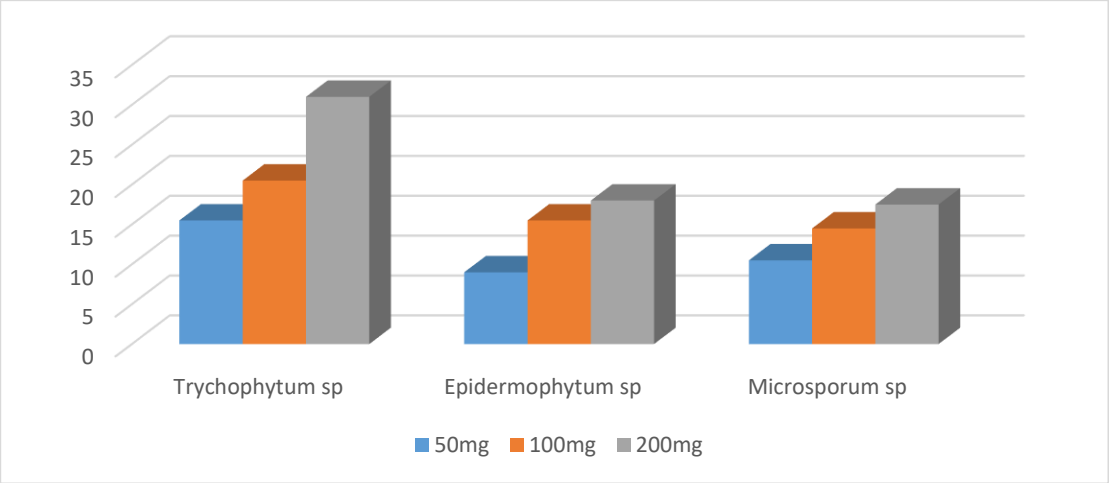

Figure 1: Zones of Inhibition after Sample Treatment

Table 4: Minimum Inhibitory Concentration of Garlic Extract against Dermatophytes

Organisms	MIC
Trichophytum sp	50mg
Epidermophytum sp	50mg
Microsporum sp	50mg

6. Discussion

A public health concern is the prevalence of dermatophytic infection, particularly in youngsters. This is a result of the pathogens developing resistance to antifungal treatments and the adverse effects of the medications used to treat fungal illnesses. Thus, there is a huge need for more effective and safer chemotherapeutic alternatives. Many regions of the world have long been using medicinal plants to treat skin conditions (Balakumar et al., 2011). Records suggest that garlic has been utilized as a traditional remedy worldwide from ancient times (Bhadauria & Kumar, 2011). There are several findings on the antiviral, antifungal, and antibacterial properties of garlic against various pathogens (Mercy et al., 2014).

In this study, the antidermatophytic activity of garlic was carried out on three dermatophytic isolates which includes *Tricophyton* sp, *Epidermophytum* sp and *Microsporum* sp. The extract which

was introduced in concentrates on the isolates showed varying inhibitory effect on the isolates. The highest effect was observed on *Trichophytum* sp (31.00±1.41 mm) at a concentration of 2000mg followed by *Epidermophytum* sp had 18.00±0.00 mm, and *Microsporum* sp 17.50±0.71 mm, respectively at 200mg. This result is in agreement with the findings of Khodavandi *et al.* (2010), who reported the activity of garlic against clinical isolates of dermatophytes which includes T. rubrum, *T. mentagrophytes, T. verrucosum, M. canis and E. flocossum*. At a concentration of 100mg, the effect observed on *Trichophytum* sp, *Epidermophytum* sp, *Microsporum* sp were 20.50±2.12 mm, 15.50±0.71 mm, and 14.50±2.12 respectively.

The effects observed at 50mg were 15.50±0.71mm for Trychophytum sp, 10.50±0.71 mm on *Microsporum* sp and least 9.00±0.00mm on *Epidermophytum* sp at a concentration. The diameter of zones of inhibition exhibited by the extracts against test fungi indicates varying degrees of susceptibility at various concentrations, this is comparable to a study by Ghahfarokhi *et al*. (2006) who demonstrated that aqueous extracts of garlic and onions on Malassezia furfur (25 strains), *C. albicans* (18 strains) and *Candida* spp (12 strains) as well as 35 strains of various dermatophytes were able to inhibit the growth of all fungi tested in a dose dependent manner.

7. Conclusion

The findings of this study showed that the extracts of garlic had a marked significance in inhibiting the test organisms. As the findings of this study compared favourably with previous studies on antifungal activity of garlic, the plant might be a promising source of drugs for the treatment of dermatophytic infections. Further work on this study may help to design a new drug against dermatophytosis.

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