

Anti-dermatophytic Activity of garlic (*Allium sativum*) extracts on some Dermatophytic fungi

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ABSTRACT

Dermatophytic infection is a common infection that constitutes public health problem among children. Anti dermatophytic activity of aqueous, ethanolic and methanolic extracts of garlic (*Allium sativum*) was investigated against isolates of dermatophytic fungi obtained from sixty primary school children in Aba. The well in agar diffusion technique was used to determine the sensitivity patterns of the test organisms. The results were compared with the activity of a known antifungal drug nystatin. The isolates included *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporium gypseum*, *Trichophyton verrucosum* and *Epidermophyton floccosum*. The result of the antifungal activity of garlic showed high but varied levels of antifungal effectiveness on the different species of the dermatophytes at four different concentrations of 12.5 %, 25 %, 50 % and 100 % used. *T. rubrum* was the most susceptible followed by *M. gypseum*, *T. mentagrophytes*, *T. verrucosum* and *E. floccosum* respectively. The diameter zones of inhibition exhibited by the extracts against test fungal species ranged between 4.50 mm and 30.67 mm. Further purification and extraction of active principle of garlic would give an antidermatophytic activity comparable to standard antifungal drugs.

Keywords: Dermatophytic; antifungal activity; extracts; *T. mentagrophytes*; *T. rubrum*; *M. gypseum*; *T. verrucosum*; *E. floccosum*

1. INTRODUCTION

Dermatophytic infections are common disorders worldwide and have been known to impact negatively on health and well being of children. A group of closely related fungi comprising of 40 identified species in the dermatophytic genera that include *Trichophyton*, *Microsporium* and *Epidermophyton* are documented in literature as potential etiological agents of dermatophytosis (Nweze, 2010; Adefemi *et al.*, 2011). Humid weather, over population and poor hygiene are the ideal conditions for the growth of dermatophytes (Vaijayantimala *et al.*, 2001). Dermatophytes represent the prevailing type of fungi that cause infection of the skin, hair and nails (Ameen, 2010; Seebacher *et al.*, 2008). These infections lead to a variety of clinical manifestations including tinea capitis, tinea pedis, tinea corporis, tinea cruris and majocchi's granuloma. Though these dermatophytes respond to treatment with conventional antifungal agents, these diseases have a tendency to reoccur in the same area or other ones

(Natarajan *et al.*, 2003). The use of plants as a source of medicine to treat infectious disease dates back to history of mankind as a result of which nearly all cultures and civilization from ancient times to the present day have used herbal medicines to cure infections (Lino and Deogracious, 2006; Sofowura, 2008). Due to lack of efficacy, side effects and resistance associated with some of the existing drugs, much of the attention has been paid to plant extracts to treat fungal infections. *Allium sativum* (Garlic) is a bulb belonging to the lily family, *Liliaceae*. It is commonly known as garlic and is found in any tropical countries. It contains aromatic sulphur based compounds, which contribute to the characteristics taste and odour. (*Allium sativum*) has been known to have inhibitory activity on various pathogenic bacteria, viruses and fungi. Antimicrobial activity of garlic is attributed to its key component allicin. Allicin is unstable, once it is generated it readily decomposes to produce diallyl sulphide, diallyl disulphide, diallyl trisulphide, allyl ethyl trisulphide, dithiis and ajoene (Jabar and Al-Mossani, 2007). The present study was done to investigate the antidermatophytic activity of aqueous, ethanolic and methanolic extracts of garlic on some dermatophytes isolated from primary school children in Aba, Nigeria.



Photo 1. *Allium sativum*.

2. MATERIALS AND METHODS

2. 1. Plant Collection and Identification

Fresh bulbs of garlic (*Allium sativa*) were bought from a local market in Aba, Abia State, Nigeria. They were identified in the department of biology/microbiology, Abia State Polytechnic, Aba, Abia State, Nigeria.

2. 2. Extraction Procedure

The bulbs of garlic were dehusked, cleaned with sterile distilled water, air dried and ground into powder. The extraction was done using method described by Doherty *et al*

(2010). The ground sample was extracted using water, ethanol and methanol as solvents. 100 g of powdered sample was extracted with 1000 ml distilled water, 70 % ethanol and methanol respectively. The sample was soaked overnight for 24 hours, the sample was filtered with muslin cloth. The extract was collected in a round bottom flask, concentrated using a rotary evaporatory and then oven dried at 40 °C.

2. 3. Reconstitution of Extract

The dried extracts were reconstituted by dissolving 1 g of extract in 1ml of water. This extract was considered as 100 % concentration of the extract. The concentrations of 75 %, 50 %, 25 % and 12.5 % were made by diluting the concentrated extract with appropriate volume of sterile distilled water.

2. 4. Specimen Collection

The specimens were collected from different parts of the body of various school children. Sixty (60) children were sampled. The procedure was carried out using sixty (60) new surgical blades for each individual. Specimens were collected by scraping affected spots into clean sheets of paper which were then transferred into sterile containers that had been properly labelled with respect to each individual's data; these were brought to the laboratory for inoculation.

2. 5. Clinical Appearance of Specimen

The lesions on the body of the individuals had various appearances. These ranged from the formation of dense to flat mass of skin which could be black or reddish in colour. Others were a mixture of black and red lesions. The shapes of the lesions were also variable. Some had blisters, some were circinate, these were also dry, irregular, and scaly with thin marginated epidermis with central clearing of the lesion. Some other lesions were diffused having broken hairs which were grey to white in appearance.

2. 6. Specimen Inoculation

Sabouraud Dextrose Agar (SDA) was prepared and poured into sixty (60) sterile Petri dishes and allowed to solidify. The media was then inoculated with each of the specimen after which the culture was incubated at room temperature for growth to occur. Subculturing into fresh SDA agar was carried out after about four days of incubation. The plates were then incubated at room temperature for about four days. This was followed by macroscopic and microscopic examination.

2. 7. Antifungal Assay

The antidermatophytic activity of ethanolic, methanolic and aqueous extracts of garlic was done using agar diffusion method as described by Lino and Deogracious (2006). Nutrient agar was poured in sterile petri dishes and allowed to solidify. 1 ml of the test culture was dropped on the solidified agar and the organism was spread all over the surface of the agar using a spreader. Wells of approximately 6 mm in diameter were made on the surface of the agar medium using a sterile cork borer. Plates were turned upside down and wells labelled with a marker. Each well was filled with 0.2 ml of the extracts. Nystatin was used as control for cultures at a concentration of 1mg/ml. The plates were incubated at 28 °C for 24 hours. The inhibition zones surrounding the agar wells were measured in mm.

3. RESULTS

The antifungal activities of the aqueous, ethanolic and methanolic extracts of garlic inhibited the fungi with zones of inhibition ranging from 4.50-30.67 mm. The zones of inhibition decreased with decrease in the concentration of the extract. The aqueous extract exhibited lower zones of inhibition (Table 1). Methanolic extract showed high inhibitory zones than ethanolic extract (Tables 2 and 3) The largest zones of inhibition occurred with *Trichophyton rubrum* followed by *M. gypseum* and *T. mentagrophytes* which indicated that they were more sensitive to the extract than other organisms. The results are comparable with results of standard drug, nystatin used.

Table 1. Antidermatophytic activity of aqueous extract of garlic on dermatophytes.

| ORGANISMS | Zones of inhibition (mm) at different concentrations | | | | Nystatin |
|--------------------------|--|-------|-------|-------|----------|
| | 100% | 50% | 25% | 12.5% | |
| <i>T. rubrum</i> | 18.33 | 15.33 | 10.00 | 7.67 | 31 |
| <i>T. mentagrophytes</i> | 18.00 | 14.33 | 9.67 | 5.67 | 30 |
| <i>E. floccosum</i> | 6.00 | 5.00 | 4.67 | 4.50 | 25 |
| <i>M. gypseum</i> | 16.53 | 12.33 | 8.67 | 5.33 | 30 |
| <i>T. verrucosum</i> | 15.67 | 12.00 | 8.00 | 5.00 | 28 |

Table 2. Antidermatophytic activity of ethanolic extract of garlic on dermatophytes.

| ORGANISMS | Zones of inhibition (mm) at different concentrations | | | | Nystatin |
|--------------------------|--|-------|-------|-------|----------|
| | 100% | 50% | 25% | 12.5% | |
| <i>T. rubrum</i> | 26.33 | 24.69 | 19.00 | 14.67 | 31 |
| <i>T. mentagrophytes</i> | 24.00 | 18.33 | 13.67 | 8.67 | 30 |
| <i>E. floccosum</i> | 12.00 | 10.67 | 8.00 | 4.50 | 25 |
| <i>M. gypseum</i> | 25.67 | 23.67 | 19.00 | 14.67 | 30 |
| <i>T. verrucosum_</i> | 23.67 | 22.00 | 17.33 | 8.67 | 28 |

Table 3. Antidermatophytic activity of methanolic extract of garlic on dermatophytes.

| ORGANISMS | Zones of inhibition (mm) at different concentrations | | | | |
|--------------------------|--|-------|-------|-------|----------|
| | 100% | 50% | 25% | 12.5% | Nystatin |
| <i>T. rubrum</i> | 30.67 | 28.00 | 26.33 | 21.33 | 31 |
| <i>T. mentagrophytes</i> | 28.33 | 24.00 | 20.00 | 14.67 | 30 |
| <i>E. floccosum</i> | 13.33 | 10.06 | 7.67 | 5.33 | 25 |
| <i>M. gypseum</i> | 29.33 | 25.33 | 22.00 | 15.33 | 30 |
| <i>T. verrucosum</i> | 24.67 | 22.33 | 19.33 | 13.33 | 28 |

4. DISCUSSION

Occurrence of dermatophytic infection is a public health problem especially in children. This is because of the development of antifungal drug resistance of the pathogens and side effects exhibited by the drugs used for fungal diseases. Hence there is a great demand for safer alternative and effective chemotherapeutic agents. Use of medicinal herbs in the treatment of skin diseases including mycotic infection is an age old practice in many parts of the world (Balakumar *et al.*, 2011). A review of literature indicates that garlic was used as a folk medicine all over the world from ancient times (Bhadauria and Kumar, 2011). A number of reports are available on antifungal, antibacterial and antiviral activities of garlic on different microorganisms (Gulsen and Erol, 2010). Harris *et al.* (2001) published a mini review of the antimicrobial properties of garlic.

A wide spectrum of antidermatophytic activity of aqueous, ethanolic and methanolic extracts of garlic against five dermatophytes isolated from 60 primary school children was observed in this study. This substantiates work by Aala *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. floccosum*. Narula and Sareen (2011) reported the effect of natural antifungals on keratinophilic fungi isolated from soil. The results showed that garlic extract was the most effective antifungal agent. Gherbawy (1996) studied the response of keratinolytic and keratinophilic fungi to garlic extract and onion oil treatments revealed that all keratinophilic fungi were sensitive to garlic extract and onion oil. Kader *et al.* (1995) studied the effect of some medicinal plants on the growth of some dermatophytes. Of all the extracts the extract *Allium sativum* inhibited the growth by 47.5-100 %, *Nigella sativa* inhibited growth by 35.13-100 % and *Lawsonia alba* inhibited growth by 21.87-100 %. Ami and Kapadnis (2005) investigated the antifungal activity of garlic against two important dermatophytes, *T. rubrum* and *T. mentagrophytes*.

It was clear from this work that the solvent of extraction affected the degree of antidermatophytic activity of garlic. Ethanol and methanol are organic solvents and will dissolve organic compounds better hence liberate active component required for antifungal activity

The diameter of zones of inhibition exhibited by the extracts against test fungi showed 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.

The results in Table (1, 2 and 3) showed that the *T. rubrum* was most sensitive for garlic extracts. These results are agreed with the researches that showed that *T. rubrum* was the most susceptible dermatophytes for some plant extract (Silva *et al.*, 2005; Feesin *et al.*, 2001) while some reported *T. mentagrophytes* (Tadeq *et al.*, 2005) indicating the specificity of each plant extract and fungus.

The results of the study indicate that garlic has a broad spectrum of antidermatophytic activity and it is comparable to nystatin a standard antifungal agent.

5. 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 treatment of dermatophytic infections. Further work on this study may help to design a new drug against dermatophytosis

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