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A Comparison of the Antifungal Properties of Onion (*Allium cepa*), Ginger (*Zingiber officinale*) and Garlic (*Allium sativum*) against *Aspergillus flavus*, *Aspergillus niger* and *Cladosporium herbarum*

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ABSTRACT

Phytochemicals present in medicinal plants have health benefits and antimicrobial activity against some pathogenic bacteria. However, little research has been undertaken on the antifungal activity of these extracts. This research aim at testing the antifungal activity of organic ethanol extracts of onion (*Allium cepa*), ginger (*Zingiber officinale)* and garlic (*Allium sativum*) against three fungal isolates (*A. flavus, A. niger* and *C. herbarium*) in Potato Dextrose Agar (PDA). Filtered plant extracts were obtained using ethanolic extraction method. Antifungal sensitivity testing was undertaking using the pour plate technique and results obtained by measuring diameter of fungal growth over a 7 day incubation period. All organic plant extracts inhibited growth resulting in a marked significant difference ($p<0.01$) in growth diameter of fungi on media with extracts compared with same fungi on Potato Dextrose Agar without extracts. Ginger had the highest antifungal activity on all test fungi with a mean diameter of 1.40 cm followed by garlic (1.70 cm) and onion (1.80 cm) respectively whilst *A. niger* (2.54 cm) showed the highest resistance to the plant extracts followed by *A. flavus* (2.50 cm) and *C. herbarum* (1.18 cm). All plant extracts inhibited any observable growth pattern in *C. herbarum* for a 2 day period and <1 cm growth diameter in *A. Flavus* and *A. Niger* whilst the least growth measurement after day one of incubation in PDA only was >2.0 cm. This study confirms the antifungal potential of these plant extracts on the test fungi and suggests the possibility of employing them in food preservation were spoilage is mainly caused by fungi.

Key words: Antimicrobial activity, plant extracts, potato dextrose agar, growth diameter, fungi, antifungal activity

INTRODUCTION

Many research investigations have demonstrated the antimicrobial efficacy of several constituents of some higher plants (Rocio and Rion, 1982; Habtemarian *et al*., 1993). Grape fruit extracts possess antifungal, antiviral and antibacterial properties (Sims, 2001). Conventional antibiotics are sometimes associated with side effects whereas phytochemicals (plant extracts) have been found to have fewer side effects, better patient tolerance, relatively less expensive and a long history of use and renewability in nature (Vermani and Garg, 2002). The ubiquitous nature of microorganisms such as fungi in the environment makes human contact with them unavoidable. The high temperatures of the tropics coupled with lack of basic infrastructures and unsanitary production conditions prevailing in most developing countries predispose many food products, fruits and vegetables to spoilage. Several outbreaks of aflatoxicosis attributed to *A. flavus* have been documented in rural human populations in tropical countries (Peraica *et al*., 1999). Some strains of *A. niger* produces a potent mycotoxin called ochratoxins A; a human carcinogen found in grains and wine products (Samson *et al*., 2004; Schuster *et al*., 2002). Additionally, many fungi are parasites on plants and animals (including humans) causing serious diseases in humans such as aspergilloses, candidoses, coccidioidomycosis, mycetomas, among others. Furthermore, persons with immuno-deficiencies are particularly susceptible to diseases by *Aspergillus*, *Candida* and *Cryptoccocus* (Hube, 2004; Brakhage, 2005; Nielsen and Heitman, 2007). *Candida herbarum* is the most important allergenic species and has been shown to have the ability of triggering allergic reactions in sensitive individuals. Prolonged exposure to elevated spore concentrations can elicit chronic allergy and asthma (Samson *et al*., 2001).

Numerous naturally occurring phytochemicals are present in plant tissues and many studies have evaluated their antimicrobial activities in several plant extracts as Garlic (*Allium sativum*) against bacteria (Cavallito and Bailey, 1994), fungi (Adetumbi *et al*., 1986) and viruses (Weber *et al*., 1992). Ginger (*Zingiber officinale*) however, is known to have analgesic, sedative, cardiotonic and antibacterial effect (Hibert, 2006), eliminate *E. coli* and *B. cereus* bacteria (Wood, 1988) as well has angiogenic effects (Kim *et al*., 2005). Onion (*Allium cepa*) also exhibit antimicrobial effect against *B. subtilis*, *Salmonella* sp. and *E. coli* (Winston, 2008) and aflatoxin producing molds (Sharma *et al*., 1979). The present study seeks to report and compares the antifungal effectiveness of garlic, ginger and onion on the test species of *A. flavus*, *A. niger* and *C. herbarum* and to ascertain their potential as both antimicrobial and preservation agents.

MATERIALS AND METHODS

Materials, laboratory location and period of research: The research material ginger, onion and garlic were obtained from Pedu Market in the Cape Coast Metropolis of Ghana whilst laboratory activity was undertaken at the Laboratories of Department of Laboratory Technology and Biological Science Laboratory of the University of Cape Coast, in Cape Coast, Ghana between September, 2008 to March, 2009.

Plant extraction: One hundred gram of cleaned, air dried plant extracts of ginger, garlic and onions obtained were blended separately and individually soaked in 100 mL of ethanol for 24 h in a sterile glass container. The pulp obtained was shaken vigorously to allow for proper extraction of active ingredients. The crude extract was then filtered using sterile Whatmans No. 1 filter paper. The filtered extracts were then stored in the refrigerator at 4°C.

Fungal isolation: Test fungi (*A. flavus*, *A. niger* and *C. herbarum*) were obtained from the Department of Biological Sciences, University of Cape Coast, Cape Coast, Ghana.

Antifungal screening test: The test was carried out on PDA agar plates using the pour plate technique. One milliliter of organic extract of onion, garlic and ginger was dispensed separately into Petri dishes and mixed with cooled molten PDA. Ethanol only was used as positive control and PDA without any extracts as negative control for each extract analysis. Thus for each organic extract 5 Petri dishes were prepared made up of 3 plates for each fungal isolate and one each for controls (ethanol only and PDA without extracts). A streak of the pure cultures of the test fungi were then transferred unto the Petri dishes of plant extracts, ethanol and PDA only using a sterile inoculation needle. The plates were covered and incubated at room temperature at 28±3°C for a day after which diameter of growth of the test fungi were measured horizontally, vertically and diagonally and mean values calculated. Measurement of diameter was made at daily intervals for six consecutive days. For each organic extract the test was repeated in triplicates and mean values calculated.

Statistical analysis: Data obtained in the study were statistically analyzed using Statview. The means were separated using double-tailed Paired Means Comparison.

RESULTS

All plant extracts were effective in inhibiting any observable growth pattern in *C. herbarum* (Fig. 1) for a 2 day period with <1cm growth diameter in *A. flavus* (Fig. 2) and *A. niger* (Fig. 3) whilst the least growth measurement after day one of incubation in PDA only was >2.0 cm (Fig. 1-3). This growth pattern continued over the incubation period reflecting in significant differences ($p<0.01$) in paired means of all three extracts against PDA only. The best extracts antifungal property was expressed in *C. herbarium* with 2 days of growth inhibition after incubation. This was almost the same as in the positive control (ethanol). There was no significant difference ($p>0.05$) between ethanol and plant extracts inhibitions in *C. herbarum* indicating an

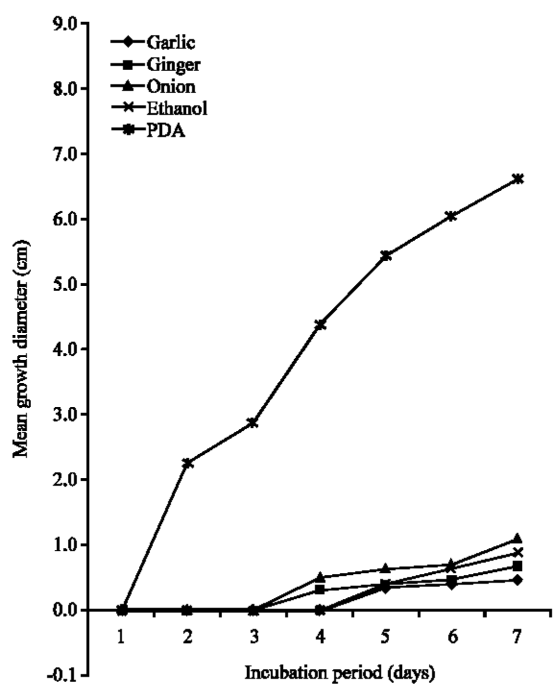

Fig. 1: Mean growth diameter of *C. herbarum* on media over a 7 day incubation period

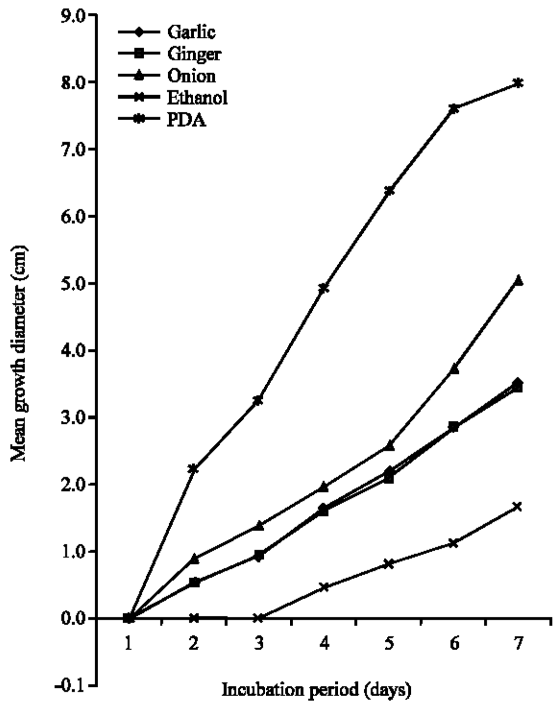

Fig. 2: Mean growth diameter *A. flavus* on media over a 7 day incubation period

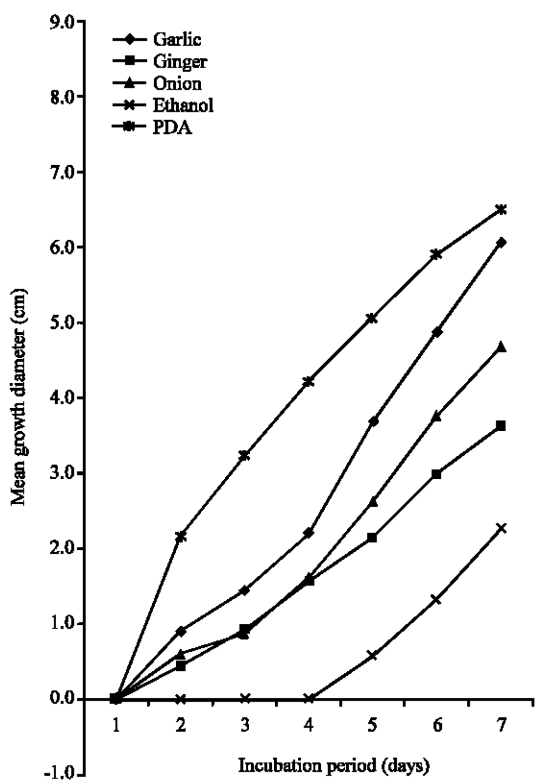

Fig. 3: Mean growth diameter of *A. niger* on media over a 7 day incubation period

equivalent antifungal property. Amongst the extracts, ginger maintained the highest measure of fungal growth inhibition (1.40 cm) whilst *A. niger* showed the highest level of extracts resistance (2.54 cm).

All extracts inhibited growth of *C. herbarum* after 2 days of incubation whereas there was a steady growth on PDA only.

DISCUSSION

Plant products, particularly spices and extracts of various plant parts have been used extensively as natural antimicrobials and antioxidants. Alam *et al*. (2002) reported high levels of inhibition of spore/conidia germination of some fungal species using extracts of rice, wheat straws and tobacco leaf. The above results clearly confirm the fact that soluble extracts of ginger, garlic and onion have antifungal properties and are able to inhibit the growth of the fungi *A. niger*, *A. flavus* and *C. herbarum* albeit to different extents. This confirms in-vitro activity of some plant extracts including garlic, ginger and onion on seed-borne fungi of wheat such as the *Aspergillus* sp. (Hasan *et al*., 2005). The antifungal activity of certain herbs and plant species against *Aspergillus* sp. has been documented in several research works (Bullerman, 1974; Bullerman *et al*., 1977; Azzouz and Bullerman, 1982) which was confirmed in the paired mean comparison of plant extracts on growth of all test fungi compared with PDA without plant extracts being highly significant ($p \leq 0.01$).Inhibitory activity of extract of onion on *A. flavus* and *A. niger* was highly significant ($p<0.0032$) and ($p<0.0041$), respectively confirming research work on the antifungal activity of onion on *Aspergillus flavus* (Sharma *et al*., 1979). The antifungal activity of garlic is in agreement with results of Dankert *et al*. (1979), who found its extracts very effective in inhibiting the growth of *Aspergillus* species. Interactive activity among the plant extracts showed no significance. Ginger had the highest antimicrobial activity on all test fungi with a mean diameter of (1.40 cm) followed by garlic (1.70 cm) and onion (1.80 cm), respectively. The strong inhibition potential of ginger is attributed to fact that it contains over 400 different compounds a mixture of both volatile and non-volatile chemical constituents such zingerone, shogaols and gingerols, sesquiterpenoids (**β**-sesquiphellandrene, bisabolene and farnesene) and a small monoterpenoid fraction (**β**-phelladrene, cineol and citral) (Chrubasik *et al*., 2005; Grzanna *et al*., 2005). These several chemical constituent increases its antimicrobial effectiveness. Additionally, differences in concentrations of active ingredients in the plants could account for their antimicrobial potential disparities. Garlic contains a higher concentration of allicin than onions increasing its antimicrobial activity comparatively. Additionally, allicin is highly volatile as compared with gingerols and shogaols in ginger and thus could be lost by diffusion during the extracts preparation process leading to a reduction in its antimicrobial effectiveness. This conforms to earlier study by Azu and Onyeagba (2007), who reported a correlation between antimicrobial activity of plant extracts and their concentrations.

The test fungi expressed different susceptibility trend against the plant extracts with *C. herbarium* (1.18 cm), *A. flavus* (2.50) and *A. niger* (2.54) in that order. Although the exact mechanism that influences the differences in the susceptibility pattern is not known, it is believed that mycelia cell wall thickness plays a significant role. The adaptation of mycelia for the efficient extraction of nutrients, due to the high surface area to volume ratios is also potentially harmful in absorbing antimicrobial agents and thus inhibiting growth when present in the medium (Moss, 1986).

The marked growth inhibition of *C. herbarum* by all plant extracts is significant in the quest to use plant extracts to prevent fungal infections and contamination of food which is preferable to artificial compounds which can be toxic at certain concentrations. Hence the use of plant extracts such as ginger, garlic and onions which are natural meat spices could be the natural means of controlling this organism.

CONCLUSION

Phytochemicals can be effectively employed as antimicrobial agents, specifically antifungal to control growth and prevent colonization and spoilage of food and other plant products with the attendant financial losses by fungi. The use of ginger, garlic and onion in controlling *C. herbarum* could help prevent cold meat spoilage and preserve meat for longer periods against fungal contamination.

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