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Extracts of *Aloe vera* and *Allium sativum*: potent inhibitors of pathogenic fungi

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Abstract

Agar diffusion method was employed, in the evaluation of the potential antifungal activity of methanol extracts of *Aloe vera* and *Allium sativum*, against pathogenic *Aspergillus niger* and *Candida albicans*. Phytochemical evaluations showed the presence of tannins, saponins, flavonoids, and terpenoid in *Aloe vera*, while saponins, alkaloids, and flavonoids were found in *Allium sativum*. Plant extract concentration used in this study ranged from 5 to 100 mg/ml. *A. vera* showed the greatest antifungal effect against *C. albicans* and *A. niger* at 100 mg/ml with a zone of inhibition of 23 mm and 26 mm respectively. *A. sativum* exhibited a greater antifungal potential at extract concentration of 100 mg/ml with 42 mm and 37 mm zone of inhibition against *A. niger* and *C. albicans*. The phytochemical contents of these plants were implicated for the antifungal potentials.

Keywords: Antifungal activity; Pathogenic fungi; *Aloe vera*; *Allium sativum*; Zone of inhibition

1. Introduction

Fungi infection refers to the invasion of tissues by specie(s) of fungi. Pathogenic fungi are linked to many human diseases and over 300 fungi are implicated in diseases of humans and other organisms [1]. An important characteristic of fungi is the possession of cell wall composed of chitin [2] unlike plant cell wall which contains cellulose. Fungi are therefore classified differently from animals, bacteria and plants. Human fungi infections particularly in immunocompromised persons are very challenging problem, because the therapeutic options are hampered by serious drawbacks, such as development of drug resistance and toxic side effects [3].

The ubiquitous filamentous ascomycete fungus -*Aspergillus niger* is implicated in opportunistic infections of humans and due to the aerosolized nature of *Aspergillus* spores, individuals are constantly exposed to this disease causing microorganism. The production of mycotoxins by *Aspergillus* causes diseases of varying nature; inducing allergic responses and localized or systemic infections (mycoses) such as serious lung disease called Aspergillosis [4]. Furthermore, studies have implicated exposure to mycotoxins to mucus membrane irritation, skin rashes, dizziness, nausea, and immunosuppression [5]. *Candida* species on the other hand are serious and important human pathogen which causes opportunist infection in immune compromised hosts such as AIDS patients, tissue/organ transplant patient, cancer sufferers etc. A great percentage of these systemic infections are difficult to treat and often result to death.

The discovery of new therapeutic agents requires the use of natural products such as different plants and plant parts extracts. Studies have shown that natural product from tea tree oil (TTO) are used in antifungal therapy such as the

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treatment of vaginal and oral candidiasis [5]. Drugs derived from natural sources play significant roles in the prevention and treatment of human diseases and as such plants and plant products are widely exploited in traditional medicine.

An important natural product is *Allium sativum* (garlic) which extract use in the treatment of malignant tumor dates back to Egyptian civilization [6]. Mendham [7] reported complete attenuation of bacteria activities under laboratory conditions. Garlic exhibits antifungal, antibacterial and antiviral properties by inhibiting growth and metabolism of these infectious agents [8]. The inhibitory activities on infectious agents are enhanced by garlic stimulatory ability of natural killer cells to destroy tumor cells and foreign invaders [9]. *Aloe vera* has potent antifungal, antibacterial and antiviral properties [10]. The study by Rahmani et al [11] showed the bacteriostatic potentials of *Aloe vera* against *Staphylococcus aureus*, *Streptococcus pyogenes* and *Salmonella paratyphi*. The drawbacks associated with the use of synthetic antifungal drugs leads to a clear demand for new therapeutic approaches, with interest in the application of natural products. It was against these drawbacks that this study determined the phytochemical constituent of *Allium sativum* and *Aloe vera*, and assayed the activities of the extracts on some pathogenic fungi compared to some standard antifungal drugs.

2. Material and methods

2.1. Sample collection and preparation

Fresh samples of *Aloe barbadensis* (*Aloe vera*) and *Allium sativum* (Garlic) were purchased from Ekeonuwa Market in Owerri, Imo State of Nigeria. The plants were identified by Dr. M.C. Duru of Department of Biology, Federal University of Technology Owerri (FUTO). The fresh *Aloe vera* leaves and garlic cloves were mashed with an electric blender EM-242. Fifty grams (50 g) of each of the mashed samples were soaked in 200 ml of 95% methanol in a conical flask for 72 hrs. This was filtered using Whatman filter paper. The filtrates were concentrated with rotary evaporator to dry powder. The dried extracts were stored in a sterile screw capped bottle and kept at 4 °C in a refrigerator [12].

2.2. Isolation of microorganisms

Candida albicans and *Aspergillus niger* were isolated from high vaginal swab and wound swab of patients admitted at the Federal Medical Centre Owerri, Imo State. Organisms were maintained on Sabouraud Dextrose Agar (SDA) and incubated for 4 days at 25 °C. Fungal growth were harvested and washed with normal saline and finally suspended in 100 ml sterile normal saline.

2.3. Preparation of molten agar medium

Two grams (2 g) of SDA was dispersed in 1000 ml of distilled water under aseptic condition and sterilized by autoclaving at 115 °C for 15 minutes. Using a wire loop; fungal isolates from the SDA slants were introduced into 2 ml distilled water. Then, 0.5 ml of the suspension and 19.5 ml of molten SDA were poured into a sterile petri dish at 45 °C, mixed and allowed to solidify. *In-vitro* antifungal activity of the extract was done using pour plate method [12]. Finally, using a sterile cork burner, 5 mm wells were bored in the solidified Agar and 0.5 ml of each of the graded extract was poured into the wells.

2.4. Experimental design

All set-ups were done in duplicates and a control was prepared with distilled water without the extracts. These were incubated for 5 days at 28 °C.

2.5. Assessment of zone of inhibition

The holes were monitored closely for the development of clear zones around the extract. The antifungal activities of the extracts were assessed by measuring the diameter of the zone of inhibition. The sensitivity of the microorganisms to the plant extract were evaluated by measuring the diameter of inhibitory zones and disk values less than 8 mm (< 8 mm) were considered not active against microorganisms.

2.6. Phytochemical analysis

Phytochemical analyses of *Allium sativum* (garlic) and *Aloe vera* for the presence of flavonoids, tannins alkaloids, terpenoid, saponins, sugar and glycosides were done using the method as described by Harborne [13].

3. Results and discussion

Phytochemicals are known to possess medicinal properties and health promoting effects [14-17]. This study showed that *Aloe vera* and *Allium sativum* contain saponins, flavonoids, and sugar (Table 1). Also, *Aloe vera* showed presence of Tannins and Terpernoids, while alkaloids were present in *Allium sativum* (Table 1). The antifungal activities of *Aloe vera* and *Allium sativum* can be attributed to the pharmacological effect of these phytochemicals. Tannins, flavonoids and saponins possess antimicrobial activities [18-19]. At the cell wall level, flavonoids can exert a variety of biological effects [20], linked to specific interactions with molecular targets.

Table 1 Qualitative phytochemical content of *Aloe vera* and *Allium sativum*

Extracts	Tannins	Saponins	Alkaloids	Flavonoids	Terpernoid	Sugar
<i>Aloe vera</i>	+	+	+	+	+	+
<i>Allium sativum</i>	-	+	+	+	-	+

+ = present, - = absent

Measurement of zone of inhibition has been used to evaluate the antimicrobial or antifungal activities of various plant extracts. In this regard, the pour plate method [12] is widely used to assess the potential toxicity of toxicants to microorganisms including bacteria.

The results of the effect of methanol extract of *A. vera* and *A. sativum* to pathogenic swab isolates are presented in Figures 1 and 2. In this study the microbial isolates showed the presence of *Candida albicans* and *Aspergillus niger* and the effective antifungal activities of the plant extracts. These observed antimicrobial activities of the methanol extracts of *A. vera* and *A. sativum* indicated dose dependent effects with respect to the zone of inhibition as shown in Figures 1 and 2. The result shown in Figure 1 indicated that, at concentrations of 5-25 % *A. vera* showed a zone of inhibition of less than 8 mm (≤ 8 mm) in *C. albicans* and *A. niger* and these were considered not active against pathogenic microorganisms. However, higher concentration (50-100 %), extracts from *A. vera* exerted a strong inhibitory effect on *C. albicans* and *A. niger* in a dose dependent manner. The rate of increase in inhibition of *A. niger* was higher compared to *C. albicans*.

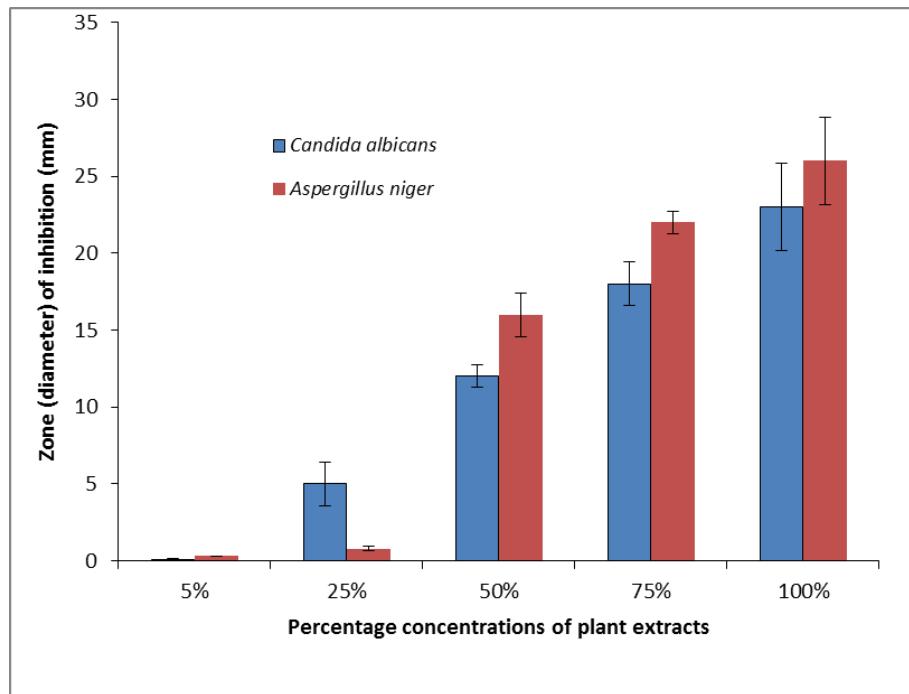


Figure 1 Mean antifungal effects of graded concentrations of extracts *Aloe vera* on *Candida albicans* and *Aspergillus niger*

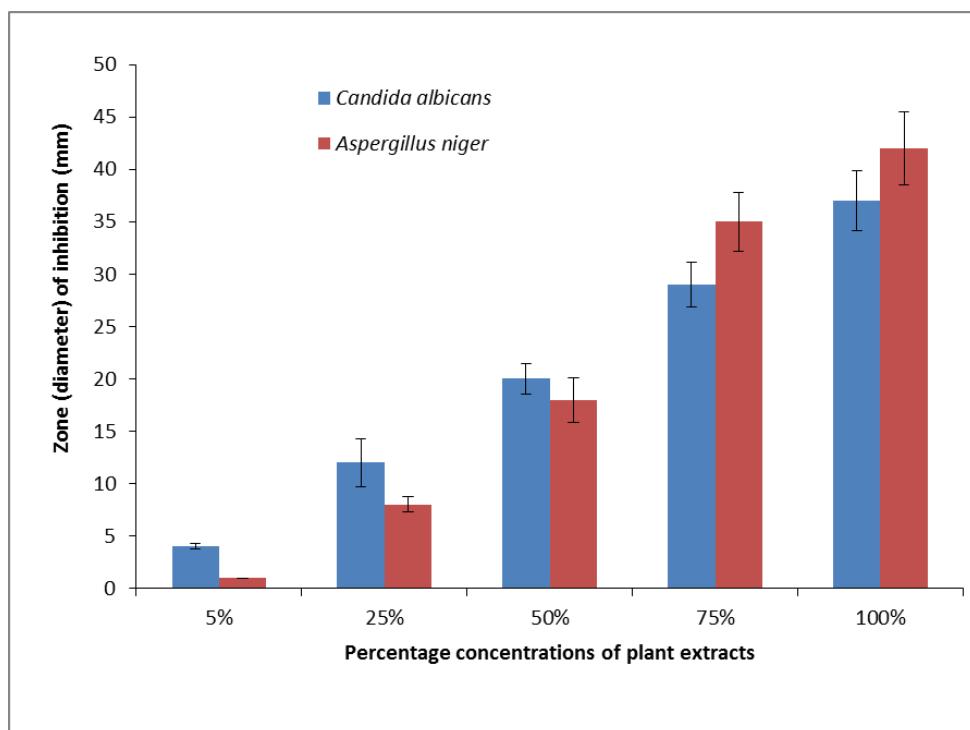


Figure 2 Mean antifungal effects of graded concentrations of *Allium sativum* on *Candida albicans* and *Aspergillus niger*

Similar antifungal effect was exerted by the methanol extract of *Allium sativum* on *C. albicans* and *A. niger*. The inhibitory effects on the activities of these pathogenic microbes were significant when compared to *A. vera* extracts. With extract concentration of 25 %, *A. sativum* exerted a greater inhibitory effect on *C. albicans* and *A. niger* and these were considered active against microorganisms. Furthermore, at 100 % extract concentration, *Allium sativum* showed a zone of inhibition almost twice that of *A. vera*. Antifungal activities of the methanol extract of *Allium sativum* increased linearly with increase in extract concentration. The growth inhibition zone measured, ranged from 4-37 mm in *C. albicans* and 1 - 42 mm in *A. niger*. This was comparatively higher than that recorded by *Aloe vera*.

These antimicrobial activities can be attributed to the phytochemical composition of these plants. Plant extracts have been applied in traditional medication to treat microbial infections and diseases from ancient times [21]. The pharmacological potentials of plants for the treatment of various diseases are implicated to the antioxidative effects [22-23], of the phytochemicals which exhibits strong antimicrobial effect [24]. The presence of alkaloids in *Aloe vera* and *Allium sativum* confers strong antimicrobial potentials and parasitic repelling effects [25]. It is possible that the basic, low-molecular weight nitrogen-containing alkaloid, have an inhibitory effect on fungi enzyme activities or was able to degrade the cell walls of *C. albicans* and *A. niger* made of chitins. The flavonoid contents of *A. vera* and *A. sativum* may be implicated in the antimicrobial and antifungal effects [26- 28].

The level of antimicrobial activities displayed by the crude extracts of plants used in this study could be explained by the presence of phytochemicals. Despite the crude extracts of the plants used in this study, the extracts nonetheless elicited high level of antimicrobial potentials comparable to conventional antibiotics. The positive antimicrobial potentials obtained may be due to synergy of the constituent phytochemicals. These results lend credence to the use of natural plant extracts in disease treatment. The study by Paul [29] indicated extracts of *Allium sativum* to be effective against specific microorganisms notorious for developing resistant strains. Therefore, natural plant products can circumvent the development of resistant strains of microorganisms usually observed in the use of synthetic antibiotics.

4. Conclusion

This study has shown that extracts *Aloe vera* and *Allium sativum* possess antifungal potentials and hence it can be used to formulate herbal mixtures for the treatment of pathogenic infections.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

This study was approved by the Research and Ethics Committee of the Department of Biology, Federal University of Technology Owerri, Nigeria.

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