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Short Communication

THE EFFICACY OF CRUDE EXTRACT OF *ALOE SECUNDIFLORA* ON *CANDIDA ALBICANS*

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Abstract

In- vitro studies on the efficacy of crude extracts of *Aloe secundiflora* on *Candida albicans* was conducted. Five mature leaves of *Aloe secundiflora* were collected and the crude extract was prepared, then autoclaved. The extract was then tested on *Candida albicans* grown on solid media. The results from these studies revealed complete inhibition of *Candida albicans* growth caused by *Aloe secundiflora* crude extract on solid media. The findings from this study suggest that the crude extract of *Aloe secundiflora* can inhibit the growth of *Candida albicans*. Further studies are required to establish the *in-vivo* activity of the crude extract, the active ingredient, dosage and safety of *Aloe secundiflora*, before recommending for clinical use.

Keywords: *Aloe secundiflora*, alternative medicine, opportunistic infections, crude extract

Introduction

Medicinal plants in many African countries including Tanzania play a crucial role in provision of primary health care including treating people living with incurable diseases such as cancer and HIV/AIDS opportunistic diseases. HIV/AIDS pandemic is currently among the highest socio-economic challenges that face Tanzania and other developing countries, as it affects mostly the young and most economically productive population. This translates to loss of skills, talents, expertise and man-hours (TACAIDS, 2006). Majority of people living with HIV/AIDS are susceptible to fungal and bacterial opportunistic infections that result from immunosuppression (UNAIDS, 2006). World Health Organization (WHO) reported that, people with advanced HIV infection are vulnerable to infections and malignancies that are called 'opportunistic infections' because they take advantage of the opportunity offered by a weakened immune system. Candidiasis is the second most common cause of vaginal irritation, or vaginitis, and can also occur on the male genital organ. In immunocompromised patients, the *Candida* infection can involve the esophagus and can become systemic, causing a much more serious condition called fungemias. The drugs required in treatment of Candidiasis (HIV/AIDS opportunistic infection) are very expensive and majority of the suffers are poor and are unable to meet the high costs of medications. There is therefore the need to search for some alternative treatment of Candidiasis through the potential use of *Aloe secundiflora* which are readily available and.

Previous studies have shown some antibacterial activity of crude extract of *A. secundiflora* (Waihenya et al., 2002). The aim of this study therefore is to establish the bioactivity of crude extract of *Aloe secundiflora* in the growth inhibition of *Candida albicans*.

Materials and Methods

Preparation of plant extracts

Aloe secundiflora (BJH 4828 i.e. B. J. Harris 24.6.1970) leaves were collected from Sokoine University of Agriculture *Aloe* garden (Figure 1). The sap was collected after five mature leaves were transversely cut using a knife and the leaves were arranged in container with 100 ml of distilled water to hasten the process of extraction. The

extraction was done for 30 minutes. The extracted sap was kept in refrigerator at 4 ºC in sterile screw capped containers until use.

Figure 1: *Aloe secundiflora* plant at the Sokoine University of Agriculture.

Test microorganism

Candida albicans was acquired from the Department of Molecular Biology and Biotechnology, University of Dar-es salaam in Malt Extract Agar slant (Courtesy of Dr. Kenneth Hosea).

Procedures of culturing the organism on fresh media

Work surface was sterilized with 70% ethanol, 500ml of Medium (Malt extract agar) was prepared according to manufacture's instructions, autoclaved at 121 °C, 15 minutes, medium was poured into sterile Petri dish up to a level of approximately 4mm that is about 20 ml of molten agar and left to solidify. Dishes with solidified agar was incubated for 24 hrs for sterility check up, *Candida albicans* from slant was streaked on media by using sterile wire loop and the streaked, dish incubated for 24 hrs at 37 °C. Formed colonies were identified culturally and stored in the refrigerator at 4 °C to stop further growth of *Candida albicans*.

Antimicrobial susceptibility testing.

A loopful of *Candida albicans* from a Petri dish was inoculated into 100ml of prepared sterile malt extract broth (in flask), then incubated at 37 °C for 24 hrs. On the agar media, four equidistant wells of 5mm in diameter and 4mm in depth were prepared using a sterile cock borer. To each five petri dishes contains about 15-20ml of sterile Malt extract agar, 0.2 ml of inoculum was poured on agar and plate tilted and distributed inoculum evenly on agar surface. Wells were labeled to correspond with the volume (20 , 80 µl and 100 µl respectively) of the crude extracts of *Aloe secundiflo*ra and standard (Ketoconazole, 40µl and Fluconazole 40 µl). Wells was filled with the plant extracts and control, and kept in the refrigerator to allow extract to diffuse in agar for 8 hrs, then sealed and incubated at 37 °C for 24 hrs.

Data analysis

Data on zones of inhibition (in mm) was analyzed using Statistix® 9 (Analytical software 2008). Analysis of variance (ANOVA) and comparison of means was conducted.

Results and Discussion

It was observed that *Aloe* crude extract obtained from five mature leaves showed antimicrobial effects on *Candida albicans* by complete zones of inhibition on the growth medium (Figure 2).

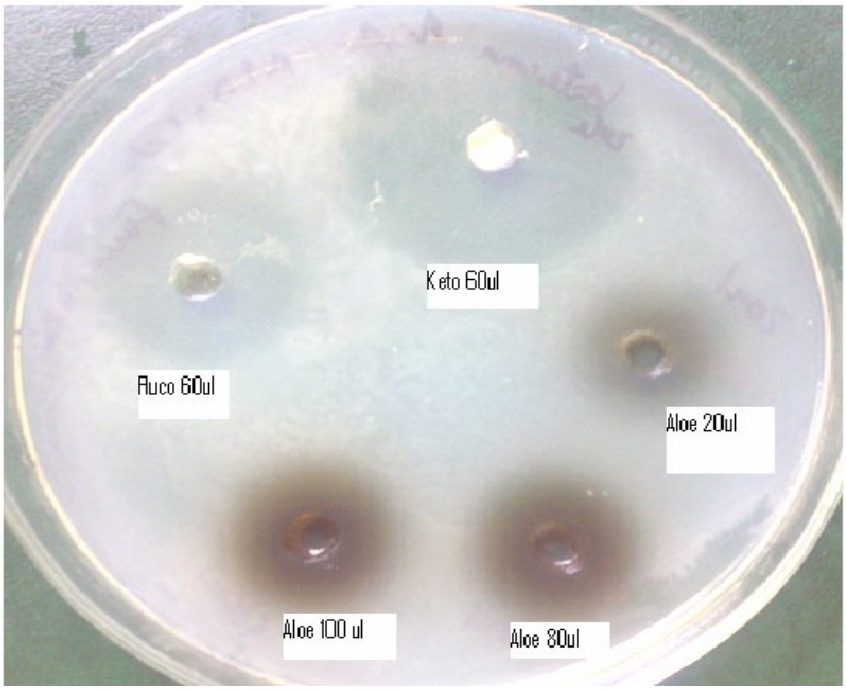

Figure 2: Zones of inhibition caused by various volumes of *Aloe secundiflora* and standard drugs.
Key: Keto = Ketokonazole
Aloe 20µl, 80µl, 100µl = Different volumes for crude Aloe secundiflora sap.
Fluc = Fluconazole

Table 1: Analysis of variance for volume of *Aloe secundiflora* and zone of inhibition

Volume used (µl)	Mean zone of inhibition ± Std deviation
20	11.46 ± 0.69^{a}***
80	15.24 ± 0.72^{b}**
100	16.66 ± 1.09^{c}***

Means in a column not sharing the same superscript are significantly different at **P<0.001; ***P<0.0001

There was a significant variation in the zone of inhibition at the different treatment volume level from each other at (P<0.001 to 0.0001). There was an increase of the zone of inhibition with increase in the volume of the crude extract. The highest zone of inhibition of 16.66 ± 1.09 was recorded at a maximum volume of 100µl, followed by 80µl and 20µl respectively The results from this experiment indicate that *Aloe secundiflora* has antifungal effect on *Candida albicans* that was tested under the experiment. This antifungal effect has been shown by complete inhibition of the area diffused by the *Aloe* sap **(Figure 2).**

This study is the first preliminary investigation so far in Tanzania, on the *in vitro* studies of *Aloe secundiflora* crude extract on *Candida albicans*. Most previous reports have been on bacteria and viruses Waihenya (2002). In this study, crude sap from five leaves of *A. secundiflora* exhibited clear zones of inhibition against *C. albicans* indicating a growth inhibition on the fungi. Waihenya (2002) found *A. secundiflora* crude extract to be effective against various numbers of bacteria such as *Pseudomonas* species, *Escherichia coli*, *Staphylococcus aureus*, *Proteus* species, *Pasteurella* species, and *Streptococcus species*. Rajabu (2004-Unpublished report) revealed that sap obtained from 4-5 mature leaves of *A. secundiflora* inhibited the growth of *Bacillus subtilis* and *Salmonella gallinarum*.

In- vivo studies involving patients with oral thrush would be useful in determining the usefulness of the crude *Aloe* extract in treatment of Candidiasis. In a study conducted to determine the effect of aloe in chickens infected with *Salmonella gallinarum*, it was revealed that there was a delay of clinical signs and lowered severity of the disease (fowl typhoid) among the aloe treated group as compared to untreated group (Waihenya et al., 2002). Bland (1985) investigated the effect of *Aloe* when consumed orally in patient suffering from an inflammatory bowels disease. The *Aloe* was given at the rate twice ounces three times daily for a week and able to rebalance the regulating gastrointestinal motility, increase stool specific gravity to 0.37 and decrease stool transit leading to curing diarrhea. From these finding it was suggested that *Aloe* could inhibit diarrhea and hence effective against some disease causing agents.

Further research should be directed in determining the active ingredients present in *Aloe* crude extract and various concentrations to determine which concentration may give excellent response. This would be a pre-requisite before clinical trials on human patients is attempted.

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