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Screening extracts of *Asparagus setaceous* Kunth and *Caesalpinia volkensii* Harm for Anti-Candida activity

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ABSTRACT

A number of plants have been investigated and demonstrated to possess antifungal activity. In this study, the organic and aqueous extracts of *Asparagus setaceous* (Liliaceae) and *Caesalpinia volkensii* (Caesalpinaceae) were investigated on their antifungal activity against the fungus *Candida albicans* using the disk diffusion method. In addition the minimum inhibition concentrations of the extracts were also determined. The organic extracts demonstrated negligible anti-candida activity while the aqueous extracts of both the plants were devoid of anti-candida activity. The extracts cannot therefore be used in the management of fungal infections caused by species of Candida

INTRODUCTION

Invasive fungal infections have emerged as major causes of morbidity and mortality. During the last decades, the frequency of life threatening infections has increased drastically along with the number of potentially invasive species [1,2]. Numerous studies have shown an association between increased prevalence of HIV infection and the occurrence of opportunistic fungal infections [3]. Among the different HIV associated fungal infections, oral mucosal lesions caused by Candida species are by far the most frequent manifestation. Up to 90% of HIV- infected individuals suffer at least one episode during the course of their disease, and the incidence and severity of the episodes increase with decreasing immunity. *Candida albicans* is the causative agent accounting for more than 90% of cases. However, other Candida species such as *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei* may also cause symptomatic oral candidiasis in HIV-positive individuals [4]. Other fungal infections seen in HIV- infected individuals include cryptococcosis due to *Cryptococcus neoformans* and Aspergillosis due to *Aspergillus flavus*, *A. fumigatus* and *A. niger* [4]. Oral candidiasis is usually treated by topical antifungal agents that include nystatin, miconazole, fluconazole, itraconazole and amphoterin B [5]. However, the management of Candida infections faces a number of problems including limited number of effective antifungal agents, toxicity of the available antifungal agents, resistance of Candida to commonly used antifungal, relapse of Candida infections, and the high cost of antifungal agents. When relapses occur, the infections tend to be refractory to treatment [1,2] The difficulties associated with the management of Candida infections necessitate the discovery of new antifungal agents in order to widen the spectrum of activity against Candida and combat strains expressing resistance to the available antifungal agents. In this case also, plant derived natural products may offer potential lead to new compounds, which act on these fungi. This study was therefore conducted to investigate possible antifungal property of extracts of *Asparagus setaceous* and *Caesalpinia volkensii*. Extracts from the two plants have been used in the management of infectious diseases in the traditional medicine practices [3, 6, 7, 8, and 9]

MATERIAL AND METHODS

Plant materials and their collection

Plant materials were colleted from Gatundu [1°3'0"S: 36°54'0"E] located in Central province of Kenya. Gatundu is approximately 40km north of Nairobi. Aerial part and root of *Asparagus setaceous* and the leaves, stem and root of *Caesalpinia volkensii* were collected. The plants were identified in the herbarium, Department of Botany JKUAT, where voucher specimens were deposited. The plant materials were dried under shade at temperature below 30°C and pulverized in a hammer mill fitted with a sieve of 0.5mm pore.

Preparation of Plant extracts

Preparation of organic and aqueous extracts was carried out as described in Oduor and Ogila, 2012. [10]

Screening of *A. setaceous* and *C. volkensii* crude extracts for antifungal activity

Antifungal activity of the plant extracts of *A. setaceous* and *C. volkensii* was tested by the disc-diffusion method. The minimal inhibition concentrations (MIC) of the extracts were also determined. The fungus C*andida albicans* were used in the study. The organism was obtained from a culture collection maintained in the Department of Botany, JKUAT.

Preparation of inoculums

Stock cultures were maintained on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Sabouraud dextrose broth (SDB) for fungi to reactivate them by culturing overnight at 37◦ C. Cultures were diluted with fresh SDB and compared with McFarland standard to achieve values corresponding to $2x10^7$ spores/ ml for fungal strain.

Screening of the extracts for anti-Candida activity

This was carried out as described by Runyoro *et al*. [4] with modification. Sabouraud dextrose agar was used to prepare the culture medium according to the manufacturer's direction. *Candida albicans* (ATCC 90028) kept in the department of Botany, JKUAT, was aseptically inoculated on petri-dishes containing autoclaved, cooled and settled medium. The petri dishes were incubated at 31^0C for 48h to give white round colonies against a yellowish background. These were aseptically subcultured on SDA slants. *Candida albicans* colonies from SDA slants were suspended in sterilized 0.9% Sodium chloride solution (normal saline), which was compared with McFarland solution. The microbial suspension (1ml) in normal saline was added to 74ml of sterile medium, kept at 45^0C to give concentration of 2 X 10^7 cells/ml. Sterilized petri dishes (9cm diameter) were inoculated with 1ml of the above solution SDA sterilized in a flask and cooled. It was then distributed by pipette (15ml) into each inoculated petri dish and swirled to distribute the medium homogenously. Disks injected with extracts were applied on the solid Sabouraud dextrose agar medium by pressing slightly. The treated petri-dishes were placed at 4^0C for 1 2 h and then incubated for 48h at 37^0C. Standard antibiotic disks (ampicilin 25µg, tetracycline (100µg), nitrofurantoin (200µg), nalidixic acid (30µg), streptomycin (25µg), sulphamethoxale (200µg), cotrimoxale (25µg) and gentamicin (10µg) were also included and tested for their anticandidal activity. The treated petri-dishes were placed at 4^0C for 1 2h and then incubated at 35^0C for 18 24h. The discs were tested in triplicate. At the end of the period, the inhibition zones formed on the media were measured with a transparent ruler in millimeters.

Determination of the minimum inhibitory concentration (MIC)

Five concentrations of each extract (3.125, 6.25, 12.5, 25 and 50 mg/ml) were prepared. The antimicrobial effect of each concentration was measured. The various concentrations were loaded onto 6 mm disks which were then pressed onto already prepared SDA plates. The inoculated plates were incubated at 35◦C for 48h. MICs were determined after 48h for *C. albicans*. Zones of inhibition were measured at the end of the incubation period. The MICs were determined as the lowest concentrations of extracts inhibiting the visible growth of each organism on the agar plate.

Table 1: Anti-candida activity of extracts of *Caesalpinia volkensii* and *Asparagus setaceous*

Group	*C. albicans*
DCVL	2.67±0.33
DCVS	-
DCVR	4.33±0.33
DASA	2.33±0.33
DASR	1.33±0.33
MCVL	1.33±0.33
MCVS	2±0.58
MCVR	1.67±0.33
MASA	1.3±0.33
MASR	1.33±0.33
ECVL	1.67±0.33
ECVS	1.33±0.33
ECVR	0.67±0.33
EASA	2±0.58
EASR	1.33±0.33
HCVL	3±0.58
HCVS	1±0.00
HCVR	1.33±0.33
HASA	2±0.58
HASR	2±0.58
ACVL	-
ACVS	-
ACVR	-
AASA	-
AASR	-

RESULT

The results of the antifungal effects of the different parts of the two plant extracts against *Candida albicans* tested are shown in table 1. Dichloromethane extracts of *C. volkensii* leaf demonstrated antifungal activity against *C. albicans* (inhibition zone 2.7mm). Dichloromethane extracts of *C. volkensii* stem did not show any antifungal activity against *C. albicans*. Amongst all the extracts tested in this study dichloromethane extracts of *C. volkensii* root demonstrated the highest antifungal activity with

an inhibition zone of 4.3mm. Dichloromethane extracts of *A. setaceous* aerial part showed slight antifungal activity against the fungus *C. albicans* (inhibition zone 2.3mm). Dichloromethane extracts of *A. setaceous* root showed minimal antifungal activity against *C. albicans* (inhibition zone 1.33mm).

Methanolic extract of *C. volkensii* leaf showed minimal activity against *C. albicans* (inhibition zone 1.33mm). Methanolic extract of *C. volkensii* stem was active against *C. albicans* (inhibition zone 2mm). Methanolic extract of *C. volkensii* root also demonstrated antifungal activity against *C. albicans* (inhibition zone 1.67mm). Methanolic extract of *A. setaceous* aerial part had less antifungal activity against *C. albicans* (inhibition zone 1.3mm). Methanolic extract of *A. setaceous* root demonstrated less antifungal activity against *C. albicans* (inhibition zone 1.33mm).

The ethanolic extracts of *C. volkensii* leaf demonstrated antifungal activity against *C. albicans* (inhibition zone 1.67mm). The ethanolic extract of *C. volkensii* stem showed minimal activity against *C. albicans* (inhibition zones 1.33mm). The ethanolic extract of *C. volkensii* root extract also showed minimal antifungal activity against *C. albicans*. The ethanolic extract of *A. setaceous* aerial part also showed minimal activity against *C. albicans* (inhibition zone 2mm). Ethanolic extract of *A. setaceous* root had minimal antifungal activity against *C. albicans* (1.33mm).

The hexane extracts of *C. volkensii* and *A. setaceous* exhibited antifungal activity against the test microbe with inhibition zones ranging from 1mm to 6.67mm. The hexane extracts of *C. volkensii* leaf extract demonstrated antifungal activity against *C. albicans* (inhibition zone 3mm). The hexane extracts of *C. volkensii* stem had less antifungal activity against *C. albicans* (inhibition zone 1mm). The hexane extracts of *C. volkensii* root showed less antifungal activity against *C. albicans* (inhibition zone 1.33mm). The hexane extracts of *A. setaceous aerial* part had antifungal activity against *C. albicans* (inhibition zone 2mm). The hexane extracts of *A. setaceous aerial* root had less antifungal activity against *C. albicans* (inhibition zone 2mm).

Table 2: Antifungal activity of standard antibiotics against Candida albicans. The results are expressed as mean inhibition zones ± SEM of three replicates

Antibiotics	*C. albicans*
A	-
T	5.07±0.07
NF	-
NA	-
S	5.87±0.09
SX	-
Co	-
G	16.33±0.2

KEY: A= Ampicilin 25µg, T= Tetracycline (100µg), NF= Nitrofurantoin (200µg), NA= Nalidixic acid (30µg), S= Streptomycin (25µg), SX= Sulphamethoxale (200µg), Co= Cotrimoxale (25µg) and G=Gentamicin (10µg).

Table 3: Minimum Inhibitory concentration of dichloromethane extracts of *C. volkensii* and *A. setaceous* against Candida albicans

Plant Extract	Conc Mg/ml	*C. albicans*
DCVL	50	2.67±0.33
	25	2.33±0.33
	12.5	1.33±0.33
	6.25	-
	3.2	-
DCVS	50	2.33±0.33
	25	1.33±0.33
	12.5	-
	6.25	-
	3.2	-
DCVR	50	4.33±0.33
	25	2.33±0.33
	12.5	1.33±0.33
	6.25	-
	3.2	-
DASA	50	3.33±0.33
	25	2.33±0.33
	12.5	-
	6.25	-
	3.2	-
DASR	50	1.33±0.33
	25	1±0.00
	12.5	-
	6.25	-
	3.2	-

From the result of the antifungal activity of the plant extracts presented in table 1, the aqueous extracts were generally found to be inactive against *C. albicans*. All the aqueous extracts lacked antifungal activity against *C. albicans*. The most resistant microbes to aqueous extracts were *C. albicans.*

According to the data presented in table 2, ampicilin lacked any activity against *C. albicans*. Tetracycline was observed to be active against *C. albicans*. Nitrofurantoin completely lacked antifungal activity against *C. albicans*. Nalidixic acid also completely lacked any form of antifungal activity against *C. albicans*. Streptomycin was one of the three standard antibiotics among the tested antibiotics that demonstrated antifungal activity against *C. albicans* giving an inhibition zone of 5.8mm. Sulphamethoxale was observed to lack activity against *C.*

Table 4: Minimum Inhibitory concentration of methanolic extracts of *C. volkensii* and *A. setaceous* against Candida albicans. Values are reported as mean inhibition zones (mm) ± SEM of three replicates

Plant Extract	Conc Mg/ml	*C. albicans*
MCVL	50	2.33±0.33
	25	1.33±0.33
	12.5	-
	6.25	-
	3.2	-
MCVS	50	2.33±0.33
	25	1.33±0.33
	12.5	-
	6.25	-
	3.2	-
MCVR	50	2.33±0.33
	25	1.67±0.33
	12.5	-
	6.25	-
	3.2	-
MASA	50	2.67±0.33
	25	2.33±0.33
	12.5	1.67±0.33
	6.25	-
	3.2	-
MASR	50	2.00±0.58
	25	1.33±0.33
	12.5	-
	6.25	-
	3.2	-

Table 5: Minimum Inhibitory concentration of ethanolic extracts of *C. volkensii* and *A. setaceous* against Candida albicans. Values are reported as mean inhibition zones (mm) ± SEM of three replicates

Plant Extract	Conc Mg/ml	*C. albicans*
ECVL	50	1.67±0.33
	25	1.33±0.33
	12.5	-
	6.25	-
	3.2	-
ECVS	50	2.33±0.33
	25	2±0.00
	12.5	-
	6.25	-
	3.2	-
ECVR	50	1.67±0.33
	25	1.33±0.33
	12.5	-
	6.25	-
	3.2	-
EASA	50	2±0.58
	25	1.33±0.33
	12.5	-
	6.25	-
	3.2	-
EASR	50	2.33±0.33
	25	1.33±0.33
	12.5	-
	6.25	-
	3.2	-

albicans. A similar pattern was observed with the antibiotic cotrimoxale which also lacked antifungal activity. Gentamicin had the greatest antifungal activity amongst the three antibiotics showing antifungal activity (inhibition zone 16mm).

The minimum inhibitory concentrations of *C. volkensii* and *A. setaceous* extracts against *C. albicans*

MIC was taken to be the lowest concentration of the extracts that completely inhibited the growth of the microorganism. Data are reported as mean inhibition zones (mm) ± SEM of three replicates. Dichloromethane leaf extracts of *C. volkensii* had a MIC value ranging from 12.5mg/ml to 25mg/ml. With *C. albicans* it had a MIC of 12.5mg/ml. The dichloromethane extracts of *C. volkensii* stem showed MIC of 25mg/ml for *C. albicans.* The dichloromethane extracts of *C. volkensii* root was active at most of the concentrations tested with MIC of 12.5mg/ml against *C. albicans*. Dichloromethane extract of aerial part of *A. setaceous* had MIC of 25mg/ml for *C. albicans*. Dichloromethane root extract of *A. setaceous* had MIC of 25mg/ml for *C. albicans* (Table 3).

For the methanolic extracts, the *C. volkensii* leaf had MIC of 25mg/ml for *C. albicans*. The MIC for the methanolic extract of *C. volkensii* stem was 25mg/ml for *C. albicans*. *C. volkensii* root extracts MIC was 25mg/ml for *C. albicans*. Methanolic extract of *A. setaceous* aerial parts had MIC of 12.5mg/ml for *C. albicans*. *A. setaceous* root extract had a MIC of 25mg/ml for *C. albicans* (Table 4).

The MIC for the ethanolic extracts of *C. volkensii* leaf was

Table 6: Minimum Inhibitory concentration of hexane extracts of *C. volkensii* and *A. setaceous* against test microbes. Values are reported as mean inhibition zones (mm) ± SEM of three replicates

Plant Extract	Conc. Mg/ml	*C. albicans*
HCVL	50	5.33±0.33
	25	3.33±0.33
	12.5	2.67±0.33
	6.25	1.67±0.33
	3.2	-
HCVS	50	1.33±0.33
	25	-
	12.5	-
	6.25	-
	3.2	-
HCVR	50	2.33±0.33
	25	1.67±0.33
	12.5	1.33±0.33
	6.25	-
	3.2	-
HASA	50	3.33±0.33
	25	2.33±0.33
	12.5	-
	6.25	-
	3.2	-
HASR	50	3.67±0.33
	25	2±0.58
	12.5	-
	6.25	-
	3.2	-

Table 6: Minimum Inhibitory concentration of hexane extracts of *C. volkensii* and *A. setaceous* against test microbes. Values are reported as mean inhibition zones (mm) ± SEM of three replicates

Plant Extract	Conc Mg/ml	*C. albicans*
ACVL	50	-
	25	-
	12.5	-
	6.25	-
	3.2	-
ACVS	50	-
	25	-
	12.5	-
	6.25	-
	3.2	-
ACVR	50	-
	25	-
	12.5	-
	6.25	-
	3.2	-
AASA	50	-
	25	-
	12.5	-
	6.25	-
	3.2	-
AASR	50	-
	25	-
	12.5	-
	6.25	-
	3.2	-

25mg/ml for *C. albicans*. For the ethanolic stem extracts of *C. volkensii*, the MIC was 25mg/ml for *C. albicans*. With the root extracts of *C. volkensii*, MIC was 25mg/ml for *C. albicans*. The MIC for ethanolic extracts of *A. setaceous* aerial part was 25mg/ml for *C. albicans* while for the ethanolic root extracts of the same plant, MIC was 25mg/ml for *C. albicans* (Table 5)

The hexane extracts of both *C. volkensii* and *A. setaceous* had low MIC with ranging from 3.2mg/ml to 25mg/ml. For the hexane leaf *C. volkensii* extract, it was 6.25mg/ml for *C. albicans*. With the stem extracts of *C. volkensii*, the extract was only active against *C. albicans* at the highest concentration tested (50mg/ml). For the hexane root extract of *C. volkensii*, MIC was 12.5mg/ml for *C. albicans*. The MIC for hexane *A. setaceous* aerial parts was 25mg/ml for *C. albicans*, while the same values for hexane root extracts of *A. setaceous* was 25mg/ml for *C. albicans* (Table 6).

Aqueous extracts were largely inactive and the extract lacked activity against *C. albicans* at all the tested concentrations (Table7).

DISCUSSION

All the dichloromethane extracts exhibited some form of antifungal activity against *C. albicans* with the dichloromethane root extract of *C. volkensii* showing the highest activity. All the methanolic extracts of the two plants had some form of activity against *C. albicans* albeit a weak one. The ethanolic root extracts of *C. volkensii* demonstrated weak activity against the fungus. The ethanolic root extract of *A. setaceous* was found to show

some form of activity against the fungus *C. albicans*. Ethanolic root extract of *C. volkensii* exhibited activity against the tested fungus. The hexane extracts of all the plant parts showed activity against the fungus *C. albicans* with the *C. volkensii* leaf extract being the most active. All the aqueous extracts lacked antifungal activity against *C. albicans*. *C. albicans* was generally more resistant to most of the standard antibiotics. Could it be possible that the antifungal property of some of the extracts could be attributed to saponins, a special class of glycosides which have soapy characteristics and reported to be active antifungal agent [11] Indeed, from the root of *Asparagus filicinus*, a folk medicine used in treatment of bronchitis, pneumonia and coughs, three saponins have been isolated and demonstrated to possess antiprotozoal and antifungal activity [12]. The interesting aspect is that all the aqueous extracts had saponins (data not shown) yet they failed to exhibit any antifungal activity. This may be an indicator that apart from the saponins, there may be other phytochemicals responsible for the observed antifungal activity in the non aqueous extracts. Extraction of aqueous extracts by boiling could also have altered the structure and hence the activity of the resulting saponins. Runyoro *et al*. [4] showed that aqueous methanolic extract of *Asparagus africanus* Lam aerial parts had some form of activity against *C. albicans* with an inhibition zone of less than 4mm. In the same study, a number of plants belonging to different families were demonstrated to be active against *C. albicans*. *Biden pilosa*, young shoots of *D. minutiflora*, *Sida acuta* and *P. umbellate* showed activity against *C. albicans* [13].

CONCLUSION

The organic extracts of *A. setaceous* and *C. volkensii* have demonstrated negligible antifungal activity while the aqueous extracts completely lacked antifungal activity against *Candida albicans*. The extracts should be subjected to further investigations especially on their immunomodulatory properties which may account for their therapeutical effects as claimed by patients utilizing these extracts.

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