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In vitro Antifungal activity of leaf extracts from *Aloe secundiflora*, *Bulbine frutescens*, *Vernonia lasiopis* and *Tagetes minuta* against *Candida albicans*

Rachuonyo HO^{1*}, Gatheri GW², Ogola PE³, Arika WM³, Wambani JR⁴, Kebira AN¹ and Kamau DN¹

¹Department of Microbiology, Kenyatta University, P.O Box 43 844-00100, Nairobi, Kenya

²Department of Plant sciences, Kenyatta University, P.O Box 43 844-00100, Nairobi, Kenya

³Department of Biochemistry and Biotechnology, Kenyatta University, P.O Box 43 844-00100, Nairobi, Kenya

⁴Department of Medical Laboratory Sciences, Kenyatta University, P.O Box 43 844-00100, Nairobi, Kenya

Abstract

Crude leaf extracts from medicinal plants were screened for *in vitro* antifungal properties against clinical isolate of *Candida albicans*. The antifungal test was carried out using the Kirby Bauer method. All the extracts from the plants tested showed antifungal activity against clinical isolate of *Candida albicans* with the extract from *Vernonia lasiopis* being more active as compared to the others. The minimum inhibitory concentration ranged from 4.0 to 8.9 mg/ml. The standard antifungal used as positive control was fluconazole (15 µg/ml) whereas dimethyl sulphoxide and distilled water were used as negative control. Preliminary screening for the presence of phytochemicals was also done to determine if there was presence of any, flavonoids, saponins, tannins and alkaloids.

Keywords: *Candida albicans*; Phytochemicals; Fluconazole; Dimethyl sulphoxide

Introduction

The use of medicinal plants (herbs) to treat diseases is almost universal among non-industrialized societies, and is often more affordable than purchasing expensive conventional drugs [1]. Medicinal plants are used by almost 80% of the world's population for their basic health care because of their low cost and ease in availability [2]. The use of herbal medicinal plants has always played a positive role in the control or prevention of diseases such as diabetes, heart disorders and various cancers [3]. Medicinal plants have been used in production of various drugs singly or in combination and even as principal raw material for the production of other conventional medicines [4]. *Candida albicans* is a normal micro biota mainly found in the mucosal cavity, vagina and gastrointestinal tract of an individual [5]. They are yeast like fungus that are commensals in healthy humans but can cause systemic infections in immunocompromised individuals [6]. Incidence of fungal infections has led to increased antimicrobial resistance hence making a few antifungal agents active [7,8]. There has been an increase in resistance by *Candida albicans* to conventionally produced antimicrobials recently, leading to the search of a new antifungal agent [9,10]. Herbal extracts from medicinal plants have been used to effectively treat infectious diseases such as candidiasis in developing nations [11-13]. *Tagetes minuta* L. is also known as Southern Cone Marigold, Stinking Roger or black mint [14]. The genus comprises of 56 species which grow either annually or perennially and mostly herbaceous plants [15]. *Tagetes minuta* extracts have been used as medicinal tea in some areas [15]. The total extracts from leaves, flowers, stem and other parts of the plant have shown antibacterial activity as well as antifungal properties [16]. Extracts from the other common species have also been used as medicine in treating various illnesses such as stomach problems and intestinal disorders [17]. The genus *Aloe* belongs to Aloaceae (liliaceae) family which has around 360 to 400 different species [18]. *Aloe secundiflora* leaf components have been found to contain a credited for antibacterial, antifungal, antiviral, antihelmintic and immunomodulatory medicinal properties [19,20]. Its crude extracts have also shown some level of antifungal activity against *Candida albicans* [21]. *Bulbine* is a genus of plants in the family xanthorrhoeaceae and sub family asphodeloideae and its members are well known for their medicinal value [22]. The most common species

is *Bulbine frutescens* which is popularly grown in flower gardens [23]. Many species have bulb shaped tuber and they are chiefly found in South Africa have been found to contain antibacterial and antifungal properties and are used for blood cleansing, treatment of ringworms and gravel rush by some local communities such as the Xhosa [24]. A decoction of bulbs and roots of some of the species has been used in the treatment of some of the venereal diseases in women and stomach upsets [23]. *Vernonieae* is a tribe which has about 1300 species and in the family Asteraceae (Compositae) which mostly contains herbaceous plants [25]. *Vernonia lasiopis* decoctions from the stems and leaves have been traditionally been found to contain antifungal, anthelmintic, antiparasitic and antibacterial activity and has been used by herbalists in East Africa to treat, malaria, worms and gastrointestinal problems [26]. The main aim of the study was to establish the antifungal activity of the crude extracts from the medicinal plants against *Candida albicans*.

Materials and Methods

Plant material collection

The fresh plant material of *Aloe secundiflora*, *Bulbine frutescens*, *Vernonia lasiopis* and *Tagetes minuta* were collected at Kenyatta University Arboretum. Voucher specimens were prepared and deposited in the university herbarium in Plant Sciences Department for future reference. The plants were brought to the laboratory and thoroughly washed in running water to remove debris and dust particles and then rinsed using distilled water and finally air dried.

***Corresponding author:** Rachuonyo HO, Department of Microbiology, Kenyatta University, P.O Box 43 844-00100 Nairobi, Kenya, Tel: +254715407214; E-mail: hilton@yahoo.com

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Preparation of plant extract

The air dried plant materials were tause into powder and soaked in methanol for 72 hours, placed in a Gallenkamp shaker at 65 revolutions per minute. The contents were homogenized and filtered using whatman filter paper no. 1. The filtrate was then poured into a round bottom flask and concentrated using a vacuum evaporator and stored in a labelled amber glass bottle at room temperature away from light and heat before being used for antifungal efficacy test.

Antifungal susceptibility testing

Clinical isolate of *Candida albicans* obtained from Kenyatta University Health Centre Laboratory, Nairobi was used for the susceptibility test. It was tested against methanolic extracts of *Tagetes minuta*, *Aloe secundiflora*, *Bulbine frutescens* and *Vernonia lasiopis*. The pure culture of clinical isolate *Candida albicans* was concentrated by comparing it with a 0.5 McFarland standard. Discs of 6 milliliters were prepared from whatman no.1 filter paper. The discs were sterilized by autoclaving. After sterilization the moisture discs were dried on hot air oven at 50°C [27]. The various solvent extracts discs were prepared were impregnated with the extracts from the highest concentration of 1000 mg/ml to the lowest concentration of 1 mg/ml [2]. The antimicrobial efficacy test was carried out using Kirby Bauer method [18]. Potato dextrose agar (PDA) was used in the spread plate technique where the clinical isolate of *Candida albicans* was spread using sterilized cotton wool swabs and exposed to extracts impregnated discs in milligrams per microliter from *Aloe secundiflora*, *Tagetes minuta*, *Vernonia lasiopis* and *Bulbine frutescens*. The discs were placed with equal distance between them on agar plates inoculated with *Candida albicans*. Positive control discs containing fluconazole were used as well as negative control discs impregnated with dimethyl sulphoxide (DMSO) and distilled water. The plates were incubated at 37°C for 24 hours. Zones of inhibition were measured in millimetres and their average determined. The experiment was carried in duplicates and the diameter of zones of inhibition formed measured. Minimal inhibitory concentration (MIC) was determined using the broth tube method [28]. 100 µl of 250 mg/ml of methanol extract was added to 100 µl of sterile peptone water in the first well of the 96 well micro plate and mixed well with a micropipette. 100 µl of this dilution was transferred subsequently to wells two folding each dilution of the original extract. This was done to the extracts of *Aloe secundiflora*, *Bulbine frutescens*, *Vernonia lasiopis*, and *Tagetes minuta*. An inoculum of 100 µl (0.5 McFarland standard) of overnight clinical culture of *Candida albicans* was added in each of the wells. Triplicate of each micro plate was made and the procedure repeated. The plates were then incubated at 37°C for 24 hours. After incubation 40 µl of 0.2 mg/µl of INT were added in each of the wells and the plates examined after an additional 60 minutes of incubation. Growth was indicated by a red colour (conversion of INT to formazan). The lowest concentration at which the colour was apparently invisible as compared to the next dilution was taken as the minimum inhibitory concentration [29]. Minimum fungicidal concentration (MFC) was determined according whereby; 100 µl of suspension was taken from micro plate wells that demonstrated no growth and inoculated on agar plates. The plates were incubated at 37°C for 24 hours. In the case where there was fungal growth and also not greater than the minimum inhibitory concentration was used to determine maximum fungicidal concentration [29].

Phytochemical analysis

Presence of saponins, tannins, flavonoids and alkaloids in the crude extract were determined according to the method defined [30].

Tannins: Each of the extracts was weighed to 0.5 mg and dissolved in 1 ml of distilled water. Filtration was carried out after 2 ml of FeCl₃ was added. If there was presence of a blue or black precipitate then it indicated the presence of tannins.

Flavonoids: Each of the extracts was weighed to 0.5 mg and dissolved in 1 ml of ethanol and filtered. 2 ml of 1% HCL and magnesium ribbon was added to the filtrate. If there was formation of a pink or red colour it indicated the presence flavonoids.

Alkaloids: Each of the extracts was weighed to 0.5 mg and dissolved in 1 ml of methanol and filtered. 1% HCL was added to the filtrate and the solution heated. Mayor's reagent was added drop wise and if there was formation of any colored precipitate it indicated the presence of alkaloids.

Saponins: Each of the extracts was weighed to 0.5 mg and dissolved in 1 ml of methanol and filtered. Distilled water was added and shaking done for a few minutes. If there was persistence frothing then it indicated the presence of saponins.

Results and Discussion

All the extracts from the plant leaves showed a considerable level of antifungal activity against clinical isolate of *Candida albicans* [21,31,32]. The plant extract from *Vernonia lasiopis* proved to be more active against *Candida albicans* in low concentrations whereas *Aloe secundiflora* was less active. The standard antifungal control used (fluconazole 15 µg/ml) formed a desirable zone of inhibition of 28 mm. Among the plant extracts *Vernonia lasiopis* produced the highest zone of inhibition against *Candida albicans* approximately 20 ± 1.86 mm in diameter. There were no zones of inhibitions formed by the negative controls used (dimethyl sulphoxide and distilled water) (Table 1). The results obtained from the study showed that the antifungal activity against *Candida albicans* by the plants extracts may be due to the presence of phytochemicals in the extracts namely flavonoid, tannins, saponins and alkaloids (Table 2). Tannins bioactive components such as catechin and pyrogallol have been known to contain antifungal capabilities and they are toxic to most microbes [33]. The flavonoids are also known for their antimicrobial activity of inhibiting the synthesis of the nucleic acids, tempering with integrity of the cytoplasmic membrane function and the energy metabolism process [34].

Plant extracts	MIC (mg/ml)	MFC (mg/ml)	Zone of Inhibition (mm)
<i>Tagetes minuta</i>	6.2	8.7	15 ± 1.68
<i>Aloe secundiflora</i>	8.1	9.0	17 ± 0.38
<i>Bulbine frutescens</i>	6.9	8.0	18 ± 0.21
<i>Vernonia lasiopis</i>	4.0	5.5	20 ± 1.86
Fluconazole	-	-	28
DMSO ₄	-	-	-
Distilled water	-	-	-

Key: DMSO₄ - Dimethyl sulphoxide and Distilled water (negative control), Mean of duplicates ± Standard error, MIC - Minimum inhibitory concentration, MFC - Minimum fungicidal concentration, Fluconazole 15µg/ml (positive control).

Table 1: Antimicrobial activity of the plant leaf extracts against *Candida albicans*.

Name of test	Plants leaf extracts			
	<i>T. minuta</i>	<i>A. secundiflora</i>	<i>B. frutescens</i>	<i>V. lasiopis</i>
Saponins test	+	+	+	+
Tannins test	+	+	+	+
Alkaloids test	+	+	+	+
Flavonoids test	+	+	+	+

Key: (+) present

Table 2: Phytochemical tests on the plant extracts.

Conclusion

In conclusion, the extracts from the plants proved to be active against *Candida albicans*. This shows that the extracts from the plants could be used as an antifungal agent against *Candida albicans* and other fungal pathogens. The bioactive components of the screened phytochemicals need to be determining so as to know the compounds responsible for the antifungal activities. This will aid as a natural source of treating fungal infections and also provide raw materials that can be used in the manufacturing of conventionally used antifungal agents.

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