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In vitro antifungal activity of *Embelia schimperi* (Vatke) and *Conyza floribunda*

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Abstract

The study aimed to investigate antifungal activity of 2 medicinal plants namely *Embelia schimperi* and *Conyza floribunda* used in Tanzania to treat infectious diseases. Micro-dilution method was used to evaluate antifungal activity of plant extracts against *Cryptococcus neuformans* and *Candida albicans*. The study revealed that tested extracts have different levels of antifungal activity with minimum inhibition concentration (MIC) range of 0.78 mg/mL to >25 mg/mL. The *Conyza floribunda* root chloroform extract (CFRC) inhibited both *C. albicans* and *C. neoformans* at MIC value of 0.78 mg/mL. *Conyza floribunda* leaf ethyl acetate (CFLE) and *Embelia schimperi* stem methanolic extract (ESSM) exhibited antifungal activity at MIC value of 0.78 mg/mL against *C. neoformans*. The results obtained suggest that CFRC, CFLE and ESSM is a potential source of antifungal secondary metabolites.

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Introduction

Over the last two decades, there have been increases in reported cases of opportunistic fungi infections worldwide from both immune-compromised patients, hospitalized serious patient as well as healthy individuals (Walsh and Groll, 1999; Eggimann *et al.,* 2003; Warnock, 2004). Factors identified as weak immune system, nutrient deficiency, prolonged use of antibiotics, organ transplant, malignancies, use of catheters, immunosuppressive agents, hormone disorder and cancer therapy have been considered to accelerate the problem (Eliopoulos *et al*., 2002, Martins *et al*., 2014;). *Candida albicans* is the most common opportunistic fungi which accounts for 50% - 90% of human candidiasis, followed by *Cryptococcus* which causes symptomatic Cryptococcosis (Vázquez-González *et al.,* 2013). Despite the facts that *Candida albicans* is a normal flora in genitourinary and gastrointestinal tracts of human body, they can cause serious infections such as endocarditis, meningitis and peritonitis in immune-compromised patients (Martins *et al*., 2014). It is estimated that; there are more than 400,000 cases of life threatening infections from *C.albicans* each year globally (Brown *et al*., 2012). Both *Candida* and *Cryptococcus* have continued to cause mortality and morbidity to HIV/AIDS patients, since the emergence of HIV. Cryptococcal meningitis has become one of the problematic diseases for HIV patients. Although antiretroviral therapy (ART) has reduced the problem in developed countries, but for the developing countries where there is a high number of HIV patients; Cryptococcal meningitis remains as one of the most problematic HIV-related opportunistic infections (Sloan and Parris, 2014; Jackson and Hosseinipour, 2010). It is approximated that each year there is about one million cases and half million deaths from Cryptococcal meningitis among HIV patients globally (Park *et al*., 2009). In Africa the Cryptococcal meningitis remains a big challenge as it is responsible for about 10% to 20% deaths among HIV-infected patients (Jackson and Hosseinipour, 2010). The available antifungal drugs suffer drawbacks such as side effect induced by antifungal drugs to patients and resistance reported by pathogenic fungi (Perfect and Cox, 1999; Organization, 2014). Therefore, there is need to search for a new antifungal agent from medicinal plants that could possibly display unique mechanism of actions. This paper reports antifungal activity of *Embelia schimperi* and *Conyza floribunda* against *Candida albicans* and *Cryptococcus neoformans*.

Materials and methods

Sample collection and preparation

Fresh sample of *Embelia schimperi* (leaves, stem and fruits) and *Conyza floribunda* (leaves, stem and roots) were collected in February 2015 from Arusha and Kilimanjaro regions in Tanzania. Plants were identified by Mr Emmanuel Mboya from National Herbarium of Tanzania (NHT) at Tropical Pesticides Research Institute (TPRI). The plant specimens for *Embelia schimperi* and *Conyza floribunda* coded as ES-EG12 and CF-EG15 respectively were kept at Nelson Mandela African Institution of Science and Technology. The samples were air dried at room temperature for three weeks then pulverized to fine powder and stored in airtight containers for further extraction process.

Preparation of extracts

Powdered 250g of each plant part was sequentially extracted using chloroform, ethyl acetate and methanol for 48hrs. The extracts were filtered by What man no.1 filter paper and solvents were removed by vacuum through rotary evaporator. The obtained extracts were stored in refrigerator at 4°C.

Test microorganisms

Two species of fungi, *Cryptococcus neuformans* (clinical isolate) and *Candida albicans* (ATCC90028) were obtained from Muhimbili University of Health and Allied Sciences (MUHAS).

Antifungal activity assay

The antifungal activity of extracts were conducted according to Eloff (1998) with minor modification. The 100 mg of extracts were dissolved in 1 mL of Dimethyl Sulfoxide (DMSO) to make a stock solution of 100 mg/mL. The 50 µL of Saborauds dextrose

broth were initially placed in each plate wells, followed by adding of 50 µL of plant extracts into the wells of the first rows, which made a total volume of 100 µL for each well of the first rows. Subsequently mixing was conducted in the first rows, 50 µL of mixture from each of the first rows were shifted to the second rows and the process was repeated downward along the column until the last rows where 50 µL of the mixture were discarded. 0.5 Mac Farhland standard turbidity were prepared, where 50 µL of microbes (fungi) were added into each plate. For positive control 50 µL of Fluconazole were used, while 50 µL of DMSO were added into negative control row and the row containing fungi and Saborauds dextrose broth were used to monitor the growth of fungi. After that the plates were incubated at 37°C for 24hrs. MICs of each extract were determined by adding 30µL of 0.02% *p*-iodonitrotetrazolium (INT) chloride dye in each well followed by incubation for 1hr at 37°C. Fungi growth was indicated by a change of color to pink. The wells which had no change in color after the addition of INT indicated no growth and they were taken as Minimum Inhibitory Concentration (MIC).

Results

In the present investigation, the antifungal activity of 2 medicinal plants was determined at different concentrations. The results are summarized in Table 1.

Table 1. Antifungal activity of *Embelia schimperi* and *Conyza floribunda*.

Plants	Minimum Inhibitory Concentration (MIC) mg/mL			
	Parts	Solvent	*C.albicans*	*C.neuformans*
C. floribunda	Leaf	chloroform	1.56	3.12
		ethyl acetate	1.56	0.78
		methanol	1.56	1.56
	stem	chloroform	1.56	3.12
		ethyl acetate	6.25	3.12
		methanol	1.56	3.12
	root	chloroform	0.78	0.78
		ethyl acetate	NT	NT
		methanol	6.25	3.12
E. schimperi	Fruits	chloroform	>25	>25
		ethyl acetate	>25	>25
		methanol	>25	>25
	Leaf	chloroform	>25	12.5
		ethyl acetate	>25	25
		methanol	6.25	6.25
	Stem	chloroform	12.5	>25
		ethyl acetate	NT	NT
		methanol	3.12	0.78
Fluconazole			0.19	0.19

Key: NT-Not tested.

The tested extracts displayed varied antifungal potencies with minumum inhibitory concentration (MIC) range of 0.78 mg/mL to >25 mg/mL. The highest antifungal activity was exhibited by *Conyza floribunda* extracts which demonstrated MIC range of 0.78 mg/mL to 6.25 mg/mL. *Embelia schimperi* had wider range of antifungal activity with MIC range of 0.78 mg/mL - > 25 mg/mL. *Conyza floribunda* root 47 hloro form was the most active, exhibited antifungal activity with MIC value of 0.78 mg/mL against *Candida albicans* and *Cryptococcus neuformans*. *Conyza floribunda* leaf ethyl acetate was the second, it inhibited the growth of *C. albicans* and *C. neuformans* with MIC values of 1.56 and 0.78

mg/mL respectively. *Embelia schimperi* stem methanolic extract was the most active among the tested *E. schimperi* extracts. It inhibited the growth of *Candida albicans* and *Cryptococcus neuformans* with MIC values of 3.12 and 0.78 mg/mL respectively. The rest of the *E. schimperi* exracts displayed MIC range of 12.5 - ≥ 25 mg/mL.

Discussion

The current study has found that the extracts of *C.floribuda* possess strongest activity against *C. albicans* and *C. neoformans* compared to other extracts. The present findings are in line with previous investigation conducted from *C.floribunda* grown in Kenya indicated that it possesses antifungal activity against *Candida albicans*, *Trichophyton mentagrophytes* and *Microsporum gypsium* (Manguro *et al.,* 2010). The phytochemical analysis from previous study revealed that the principle compounds from *C.floribunda* are (24S)-ethylcholesta-5, 22E, 25-trienene 3-O-glucopyranoside, cyasterone, 3-oxofriedooleanane and betulinic acid, exhibited antibacterial and antifungal activities (Manguro *et al.,* 2010). In addition previous investigation has reported antifungal and antibacterial properties from *Conyza bonariensis* which belong to the same genus with *Conyza floribunda* (Shah *et al.*, 2013). The current and previous results indicate that member of genus Conyza are very rich in medicinal value against both fungi and bacteria.

The stem bark, leaves, root and fruits of *Embelia schimperi* on the other hand are used among African communities for management of various illnesses (Kokwaro, 1993). For instance, in Tanzania concoction resulted from crushed leaves are used as disfectant while fruits are used as antihelmentic by the Masai communities. In Ethiopia fruits are also used as antihelmentic (Debebe *et al.,* 2015). The pytochemical investigation of *E.schimperi* growing in Kenya revealed the presence of benzoquinones and oleanane triterpenes. These compounds were established to exhibit antibacterial activities against *Rhodococcus* sp, *Escherichia coli*, *Pseudomonas putida*, *Bacillus subtilis* (Machocho *et al.*, 2003). However, they were inactive against pathogenic fungi in which the disc diffusion method was employed (Chepkwony *et al.,* 2011, Awino *et al.*, 2008). Apparently the current study has revealed methanolic stem bark extract exhibited strong activity against *C. albicans* and *C. neoformans*. It is therefore suggestive that geographic separation of *Embelia schimperi* coupled with local microbial challenges might have influenced the production of unique secondary metabolites with antifungal properties.

Conclusion

The tested plant extracts exhibited a different level of antifungal activities which validate the tradition uses of these plants in management of infectious diseases. The strong antifungal activities shown by *Conyza floribunda* toward *C. neuformans* and *C. albicans* indicate that in future this plant can be potential source to develop antifungal drugs.

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