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Antifungal Activity of *Ziziphus mucronata* and *Erythrina abyssinica* Bark Crude Extracts on *Cryptococcus neofomans and Candida albicans* Species

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Authors' contributions

This work was carried out in collaboration between all authors. Author TEM designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors TEM, FTT and JC managed the literature searches, analyses of the study performed the spectroscopy analysis. Authors JC and FTT managed the experimental process and author FTT identified the species of plant. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The study aims at extraction, characterization and identification of bioactive compounds with antifungal properties from the plant species *E. abyssinica* and *Z. mucronata* against *Cyptococcal neoformans* and *Candida albicans.*
Study Design: Phytochemical screening of crude plant extracts and determination of their minimum inhibitory concentration using Agar disc diffusion method.
Place and Duration of Study: Pharmaceutical Technology Lab, Harare Institute of Technology, February 2014 and May 2015.
Methodology: In this study crude bark extracts of *Ziziphus mucronata* and *Erythrina abyssinica*

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from Zimbabwe were test for antifungal activity using the disc diffusion method against *Cryptococcus neoformans*, *Candida albicans* with fluconazole as the positive control. Phytochemical screening tests on the crude extracts were carried out to identify phytoconstituents.
Results: Effective minimum inhibitory concentrations against *C. albicans* were found to be 20% w/v and 10%w/v for *Ziziphus mucronata* and showed moderate growth at a 5% w/v concentration. *Erythrina abyssinica* had effective minimum inhibitory concentrations at 25% w/v and 12.5% w/v with moderate fungal growth observed at 6.25% w/v. The same concentration ranges for both crude extracts showed similar antifungal activity for *C. neoformans*. Both crude bark extracts tested positive for tannins, saponins, flavonoids and alkaloids which contribute to the antifungal activity of the plant species.
Conclusion: *Candida albicans* and *Cryptococcus neoformans* are the most common fungal species that cause opportunistic infections to occur in HIV and AIDS. The effects produced by these plants have proven that they can be used to develop pharmaceutical agents alleviate the symptoms associated with these infections. The crude plant extracts were found to be active against *Candida albicans* and *Cryptococcus neoformans*. Both crude bark extracts tested positive for tannins, saponins, flavonoids and alkaloids which contribute to the antifungal activity of the plant species.

Keywords: Ziziphus mucronata; Erythrina abyssinica; cryptoccocal meningitis; Candida albicans; anti-fungal.

1. INTRODUCTION

Cryptococcal meningitis is an opportunistic central nervous system (CNS) infection caused by *Cryptococcus neoformans*, a ubiquitous encapsulated fungus. It often develops in immune-compromised people, especially AIDS patients. It is the third most frequent opportunistic infection of the central nervous system in HIV-infected individuals. In patients with HIV/AIDS, the yearly incidence rate is between 2 and 7 cases per 1,000 people. It is far more common in HIV/AIDS patients in sub-Saharan Africa, where these patients have a mortality rate that is estimated to be 50 to 70% [1–3].

Cryptococcus neoformans, a type of yeast found worldwide can cause pulmonary and central nervous system (CNS) infections that can potentially spread to other areas of the body. This infection is called *cryptococcosis*. HIV/AIDS patients are especially vulnerable to developing the infection. If the infection spreads from the lungs to the CNS (brain and spinal cord) of an HIV patient, the condition is considered an AIDS-defining illness. This means the patient's condition has progressed to AIDS [1–3].

Most infections develop after the yeast has been inhaled into the lungs. The fungus strongly resists phagocytosis. This means the immune system cells have to work hard to engulf the organism. Cryptococcosis usually starts with a pulmonary (lung) infection, which then spreads to the CNS. If left untreated, the infection may continue to spread to other organs in the body, including the skin, prostate and medullary cavity of the bones.

It may also complicate organ transplantation, reticuloendothelial malignancy, corticosteroid treatment, or sarcoidosis. Common symptoms of pulmonary involvement include fever, general feeling of discomfort, dry cough, pain in the membrane surrounding the lungs, and rarely, hemoptysis (blood in sputum). The presentation in cryptococcosis varies with the site of infection and the patient's immune status [4,5].

Globally, it has been estimated that approximately 957,900 cases of cryptococcal meningoencephalitis occur each year, resulting in more than 600,000 deaths. The region with the highest number of estimated cases in 2006 was sub-Saharan Africa (720,000 cases; range, 144,000 to 1.3 million), followed by South and Southeast Asia (120,000 cases; range, 24,000 to 216,000) [1,2]. Although the incidence of cryptococcal meningoencephalitis has declined in patients who have access to antiretroviral therapy (ART), cryptococcal disease remains a leading cause of mortality in the developing world where access to ART is limited. A low CD4 cell count is the main predictor of risk of cryptococcal meningoencephalitis; the vast majority of cases occur among AIDS patients with a CD4 count <100 cells/microlitre [6,7].

The disease epidemic is commonly managed by initiation of ART to boost CD4 cell count and reduce risk of occurrence due to poor immune response from the hosts' body. Treatment or

prophylaxis of cryptoccocal meningitis cases may also be employed by the use of amphotericin B (Ambisome©) or fluconazole. Due to its excellent penetration into cerebrospinal fluid, fluconazole is the agent of choice for the treatment of cryptococcal meningitis and for prophylaxis of cryptococcosis in AIDS patients. Although fluconazole is generally less effective than either ketoconazole or itraconazole against nonmeningeal coccidiomycosis, it is the preferred treatment for coccidioidal meningitis [1,3,7].

Candida albicans are a fungal species responsible for yeast infections of the vaginal and oral mucosa especially as an opportunistic infection in diseases like HIV/AIDS and uncontrolled diabetes. The species will normally exist of the human skin but weakening immune system or homeostatic control for the body may lead to their overgrowth and subsequent infection [8]. Oropharyngeal candidiasis develops in the mouth and throat and can be invasive leading to ulceration of the upper gastrointestinal tract and systemic infection from the candida species. Vaginal candiasis is often associated with white discharge, irritation and burning sensation [8]. It occurs as a result in the imbalance of vaginal bacterial flora due to a weakened immune system, imbalance between estrogen and progesterone hormones in the menstrual cycle and poor sugar dietary control.

Antifungal drug resistances have become a major problem in late-stages of AIDS patients. About 5-10% of oral candidiasis is now intractable with the antifungal drug fluconazole and up 33% of oral *Candida* isolates from AIDS patients are resistant to fluconazole. Resistance appears to be correlated with the total cumulative dose, which is a reflection of long-term prophylaxis or therapy [1,2].

Antifungal drug resistance has recently been identified in systemic candidiasis from bone marrow transplant patient and is starting to appear in strains of *Cryptococcus neoformans* from AIDS patients with a history of cryptococcal infection who are prophylaxed to prevent reactivation [4]. Due to the limitations of conventional drugs and high rate of mortality in AIDS patients due to cryptoccocal induced infections indigenous knowledge systems can be explored to screen for plants with traditional claims of antimicrobial activity and help develop newer drug agents with greater access for patients and lower cost of treatment [1,9,10].

Ziziphus mucronata is a dense leafy small to medium-size tree, which is 2-5 m tall. This plant belongs to family *Rhamnaceae* and genus *Ziziphus*. *Z. mucronata* is found in tropical area and along moist riverbanks. The Latin name '*Ziziphus'* means thorny and '*mucronata'* refers to the pointed leaves of this species. The genus *Ziziphus* is of some historical importance. It is believed that Christ's crown was made from Z. *spina-christi* Willd., a species which closely resembles *Z. mucronata* but grows from central Africa northwards [9]. This plant is widely distributed in southern Africa. In Namibia, *Z. mucronata* is widespread throughout Namibia, except in the Namib Desert, north-west of Etosha and a few areas in the southeast [11] recorded *Z. mucronata* to be the most widely distributed tree in South Africa. This plant flowers between March and June producing small, round, red-brown berries with thin dry flesh which appears all year round but is mostly common between December and June [11,12]. Local names for *Z. mucronata* includes buffalo thorn (English), *Omukaru* (Herero), (Damara/Nama), *omukekete* (Kwanyama), *mukalu* (Silozi) and *omusheshete and muchecheni* in shona.

The leaves and fruits of *Z. mucronata* are a source of food for livestock, especially goats. The fruits are also edible to humans. In Ovamboland, the dried fruits are fermented and distilled to make alcoholic liquor called *ombike* (Oshindonga), which is sold by the locals, serving as a source of income. The woods of *Z. mucronata* are a fuel source in rural areas [11,9].

Buffalo thorn is widely known as a medicinal plant. Its leaves and roots have been used to treat diarrhoea, tumor, cough, chest complaints, dysentery, sores, glandular swellings, skin diseases, open and swollen wounds, ear inflammation, asthma, syphilis, gonorrhea, lumbago, measles, as well as rheumatic pains and fever [10,9].

Other reports include the use of the roots by the Bambara and Malinke tribes in Tanzania for the psychiatric treatment, and the use of the leaf juice to prevent abortion.

Several other *Ziziphus* species are known for their medicinal properties. Strong sedative effects have been reported from *Z. vulgaris* and *Z. jujube* [11]. *Z. mauritiana* and *Z. abyssinica* have a range of medicinal uses which include wounds, fever, abdominal pains, venereal

diseases and diarrhoea treatment; diuretic effects has also been reported for the two plants, just to mention a few [9,11].

A number of active ingredients have been isolated from leaves, roots, stems and seeds of *Ziziphus* species. Among them are cyclopeptide alkaloids, sterols, triterpenoids, saponins and tannins.

The genus *Erythrina*, a member of the family *Fabaceae* and subfamily *Papilionideae* comprises of over 110 species of trees, shrubs and herbicaceous plants that are widely distributed throughout the tropical warm regions of the world. *Erythrina abyssinica* is medium-sized tree, usually 5-15 m in height, deciduous, thickset, with a well-branched, rounded, spreading crown ;trunk short; bark yellow-buff when fresh, otherwise grey-brown to creamy brown, deeply grooved, thickly corky and often spiny; when damaged the tree exudes a brown, gummy sap [9].

Seven species of *Erythrina* are found in eastern and southern Africa, that is, *E. Caffra* T*, E. decora, E. humeana*, *E. livingstoniana, E. lysistemon, E. abyssinica and E. Latissima* [9]. *Erythrina* species are known to produce flavonoids, isoflavonoids, pterocarpans, terpenoids, saponins and alkaloids. The alkaloids produced are of the erythrina type, some of which has been shown to have curare-like activity on the central nervous system [13,9].

1.1 Traditional Uses

Traditionally, Pounded parts are used in a steam form in Kenya to treat diseases such as anthrax, and the bark is boiled with goat meat for treating gonorrhoea. The bark of the green stem may also be pounded and then tied into a fine piece of cloth and the liquid from it squeezed into the eyes to cure inflammation of the lids [9]. The bark may be roasted until black, powdered, and applied to burns and general body swellings. A decoction is taken orally as an antihelmintic and to relive abdominal pains. The Powdered root is used for syphilis, anthrax, and snakebites. The leaves have been used to cure skin diseases in cattle. The bark of young stems is used to treat trachoma which is an infectious disease of the eyelid caused by *Chlamydia trachomatis* [10,9]. It is also roasted and applied to burns and swellings. The following flavonoids have been isolated from the roots of *E. abyssinica*; abyssinone, cristacarpin, erythrabyssin II, phaseollidin and phaseollin.

1.2 Mechanisms of Action of Bioactives

Ziziphus mucronata and *Erythrina abysnicca* act against bacteria and fungi due to the inhibition of cell wall through pore formation in the cell and leakage of cytoplasmic constituents by the bioactive components of the extract [10,9].

Phytochemical compounds such as tannin coagulate the wall proteins, saponins facilitated the entry of toxic material or leakage of vital constituents from the cell. Flavonoids inhibit the activity of enzymes by forming complexes with fungal cell walls, extracellular and soluble proteins, more lipophilic flavonoids disrupt cell wall integrity of microbial membranes at low concentrations [14,9].

2. METHODOLOGY

2.1 Extraction of Plants Material

2.1.1 Materials

Weighing balance, rotary evaporator, methanol, acetone, flasks, polyfilm, separating funnels and filter papers.

2.1.2 Methods

Plant samples were collected from Chikurubi Maximum Prison on the 11th of February 2015. The samples were authenticated at the herbarium at Harare Botanical gardens. About 100 g of *Ziziphus mucronata* bark extract was soaked in 1000 mls of hexane for 72 hours. A mass of 200 g *Erythrina abyssinica* bark extracts was soaked in 1000 mls of hexane for 72 hours. The bark residues were separated from the solvents by filtration using a filter paper, funnel and collecting bottle. The solutions with the crude extracts were then put in the rotary evaporator. A rotary evaporator was used to concentrate the crude extracts separately. The extracts were separately air dried in a fume to remove excess solvent. The dried crude extracts were crushed into powder and weighed on a balance. They were put in sample bags, a labelled and kept for later use.

2.2 Determination of the MIC of Crude Extracts

Agar diffusion method.

2.3 Preparation of Fungal Cultures

2.3.1 Materials

Petri dishes, potato dextrose agar, sabouraud agar, nutrient broth, C*ryptococcus neoformans, candida albicans,* fluconazole and distilled water.

2.3.2 Methods

Fungal strains were obtained from South Africa by the Harare Institute of Technology in 2012. Three different culture media were prepared.

The first medium was a nutrient broth which was prepared by dissolving 13 g of nutrient broth powder in 200 ml of water.

The mixture was dissolved on a hot plate and sterilised by autoclaving. The solution was cooled to room temperature. The fungal strain was taken and dropped into the solution and incubated at 37℃ for 72 hours. Sabouraud agar was also prepared by dissolving 6,5 g of sabouraud powder in 200 mls distilled water. The solution was mixed on a hot plate, sterilised and poured in sterile petri dishes before it had cooled and solidified. Potato dextrose agar which is the last medium was prepared by dissolving 6.5 g of potato dextrose powder in 200 mls of distilled water. The solution was then sterilized and poured in sterile plates before solidifying. The fungi were swabbed on one plate sabouraud agar plate and one potato dextrose plate. The rest were kept for later use. The cultured plates were incubated for 72hours at 37℃.

2.4 Preparation of Solutions by Serial Dilutions

2.4.1 Methods

Fluconazole solution was prepared by dissolving 1 gram of fluconazole powder in 5 mls of distilled. This solution was the positive control of the experiment.

The stock solution for *Ziziphus mucronata* was prepared by dissolving 1 g of the crude plant extract in 5 mls distilled water. The serial dilutions for this stock solution were done as shown in the following Table 1. The discs were prepared and soaked in the respective solutions.

The stock solution for *Erythina abyssinica* was prepared by dissolving 1 g of crude extract in 4 mls of distilled water.

The serial dilutions to prepare different solutions from the stock solution were prepared as shown in the Table 2. The discs were prepared and soaked in the different solutions for use in determining the MIC.

Serial dilutions were also made using the crude extracts separately, starting with a stock solution of 20% for the *Ziziphus mucronata* and 25% for the *Erythrina abyssinica* extract. The disc diffusion method was used to determine the MIC of the different strengths of the extracts. The plates were incubated at 37℃ for 72 hrs. The different concentrations made were as shown in the Table 2.

2.5 Testing for the Presence of Bioactive Compounds

2.5.1 Sodium hydroxide test for flavonoids

About 5 mg of the compound is dissolved in water, warmed and filtered. 10% aqueous sodium hydroxide is added to 2 ml of this solution. This produces a yellow coloration. A change in color from yellow to colorless on addition of dilute hydrochloric acid is an indication for the presence of flavonoids.

2.5.1.1 Tannins

Two methods were used to test for tannins. First, about 1 ml of the ethanol extract was added in 2 ml of water in a test tube. 2 to 3 drops of diluted ferric chloride solution was added and observed for green to blue-green (cathechic tannins) or a blue-black (gallic tannins) coloration.

Secondly, 2 ml of the aqueous extract was added to 2 ml of water, a 1 to 2 drops of diluted ferric chloride solution was added. A dark green or blue green coloration indicates the presence of tannins.

2.5.1.2 Test for saponins

To 1 ml of aqueous extract was added few volume of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth for 20 min.

Table 1. Preparation of *Z. mucronata* solutions

2 ml 20% solution +2 ml distilled water = 4 mls 10% solution
2 ml 10%solution +2 ml distilled water = 4 mls 5% solution
2 ml 5% solution + 2 ml distilled water = 4 mls 2,5% solution

2.5.1.3 Test for alkaloids

Three methods were used to test for alkaloids.

- First, evaporate 10 ml of concentred etheric solution, the dry residue was added to 1.5 ml HCl (2%) acid solution. After that, 1 to 2 drops of Mayer's reagent and Wagner was added, and the yellow- white precipitate indicates the presence of the alkaloidal base.
- Second, evaporate 20 ml of ethanol extract, the dry residue dissolved in 5 ml of HCl (2N) and filtered. A few drops of Mayer's reagent and Wagner was added, the presence of precipitate indicates the alkaloids.
- Three, to 15 ml of the aqueous extract was added 2 ml of a ammonium hydroxide, NH4OH 10%v/v solution (pH=7). The alkaloids were extracted 3 times with 10 ml chloroform. The chloroform layer was washed 3 times with 2 ml of hydrochloric acid 10%v/v solution. This was divided into two portions. Mayer's reagent was added to one portion and Wagner's reagent to the other. The formation of a brown or white precipitate was regarded as positive for the presence of alkaloids.

3. EXPERIMENTAL RESULTS AND ANALYSIS

3.1 Extraction of Plants Material

3.1.1 Results

From the first extractions, the percentage yields for each of the plants were calculated as follows:

3.2 Determination of the MIC of Crude Extracts

After 72 hours, the plates were observed. The plates that were cultured using the agar to agar transfer method which did not produce desired results. The growth of the fungi on those plates was not measurable. The plates that were cultured using the broth to agar method showed significant growth that could be measurable. The agar plates that were made using sabouraud agar produced a marked growth as compared to those that were made using potato dextrose agar.

3.3 Preparation of Fungal Cultures

From the two types of agar that were used, sabouraud agar was the most effective for the fungal strains compared to those in potato dextrose agar.

Table 2. Preparation of *E. abyssinica* solutions

2 ml 25% solution +2 ml distilled water = 4 mls of a 12.5% v/v solution
2 ml 12.5%solution +2 ml distilled water = 4 mls of a 6.25%v/v solution
2 ml 6.25% solution + 2 ml distilled water = 4 mls of a 3,125%v/v solution

Table 3. *Ziziphus mucronata* yield

Mass of *Z. Mucronata* plant material	100 g
Mass of crude extract	5 g
% yield	$(\text{mass of crude extract} \div \text{mass of plant material}) \times 100$ = (5 g/100 g) x 100 = 5% yield

Table 4. *Erythrina abyssinica* yield

Mass of *E. abyssinica* plant material	200 g
Mass of crude extract	4 g
% yield	$(\text{mass of crude extract} \div \text{mass of plant material}) \times 100$ = (4 g/200 g) x 100 = 2% yield

3.4 Preparation of Solutions by Serial Dilutions

Table 5. Difference strength of each extract solution

	Name of solution	Strength in %w/v
Negative control	Distilled water	
Positive control	Fluconazole	20%
Test solution1	*Ziziphus mucronata*	20%
	Ziziphus mucronata	10%
	Ziziphus mucronata	5%

	Name of solution	Strength in %w/v
Test solution 2	*Ziziphus mucronata*	2.5%
	Erythrina abyssinica	25%
	Erythrina abyssinica	12,5%
	Erythrina abyssinica	6.25%
	Erythrina abyssinica	3,125%

Fig. 1. MIC determination by disc diffusion method

3.4.1 MIC in *Cryptococcal neoformans*

Table 6. Effects of *Z. mucronata* extracts, fluconazole and water on *C. neoformans*

Concentrations	Level of fungal growth	Zone of inhibition
Distilled water	Marked growth	0 mm
Fluconazole 20% solution	No growth	5 mm
20% *Ziziphus mucronata*	No growth	6±0.5 mm
10% *Ziziphus mucronata*	No growth	4 mm
5% *Ziziphus mucronata*	Moderate growth	2,5 mm
2.5% *Ziziphus mucronata*	Minimal growth	1.5 mm

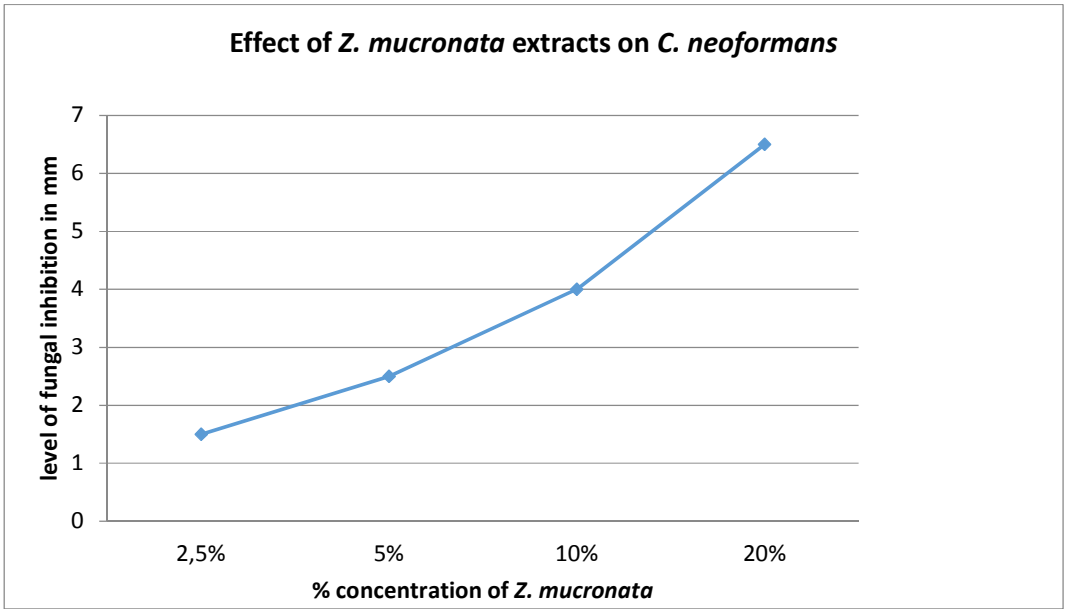

Fig. 2. Effect of *Z. mucronata* extracts on *C. neoformans*

Table 7. Effects of *E. abyssinica* extracts, fluconazole and water on *C. neoformans*

Concentrations	Level of fungal growth	Zone of inhibition
Distilled water	Marked growth	0 mm
Fluconazole 20% solution	No growth	5 mm
25% *E. abyssinica*	No growth	3 mm
12.5% *E. abyssinica*	No growth	1±0,5 mm
6.25% *E. abyssinica*	Moderate growth	1 mm
3.125% *E. abyssinica*	Marked growth	<1 mm

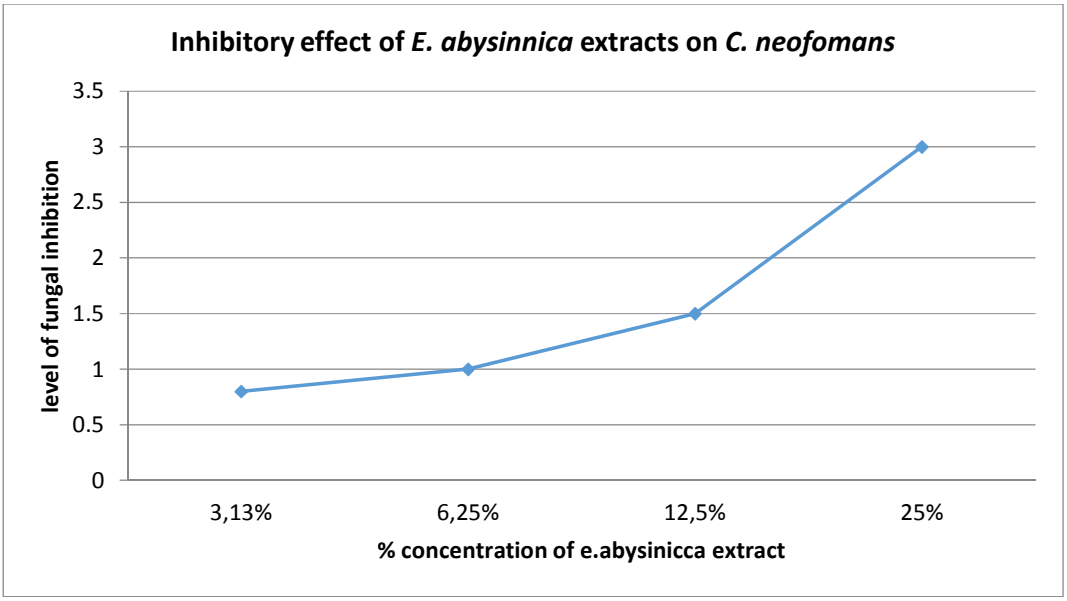

Fig. 3. Effects of *E. abysinnica* on *C. neoformans*

3.4.2 MIC in *Candida albicans*

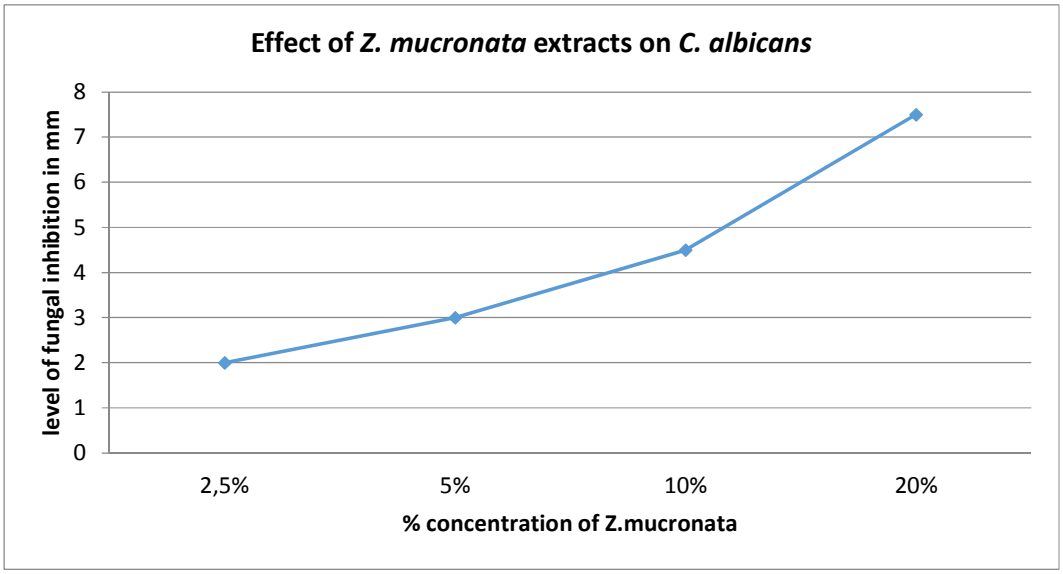

Fig. 4. Effect of *Z. mucronata* extracts on *C. albicans*

Table 8. Effects of *Z. mucronata* extracts, fluconazole and water on *C. albicans*

Concentrations (w/v)	Level of fungal growth	Zone of inhibition
Distilled water	Marked growth	0mm
Fluconazole 20% solution	No growth	6mm
20% *Ziziphus mucronata*	No growth	7,5mm
10% *Ziziphus mucronata*	No growth	4,5mm
5% *Ziziphus mucronata*	Moderate growth	3mm
2.5% *Ziziphus mucronata*	Minimal growth	2mm

Table 9. Effects of *E. abyssinica* extracts, fluconazole and water on *C. albicans*

Concentrations (w/v)	Level of fungal growth	Zone of inhibition
Distilled water	Marked growth	0 mm
Fluconazole 20% solution	No growth	5 mm
25% *E. abyssinica*	No growth	3 mm
12.5% *E. abyssinica*	No growth	1±0,5 mm
6.25% *E. abyssinica*	Moderate growth	1 mm
3.125% *E. abyssinica*	Marked growth	<1 mm

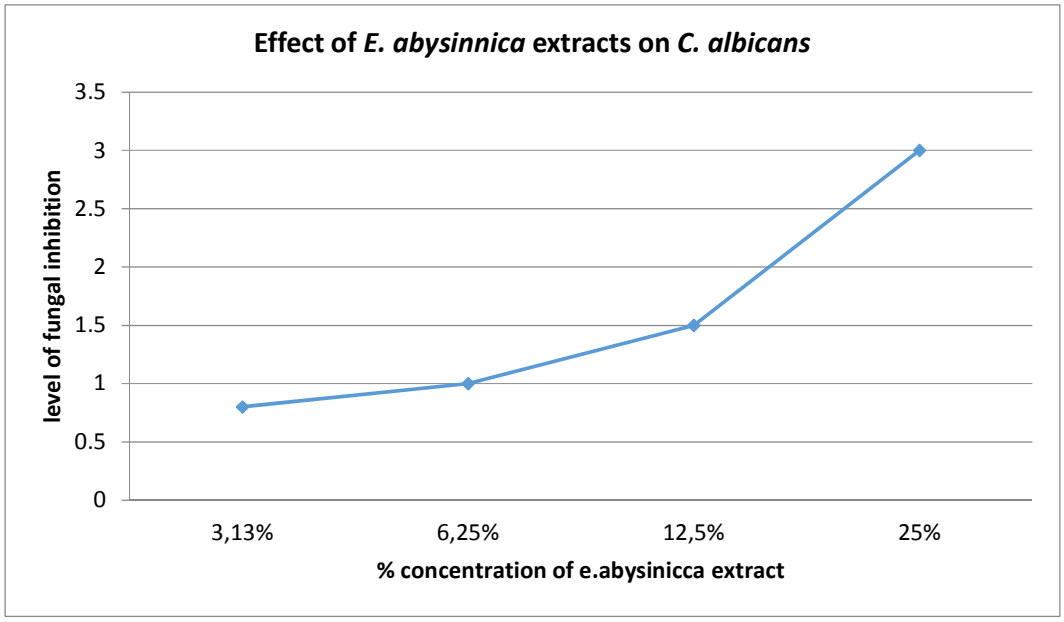

Fig. 5. Effects of *E. abysinnica* on *C. albicans*

The MIC was the minimum concentration inhibiting growth on macroscopically visible colonies on drug containing plates yet there was no growth on the control plates.

The readings were taken after 72 hours. From the graphs shown above, the extracts were more potent in the *candida albicans* strains.

3.5 Testing for the Presence of Bioactive Compounds

All the tests that were done confirmed presence of the bioactives as shown in the tables that follow:

Table 10. The active compounds found in *E. abyssinica* bark crude extract

Name of test	Bioactive being tested	Results	Conclusion
	Tannins	blue green colour	Positive
Foam test	Saponins	A 2 cm layer was formed	Positive
Mayer's reagent	Alkaloids	A cream precipitate was observed	Positive
	Flavonoids	change in color from yellow to colorless	positive

Table 11. The active compounds found in *Z. mucronata* bark crude extract

Name of test	Bioactive being tested	Results	Conclusion
	Tannins	Blue green colour formed	positive
Foam test	Saponins	A 2 cm layer was formed	Positive
Mayer's reagent	Alkaloids	A cream precipitate was observed	Positive
	Flavonoids	Change in color from yellow to colorless	Positive

4. DISCUSSION

Traditionally *Ziziphus mucronata* and *Erythrina abyssinica* have been used for the treatment of topical fungal infections and showed activity against fungal species which commonly infect the skin as dermal infections or as a disseminated form of a systemic fungal disease [12]. *In vitro* antifungal activity was comparable to that of fluconazole an allopathic medicine commonly used in the management of cryptoccocal meningitis. This shows the pharmaceutical importance that *Z. mucronata* and *E. abyssinica* in developing newer generation of antifungal agents from indigenous knowledge systems. Hexane served as a good medium for extraction showing inhibitory effect for both crude extracts [13,12]. The solvent used was also able to extract tannins, saponins, alkaloids and flavonoids which contribute to the plants antifungal activity. The exact mechanism of action is not well understood for the antifungal activity though tanins and saponins are known to interfere with the cell walls proteins and facilitate the diffusion of other phytochemical constituents which actively inhibit fungal growth. Flavonoids are polyphenolic compounds capable of producing cytotoxic through interaction with the fungal membrane as well as having inhibitory effect on enzymes [14]. Alkaloids have always shown broad spectrum antifungal activity and structure elucidation has been carried out for some plant extracts including *Kopsia hainanensis* indicating indole ring structure responsible for alkaloid antifungal activity [9]. The MIC values of both *Z. mucronata* and *E. abyssinica* at different concentrations indicate the extent of inhibitory that the plant extracts have against clinically important fungal species. At 20%w/v of the crude extract *Z. mucronata* showed greater antifungal activity than *E. abyssinica* and fluconazole against *Candida albican* and *Cryptococcus neofomans* and had greater zone of inhibition than *E. abyssinica* at comparable MIC values.

With the emergence of drug resistance to antifungal agents and high rate of fungal related diseases leading to mortality in HIV positive patients the activity of *E. abyssinica* and *Z. mucronata* have shown the importance of indigenous knowledge systems in developing new therapeutic agent [10,12]. The need to investigate medicinal plants traditional claims for targeting specific disease groups or mechanisms of social and economic importance remains integral for drug development. This study chose to focus on *Cryptococcus neofomans* the agent responsible for cryptoccocal meningitis as it continues to be a burden in HIV/AIDS management accounting for 1 out of every 3 HIV related death with short term mortality been very high. Further studies must be carried out for structure elucidation of compounds with antifungal activity and preformulation looking at possible nanoencapsulation of the extracts to optimize oral bioavailability as a potential oral or intravenous antifungal agent.

5. CONCLUSION

Candida albicans and *Cryptococcus neoformans* are the most common fungal species that cause opportunistic infections to occur in HIV and AIDS. The effects produced by these plants have proven that they can be used to alleviate the symptoms associated with these infections. The crude plant extracts were found to be active against *Candida albicans* and *Cryptococcus neoformans*. This means that they can be used for antifungal activity. *Z. Mucronata* had greater antifungal activity as compared to *E. Abyssinica* though they both had the same types of bioactives. Effective minimum inhibitory concentrations against *C. albicans* were found to be 20%w/v and 10%w/v for *Ziziphus mucronata* and showed moderate growth at a 5% w/v concentration. *Erythrina abyssinica* had effective minimum inhibitory concentrations at 25% w/v and 12.5%w/v with moderate fungal growth observed at 6.25%w/v. The same concentration ranges for both crude extracts showed similar antifungal activity for *C. neoformans*. Both crude bark extracts tested positive for tannins, saponins, flavonoids and alkaloids which contribute to the antifungal activity of the plant species.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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