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Ethnobotanical survey and anti-candidal activity of plant species used for oral candidiasis

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Background: Oral candidiasis is caused by *Candida albicans*, which is most prevalent in immunocompromised patients.

Aim: The study aimed to investigate the antifungal activity of plant species used for oral candidiasis against *C. albicans*.

Setting: The study was conducted in Aganang Local Municipality, Capricorn District, Limpopo province, South Africa.

Methods: A survey was conducted using a semi-structured questionnaire supplemented with guided field walks with traditional health practitioners to gather information on medicinal plants used to treat oral candidiasis. Nine plant species (*Artemisia afra* Jacq. ex Willd., *Blepharis subvolubilis* subsp. *subvolubilis* C.B. Clarke, *Enicostemma axillare* [Lam.], *Helichrysum caespititium* [DC.] Harv., *Solanum incanum* L., *Waltheria indica* L., *Ximenia caffra* Sond. var. *caffra*, *Ximenia caffra* Sond. var. *natalensis* and *Ziziphus mucronata* Willd.) were investigated for antifungal activity. The plant material were extracted with solvents of varying polarities: acetone, dichloromethane, ethyl acetate, ethanol, hexane, methanol, and water. The Micro-dilution and bioautography assays were used to determine the antifungal activity of the plant extracts.

Results: Leaf extracts of *A. afra* and *S. incanum* were more active against *C. albicans* with MIC values of 0.02 mg/mL. Bioautography assay demonstrated active compounds in *S. incanum*, *W. indica* and *X. caffra var. caffra* extracts developed in Benzene: Ethanol: Ammonia hydroxide (BEA).

Conclusion: An ethnobotanical survey is a worthy starting point in selecting potential plant species for ethnopharmacological studies.

Contribution: The effectiveness of oral administrations of the medicinal plants was confirmed by the excellent antifungal activity of the aqueous extracts.

Keywords: medicinal plants; candidiasis; ethnobotanical survey; antifungal activity; minimum inhibitory concentration.

Introduction

Oral thrush (Oral candidiasis) is a condition where the fungus accumulates in the mouth's lining. It is characterised by an overgrowth of *Candida* species in the epithelium of the oral mucosa (Melkoumov et al. 2013). More importantly, it reduces the quality of life and increases mortality in infected patients, leading to life-threatening systemic infections. The occurrence of oral candidiasis is a sign of impaired local or systemic defence mechanisms (Magare & Awusthi 2014) and imbalances in the immune system (Oro et al. 2015). The symptoms of oral candidiasis include creamy white lesions on the tongue, inner cheeks, and sometimes on the roof of the mouth, gums and tonsils, and slightly raised lesions with a cottage cheese-like appearance. Asymptomatic characteristics include burning, change of taste, painful sensation, and swallowing difficulty. Infected people often lose weight because of sore throat, which prevents them from eating (Sanne 2001). Oral candidiasis is severe in immunocompromised patients and people receiving treatment for human immunodeficiency virus (HIV) (Bonifait et al. 2012). The high incidence of oral candidiasis in HIV and/or acquired immune deficiency syndrome (AIDS) patients has made candidiasis a leading fungal infection (Jankowaska et al. 2001; Vazquez 2000).

There are several methods for preventing oral thrush, although there is no reliable evidence for its effective treatment (Clarkson, Worthington & Eden 2004). Treatment of oral candidiasis includes

the use of polyenes, azoles, and echinocandins. However, the extensive usage of these antifungal agents is associated with adverse effects, which may result in organ damage (Gupta, Dubey & Kumar 2016). Furthermore, these treatments face challenges such as drug resistance, prohibitive cost, and adverse effects that are usually caused by the toxicity of antifungal agents. These complications necessitate research on the bioactive compounds of natural products or traditional medicine to control infectious diseases. The bioactive compounds produced by plants could provide lead to new, effective, and safe antifungal agents.

The emerging resistance of *Candida* strains to the available antifungals is a public health issue (Sanguinetti, Posteraro & Lass-Flörl 2015). This resistance may have arisen from the extensive use of limited antifungal agents or the improper handling of the antifungals (Garza et al. 2017). Furthermore, the ability of *C. albicans* to form biofilms contributes to its resistance to antifungal drugs (Cretton et al. 2016). Other challenges in the management of oral candidiasis include a limited number of antifungal agents (Feldmesser 2003), toxicity, and low efficacy rate (Kathiravan et al. 2012; Mehta et al. 2002).

Documenting indigenous knowledge through ethnobotanical studies is important for the sustainable utilisation of medicinal plants in plant discovery (Mbunde et al. 2017). Documentation of this information may also play a key role in conservatory aspects of potential plant species with proven biological activities. In this article, we investigate medicinal plant species used for the treatment of oral candidiasis in Aganang Local Municipality of Capricorn District, Limpopo province. The antifungal activity of selected medicinal plants used to combat candidiasis in Aganang Local Municipality was also investigated. Pharmacological screening of plants is an important means for validating the safety and efficacy of medicinal plants and may result in the discovery of new, safe, and effective drugs that could combat fungal infections in humans and animals.

Research methods and design

Study area

The study was conducted in Aganang Local Municipality, Capricorn District, Limpopo province, South Africa (Figure 1). The region lies between 23°40′S and 29°5′E, covering an area of 1881 km^2. It is a rural municipality situated 45 km west of Polokwane city with a human population size of 131 164, and four traditional councils with 19 wards (Statistics SA 2011). It receives rainfall during summer (between November and May) with mean annual precipitation ranging from 454 mm to 500 mm (Mucina et al. 2006). Annual temperatures range between 26 °C and 32 °C in summer and between 7 °C and 24 °C in winter.

Ethnobotanical survey

Traditional health practitioners and local people were interviewed in March 2016 after permission was granted from Bakone Ba Matlala A Thaba Traditional Council and the headmen of each village. The snowball method was used to select traditional health practitioners and people who have knowledge of the use of medicinal plants. All participants were requested to sign a consent form before conducting the interview. Data were obtained using semi-structured questionnaires and guided field walks with the traditional health practitioners. A questionnaire was designed to gather information on the names of plants used for the treatment of oral candidiasis, the source of these plants, the parts of plants used, methods of preparation of medications, and other information. Collated information was analysed using descriptive statistics.

Plant collection

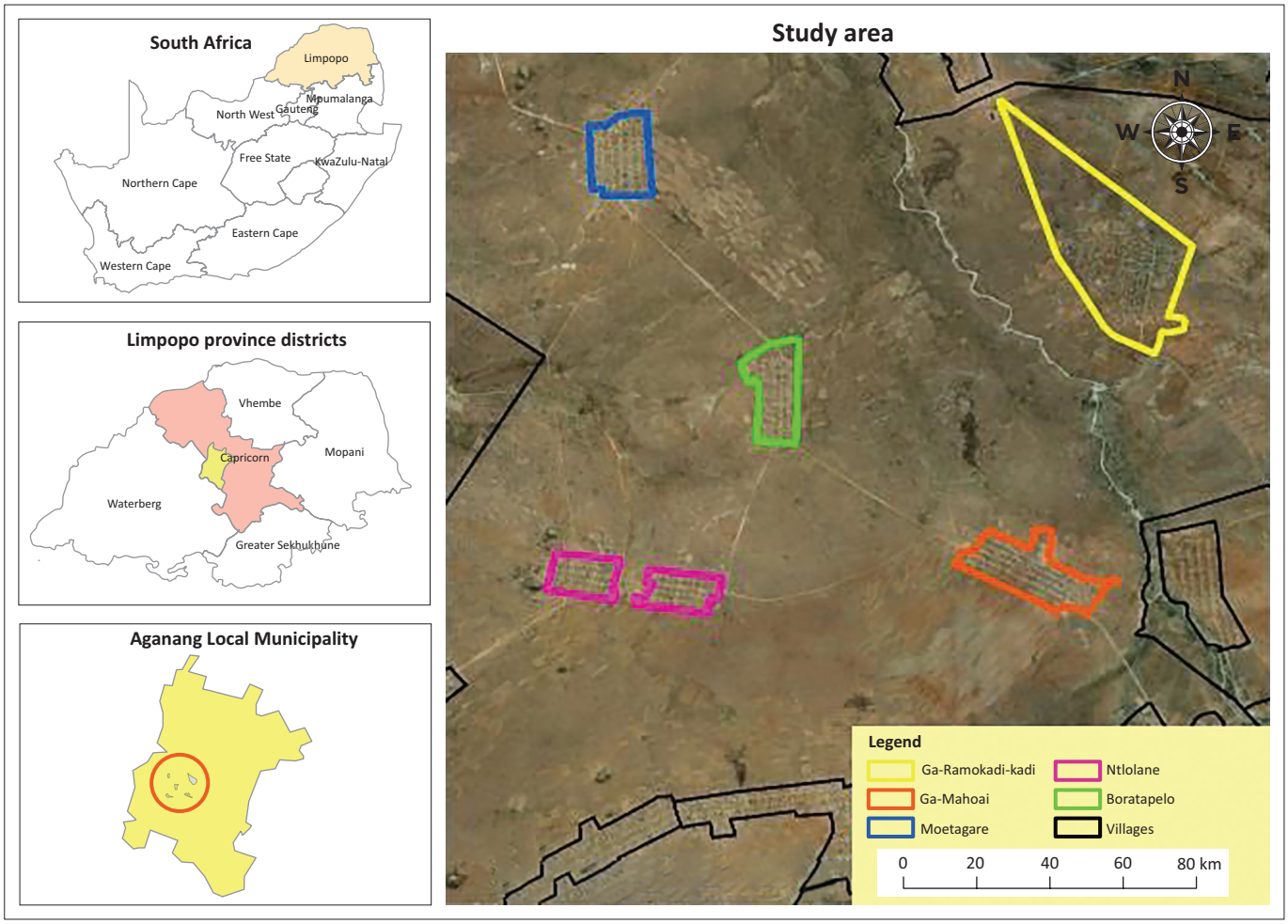
Plants were collected during March–May 2016 from Boratapelo, Ntlolane, and Vlakfontein villages in Aganang Municipality and the University of Limpopo with the help of traditional health practitioners. Literature and the University of Limpopo herbarium were used to identify the plant species. Voucher specimens of the plant species were prepared and deposited at the herbarium.

Plant extracts preparation

Plant materials such as the roots, leaves, and whole plants were dried at room temperature (25°C) in the shade for 4 weeks. The dried material was ground to a fine powder using a laboratory grinding mill and stored in airtight bottles. Each finely ground powder (4 g) was extracted with 40 mL solvents of varying polarities: acetone, dichloromethane, ethyl acetate, ethanol, hexane, methanol, and water. The extracts were shaken with a Labcon Platform shaker at 120 rpm for 10 min and then centrifuged at 2000 rpm for all solvents. The supernatants were filtered into labelled, weighed glass vials. The extracts were placed under a stream of air to evaporate the solvents. The process was repeated three times and the extracts were combined. Aqueous extracts were frozen in a deep freezer. The crude extracts were re-dissolved in acetone prior to biological assay.

Fungal strains and inoculum quantification

Candida albicans (ATCC 10231) were obtained from the culture collection of the Department of Veterinary Tropical Diseases at the University of Pretoria. The final inoculum concentration was adjusted to approximately 1.0×10^6 cells/mL (Aberkane et al. 2002). For the quantification of fungi, the haemocytometer cell-counting method described by Aberkane et al. (2002) was used for counting the number of cells for each fungal culture. Filamentous fungal colonies were enumerated by using a haemocytometer. The inoculum of each isolate was prepared by growing the fungus on sabouraud dextrose (SD) agar slants for 7 days at 35 °C. The slants were rubbed with a sterile loop to collect conidia or yeast cells and transferred to a sterile tube with fresh SD broth (50 mL). The sterile tubes were shaken for 5 min and



Source: Tlaamela, D.M. & Mahlo, S.M., 2021, 'A survey of plant species used in traditional medicine for the treatment of various ailments in Aganang Local Municipality, Limpopo Province', Indilinga African Journal of Indigenous Knowledge Systems 20(1), 69–80; Tlaamela, D.M., Mahlo, S., Abdalla, M. & McGaw, L., 2023, 'Antifungal activity and toxicity of bioactive compounds isolated from the leaf of Ximenia caffra Sond. var. natalensis', Journal of Medicinal Plants for Economic Development 7(1), a219. https://doi.org/10.4102/ jomped.v7i1.219

FIGURE 1: Map of Aganang Local Municipality.

appropriate dilutions were made in order to determine the number of cells by microscopic enumeration using a haemocytometer (Neubauer chamber; Merck S.A). The final inoculum concentrations were adjusted to approximately 1.0×10^6 cells/mL.

Antifungal activity of plant extracts against *Candida albicans*

Nine plant species (*Artemisia afra, Blepharis subvolubilis* subsp. *subvolubilis, Enicostemma axillare, Helichrysum caespititium, Solanum incanum, Waltheria indica, Ximenia caffra* Sond. var. *caffra, Ximenia caffra* Sond. var. *natalensis* and *Ziziphus mucronata*) were selected for further phytochemical analysis and microbiological assays. The micro-dilution assay described by Eloff (1998), modified for antifungal activity testing by Masoko, Picard and Eloff (2005), was used to determine the minimum inhibitory concentrations (MICs) of different plant extracts. Each plant extract (10 mg/mL) was serially diluted (50%) with distilled water in a 96-well microtitre plate and fungal culture (100 µL) was added to the well. Amphotericin B was used as a positive control and 100% acetone as a negative control. After overnight incubation, 40 µL of p-iodonitrotetrazolium violet (INT) of 0.2 mg/mL was added to the microplate wells as an indicator of fungal growth. The covered plates were incubated at 37°C for 24 to 72 h. The experiments were repeated three times to confirm the results.

Bioautography assay

Thin layer chromatography (TLC) plates were loaded with 10 µL of each of the plant extracts and developed using different eluent solvent systems: chloroform: ethyl acetate: formic acid: 20:16:4 [CEF], ethyl acetate: methanol: water: 40:5.4:4 [EMW] and benzene: ethanol: ammonia hydroxide: 90:10:1 [BEA] (Kotze & Eloff 2002). The chromatograms were dried under a stream of air overnight to evaporate the solvents. The developed plates were sprayed with an overnight culture of *C. albicans* until they were completely wet. The plates were incubated at 37°C in a clean chamber at the humidified chamber overnight and further sprayed with a solution of p-iodonitrotetrazolium (INT) violet and incubated for 2–6 h for fungal growth. White areas indicated where the reduction of INT to the coloured formazan did not take place because of the presence of compounds that inhibited the growth of the fungi. The experiments were repeated three times to confirm the results.

Ethical considerations

A permit was obtained from the local authorities and the traditional council of Aganang Local Municipality. The traditional health practitioners were requested to sign a consent form approved by the University of Limpopo Research Ethics Committee prior to the ethnobotanical survey (TREC/385/2017: PG). All traditional health practitioners involved in the study participated freely. The information that the participants shared with us was protected and respected to ensure confidentiality.

Data analysis

The collected data were captured in MS Excel 2018 and analysed using descriptive and inferential statistics such as percentages and frequencies. The frequency index was calculated using the equation:

$$FI = FC/N \times 100, \quad \text{[Eqn 1]}$$

where:

FC is the number of traditional health practitioners who indicated the use of the plant, N is the total number of informants.

The frequency index is directly proportional to the number of informants (Madikizela et al. 2012).

Results

Ethnobotanical survey

Twenty participants were interviewed from selected villages in Ga-Matlala, Limpopo province. A survey revealed that 12 plant species belonging to 10 plant families were used to treat oral candidiasis (Table 1). The most preferred families were Ximeniaceae and Asteraceae, both with a prevalence of 16.7%. Asteraceae was the dominant family in several studies (Afolayan, Grierson & Mbeng 2014; Fenetahun et al. 2017; Maema, Mahlo & Potgieter 2016). Most of the identified plant species were herbs (45.4%), followed by trees (36.4%) and shrubs (18.2%). Similar results for herbs were obtained from several studies (Ahmed 2016; Fenetahun et al. 2017). Trees were the dominating life forms in several studies (Maema et al. 2016; Masevhe et al. 2005). Traditional health practitioners used various parts of plants to prepare their remedies such as roots (43%), leaves (21.4%), fruits, whole plants (14.3% each), and branches (7%). The roots were also dominant in previous studies (Maema, Mahlo & Potgieter 2016; Masevhe, McGaw & Eloff 2015). Leaves were the most used plant parts in several studies (Ahmed 2016; Eddouks, Ajebli & Hebi 2017). The most dominating method of preparation was decoction (50%), followed by burning (28.6%), chewing (14.3%), and grinding (7.1%).

Discussion

Plant extraction

Methanol extracted large quantity of plant material (34.66%), followed by acetone (16.23%) except for *H. caespititium*, where acetone yielded the lowest quantity (11%). The highest yield was obtained from methanol extracts of *X. caffra* var. *caffra* (40%) followed by *E. axillare* (36.5%) (Figure 2). Other researchers found that acetone extracted large quantities of plant materials compared to other solvents (Eloff 1998; Mahlo, McGaw & Eloff 2010). In this study, the lowest yield (0.2%) was obtained from the hexane extract of *B. subvolubilis* subsp. *subvolubilis*. The ethanol extracts of *X. caffra* var. *caffra* and *E. axillare* extracted the same amount of plant material (22%). In general, the lowest yield was obtained (0.2% – 5%) from the extracts *S. incanum*.

Determining anti-candidal activity

Micro-dilution

The antifungal activity of nine plant extracts (10 mg/mL) was determined against *C. albicans.* All plant extracts were active against the tested fungal pathogen (Table 2, Table 3, Table 4, Table 5, Table 6 and Table 7). Excellent anti-*Candida* activity was observed in the extracts of *A. afra* and *S. incanum* with an MIC value of 0.02 mg/mL. Amphotericin B was more active against the tested fungal pathogen with MIC value of 0.02 mg/mL. Other researchers reported that methanol leaf extracts of *S. incanum* were inactive against *C. albicans* (Hamza et al. 2006). Good antifungal activity was found in extracts of *A. afra* against *Cryptococcus neoformans* (Suliman, Van Vuuren & Viljoen 2010). However, aqueous extracts of *A. afra* (Hübsch et al. 2014) and *S. incanum* leaves (Mabona et al. 2013) previously showed moderate activity against *C. albicans.*

Acetone, hexane and ethyl acetate leaf extracts of *W. indica* demonstrated excellent antifungal activity against *C. albicans* with MIC value of 0.02 mg/mL. The dichloromethane extract had good antifungal activity against *C. albicans* with MIC value of 0.08 mg/mL. Similar results for the dichloromethane (DCM) leaf extracts of *W. indica* were obtained by Cretton et al. (2016). Nciki et al. (2016) found similar results for poorly active aqueous extracts. Acetone and DCM extracts of *Z. mucronata* exhibited good antifungal activity against *C. albicans* with MIC value of 0.02 mg/mL, while ethanol, ethyl acetate, methanol, hexane, and aqueous extracts were active with MIC value of 0.08 mg/mL. Similar results for acetone extracts against *C. albicans* were reported by Shikwambana and Mahlo, 2020. Ilonga et al. (2012) noted that DCM extracts of *Z. mucronata* were poorly active, while ethanol, hexane, and methanol extracts had moderate antifungal activity. However, acetone and hexane extracts of *Z. mucronata* were inactive against *C. albicans* in a study conducted by Samie et al. (2010). Furthermore, previous studies reported poor antifungal activity of the aqueous extracts of its leaves (Mabona et al. 2013; Nciki et al. 2016).

Noteworthy antifungal activities of *X. caffra* var. *caffra* were observed in DCM, ethanol, hexane, ethyl acetate, methanol, and aqueous extracts with MIC value of 0.02 mg/mL. Acetone extracts also had good antifungal activity with an MIC value of 0.04 mg/mL. Similar results for

TABLE 1: Medicinal plants used for the treatment of oral candidiasis in Aganang local municipality.

Scientific name and family	Voucher specimen #	Vernacular name	Part/s used	Preparation method	Mode of administration	Frequency index (%)	Availability status (Red list of South African plants 2017)	Other medicinal uses	References
Artemisia afra Jacq. ex Willd. (Asteraceae)	Tlaamela 05	Lengana	Leaves	Chew and/or decoction	Oral	5	Least concern	Constipation, intestinal worms, coughs, colds, fever, flu, ear ache	Verschaeve and Van Staden (2008), Coopoosamy and Naidoo (2012)
Blepharis subvolubilis subsp. *subvolubilis* C.V. Clarke (Acanthaceae)	Tlaamela 06	Sehlabatshukudu	Roots	Decoction	Oral	25	Least concern	No report	-
Carpobrotus edulis N.E.Br. (Aizoaceae)	Tlaamela 08	Mochips or Ino	Leaves	Chew	Oral	25	Least concern	Sore throat, mouth infections	Motsei et al. (2003), Otang, Grierson and Ndip (2012)
Helichrysum caespititium (DC.) Harv. (Asteraceae)	DK 08	Senyabotse	Roots or branches	Decoction	Oral	25	Least concern	Cough, blocked nose, chest colds, headache, wound dressing	Mathekga (2001), Phungula (2015)
Enicostemma axillare (Lam.) A. Raynal. (Gentianaceae)	DK 04	Makgonatsohle	Whole plant	Decoction	Oral	10	Least concern	Boils, blood purifier, itches, appetite loss, stomach ache	Deore et al. (2008), Saranya et al. (2013)
Solanum incanum L. (Solanaceae)	DK 07	Thola	Roots	Decoction mixed branches with *Waltheria indica* L.	Oral	5	Least concern	Stomach problems, chest pains, tonsillitis, skin disorders	Kambizi and Afolayan (2001), Mabona and Van Vuuren (2013)
Waltheria indica L. (Malvaceae)	DK03	Mohutasela	Whole plant	Decoction mixed with roots *Solanum incanum*	Oral	20	Least concern	Skin disorders, urinary tracts infections, burns, wounds, diarrhoea, consumed as a vegetable	Nciki et al. (2016), Magwede et al. (2019)
Warbugia salutaris (G. Bertol.) Chiov. (Canellaceae)	DK 06	Molaka	Roots	Burnt or ground and drunk	Topical or oral	30	Endangered	Abdominal pains, respiratory complaints, stomach ulcers, toothache, venereal diseases	Mabogo (1990)
Ximenia caffra Sond. var. *caffra* (Ximeniaceae)	DK 01	Motshidi	Fruit	Burnt, ground and mixed with lotion	Topical	65	Least concern	Diarrhoea, venereal diseases, blood in faeces, fever, cough, infertility, headache, indigestion and scurvy	Mulaudzi et al. (2011), Nciki et al. (2016)
Ximenia caffra Sond. var. *natalensis* (Ximeniaceae)	DK02	Motshidimphiswane	Fruit	Burnt, ground and mixed with lotion	Topical	35	Least concern	No literature recorded	-
Zanthoxylum humile (E.A. Bruce) P.G. Waterman (Rutaceae)	Tlaamela 41	Monokwane	Roots	Burnt	Topical	20	Least concern	Mouth anaesthetic, burns, pains	Ribeiro et al. (2010)
Ziziphus mucronata Willd. (Rhamnaceae)	Tlaamela 42	Mokgalo	Roots	Decoction	Oral	15	Least concern	Fertility enhancement, sores, burns, dysentery, boils, glandular swellings, diarrhoea, coughs and chest problems	Mabogo (1990), Maema et al. (2016)

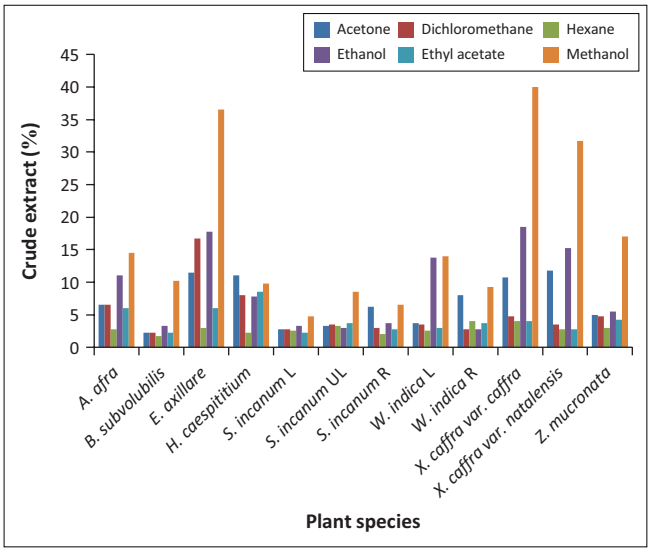
aqueous extracts were reported (Nciki et al. 2016). A previous study by Mulaudzi et al. (2011) also reports ethanol extracts were active against *C. albicans*. However, the weak anti-candidal activity of the aqueous extract was reported by Naidoo et al. (2013). Samie et al. (2010) reported poor antifungal activity of the root hexane extracts against *C. albicans*.

Acetone, DCM, hexane, and ethyl acetate extracts of *E. axillare* had good anti-candidal activity, while aqueous and methanol extracts had moderate activity. Previous studies reported good antifungul activity of ethyl acetate, methanol and aqueous extracts of *E. axillare* against *C. albicans* (Deore et al. 2008). Excellent antifungal activity of *H. caespititium* was observed in DCM, hexane, methanol, and aqueous extracts. Acetone and ethyl acetate extracts had moderate antifungal activity and ethanol extracts had weak activity against *C. albicans*. However, acetone extracts had good activity against other fungal pathogens such as *Aspergillus niger, Cladosporium* sp., and *Phytopthora capsica* (Mathekga 2001).

Moderate antifungal activity was observed in ethyl acetate and ethanol extracts and weak anti-*Candidal* activity in methanol and aqueous extracts. Noteworthy antifungal activity of *X. caffra* var. *natalensis* was observed in acetone, DCM, hexane, ethanol, ethyl acetate, and methanol extracts. Based on the literature, there is a lack of information on the biological activities of *X. caffra* var. *natalensis*. This might be attributed to the fact that many researchers do not separate *X. caffra* into their specific varieties. The currently available varieties of *X. caffra* were classified in a study conducted by Maroyi (2016). This indicates that more research is required to ascertain their other biological activities, and identification

of potential compounds, particularly those found in extracts with strong antifungal activity.

As a result of the relatively slow growth of fungi, the MIC value of plant extracts against *C. albicans* was determined after 24 and 48 h of incubation. Furthermore, a significant factor influencing the susceptibility of test organisms is the duration of incubation. Noticeably, some plant extracts' antifungal activity decreased with longer incubation times. Ethanol and ethyl acetate extracts of *A. afra* lost activity from 0.02 mg/mL to 0.16 mg/mL. Similar activity loss was observed in the methanol extract of *X. caffra* var. *natalensis*.



Note: The legend of this table does not distinguish between different plant extracts and solvents used.

FIGURE 2: Mass of crude extract (%) obtained from 4 g of powdered plant material.

The reduction of antifungal activity after 48 hours of incubation may be attributed to prolonged incubation time for sufficient growth of *C. albicans*. Prolonged incubation also has little effect on the activity of Amphotericin B, but consistently raises the MIC value (Tornatore et al. 1997). In contrast, the roots of *W. indica* gained activity after 48 h incubation in acetone, dichloromethane, and ethanol extracts. These extracts had excellent antifungal activity with MIC values of 0.02 mg/mL. No change of activity was observed in *B. subvolubilis* subsp. *subvolubilis*, *E. axillare*, *H. caespititium*, *S. incanum*, *W. indica* leaves and *X. caffra* var. *caffra*.

Hexane and DCM extracts exhibited strong antifungal activity against *C. albicans*. Noticeably, aqueous extracts had the lowest anti-candidal MIC values in most plant extracts, except for *B. subvolubilis*, *X. caffra* var. *natalensis* and *W. indica* (1.25 mg/mL, 0.156 mg/mL, and 1.25 mg/mL, respectively). This was of great interest because traditional health practitioners and local people use water extracts such as infusions and decoctions. However, poor activity of the aqueous extracts was found against microorganisms (Eloff, Katerere & McGaw 2008; Masevhe et al. 2015). Moreover, different parts of the plant exhibit varying antifungal activities. Based on our findings, the leaves of *S. incanum* had the lowest MIC values than the roots.

The roots of *W. indica* also had the highest MIC values than the leaves, especially in acetone, ethanol, and aqueous extracts. The similarities were only found in DCM extracts with 0.08 mg/mL. Moreover, it was observed that plant species from different geographical regions exhibit

TABLE 2: The minimum inhibitory concentrations (mg/mL) and total activity (mL/g) of different plant extracts against *Candida albicans*.

Time (h)	*Artemisia afra* (L)							*Blepharis subvolubilis* subsp. *subvolubilis* (L)							Amp B
	A	D	H	EtOH	EA	M	H_2O	A	D	H	EtOH	EA	M	H_2O	
MIC values (mg/mL)															
24	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.16	0.156	1.25	1.25	0.02
48	0.02	0.02	0.02	0.16	0.16	0.02	0.02	0.02	0.02	0.02	0.16	0.156	1.25	1.25	0.02
Average	0.02	0.02	0.02	0.08	0.08	0.02	0.02	0.02	0.02	0.02	0.16	0.156	1.25	1.25	0.02
Total activity (mL/g)															
24	3250	3250	1375	5500	3000	7250	-	1125	1125	875	208	144	82	-	-
48	3250	3250	1375	705	384	7250	-	1125	1125	875	208	144	82	-	-
Average	3250	3250	1375	1250	681	7250	-	1125	1125	875	208	144	82	-	-

Note: The results are the mean of three replicates and the standard deviation was zero. Amphotericin B (Amp B) was used as a positive control.
A, acetone; D, dichloromethane; H, hexane; EtOH, ethanol; EA, ethyl acetate; M, methanol; H_2O, water.

TABLE 3: The minimum inhibitory concentrations (mg/mL) and total activity (mL/g) of different plant extracts against *Candida albicans*.

Time (h)	*Enicostemma axillare* (W)							*Helichrysum caespititium* (W)							Amp B
	A	D	H	EtOH	EA	M	H_2O	A	D	H	EtOH	EA	M	H_2O	
MIC values (mg/mL)															
24	0.02	0.02	0.02	0.02	0.02	0.02	0.08	0.63	0.02	0.02	1.25	0.63	0.02	0.02	0.02
48	0.02	0.02	0.02	0.02	0.02	0.02	0.08	0.63	0.02	0.02	1.25	0.63	0.02	0.02	0.02
Average	0.02	0.02	0.02	0.02	0.02	0.02	0.08	0.63	0.02	0.02	1.25	0.63	0.02	0.02	0.02
Total activity (mL/g)															
24	5750	8375	1500	8885	3000	18 250	-	176	4000	1125	62	136	4875	-	-
48	5750	8375	1500	8885	3000	18 250	-	176	4000	1125	62	136	4875	-	-
Average	5750	8375	1500	8885	3000	18 250	-	176	4000	1125	62	136	4875	-	-

Note: The results are the mean of three replicates and the standard deviation was zero. Amphotericin B (Amp B) was used as a positive control.
A, acetone; D, dichloromethane; H, hexane; EtOH, ethanol; EA, ethyl acetate; M, methanol; H_2O, water.

TABLE 4: The minimum inhibitory concentrations (mg/mL) and total activity (mL/g) of different plant extracts against *Candida albicans*.

Time (h)	*Solanum incanum* (L)							*Solanum incanum* R							Amp B
	A	D	H	EtOH	EA	M	H_2O	A	D	H	EtOH	EA	M	H_2O	
MIC values (mg/mL)															
24	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.08	0.02	0.02	0.02
48	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.08	0.02	0.02	0.02
Average	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.08	0.02	0.02	0.02
Total activity (mL/g)															
24	1375	1375	1250	1625	1125	2375	-	3125	1500	1000	1875	353	3250	-	-
48	1375	1375	1250	1625	1125	2375	-	3125	1500	1000	1875	353	3250	-	-
Average	1375	1375	1250	1625	1125	2375	-	3125	1500	1000	1875	353	3250	-	-

Note: The results are the mean of three replicates and the standard deviation was zero. Amphotericin B (Amp B) was used as a positive control.
A, acetone; D, dichloromethane; H, hexane; EtOH, ethanol; EA, ethyl acetate; M, methanol; H_2O, water.

TABLE 5: The minimum inhibitory concentrations (mg/mL) and total activity (mL/g) of different plant extracts against *Candida albicans*.

Time (h)	*Solanum incanum* (L) UL							*Waltheria indica* (L)							Amp B
	A	D	H	EtOH	EA	M	H_2O	A	D	H	EtOH	EA	M	H_2O	
MIC values (mg/mL)															
24	0.02	0.02	0.02	0.04	0.02	0.02	0.04	0.08	0.08	0.02	0.16	0.02	0.31	0.02	0.02
48	0.02	0.02	0.02	0.04	0.02	0.02	0.04	0.08	0.08	0.02	0.16	0.02	0.31	0.02	0.02
Average	0.02	0.02	0.02	0.04	0.02	0.02	0.04	0.08	0.08	0.02	0.16	0.02	0.31	0.02	0.02
Total activity (mL/g)															
24	1625	1750	1625	769	1875	4250	-	480	448	1250	881	1500	447	-	-
48	1625	1750	1625	769	1875	4250	-	480	448	1250	881	1500	447	-	-
Average	1625	1750	1625	769	1875	4250	-	480	448	1250	881	1500	447	-	-

Note: The results are the mean of three replicates and the standard deviation was zero. Amphotericin B (Amp B) was used as a positive control.
A, acetone; D, dichloromethane; H, hexane; EtOH, ethanol; EA, ethyl acetate; M, methanol; H_2O, water.

TABLE 6: The minimum inhibitory concentrations (mg/mL) and total activity (mL/g) of different plant extracts against *Candida albicans*.

Time (h)	*Waltheria indica* (R)							*Ximenia caffra* var. *caffra* (L)							Amp B
	A	D	H	EtOH	EA	M	H_2O	A	D	H	EtOH	EA	M	H_2O	
MIC values (mg/mL)															
24	1.25	0.16	0.02	2.5	0.02	0.02	1.25	0.04	0.02	0.02	0.02	0.02	0.02	0.02	0.02
48	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.04	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Average	0.64	0.08	0.02	1.26	0.02	0.02	0.63	0.04	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Total activity (mL/g)															
24	64	176.28	2000	11	1875	4625	-	2756	2375	2000	9250	2000	20 000	-	-
48	4000	1375	2000	1375	1875	4625	-	2756	2375	2000	9250	2000	20 000	-	-
Average	125	312	2000	21	1875	4625	-	2756	2375	2000	9250	2000	20 000	-	-

Note: The results are the mean of three replicates and the standard deviation was zero. Amphotericin B (Amp B) was used as a positive control.
A, acetone; D, dichloromethane; H, hexane; EtOH, ethanol; EA, ethyl acetate; M, methanol; H_2O, water.

varying antifungal activity. The total activity of the crude plant extracts, except for water extract, was calculated by dividing the mass extracted from 1 g by the MIC value in milligram/millilitres (Table 1). This is an important parameter for comparing the activity of different plant extracts. It indicates the degree to which the active compounds in 1 g can be diluted and still inhibit the growth of microorganisms and assist in selecting promising plants (Eloff et al. 2008). Among all extracts, methanol extracts had the best total activity. This activity was observed in all plant extracts except for *B. subvolubilis* subsp *subvolubilis* and *W. indica* leaves. The highest total activity (20 000 mL/g) was observed in methanol extracts of *X. caffra* var. *caffra* followed by methanol extracts of *E. axillare* (18 250 mL/g). Acetone and dichloromethane extracts had the best total activity in *B. subvolubilis* susp *subvolubilis*. Shai et al. (2008) obtained the highest total activity in acetone extracts. Ethyl acetate extracts had the best activity in leaves of *W. indica*. The lowest total activity of hexane extracts was observed in *E. axillare*, *S. incanum* roots, *X. caffra* var. *natalensis* and *Z. mucronata*. The lowest total activity was observed in the ethanol extract of *W. indica* roots (11 mL/g).

Bioautography assay

The bioautography assay was used to determine the number of active compounds against *C. albicans* (Figure 3). More active compounds were present in TLC bioautograms developed in BEA, and active compounds were visible in extracts of *S. incanum*, *W. indica* and *X. caffra* var. *caffra* developed in EMW. This suggests that most of the active compounds present in the tested plant extracts are relatively non-polar. Contrasting results for active compounds in EMW and BEA were reported by Masevhe (2013). Previous studies indicated more active compounds separated with EMW and few with BEA (Masevhe et al. 2015). Noticeably, more active compounds were visible in the DCM extract. Methanol extracts had the lowest number of active compounds with one compound in *S. incanum* developed in CEF and one developed in EMW. *Ziziphus mucronata* had more antifungal compounds (19.05%), where seven of these

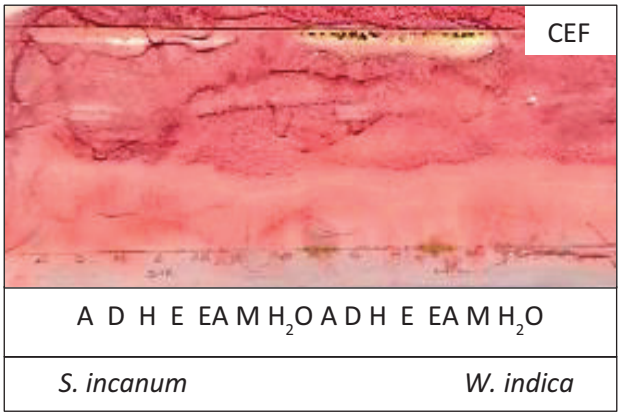
TABLE 7: The minimum inhibitory concentrations (mg/mL) and total activity (mL/g) of different plant extracts against *Candida albicans*.

Time (h)	*Ximenia caffra* var. *natalensis* (L)							*Ziziphus mucronata* (L)							Amp B
	A	D	H	EtOH	EA	M	H_2O	A	D	H	EtOH	EA	M	H_2O	
MIC values (mg/mL)															
24	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
48	0.02	0.02	0.02	0.02	0.02	0.02	0.16	0.02	0.02	0.08	0.08	0.08	0.08	0.04	0.02
Average	0.02	0.02	0.02	0.02	0.02	0.02	0.08	0.02	0.02	0.05	0.05	0.05	0.05	0.03	0.02
Total activity (mL/g)															
24	5875	1750	1375	7625	1375	15 875	-	2500	2375	1500	2750	2125	8500	-	-
48	5875	1750	1375	7625	1375	15 875	-	2500	2375	384	705	544	2179	-	-
Average	5875	1750	1375	7625	1375	15 875	-	2500	2375	612	1122	867	3469	-	-

Note: The results are the mean of three replicates and the standard deviation was zero. Amphotericin B (Amp B) was used as a positive control.
A, acetone; D, dichloromethane; H, hexane; EtOH, ethanol; EA, ethyl acetate; M, methanol; H_2O, water.

were visible in BEA, four in CEF and one in EMW. Acetone, DCM, ethanol and hexane extracts of *Z. mucronata* developed in CEF had compounds with a similar R_f value of 0.68. Runyoro et al. (2006) reported less than four inhibition zones of chloroform and methanol root extracts of *Z. mucronata* against *C. albicans*.

Acetone, DCM, hexane, and ethyl acetate extracts of *S. incanum* developed in BEA had compounds with the same R_f value of 0.26. Antifungal compounds were also observed in DCM, hexane, and ethyl acetate extracts of *X. caffra* var. *natalensis* with R_f values ranging between 0.2 and 0.27. In TLC bioautograms separated with CEF, two compounds were visible in DCM extracts of *S. incanum* with R_f values of 0.61 and 0.63. No active compounds were observed in all extracts of *H. caespititium*. However, it was observed that this plant species had good activity in the micro-dilution method. The absence of active compounds in some plant species was observed in previous studies (Mahlo et al. 2010; Masevhe et al. 2015). This demonstrates that the compounds in this plant extract inhibited fungal growth synergistically. The separation of active compounds on TLC plates, as a result, disrupted their activity. Moreover, an antifungal compound with an R_f value of 0.04 in aqueous extracts in TLC bioautogram separated with BEA, although there was good antifungal activity in other plant species. This may be because of the insolubility of some active compounds in water. Previous studies reported no active compounds for aqueous extracts (Eloff et al. 2008; Mahlo et al. 2013). Variations in bioautograms of tested plant species may result from different plant parts, types of solvent used for extraction, and geographical location from which the plant material was collected. *Waltheria indica* and *X. caffra* var. *natalensis* were selected as the most promising plant species.



Note: White areas indicate inhibition of fungal growth.
A, acetone; D, dichloromethane; H, hexane; E, ethanol; EA, ethyl acetate; M, methanol; H2O, aqueous extract; CEF, chloroform: ethyl acetate: formic acid.

FIGURE 3: Bioautograms of root extracts of *S. incanum* and leaf extracts of *W. indica* developed in CEF and sprayed with *Candida albicans*.

Conclusion

An ethnobotanical survey is an important parameter for discovering useful medicinal plants in a region. Although traditional medicinal plants are used for primary healthcare in Aganang Local Municipality, only a few plant species were recorded to be used for the treatment of oral thrush. The use of roots for medicine preparation raises concerns regarding the survival of certain plant species. However, the participants are aware of the sustainable measures of harvesting medicinal plants to ensure the future availability of useful plants. Methanol and acetone extracted larger quantities of plant material compared to other organic solvents.

In serial dilution assay, extracts of *A. afra*, *E. axillare*, *S. incanum*, *X. cafffra* var. *caffra*, *X. caffra* var. *natalensis* and *Z. mucronata* had excellent activity with MIC values ranging between 0.02 mg/mL and 0.08 mg/mL. The resistance of amphotericin B to *C. albicans* necessitates the need for new antifungal agents. The effectiveness of oral administrations of medicinal plants (decoctions and infusions) was confirmed by the excellent antifungal activity of the aqueous extracts.

More active compounds were observed in TLC bioautograms developed in BEA than in other eluent solvent systems. The absence of active compounds, particularly those that had good antifungal activity in the micro-dilution method indicates possible synergism. Following the good antifungal activity in both micro-dilution and bioautography assays, the roots of *W. indica* and the leaves of *X. caffra* var. *natalensis* were the most promising plant species.

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Competing interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors' contributions

D.T. was involved in conceptualisation, methodology, formal analysis, investigation, writing-original draft, visualisation, and data curation. S.M. was responsible for conceptualisation, methodology, formal analysis, investigation, writing original draft, visualisation, project administration, software, validation, data curation, resources, writing-review and editing, supervision, and funding acquisition. L.M. was responsible for writing-review, editing and co-supervision.

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Data availability

The data used to support the findings of this study may be released upon application to the corresponding author, S.M.

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

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