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Antifungal Activity of Medicinal Plants Used Traditionally for the Treatment of Fungal Infections and Related Ailments in South Africa

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***Abstract*—**The current study investigates the antifungal properties of crude plant extracts from selected medicinal plant species. Eight plant species used by the traditional healers and local people to treat fungal infections were selected for further phytochemical analysis and biological assay. The selected plant species were extracted with solvent of various polarities such as acetone, methanol, ethanol, hexane, dichloromethane, ethyl acetate and water. Leaf, roots and bark extracts of *Maerua juncea* Pax, *Albuca seineri* (Engl & K. Krause) J.C Manning & Goldblatt, *Senna italica* Mill., *Elephantorrhiza elephantina* (Burch.) Skeels, *Indigofera circinata* Benth., *Schinus molle* L., *Asparagus buchananii* Bak., were screened for antifungal activity against three animal fungal pathogens (*Candida albicans, Aspergillus fumigatus* and *Cryptococcus neoformans*). All plant extracts were active against the tested microorganisms. Acetone, dichloromethane, hexane and ethanol extracts of *Senna italica* and *Elephantorrhiza elephantine* had excellent activity against *Candida albicans* and *A. fumigatus* with the lowest MIC value of 0.02 mg/ml. Bioautography assay was used to determine the number of antifungal compounds presence in the plant extracts. No active compounds were observed in plant extracts of *Indigofera circinnata*, *Schinus molle* and *Pentarrhinum insipidum* with good antifungal activity against *C. albicans* and *A. fumigatus* indicating possible synergism between separated metabolites.

***Keywords*—**Antifungal activity, minimum inhibitory concentration, bioautography.

I. Introduction

MEDICINAL plants are widely used as a primary source of prevention and control of various diseases in both animals and human. In South Africa, over 30 000 species of higher plants were recorded and 3 000 of these species are used in traditional medicine [6]. Local people and traditional healers utilise indigenous, exotic or invasive plants as medicine. Almost 27 million local people in South Africa support the use of indigenous medicinal plants [19]. On the other hand, local people also use these plants as source of fire wood, furniture, timber, fencing and protection of their homesteads and for commercial purposes.

Traditional medicines are more acceptable in developing countries and are part of the cultural and religion amongst various ethnic groups. This is attributed to their accessibility and affordability [31]. South Africa has a huge diversity of tribes which is reflected in the systems of medicinal practises [36]. Some rural people prefer traditional medicines for cultural reasons cost effectiveness, acceptability, accessibility [37]. People utilise medicinal plants based on their beliefs in traditional knowledge and due to relatively poor access to clinics or primary health care facilities [35].

Indigenous knowledge rests with traditional healers and local people, and information is generally acquired from survey and interview. In most cases, traditional local knowledge is passed on from generation to generation [34]. Local people prefer to consult traditional healers since they are easily accessible and their medication is not expensive. Traditional healers are recognized by the community to provide health care by using organic substances (plants and animals) based on the social, cultural and religious background. In South Africa, the elderly people have more indigenous knowledge of the plant species that are used for medicinal purposes. Information on the indigenous knowledge on the use of medicinal plants is passed from generation to generation. Furthermore, it is crucial for young people to learn about indigenous knowledge as this information will be preserved amongst future generations [17].

In this paper, ethnobotanical survey and antifungal activity of selected medicinal plants in Makhado Local Municipality was investigated. This is important for detecting plant extracts that have the ability to inhibit growth of microorganisms. Plant extracts showing activity against tested microorganism can provide lead to the discovery of novel antifungal agent. Thus, thorough ethnobotanical survey on various plant species has been conducted. Ethnobotanical surveys play an important role in gathering information about plant species used for medicinal purpose and also could provide a lead into the discovery of the new safer and cheaper potent drugs [24]. It is important to document the indigenous knowledge of medicinal plants for future generations before it gets lost.

II. Materials And Methods

A. Description of the Study Area

The study was conducted in five selected Kutama villages (Muduluni, Tshikwarani, Ha-Madodonga, Maebane and Ha-Manavhela) situated in Makhado Local Municipality in Vhembe district of Limpopo Province (Fig. 1). The municipality lies between 23.0000°S and 29.7500°E. Almost 70% of the municipality is rural and comprised of three ethnic groups: Vhavenda, Ba-Pedi, and VaTsonga [21], [22].

B. Ethnobotanical Survey

The ethnobotanical survey was conducted in Makhado

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Local Municipality, Vhembe District, Limpopo Province. Permission to conduct a survey was obtained from Local authorities, in order to access the communities. Thirty traditional healers from selected areas were randomly selected. Data was collected using a semi-structured questionnaire and guided field work with traditional healers. A questionnaire was designed to gather information on the names of plants used for the treatment of human ailments, the source of these plants, the part/s of plants used, and methods of preparation of medications, diagnosis of different ailments and other information.

TABLE I
MEDICINAL PLANTS USED IN MAKHADO LOCAL MUNICIPALITY FOR THE TREATMENT OF VARIOUS DISEASES

Family	Scientific name	Common name	Voucher number	Plant part(s) used
Anacardiaceace	*Schinus molle* L.	Mubibiri	TC3	Leaves
Asclepladaceae	*Pentarrhinum insipidum* E.Mey.	Phulule	TC6	Roots
Asparagaceae	*Asparagus buchananii* Bak.	Mufhaladzamakole	TC7	Roots
Asparagaceae	*Albuca seineri* (Engl.&K.Krause) J.C Manning & Goldblatt	Kgofakgofane	TC8	Bulb and leaves
Capparaceae	*Maerua juncea* Pax	Mukundulela	TC15	Roots
Asparagaceae	*Albuca seineri* (Engl. & K.Krause) J.C Manning & Goldblatt	Kgofakgofane	TC8	Bulb and leaves
Fabaceae	*Senna italica* Mill.	Murundelatshotshi	TC34	Roots
Fabaceae	*Elephantorrhiza elephantina* (Burch.) Skeels	Tshisesana	TC38	Roots
Fabaceae	*Indigofera circinnata* Benth	Mutahala	TC41	Roots

Uses	Mode of preparation	Plant form	Other uses in literature	References
Flu and throat sores	The decoction of the leaves is used to steam a person suffered from flu and covered with a blanket. A small amount of the decoction is taken orally to treat sore throat.	Tree	Malaria, jaundice, diarrheal, bloating, tonsillitis	[6]-[8]; [23]
Fat	A decoction of roots is used to bath a baby to grow stronger and gain weight.	Climber		
Vomiting	A decoction of the roots is taken orally, 1 cup to prevent vomiting.	Shrub	Amenorrhoea	[1]; [22]
Wounds (*tshifula*)	The decoction of the bulb is used externally on the wound	Herb		
Flu, respiratory problem	The roots decoction is taken orally for flu.	Climber/ shrub	Tuberculosis	[2]
Wounds (*tshifula*)	The decoction of the bulb is used externally on the wound	Herb		
Back pain and diarrhoea	A decoction of roots is used externally (*u kanda*) to relief the back pains, and decoction taken orally to induce diarrhoea.	Herb	STIs	
Body cleansing, stomach problems	Infusion of roots is used for bathing, and taken orally to treat stomach problems.	Shrub	Diarrhoea, impotence, shingles	[4], [5]; [12]
Throat and mouth sores, flu, measles	The decoction of roots is gargled to treat sores, for the treatment of flu the decoction is taken orally, and for the measles treatment, a kid is bathed with the decoction.	Shrub		

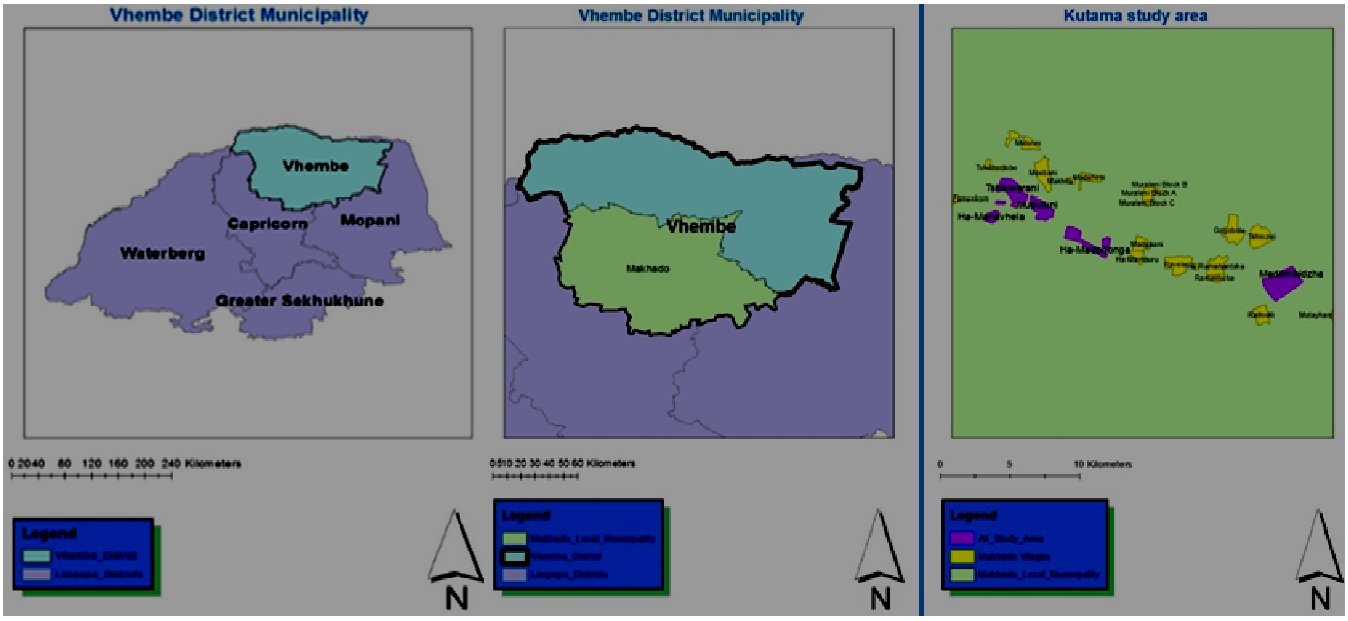


Fig. 1 Map of the study area showing Makhado Local Municipality

C. Plant Collection and Identification

Plants were collected from Soutpansberg West Mountain and five local villages in Makhado Municipality, with the help of traditional healers. Collected plants were identified using

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the literature and Larry Leach herbarium (UNIN). Voucher specimens were collected and deposited to the University of Limpopo herbarium.

D. Plant Extraction

The plant materials such as leaves, bark and roots were dried at room temperature (25 °C) for four weeks. The dried plant materials were grinded to fine powder using a laboratory grinding mill (Telemecanique/MACSALAB model 200 LAB) and stored in airtight bottles. Each finely ground plant material (4g) was extracted with 40 ml hexane, dichloromethane, acetone, ethanol, ethyl acetate, methanol and water in polyester plastic tubes, while shaking vigorously for 3-5 minutes on a shaking machine at high speed of 3500 rpm. The plants were filtered using Whatman No.1 filter paper. After centrifuging at 3500 rpm for 5 minutes, the supernatants were decanted into labelled vials. The process was repeated three times and the extracts were combined. The solvents were removed under a stream of cold air at room temperature. Aqueous extracts were dissolved in distilled water to the concentration of 10mg/ml. The crude extracts were dissolved in acetone prior to microbiological assay. Acetone was reported to be not toxic to the tested microorganisms [9].

E. Phytochemical Analysis

Thin layer chromatography was used to analysed the chemical components of different plant extracts and developed using three different eluent systems: Ethyl acetate: methanol: water: 40:5.4:4 [EMW], Chloroform: ethyl acetate: formic acid: 5:4:1 [CEF] and Benzene: ethanol: ammonia hydroxide: 90:10:1 [BEA] [13]. Ten microliters of each sample were loaded on TLC plates. Chemical components were visualized under visible and ultraviolet light (254 and 360 nm, Camac Universal UV lamp TL-600). Chemical compounds that were not visible under UV light, vanillin-sulphuric acid spray reagent were used for detection [30].

F. Determining Antifungal Activity

1. Fungal Strains and Inoculum Quantification

Candida albicans, *Cryptococcus neoformans* and *Aspergillus fumigatus* were obtained from the Department of Veterinary Tropical Diseases at the University of Pretoria. For quantification of fungi, the haemocytometer cell-counting method was used for counting the number of cells for each fungal culture [1].

G. Micro-Dilution Assay

The antifungal activity of plant extracts was determined using microplate method [9]. The plant extracts were tested in triplicate in each assay, and the assays were repeated three times to confirm results. Residues of different extracts were dissolved in acetone to a specific concentration.

The plant extracts (100µl) were serially diluted 50% with water in 96 well microtiter plates [10], and 100µl of fungal culture were added to each well. Amphotericin B was used as the reference antibiotic and 100% acetone as the negative control. As an indicator of growth, 40µl of 0.2 m/ml p-iodonitrotetrazolium violet (INT) dissolved in water were added to the microplate wells. The microplates were covered and incubated for three to five days at 35ºC at 100% relative humidity. The MIC was taken as the lowest concentration of the extract that inhibit fungal growth.

1. Data Analysis

The collected data was captured in MS Excel 2010. Data was analysed using descriptive and inferential statistics such as percentages and frequencies. Frequency index was calculated using the formula: FI= FC/N x 100, where FC is the number of traditional healers who indicates the use of the plant and N is the total number of informants. The frequency index is directly proportional to the number of informants [16].

III. Results And Discussion

A. Ethnobotanical Survey

The survey revealed sixty-three plants species commonly used for the treatment of various ailments in Vhembe District, Limpopo Province. The vernacular names of plants, family names, scientific names, plant forms, plant parts used, method of preparation and administration are represented in Table I. The most dominating families were Fabaceae 33%, followed by Celastraceae 15%, Capparaceae and Euphorbiaceae (12%). Of the sixty-three plants species identified, trees were the most predominant plant form (53%), followed by shrubs (23%), herbs (14%), and climbers (10%). Root, fruit, bark, leaves, seeds and in some instances the whole plant parts are used for the preparation of medicine while decoction and infusion were the general methods of preparation.

The mode of administration of medicine was mainly orally. The most frequency plant species used is *Warburgia salutaris* (64.7%), followed by *Sclerocarya birrea* (58.8%) and *Elaedendron transvaalensis* (52%). *S. birrea* is used to treat wounds, ulcer infertility while the roots of *Elaedendron transvaalensis* are boiled and the steam is used to treat sore eyes. *Senna italica* is used to induce diarrhoea and also relieve the backaches [14]. The most common method of preparation used by the traditional healers is decoction (65%) and infusion (35%). Decoction is a method of choice when extracting tougher and more fibrous bark and roots because they have more water-soluble chemicals [21]. Other studies reported similar results stating that decoction and infusion were the most frequently used methods of preparation of medicinal plants to treat various ailments [3].

B. Micro-Dilution Method

The antifungal activities of the plant extracts were tested against *Candida albicans*, *Cryptococcus neoformans*, *and Aspergillus fumigatus*. The minimum inhibitory concentrations (MIC) values are shown in Table II.

TABLE II
MINIMUM INHIBITORY CONCENTRATION (MIC) OF EIGHT PLANT SPECIES TESTED AGAINST FUNGAL PATHOGENS

Fungi		MIC (mg/ml)														
		Asparagus buchananii							*Albuca seineri*							AMP B
	Time(h)	Extractants														
		A	D	H	E	EA	M	H_2O	A	D	H	E	EA	M	H_2O	
C. a	24	0.04	0.04	0.08	1.25	0.02	0.02	0.04	0.02	0.02	0.02	0.02	0.08	0.02	2.5	<0.02
	48	1.25	0.32	0.02	1.25	0.02	0.02	0.04	0.02	0.02	0.02	0.02	0.02	0.02	0.62	<0.02
	72	1.25	0.32	0.02	1.25	0.02	0.02	0.04	0.02	0.02	0.02	0.02	0.02	0.02	0.32	<0.02
C. n	24	1.25	1.25	0.02	1.25	0.32	0.02	0.02	0.02	0.62	0.32	0.04	0.02	0.02	0.32	<0.02
	48	0.32	0.32	0.02	1.25	0.04	0.02	0.02	0.02	0.02	0.08	0.04	0.04	0.02	0.32	<0.02
	72	0.32	0.32	0.02	1.25	0.08	0.02	0.02	0.02	0.02	0.08	0.08	0.04	0.02	0.32	<0.02
A. f	24	0.02	0.02	1.25	0.62	0.04	2.5	2.5	2.5	0.08	0.02	0.04	0.16	0.08	0.08	<0.02
	48	1.25	1.25	0.32	0.32	0.32	0.32	2.5	0.08	0.08	0.08	0.08	0.62	0.08	0.04	<0.02
Average		0.71	0.48	0.22	1.05	0.11	0.39	0.65	0.34	0.11	0.08	0.04	0.12	0.04	0.56	<0.02

Fungi		MIC (mg/ml)														
		Elephantorrhiza elephantina							*Indigofera circinnata*							AMP B
	Time(h)	Extractants														
		A	D	H	E	EA	M	H_2O	A	D	H	E	EA	M	H_2O	
C. a	24	0.02	0.02	0.02	0.02	2.5	2.5	2.5	0.02	0.02	0.02	0.02	0.04	0.02	0.02	<0.02
	48	0.02	0.02	0.02	0.02	2.5	2.5	2.5	0.02	0.02	0.02	0.02	0.08	0.02	0.02	<0.02
	72	0.02	0.02	0.02	0.02	2.5	2.5	2.5	0.02	0.02	0.02	0.02	0.08	0.02	0.02	<0.02
C. n	24	0.16	0.02	0.04	0.08	0.08	0.02	0.62	0.04	0.02	2.5	0.04	1.25	0.02	0.02	<0.02
	48	0.16	0.02	0.04	0.08	0.08	0.02	0.02	0.04	0.02	2.5	0.04	1.25	0.02	0.02	<0.02
	72	0.08	0.02	0.04	0.08	0.08	0.02	0.02	0.04	0.02	0.16	0.04	1.25	0.02	0.02	<0.02
A. f	24	0.16	0.04	0.02	0.32	0.02	2.5	2.5	0.16	0.02	0.02	0.16	0.04	0.16	0.32	<0.02
	48	0.62	1.25	0.62	2.5	0.62	2.5	0.62	0.16	1.25	0.04	1.25	0.08	0.08	1.25	<0.02
Average		0.15	0.18	0.10	0.34	1.05	1.57	1.41	0.06	0.17	0.67	0.19	0.51	0.04	0.21	<0.02

Fungi		MIC (mg/ml)														
		Maerua juncea							*Pentarrhinum insipidum*							AMP B
	Time(h)	Extractants														
		A	D	H	E	EA	M	H_2O	A	D	H	E	EA	M	H_2O	
C. a	24	0.04	0.04	0.02	0.02	0.08	0.02	2.5	0.02	0.02	0.02	0.02	0.04	0.04	0.02	<0.02
	48	0.04	0.04	0.02	0.02	2.5	0.02	2.5	0.02	0.02	0.02	0.02	0.04	0.04	0.02	<0.02
	72	0.04	0.04	0.02	0.02	2.5	0.02	0.02	2.5	0.02	0.02	0.02	0.02	0.02	0.02	<0.02
C. n	24	0.04	0.32	0.02	0.16	0.04	0.02	0.02	0.32	2.5	0.32	2.5	2.5	0.32	0.62	<0.02
	48	0.04	0.02	0.02	0.16	0.04	0.02	0.02	0.16	1.25	0.02	2.5	0.16	0.62	0.62	<0.02
	72	0.04	0.02	0.02	0.16	0.04	0.02	0.02	0.16	1.25	0.02	0.08	0.08	0.62	0.62	<0.02
A. f	24	0.62	0.32	0.02	0.02	1.25	0.62	2.5	0.16	0.16	1.25	0.04	0.04	0.04	0.04	<0.02
	48	0.08	0.32	0.02	1.25	0.02	1.25	0.32	0.16	0.16	0.08	0.32	0.04	0.32	0.04	<0.02
Average		0.12	0.14	0.02	0.23	0.81	0.24	0.98	0.44	0.67	0.23	0.69	0.36	0.25	0.25	<0.02

Fungi		MIC (mg/ml)														
		Senna italica							*Schinus molle*							AMP B
	Time(h)	Extractants														
		A	D	H	E	EA	M	H_2O	A	D	H	E	EA	M	H_2O	
C. a	24	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.62	0.62	0.62	0.02	2.5	1.25	1.25	<0.02
	48	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.62	0.62	0.62	0.02	2.5	0.02	0.02	<0.02
	72	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.04	0.04	0.04	0.02	2.5	0.02	0.02	<0.02
C. n	24	1.25	0.02	1.25	0.62	0.02	0.04	1.25	0.02	0.08	0.16	0.08	0.04	0.32	0.32	<0.02
	48	1.25	0.02	0.16	0.02	0.02	0.02	1.25	0.02	0.16	0.04	0.08	0.04	0.02	0.02	<0.02
	72	0.16	0.02	0.16	0.02	0.02	0.02	0.02	0.02	0.04	0.04	0.08	0.04	0.02	0.02	<0.02
A. f	24	0.32	0.08	0.02	0.02	0.02	1.25	0.02	0.04	0.02	0.04	0.04	0.08	2.5	2.5	<0.02
	48	0.16	0.16	0.16	0.16	0.02	0.62	0.02	0.32	0.08	0.02	0.04	0.08	0.08	0.08	<0.02
Average		0.40	0.04	0.23	0.11	0.02	0.25	0.33	0.21	0.21	0.19	0.05	0.97	0.52	0.52	<0.02

Abbreviations: C. a- *Candida albicans*, C. n- *Cryptococcus neoformans*, A. f- *Aspergillus fumigatus*, A- acetone, D-dichloromethane, H- hexane, E- ethanol, EA-ethyl acetate, M- methanol and H_2O- water.

Of the tested plant species, hexane leaf extracts of *M. juncea*, ethyl acetate leaf extracts of *S. italica*, *A. buchananii* and *E. elephantin*a were the most active against *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus*

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with MIC values ranging between 0.02 mg/ml and 0.08 mg/ml. The leaf extracts of *S. italica* had excellent activity against *C. albicans* with the lowest MIC value of 0.02 mg/ml. Acetone, dichloromethane, hexane and ethanol extracts of *E. elephantina* had good activity against *C. albicans, C. neoformans* and *A. fumigatus* with MIC value ranging between 0.02 mg/ml and 0.04 mg/ml. [18] reported that acetone extract was active against *A. fumigatus* with MIC ranging between 0.02 and 0.08mg/ml. Hexane leaf extracts of *M. juncea* had shown good activity against *C. albicans, A. fumigatus* and *C. neoformans* with MIC value of 0.02 mg/ml.

Amongst all of the extracts tested for antifungal activity against *C. albicans*, *A. fumigatus*, *C. neoformans,* acetone, hexane, methanol and DCM leaf extracts of *A. seineri* and *M. juncea* had good activity against the animal fungal pathogens (MIC value= 0.02 mg/ml). Literature reports that methanol extracts from seven South African plants were relatively inactive against the tested pathogens [32], [33]. Ethanol extracts of *S. molle* had good activity against all tested fungal pathogen with the MIC value ranging between 0.02 mg/ml to 0.04 mg/ml. Some studies have reported antifungal activity of essential oil, ethanol and aqueous extracts of *S. molle* [29]. Hexane, methanol and aqueous leaf extracts of *A. buchananii* had shown excellent activity against *C. albicans* and *C. neoformans* with MIC values between 0.02 mg/ml and 0.04 mg/ml. Plants from genus *Asparagus* are regarded as medicine and some are used in the treatment of epilepsy [25]-[28]. In some instances, other plant species are used in ethnoveterinary medicine [20]. Dichloromethane, hexane, ethyl acetate, methanol and aqueous leaf extracts of *P. inspidum* had good activity against *C. albicans* and *C. neoformans* with MIC value ranging between 0.02 mg/ml and 0.04 mg/ml. Acetone extracts had shown moderate activity against the fungal pathogens. *A. fumigatus* was relatively sensitive compared to other tested fungi.

In general, aqueous extracts of some plant extracts had shown excellent activity against *C. albicans* with the lowest MIC value of 0.02 mg/ml. These findings support the results obtained with water extracts tested against other microorganisms [15]. Traditional healers prepared the plant extracts with water for infusion, decoction and macerations. Therefore, it would be difficult for the traditional healer to be able to extract those compounds which are responsible for antimicrobial activity in the acetone, hexane and methanol extracts [11].

IV. Conclusion

Local people and traditional healers in Vhembe District still rely on medicinal plants as a source of primary health care. Most of medicinal plants used to combat various diseases have not yet been documented. It was noted that traditional healers use bark and roots to prepare the remedies. This could lead to extinction of some of plants due to overexploitation and deforestation. Therefore, the sustainable way of plant collection should be taught to our traditional healers and local people, more especially to conserve plants that are indigenous to South Africa. They should also be encouraged to cultivate their own traditional medicine in their home gardens. The indigenous knowledge on medicinal plants should also be passed to new generation, because it was found that young generation was less knowledgeable about the medicinal plants.

The valuable knowledge regarding the folk medicinal uses of plants should be recorded before it is lost forever. In Vhembe District, no information regarding the biological activity of *A. buchananii*, *A. seineri*, *I. circinnata* and *P. insipidum* has been recorded. These plant species were investigated for the first time against fungal infections. The results from the study support the use of these plants by the traditional healers and the local people for the treatment of different ailments related to fungal infections using the water extracts. Ethnobotanical surveys and indigenous knowledge on medicinal plants should be taken into consideration because they may provide a lead to new antifungal agents.

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