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Antifungal, anti-oxidant activity and cytotoxicity of South African medicinal plants against mycotoxigenic fungi

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Abstract

Fungal strains belonging to genus *Fusarium* and *Aspergillus* are known to infect crops, resulting in threatened food security and less agricultural crop yields. The aim of the current work was to investigate the anti-mycotoxigenic activity, cytotoxic effect and antioxidant potential of selected South African medicinal plants extracts. The aqueous and organic extracts of the leaves from selected medicinal plants were investigated for their antifungal activity against various fungal strains that are known to infect crops and produce mycotoxins. Antioxidant activity, total phenolic and total flavonoids contents were also evaluated. Organic extract of *Milletia grandis* (E. Mey) Skeels revealed the lowest minimum inhibitory concentration (MIC) value of 0.01 mg/mL against *Aspergillus ochraceous, Fusarium graminearum* and *Furasium oxysporum*. Generally, organic extracts revealed significant antifungal activity compared to aqueous extracts. *Carpobrutus eludis* L. and *Warburgia salutaris* (G. Bertol) Chiov. revealed a potent cytotoxic effect yielding 50 % lethal concentration (LC_{50}) value of 0.01 mg/mL against Bovine dermis and Vero cells respectively. *Ricinus communis* L. revealed a 50 % inhibitory concentration (IC_{50}) value of

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945 μg/mL against 2, 2 diphenyl-picryl-hydrazyl (DPPH). Generally, the plant species revealed lower flavonoids compared to phenolic contents. The biological activity of the selected plant extracts may be attributed to the high phenolic contents.

Keyword: Plant biology

1. Introduction

Saprophytic fungi are a major threat to human and animal health through food spoilage and subsequent contamination with mycotoxins, during pre and postharvest [1]. Due to their ubiquitous nature, these fungal strains infect and contaminate a wide variety of food crops and are difficult to control. Poor harvesting practices, improper drying, handling, transportation and storage material used contribute to fungal growth and increase the chances of mycotoxin production, which may directly have a negative impact on the economy of the affected region [2]. High temperature, moisture content, processing and heavy rains may also favour some fungal growth [3]. In developed countries, mycotoxins levels in foods are regulated using various recent technological tools, different quality standards and recommended daily intake levels [4]. However, it is important to note that these regulations may not be applicable to most rural communities in both developed and under developed countries that rely on farming for food [5].

By estimation, the global food production should increase by 50 % to meet the demands of the world population by 2050 [6]. Since human populations may be largely determined by conditions of food producing systems, these may not be achieved due to contamination of food by mycotoxigenic fungal strains mostly belonging to the genus *Fusarium, Aspergillus* and *Penicillium* [7]. This problem is one of the universal and frequently recognized cause of several devastating infections and various lethal human diseases. These strains play a role in contamination and degradation of both quality and quantity in major agricultural commodities like maize, rice and wheat which are staple foods in most African countries. The mycotoxigenic fungal species from these genus groups are known to produce a variety of mycotoxins or secondary metabolites and one strain may produce more than one mycotoxin at once [8, 9, 10, 11]. The most common mycotoxins produced by these species include fumonisin, aflatoxin, ochratoxin, zearalenone and trichothecenes [12]. The human exposure to mycotoxins may occur mostly through direct consumption of mycotoxin contaminated crops and animal based products, inhalation and in direct contact through the skin [13].

Free radicals such as reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive chlorine species (RCS) are produced *in vivo* from various biochemical reactions including respiratory chain. These molecules are introduced

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into the body from outside environment such as ultra violet (UV) light, radiation, smoking, air pollution, unhealthy foods, stress, certain drugs and others. The accumulation of free radical molecules within human body may lead to cellular damage causing various illnesses in humans and animals [14]. The current paper aims at investigating the anti-mycotoxigenic potential of the selected South African medicinal plants against *Furasium* and *Aspergillus* species-important food contaminating fungal strains. Furthermore, to explore the antioxidant properties of such plant extracts against 2, 2diphenyl-picryl-hydrazyl (DPPH) a stable free radical. Plant extracts with good antifungal and antioxidant activity may well prevent spoilage resulting from oxidation in foodstuffs. Oxidation in foodstuffs may well be facilitated by these fungal strains through production of various toxins.

2. Methodology

2.1. Plant material and study area

The fresh and healthy leaves of the selected medicinal plants (Table 1) were randomly selected and collected from the wild within the villages around Kwa-Dlangezwa, Empangeni area, Northern KwaZulu-Natal Province. Villages such as

Table 1. Ethnobotanical uses of selected medicinal plants.

Plant names	Family	Plant part used	Diseases or symptoms treated in humans	References
Ziziphus mucronata Wild. (UNI 101)	Rutaceae	Roots and stem bark	Stomach ache, diarrhoea, Dysentery, chest pains and tuberculosis	[15, 16]
Carpobrotus eludis L. (PRU 123546)	Mesembryanthemaceae	Leaves	Diabetes, tuberculosis, sores, shingles and skin infections	[16, 17, 18]
Harpephyllum caffrum Benth (PRU 2583)	Anacardiaceae	Stem bark and roots	Acne, eczema	[19]
Milletia grandis (E. Mey) Skeels (UNI 102)	Fabaceae	Roots	Epilepsy, induce sleep and dispel worries	[20]
Solanum aculeastrum Dunal. (UNI 103)	Solanaceae	Leaves and Fruits	Dysentery, diarrhea and female infertility	[15, 16]
Ricinus communis L. (PRU 123549)	Euphorbiaceae	Leaves	Malaria and vaginal candidiasis	[21, 22]
Maesa lanceolata Forsk (PRU 120125)	Myrsinaceae	Stem bark	Malaria and veterinary use as antipoison.	[23]
Bauhinia galpinii N.E.Br. (PRU 123537)	Fabaceae	Stem bark	Diarrhoea and infertility	[24]
Waburgia salutaris (G. Bertol) Chiov. (PRU 120013)	Canellaceae	Stem bark	Malaria, pneumoniae, penial irritation and respiratory complaints	[25]
Solanum panduriforme E. Mey. (RN 19)	Solanaceae	Roots	Sexually transmitted infections	[26]

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Vulindlela, Iniwe, Esikhaleni, Mthunzini, Ongoye, Amanziamnyama and Indabayakhe were selected for the current study. Although traditional medicine generally use roots and stem bark for treatment of various infections (Table 1), the leaves are selected in the current study, as a biodiversity conservation measure against the degradation and extinction of important plant populations in the wild.

Leaves were separately collected into brown paper bags, shade dried on a well ventilated laboratory bench at room temperature to ensure efficient drying without microbial attack and then ground into powder (2 mm mesh) using hammer mill (IKA Scientific, Model MF 10 B, Germany). The powders were then stored in tightly closed plastic bottles until needed. The voucher specimen were collected, pressed and identified by botanist (Dr Monde Nyila) in the department of Life and Consumer Science, Unisa, Florida Campus. The specimen were verified at herbarium, University of Pretoria against the available vouchers, with code PRU. The other specimen were lodged at UNISA with the codes UNI. The leaves were collected into the small bags and dried at 15 °C.

2.2. Extraction of plant materials

The dried plant materials were separately extracted with hot water and 1:1 methanol: dichloromethane (AR Grade) respectively. Aqueous extracts were boiled 1:5 w/v, cooled off on a laboratory bench, filtered through Whatman's No. 1 paper and freeze dried (Labconco Corporation, Kansas City, Moussouri, USA). The organic extracts were extracted at 1:10 w/v, kept on a Desk shaking incubator (Scientific, USA) overnight at 100 rpm and then filtered through Whatman's no1 paper and then concentrated using Buchi rotary evaporator (Bibby Scientific Limited, Stone Staffordshire, UK). The resulting plant extracts of both aqueous and organic origin were weighed and then kept in the refrigerator at 4 °C.

2.3. Selected fungal strains

A total of six phyto-pathogenic fungal strains belonging to the genera such as *Fusarium* and *Aspergillus* were selected for antifungal activity tests. The selected species are *Furasium verticillioides* (PPRI 10148), *Fusarium oxysporum* (PPRI 10185) and *Fusarium graminearum* (PPRI 10340), *Aspergillus parasiticus* (PPRI 9153), *Aspergillus flavus* (PPRI 14636) and *Aspergillus ochraceous* (PPRI 6816). The fungal strains were purchased from Agricultural Research Council- Plant Protection Research Institute (ARC-PPRI) in 2015 (Pretoria, South Africa).

2.4. Anti-fungal activity

The antifungal activity of the selected medicinal plants extracts were determined using the method adopted from Eloff [27] with slight modifications. All the organisms

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were maintained on a freshly prepared Sabourand Dextrose Agar. The overnight culture of each strain was diluted with fresh Sabourand Dextrose broth (Biolab, Merck, South Africa) to a concentration of about 1.1×10^7 cfu/mL. Shortly, the minimum inhibitory concentrations (MIC) values of the selected medicinal plant extracts were determined from the stock solution of 10 mg/ml which was serially diluted two-fold in sterile distilled water. About 100 μL of the standardised fungal cultures were then added to all the wells, yielding the highest concentration of the plant extract of 0.25 mg/mL. About 40 μl of 0.2 mg/mL of the freshly prepared iodonitrotetrazolium chloride (INT) was added into the diluted plant extracts. The wells showing purplish colour were indicative of growing fungi, while colourless or greenish colour indicates that the extract inhibit the growth of the fungi and reported as minimum inhibitory concentration (MIC). The results were read after both 24 and 48 hrs incubation period.

2.5. Cytotoxicity studies

The cytotoxicity of the 1:1 methanol: dichloromethane extracts of the selected medicinal plants was determined against both the Bovine dermis and Vero cells obtained from Sigma Aldrich (Germany) using the tetrazolium-based colorimetric (MTT) assay described by Mosman [28]. Extracts were prepared at 100 mg/ml dissolved in DMSO, and dissolved in DMEM to yield final highest concentration of 1 mg/ml. Cells of a sub-confluent culture were harvested and centrifuged at 100 x g for 5 min, and then re-suspended in growth medium to a concentration of 5×10^4 cells/ml. The growth medium used was Minimal Essential Medium (MEM, Whitehead Scientific) supplemented with 0.1% gentamicin (Virbac) and 5% foetal calf serum (Highveld Biological). A cell suspension of about 200 μl was pipetted into each well of columns 2 to 11 of a sterile 96-well micro titre plate. MEM (200 μl) was added to wells of columns 1 and 12 to minimize the "edge effect" and maintain humidity. The plates were incubated for 24 h at 37 °C in a 5% CO_2 incubator, until the cells were in the exponential phase of growth. The MEM was aspirated from the cells which were then washed with 150 μl phosphate buffered saline (PBS, Whitehead Scientific) and replaced with 200 μl of the selected test acetone extracts at different concentrations in quadruplicate. The serial dilutions of the test extracts were prepared in MEM. The cells were disturbed as little as possible during the aspiration of medium and addition of test substance. The micro titre plates were incubated at 37 °C in a 5% CO_2 incubator for 48 h with test compound or medicinal plant extract. Untreated cells and positive control, doxorubicin (Pfizer Laboratories) were included.

After incubation, 30 μl MTT (Sigma, stock solution of 5 mg/ml in PBS) was added to each well and the plates incubated for a further 4 h at 37 °C. After incubation with MTT the medium in each well was carefully removed, without disturbing the MTT

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crystals in the wells. The MTT formazan crystals were dissolved by adding 50 μl DMSO to each well. The plates were shaken gently until the MTT solution was dissolved. The amount of MTT reduction was measured immediately by detecting absorbance in a micro plate reader (Varioskan Flash, AEC Amsterdam Pty Ltd) at a wavelength of 590 nm. The wells in column 1, containing medium and MTT but no cells were used to blank the plate reader. The LC_{50} values were calculated as the concentration of test plant fraction or isolated compound resulting in a 50% reduction of absorbance compared to untreated cells. Selectivity index of the extracts were calculated as follows:

Selectivity Index (SI) = LC_{50} in mg/mL/MIC in mg/mL at 48 hr incubation time [29].

2.6. DPPH assay

The DPPH free radical-scavenging activity of the organic extracts was determined using the method adopted from Brand-Williams et al [30] with slight modification. In the current study, only DPPH assay was used as the free radical is more stable than others, hence used as a representative model for anti-oxidant assay. Shortly, 3.75 mg of DPPH was dissolved in 100 mL methanol. Plant extracts were made to a solution of 5 mg/ml, dissolved in DMSO. About 150 μL of DMSO was added to 96 well plates and an equal amount of plant extracts were added in the first well and then two-fold serially diluted. DPPH solution of about 150 μL was then added to all the wells. Plates were incubated in the dark for 1hr and then later read at 517 nm using microplate reader (Varioskan Flash, AEC Amsterdam Pty Ltd). Ascorbic acid was used as a positive control, while methanol and DMSO were used as negative control. Percentages of inhibition were calculated using the formula below.

$$\text{Scavenging capacity} = OD_{sample} - OD_{sample\ blank}/OD_{control} - OD_{control\ blank} \times 100.$$

The concentration of the plant extract leading to a 50 % reduction of DPPH (IC_{50}) was then determined from the linear graphs constructed using percentage of inhibition against concentration of plant extract.

2.7. Determination of total phenolic contents

The total phenolic contents of the organic extracts of the selected medicinal plants were determined using the Folin-Ciocalteu method as described by Makkar [31], with slight amendments. Briefly, 25 μL of crude plant extract (0.5 mg/ml) was treated with 250 μL Folin-Ciocalteau reagent for 5 min. The reaction was stopped by adding about 750 μL 20 % anhydrous sodium carbonate. The volume was made up to 5 mL with distilled water and incubated in the dark at room temperature for 2 hr. After incubation, the absorbance was read at 760 nm with a

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spectrophotometer (Varioskan Flash, AEC Amsterdam Pty Ltd). The phenolic content was determined from a standard curve of different concentrations of gallic acid DMSO. The results were expressed as mg/g gallic acid equivalents (GAE).

2.8. Determination of total flavonoid content

The total Flavonoid contents of the organic extracts was determined using the method adopted from Yadav and Agarwal [32], slightly amended. Crude extracts at 0.5 mg/ml (100 μL) were dissolved in 300 μL methanol, to which 20 μL 10 % aluminium chloride was added. A further 20 μL of 1 M sodium acetate was added to the solution. The resultant solution was made up to 1 mL with distilled water. This was incubated at room temperature for 30 min. After incubation, the absorbance was read at 450 nm in a microplate reader (Varioskan Flash, AEC Amsterdam Pty Ltd). Quercetin (10 mM) was used as a standard. The flavonoid content of each extract was expressed as mg/g quercetin equivalent (QE).

2.9. Statistical analysis

The results in the current study were analysed using ANOVA. In the antifungal studies, the results were recorded as mean of the three independent experiments, while the results for the cytotoxicity, antioxidant activity and total phenolic and total flavonoids contents were reported as mean ± SE (n = 3). *P*-value of ≤ 0.05 was referred to as significant.

3. Results and discussions

3.1. Antifungal activity

Mycotoxins are secondary metabolites produced by some filamentous fungi, which can be produced on foodstuffs due to fungal growth. When such toxins are ingested by humans and animals, they may cause illnesses which may include liver and kidney malfunctioning. The important mycotoxins causing significant health hazards are aflatoxins, ochratoxin A, citrinin, *Fusarium* toxins, patulin, and zearalenone [33]. *Fusarium* and *Aspergillus* species infects a variety of agricultural commodities including maize, cereals, peanuts, sorghum, nuts, meat, eggs oil seeds and indigenous vegetables which includes 'morogo' [34, 35].

The saprophytic fungal strains from the *Fusarium* and *Aspergillus* species are the prolific producers of mycotoxins which includes fumosin and various ochra toxins respectively [36]. The results for the antifungal activity of aqueous and organic South African plant extracts are reported (Table 2). *Aspergillus parasiticus* was the most susceptible fungal strain to the organic plant extracts yielding the average MIC value of 0.22 mg/mL. Some mutant strains of *A. parasiticus* have been reported

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Table 2. Antifungal activity (MIC in mg/ml) of South African medicinal plants against agricultural related pathogenic strains.

Selected fungal strains	Extracts	Medicinal plants, Control drug and Average MIC																					
		Maesa lanceolata		*Ricinus communis*		*Waburgia salutaris*		*Bauhinia galpinii*		*Carpobrotus eludis*		*Ziziphus mucronata*		*Solanum aculeastrum*		*Solanum panduriforme*		*Harperphylum caffrum*		*Milletia grandis*		Av. MIC	Amphotericin B (Positive control)	
		24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	48 hr	24 hr	48 hr
A. parasiticus	Aq.	0.39	0.39	0.39	0.65	0.39	0.78	0.39	0.39	0.65	0.78	3.13	6.25	0.20	0.39	0.39	0.39	0.78	0.65	1.17	1.17	1.18		
	Org.	0.39	**0.01**	0.01	0.20	0.01	**0.02**	0.10	**0.10**	0.01	**0.01**	0.20	1.56	0.20	0.20	0.02	**0.02**	0.04	**0.10**	0.01	**0.02**	**0.22**	0.001	0.002
A.ochraceous	Aq.	0.78	1.56	1.56	1.56	1.56	1.56	1.30	1.56	1.56	1.56	1.30	6.25	0.39	0.39	0.78	0.78	1.30	1.56	1.30	1.30	1.81		
	Org.	0.13	0.17	0.40	0.20	0.08	0.16	0.20	0.33	0.20	0.39	0.10	0.20	0.20	1.56	0.02	**0.04**	0.02	**0.10**	0.01	**0.01**	0.32	0.003	0.003
A. flavus	Aq.	0.78	0.78	1.04	1.04	0.78	0.78	1.56	1.56	6.25	12.5	1.56	3.13	0.39	0.39	0.78	0.78	2.60	4.70	1.56	1.82	2.75		
	Org.	0.78	1.56	0.20	0.20	0.10	0.16	0.02	**0.02**	0.20	0.20	0.40	0.78	0.20	0.39	0.20	0.20	0.20	0.33	0.10	0.52	0.44	0.002	0.002
F. graminearum	Aq.	0.39	0.78	0.39	0.65	0.78	0.78	0.30	0.39	0.39	0.39	1.56	1.56	0.39	0.39	1.56	1.56	0.39	0.65	0.78	1.56			
	Org.	0.20	0.78	0.40	0.20	0.10	0.16	0.20	0.78	3.13	3.13	0.01	**0.01**	0.78	0.78	0.10	**0.10**	0.20	0.20	0.01	**0.01**	0.62	0.004	0.004
F. verticillioides	Aq.	0.78	1.56	2.08	2.61	1.56	1.56	3.13	3.13	3.13	3.13	0.78	1.56	0.39	0.39	0.78	0.78	0.78	2.61	0.78	1.56	1.89		
	Org.	0.20	0.39	0.02	0.20	0.10	0.20	0.20	0.20	0.10	0.20	1.56	1.56	0.40	0.83	0.20	0.20	0.20	0.78	0.10	0.17	0.47	0.006	0.006
F. oxysporum	Aq.	0.78	0.78	0.78	2.34	0.78	0.78	3.13	3.13	3.13	3.13	3.13	6.25	0.20	0.39	0.39	0.39	0.39	2.34	0.78	0.65	2.02		
	Org.	0.78	0.26	0.20	0.16	0.10	**0.10**	1.56	2.61	0.65	0.65	0.39	1.56	0.78	0.78	0.01	**0.02**	0.52	0.91	0.01	**0.01**	0.71	0.004	0.004
Ave. MIC (48hr)	Aq.	0.98		1.48		1.04		1.69		3.58		4.17		0.39		0.78		2.09		1.34		-	-	-
Ave. MIC (48hr)	Org	0.53		0.19		**0.13**		0.67		0.76		0.95		0.76		**0.05**		0.40		**0.12**		-	-	-

Key: Values shown in bold shows noteworthy activity at 48 hours incubation. A-*Aspergillus*, F-*Furasium*, Aq-Aqueous, Org-Organic, - not applicable, Ave- Average, MIC- Minimum Inhibitory Concentrations.

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to possess high mutation frequencies and produce higher yields of aflatoxins and are increasingly becoming resistant to several other fungicides already available in the market [37]. These strains are believed to possess two different homologous genes, cyp51A and cyp51B, encoding C-14 alpha sterol demethylase (Cyp51) and an mdr gene encoding an ATP-binding cassette protein which may be involved in multidrug resistance [38].

Generally, organic extracts exhibited better antimyco-toxigenic effect compared to the aqueous extracts of the selected medicinal plants extracts. Organic extract of *Milletia grandis* had the lowest minimum inhibitory concentration (MIC) of 0.01 mg/mL against three fungal strains such as *Aspergillus ochraceous* and *Fusarium oxysporum* and *Fusarium graminearum* at a 48 hr incubation period. A similar trend of MIC value was exhibited by organic extract of *Ziziphus mucronata* against *F. graminearum* after 48 hr incubation time. However, the organic extracts from *Solanum panduriforme* exhibited the overall MIC value of 0.05 mg/mL against the selected fungal strains at 48 hr incubation time. Recently, some authors reported the antimyco-toxigenic potential of isolated compounds such as torvoside K from *Solanum* species against both *Aspergillus* and *Furasium* species yielding MIC values ranging from 33.4 to 37.4 μg/mL [39].

The organic extract of *Harpephyllum caffrum* exhibited notable MIC value of 0.10 mg/mL against *Asperillus parasiticus* in the current study. Elsewhere, the methanol extracts from *H. caffrum* revealed MIC value of 0.63 mg/mL against similar species [40]. The differences in terms of MIC values may be attributed to choice of the extracting solvents, collection times, locality of collection and other geographical factors. *Aspergillus parasiticus* was the most susceptible organism with the overall MIC value of 0.22 mg/mL against all the selected organic extracts at 48 hr incubation period.

Besides a growing interest and an extensively increasing trend in assessing the antifungal potential of South African medicinal plants (Table 3) extracts, essential oils and isolated compounds, some of the crude organic extracts from the selected medicinal plants species relatively exhibited a potential anti-mycotoxigenic potential against selected fungal strains. *Ziziphus mucronata*, *Harpephyllum caffrum*, *Solanum panduriforme* and *Milletia grandis* revealed some activity better than most of the reported medicinal plants extracts revealing some anti-mycotoxigenic activity of 0.01−0.10 mg ml against a wide variety of pathogens.

The general consensus on the antimicrobial activity of plant extracts is that the MIC value of 0.1 mg/mL is referred to as potent and worth investigating for active compounds that may be responsible for such activities [45, 46]. When using the said benchmark, in the current study, *Waburgia salutaris*, *Bauhinia galpinii*, *H. caffrum, Solanum panduriforme*, and *M. grandis* are good candidates for investigating the relevant individual compounds which may well explain the antimicrobial activity

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Table 3. Anti-mycotoxigenic activity of some South African medicinal plants.

Medicinal plants (plant part used)	Solvents and model(s) used	Tested doses	Positive control(s) used	Activity and notable results	Experimental evidence	References used
Bucida buceras (Leaves)	Acetone, hexane, methanol and dichloromethane extracts, Bio-autography and micro dilution assay.	10 mg/ml	Amphotericin B	Acetone extract from *B. buceras* revealed lowest MIC value of 0.02 mg/ml against *Penicillium janthinellum*, *Trochoderma harzianum* and *Furasium oxysporum*.	Positive evidence, dose dependent.	[40]
Arctotis arctotoides (Shoots)	Acetone, Agar diffusion method.	10 mg/ml	None used	The extract exhibited a minimum inhibitory concentration (MIC) value of 0.35 mg/ml against *Schizophyllum commune*.	Positive evidence, dose dependent.	[41]
Terminalia brachystemma (Leaves)	Acetone, hexane, methanol and dichloromethane extracts, Bio-autography and micro dilution assay.		Amphotericin B	The dichloromethane extract revealed a MIC value of 0.08 mg/ml against *Aspergillus fumigatus.*	Positive evidence, dose dependent.	[13]
Euclea natalensis (Root bark)	Compounds isolated from ethanol extract, Agar cup diffusion assay.	0.01, 0.05 and 0.1 mg/ml	Bifonazole and metalaxyl.	β-sitosterol, 20(29)-lupene-3β-isoferulate and shinanolone exhibited a significant inhibition of *Aspergillus niger* at 0.01 mg/ml.	Positive evidence, dose dependent.	[42]
Melianthus comosus (Leaves)	Fractions made from leaves extracted with acetone, Micro dilution assay.	10 mg/ml	None used	Acetone extract revealed a MIC value of 0.02 mg/ml against *Rhizoctonia solani*. The extract further exhibited MIC value of 0.04 mg/ml against *Penicillium janthinellum*, *Penicillium expansum*, *Colletotrichum gloeosporiodes and Phytophthora nicotiana.*	Positive evidence, dose dependent.	[43]
Syzygium aromaticum, Lippia rehmannii Thymus vulgaris and *Cinnamon zeylanicum*	Essential oils, Toxic medium assay and anti-biofilm capacity.	100 to 3000 ul/l.	None used	The oils from the plant species revealed an inhibitory effect of 72−100 % against *Furasium oxysporum* at 500 µl/l.	Positive evidence, dose dependent.	[44]

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of these plants. It should be noted that the antifungal activity of these plant extracts against *Furasium* and *Aspergilllus* species was not found in the literature. The leaves of *B. galpinii* are reported to contalin quercetin-3-O-b-glactopyranoside, myricetin-3-O-bgalactopyranoside, 2″-O-rhamnosylvitexin [47].

The organic extracts from these plant species revealed MIC values of 0.1 mg/mL or less against at least two of the selected fungal strains. The antifungal activity of *H. caffrum* stem bark against *Candida albicans* has been reported [48]. In the earlier report, two pharmacologically active triterpenoids, β-sitosterol and lupeol, were isolated from the fruit of *H. caffrum* while an alkyl p-coumaric acid ester, and (+)-catechin were isolated from the stem bark [49]. Elsewhere, the ethanol extract of the leaf from *H. caffrum* revealed compounds such as ethyl gallate, quercetin-3-O-rhamnoside, hendecane, methyl linoleate, betulinic acid, 3-acetyl-methyl betulinate, lupenone and lupeol [50], while the dichloromethane extract from leaves of *W. salutaris* revealed the presence of warburganal, polygodial, muzigadial, andugandensidial [51]. It should be noted that the biological and phytochemical data on *M. grandis* is scarce in the literature. In the disk diffusion assay, extracts from *S. panduriforme* did not show any antifungal activity [52].

3.2. Cytotoxicity and selectivity index

Although the pharmacological activity of most plant extracts and isolated have been explored in the entire world, the potential cytotoxic effects of such plants is less explored. However, there is a growing trend from researchers in carrying out such work. The cytotoxicity of various medicinal plant extracts in Africa has been carried out [53, 54, 55]. The results of the cytotoxic effect and selectivity index of selected medicinal plants are reported in Table 4. The higher LC_{50} implies that it would take a large quantity of the extract to cause a toxic response, while small LD_{50} values are highly toxic and could be dangerous to human health [56]. The LC_{50} of the selected medicinal plants against the Bovine dermis and Vero cell line ranged from 0.01 to 0.68 mg/mL and 0.01−0.59 mg/mL respectively. The organic extracts from *Carpobrutus eludis, Ziziphus mucronata, Harpephyllum caffrum* and *Milletia grandis* revealed better anti-proliferative effect on Bovine dermis compared to Vero cell line. These results may well validate the use and safety efficacy of *C. eludis* in traditional medicine as it is applied to wounds and other skin related infections. Although *C. eludis* and *Waburgia salutaris* revealed some degree of toxicity (LD_{50} = 0.01 mg/mL) against Bovine dermis and Vero cell lines respectively, the selected plant species are generally none toxic. Some authors refer to the IC_{50} of 0.1 mg/mL as potentially toxic to cell lines [57]. Moreover, the American National Cancer Institute (NCI) refer to an IC_{50} of less than 0.03 mg/mL to be toxic [58]. When applying these two standards to our data, only *C. eludis* and *W. salutaris* extracts exhibited some degree of toxicity against Bovine dermis and Vero cell respectively. However, there

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Table 4. Cytotoxicity studies (LC_{50} in mg/ml) and selectivity index (SI) of South African medicinal plants against Bovine dermis and Vero cell lines.

	Medicinal plants extracts		***Waburgia salutaris***	***Bauhinia galpinii***	***Carpobrotus eludis***	***Ziziphus mucronata***	***Solanum aculeastrum***	***Solanum panduriforme***	***Harperphylum caffrum***	***Milletia grandis***	**Doxorubicin (positive control)**
	Maesa lanceolata	***Ricinus communis***									
Bovine dermis	0.09 ± 0.01*	0.12 ± 0.08	0.68 ± 0.02*	0.44 ± 0.04*	0.01 ± 0.01*	0.10 ± 0.04*	0.68 ± 0.02*	0.18 ± 0.02*	0.19 ± 0.04*	0.10 ± 0.03*	0.003 ± 0.00*
Vero	0.59 ± 0.06	0.11 ± 0.03 *	0.01 ± 0.00 *	0.11 ± 0.06	0.35 ± 0.09	0.22 ± 0.01*	0.10 ± 0.01*	0.12 ± 0.01*	0.29 ± 0.06	0.14 ± 0.04*	0.001 ± 0.00*
Fungal strains and SI values (Bovine dermis)											
A. parasiticus	**9.00**	0.6	**34.0**	**4.40**	1.00	0.06	**3.40**	**9.00**	1.90	**5.00**	
A.ochraceous	0.53	0.6	**4.25**	1.33	0.03	1.00	0.45	**4.50**	1.90	**10.0**	
A. flavus	0.06	0.6	**4.25**	**22.0**	0.05	0.25	1.74	0.90	0.58	0.23	
F. graminearum	0.12	0.6	**4.25**	0.56	0.00	**10.00**	0.87	1.80	0.95	**10.0**	
F. verticillioides	0.23	0.6	**3.40**	2.20	0.05	0.06	0.82	**9.00**	0.24	0.59	
F. oxysporum	0.35	0.75	**6.80**	0.17	0.02	0.06	0.87	0.90	0.21	**10.0**	
Fungal strains and SI values (Vero)											
A. parasiticus	**59**	0.1	0.5	1.10	**35.0**	0.14	0.50	**6.00**	2.90	**7.00**	
A.ochraceous	**3.78**	0.55	0.06	0.55	0.90	1.10	0.06	**3.00**	2.90	**14.00**	
A. flavus	0.38	0.55	0.06	**5.50**	1.75	0.28	0.26	0.60	0.88	0.27	
F. graminearum	0.76	0.55	0.06	0.55	0.11	**22.0**	0.13	1.20	1.50	**14.00**	
F. verticillioides	1.51	0.55	0.05	0.55	1.75	0.14	0.12	0.60	0.40	0.82	
F. oxysporum	2.27	0.67	0.1	0.07	0.54	0.14	0.13	**6.00**	0.32	**14.00**	

Values in bold shows a noteworthy SI value (>3.0). *P*-values ≤0.05 were significant and marked (*).

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is a need to explore the toxicity of the said plant extracts against other normal human cell line to validate their toxicity.

Phytochemically, the leaves of *C. eludis* revealed the presence of compounds such as β-amyrin, oleanolic acid, uvaol, monogalactosyldiacyl glycerol, catechin, epicatechin and procyanidin [59]. In the earlier reports, oleanolic acid isolated from other plant sources reveled an LC_{50} of >300 μg/mL against both human embryonic kidney (HEK293) and hepatocellular carcinoma (HepG2) cell lines [60]. These findings may suggest that the cytotoxic effect of the plant species may not be due to the said phenolic acid alone but other compounds or in combination with those of the known compounds.

The selectivity index (SI) of the selected plant extracts ranged from 0.00 to 34.0. In the current study, SI value of >3 indicates high selectivity [61, 62]. Plant extracts with high SI values are generally likely to be much safer for human consumption or use compared to those with lower SI values. *Waburgia salutaris* revealed highest SI value of 34.0 against *Aspergillus parasiticus* and the bovine dermis cell line. Generally SI values of *W. salutaris* were higher compared to other plant extracts, hence possess a highest safety margin. Elsewhere, the dichloromethane extract from *W. salutaris* bark exhibited SI value of 3.80 [63], thereby validating the higher selectivity index as obtained in the current study.

Generally, judging by the noteworthy SI values, the selected plant species revealed higher SI values against the Bovine dermis cell line compared to the Vero cells. These may well suggest that the plant extracts may well be applied to human skin without any harm. A contrary trend has been reported elsewhere [64].

3.3. Antioxidant activity

Free radicals play a role in decaying and deteriorating the quality and quantity of the agricultural crops. Phenolic compounds and flavonoids from various sources are believed to retard the oxidative degradation of lipids thereby improving the quality and nutritional value of foodstuffs [65]. In the current study, DPPH assay was the only selected model because of its stability and its results are more reproducible. The DPPH assay is based on the ability of the extract or compound to donate a hydrogen atom, thereby reducing the purplish color of DPPH to yellowish or colorless [66]. The results for the DPPH free radical scavenging activity of selected medicinal plants are shown in Table 5. Organic extract from *Ricinus communis* revealed the lowest IC_{50} of 945 μg/mL against a stable free radical (DPPH).

The leaves of *R. communis* are known to possess phenolic compounds such as nigrinin, caffeic acid, chlorogenic acid, p-coumaric acid, rutin, epicatechin, vitexin, gallic acid, catechin, epicatechin and procatechuic acid [67]. Generally, phenolic

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Table 5. Antioxidant, total phenolic and flavonoid contents of selected medicinal plants.

Medicinal plants	Antioxidant activity (IC_{50} μg/mL)	Total phenolic content (mg/gGAE)	Total flavonoid content (mg/gQE)
Maesa lanceolata	2252.33 ± 7.57	260.5 ± 0.52	28.94 ± 0.05*
Ricinus communis	975.07 ± 0.12	310.9 ± 6.76	33.11 ± 0.75
Waburgia salutaris	3155.68 ± 6.93	320.6 ± 1.38	19.44 ± 0.91
Bauhinia galpinii	1513.41 ± 6.71	402.1 ± 4.68	11.56 ± 0.05*
Carpbrotus eludis	2600 ± 6.42	150.9 ± 6.71	3.77 ± 0.03*
Ziziphus mucronata	2032 ± 2.11	482.8 ± 6.99	33.13 ± 0.80
Solanum aculeastrum	3216.23 ± 12.25	612.7 ± 4.68	24.33 ± 0.64
Solanum panduriforme	1847.2 ± 7.01	490.7 ± 0.02*	23.92 ± 0.92
Harpephyllum caffrum	2607.19 ± 7.57	425.9 ± 0.43	25.76 ± 0.62
Milletia grandis	1265.16 ± 9.88	576.2 ± 0.03*	26.72 ± 0.65
Ascorbic acid	68.66 ± 2.25	-	-

Results were reported as mean ± SE (n = 3).There was poor correlation between antioxidant activity and TFC ($R^2 = 0.01$). *P*-values ≤0.05 were significant and marked (*).

acids are known to possess higher antioxidant activity [68]. In the current study, a similar trend has been observed.

Elsewhere, the ethanol extract of the leaves revealed an IC_{50} of 37.32 μg/mL [69], while the methanol extract of the leaves of *R. communis* revealed a strong inhibition of DPPH, exhibiting an IC_{50} of 41.40 μg/mL [70]. The acetone extract from *M. lanceolata* also revealed a potent IC_{50} of 12.95 μg/mL [71]. It is difficult to compare the results with the current study. Besides the differences in terms of extracting solvent used, geographical area and collection times, there is a great difference in terms of the amount of DPPH used in these studies. It should be noted that the concentration of the DPPH was also at its highest (3.75 mg/100 mL in methanol).

In the current study, the IC_{50} below 1000 μg/mL are referred to as potent, between 1000 and 2000 μg/mL as moderate inhibitors of DPPH and above 2000 μg/mL as poor and inactive. Ascorbic acid was used as a control and revealed IC_{50} of 68.66 μg/mL. Organic extracts from *Milletia grandis, Solanum panduriforme* and *Bauhinia galpinii* revealed a moderate inhibition of DPPH. In the current study, plant species such as *M. grandis, S. panduriforme, B galpinii* and *R. communis*-the moderate and best inhibitors of DPPH, which also possess notable inhibition of fungal growth, are believed to be the possible inhibitors of food decaying. However, we are in the process of validating the value of these medicinal plants species, in our laboratories, applying the plant extracts directly to the maize infected with the pathogens used in the current study.

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3.4. Total phenolic and total flavonoid contents

Phenols and flavonoids represent the phytochemicals or secondary metabolites that are abundantly available within the plant kingdom. The total phenolic and flavonoids contents of the organic extracts of the selected plant species are presented in Table 5. *Solanum aculeastrum* insignificantly revealed the highest phenolic contents, yielding 612.7 mg/g gallic acid equivalents (GAE) while *Milletia grandis* significantly revealed 576.2 mg/g (*P*-value $\leq$0.05). Furthermore, *Carpobrotus eludis* insignificantly revealed the lowest phenolic content of about 150.9 mg/g gallic acid equivalents (*P*-values $\geq$0.05).

It should be noted that in the current study, *Waburgia salutaris, Bauhinia galpinii, H. caffrum, Solanum panduriforme* and *Milletia grandis* exhibited potent antifungal activity against the selected strains. The extracts from these plants also possessed higher phenolic contents which may well explain the biological activity of selected plant species. *Ziziphus mucronata* and *Ricinus communis* insignificantly revealed the highest total flavonoid contents of 33.13 and 33.11 mg/g quercetin equivalents (QE) respectively. Generally, the selected plant species revealed higher total phenolic contents compared to flavonoids contents. Elsewhere, a similar trend has been reported [72]. The biological activity of the plant species may be attributed to mostly phenolic contents than the flavonoid.

4. Conclusions

The selected medicinal plants species exhibited noteworthy antifungal activity against the selected pathogenic crop strains. *Waburgia salutaris, Bauhinia galpinii, Harpephyllum caffrum, Solanum panduriforme,* and *Milletia grandis* are good candidates for investigating the relevant individual compounds which may well explain the antimicrobial activity of these plants species. The selected plant species were generally non-toxic to selected cell line and *W. salutaris* revealed a best safety margin with SI value of 34. Furthermore, the extracts, especially *Ricinus communis*, revealed potent antioxidant activity against the stable free radical, DPPH. In our study, we conceptualize that plant species with good antifungal and antioxidant activity are potent and may be used to inhibit growth of such fungal strains by blocking decay of food stuffs (oxidation). However, we still need to measure other aspects, which may include mycelial growth inhibition and treating the infected maize and other crops with various concentrations of plant materials. Although the phenolic and flavonoid contents of the selected medicinal plants species are reported in the current paper, there is a need to further quantify those phenolic contents using other methods like GC-MS and or isolate the individual compounds, which may be singularly or in combination explain the biological activity of the reported plant species.

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Declarations

Author contribution statement

NI Mongalo: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

PM Dikhoba, SO Soyingbe: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

TJ Makhafola: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interests.

Additional information

No additional information is available for this paper.

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