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The variation in antimicrobial and antioxidant activities of acetone leaf extracts of 12 *Moringa oleifera* (Moringaceae) trees enables the selection of trees with additional uses

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ABSTRACT

Background: The aim of this study was to evaluate the variation in antimicrobial and antioxidant activities of the leaf acetone extracts of 12 *Moringa oleifera* trees harvested in order to select the best material for clonal propagation.
Methods: A two-fold serial microdilution method was used to determine the minimum inhibitory concentration (MIC) against a panel of fungal (*Candida albicans*, *Aspergillus fumigatus* and *Cryptococcus neoformans*) and bacterial (*Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa*) species. The radical scavenging capacity was determined using 2,2 diphenyl-1-picryhydrazyl (DPPH).
Results: There was a large variation in antimicrobial activities with MICs between 0.04 and 2.50 mg/ml against bacteria and from 0.16 to >2.50 mg/ml against fungi. For samples harvested in winter: trees L3 and LP2 had significant activity against *E. faecalis* (MIC 0.08 mg/ml) and *E. coli* (MIC 0.04 mg/ml). Trees L5, LP1 and LP6 had weak activity against *E. coli* (MICs 1.25 and 2.50 mg/ml), *S. aureus* (MIC 1.25 mg/ml), and *E. faecalis* (MIC 2.50 mg/ml), while other samples had moderate activity against the four bacteria (MICs 0.16–0.63 mg/ml). From samples collected in summer: L5 (MIC 0.08 mg/ml), L6 (MIC 0.08 mg/ml after 1 h incubation), LP1 (MICs 0.08 mg/ml), LP2 (MICs 0.08 mg/ml after 1 h incubation), LP4 (0.08 mg/ml) and LP5 (MICs 0.04 and 0.08 mg/ml) had significant activity against *E. faecalis* (L5, L6, LP1, LP2, LP4, and LP5), *S. aureus* (LP1, and LP5), and *E. coli* (LP2, and LP5), respectively. Other extracts had weak antibacterial activity with MICs ranging from 0.16 to 0.63 mg/ml. Most of the samples harvested in winter had moderate antifungal activity: L1, L2, L3, L4, L5, L6, LP1, LP2, and LP3 had moderate activity against *C. albicans* (ATCC strains) with MIC of 0.63 mg/ml in all cases while L2, L3 and L4 as well as L6, LP1, LP2, LP3, LP5 and LP6 against *A. fumigatus* (MICs 0.63 mg/ml) and *C. neoformans* (MICs 0.63 mg/ml), respectively. Apart from L1 (MIC 0.31 mg/ml), L2, L3 and LP6 (MICs 0.63 mg/ml in all cases) with moderate activity, all the samples collected during summer had weak activity against *A. fumigatus* (MICs 1.25–2.50 mg/ml). All the extracts had a low radical scavenging activity with the IC_{50} values ranging from 34.72 to 109.62 μg/ml, compared to the reference standard L-ascorbic acid (IC_{50} 2.41 μg/ml). This may be related to the extractant used.
Conclusion: The large variation in antimicrobial activity and antioxidant activities of 24 acetone leaf extracts of 12 *M. oleifera* trees may lead to the selection of clonal material to serve as a source of propagation materials. Successful propagation and growth of tree LP with very good activity against *E. coli* and a high total activity could provide an additional use of this valuable plant species to rural people.

1. Introduction

Moringa oleifera Lam., a member of the Moringaceae family also known as Drumstick or Horseradish-tree, is indigenous to the sub-Himalayan regions of India, Pakistan, Bangladesh and Afghanistan (Fahey, 2005). Recently, African countries, including South Africa started cultivating the plant (Muhl et al., 2011) due to its traditional medicine use for both humans and animals (Moyo et al., 2011). *M. oleifera* tree itself represents an important health care and economic resources of biodiversity. Almost all plant parts are used in traditional medicine and as vegetables for dietary purposes (Iqbal and Bhanger, 2006). Root-bark extracts are used as anthelmintic, analgesic, and astringent, and for ulcers, tumours, earache, tuberculous glands in the neck, etc. (Nikkon et al., 2003).

Due to the importance uses of *M. oleifera* in traditional medicine, many investigations have previously reported on pharmacological properties such as antifertility (Shukla et al., 1988), anti-inflammatory, antispasmodic, and diuretic activities (Cáceres et al., 1992). Many authors have used different methods to determine the antimicrobial

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activity of *M. oleifera* extracts. However, agar diffusion assays were mostly used (Abalaka et al., 2012; Bukar et al., 2010; Talreja, 2010; Gamila et al., 2004; Nikkon et al., 2003; Kasolo et al., 2011; Nepolean et al., 2009; Chuang et al., 2007). There are serious problems using agar diffusion assays to determine the antimicrobial activity of plant extracts (Eloff, 1998) and the agar diffusion data is not easily quantified. This investigation reports on antimicrobial and antioxidant activities of 24 leaf acetone extracts from 12 *M. oleifera* trees harvested in summer and winter. The results obtained could help in determining variation and the selection of plant material for propagation that would have additional uses in treating microbial infections.

2. Material and methods

2.1. Plant materials

The *Moringa* orchards (seeds sourced from different trees in the wild from Malawi) were established in 2007, at the University of Pretoria (25°45′S, 28°16′E) Agricultural Experimental farm at an altitude of 1372 m above sea level and an average annual rainfall of 674 mm. There are many variations in the growth habit of the trees. The leaves were collected twice from 12 selected trees of *M. oleifera* on the 30th August (winter) and the 25th October (summer) 2012. The selection of trees was based on their sustainable leaf production. Two sets of six trees each were selected: the first set consisted of trees bearing mainly leaves (L) while the second set bore both leaves and pods (LP). Voucher specimens (Table 2) of the plant species were deposited at the HGWJ Schweickerdt Herbarium (PRU) of the University of Pretoria.

2.2. Climatic conditions and extraction

The climatic conditions at the experimental farm during the *Moringa* production periods were recorded and the data are presented in Table 1. The mature leaves harvested from hybrid trees were dried in the shade at room temperature and milled into fine powder that was stored at 25 °C in the dark in tightly closed glass containers. The leaf powder (1 g) of each sample was extracted three times with acetone (10 ml) for 1 h under shaking condition to give the crude extracts.

2.3. Antimicrobial assay

2.3.1. Microorganisms

The following served as test organisms in this study: Bacterial microorganisms were Gram-positive *Staphylococcus aureus* (ATCC 29213) and *Enterococcus faecalis* (ATCC 29212), Gram-negative, *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). Fungal microorganisms used were: *Candida albicans*, *Aspergillus fumigatus* and *Cryptococcus neoformans* (animal isolates), and *C. albicans* (ATCC 10231). Some fungal strains used were cultured from clinical cases of fungal infectious diseases in animals, before treatment, in the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science. *C. albicans* was isolated from a Gouldian finch, *C. neoformans* from a cheetah, while *A. fumigatus* was isolated from a chicken which suffered from a systemic mycosis.

2.3.2. Minimal inhibitory concentration (MIC) determination

Fungal and bacterial cultures were taken from a 24 h incubation (37 °C) agar culture plates and inoculated in fresh Sabouraud Dextrose Broth (SDB) and Mueller Hinton Broth (MHB) (Fluka, Switzerland), respectively. The turbidity of the microbial suspension was adjusted to a McFarland standard 0.5 equivalent to concentrations of $1–5 \times 10^7$ CFU/ml for fungi and $1–5 \times 10^8$ for bacteria. The microbial suspensions were further diluted (1:100, v/v) in media to result into final inoculums of approximately 1.5×10^4 CFU/ml for fungi and 1.5×10^6 CFU/ml for bacteria.

The two-fold serial microdilution method described by Eloff (1998) was used to determine the minimum inhibitory concentration (MIC) values for extracts against bacteria and for fungi the same method as modified by Masoko et al. (2005) was used.

A 100 μl of the (10 mg/ml) extract dissolved in acetone was serially diluted two-fold in triplicate with sterile distilled water in 96-well microtitre plates and 100 μl fungal cultures in Potatoes Dextrose Broth (PDB) was added to each well. Amphotericin B and acetone were used as positive and negative controls, respectively. To indicate the growth of microorganisms, 40 μl of 0.2 mg/ml of *p*-iodonitrotetrazolium violet (INT) was added to each well. The microplates were sealed in air-tight plastic bags and then incubated at 37 °C in 100% relative humidity. MIC was taken as the lowest concentration of the extract that inhibited fungal growth after 16 and 36 h.

A 100 μl of extract dissolved in acetone at a concentration of 10 mg/ml in triplicate were serially diluted two-fold with sterile distilled water in 96-well microtitre plates and 100 μl of freshly prepared bacterial culture in MHB as described above was added to each well. Acetone was used as negative control while Gentamicin was a positive control. The microtitre plates were sealed in plastic bags and were incubated for 24 h at 37 °C. Thereafter, 40 μl of 0.2 mg/ml of INT was added to each well to indicate microbial growth. The microtitre plates were further incubated at 37 °C and minimal inhibitory concentration was determined 1 and 2 h after the addition of INT. MIC was determined as the lowest concentration inhibiting microbial growth, indicated by a decrease in the intensity of the red colour of the formazan.

2.3.3. DPPH radical scavenging assay

The antioxidant activity was determined using the method described by Du Toit et al. (2001) with slight modifications. Samples were dissolved in HPLC-grade methanol (Sigma-Aldrich, Germany) and two-fold serially diluted to concentration ranges of 1000 to 7.81 μg/ml for extracts and 40 to 0.31 μg/ml for a standard reference L-ascorbic acid (Sigma, Germany). Briefly, 40 μl of (10 mg/ml) samples was introduced in a 96-well microtitre plates (Bioster, Spain) and two-fold serially diluted in methanol. Thereafter, 160 μl of (3.7 mg/100 ml) methanolic solution of 2,2-diphenyl-1-picryhydrazyl (DPPH) was introduced in each well and after 30 min incubation at room temperature in the darkness the absorbance was

Table 1
Average monthly climatic conditions of the University of Pretoria Experimental Farm from January to December 2012.[a]

Parameters	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Average
Temp min (°C)	18	18	16	12	10	6	6	8	12	15	16	17	13
Temp max (°C)	27	28	26	22	23	18	19	20	23	24	26	25	24
Light min (%)	95	95	95	95	93	94	93	95	96	97	96	97	95
Light max (%)	96	96	96	96	94	95	94	96	95	96	95	96	95
Clouds (%)	4	4	3	2	1	1	1	1	4	4	4	4	2.75
Dry (%)	21	21	19	14	13	8	10	11	12	18	19	20	15.5
Wet (%)	18	18	16	12	10	6	7	8	11	16	16	17	13
Rainfall (mm)	74	108	53.5	11.45	0	0	0	0	141.1	108	65	142	58.7

[a] These data were provided by the weather station service at the University of Pretoria Experimental Farm.

Table 2
Extraction yields (%) of acetone leaf extracts of 12 *M. oleifera* collected in winter and summer and voucher specimen numbers in PRU.

Samples[a]	L1	L2	L3	L4	L5	L6	LP1	LP2	LP3	LP4	LP5	LP6
Winter	2.08	6.29	6.01	5.60	5.05	3.87	4.26	7.06	5.33	3.63	2.68	2.33
Summer	2.66	2.39	2.56	1.75	2.25	1.81	2.81	2.45	2.76	2.61	2.15	2.71
Voucher specimens	119823	119824	119825	119826	119827	119828	119829	119830	119831	119832	119833	119834

[a] L = tree bearing leaves, LP = tree bearing leaves and pods.

measured at 517 nm using a Multi-Mode Microplate Reader (BioTek, USA). The free radical scavenging activity of each sample and the reference standard were determined as percent of the inhibition obtained from the following formula: Radical scavenging capacity (%) = [100 − ((Ab_{sample} − Ab_{blank}) / $Ab_{control}$) × 100]. With Ab_{sample} as the absorbance of the extract with DPPH, Ab_{blank} as the absorbance of the extract without DPPH and $Ab_{control}$ as absorbance of methanol and DPPH. The concentration of samples reducing 50% of free radical DPPH (IC_{50}) was determined by plotting the percentage of inhibition against the sample concentrations. The assay was replicated three times and results are expressed as mean ± standard deviation.

3. Results

3.1. Extraction yields

Twenty-four leaf powders (1 g each) from 12 *Moringa* collected in two different seasons (Winter and Summer) were extracted in acetone (10 ml) to give extracts with the masses ranging from 17.5 to 70.6 mg. The percentage yield of extraction is presented in Table 2. It is clear that samples collected during winter had the highest yields compared to those from summer. This may be explained by the maturation of leaves during winter. The highest yield (7.06%) was obtained for the leaf and pods bearing tree sample LP2 collected in winter.

3.2. Antimicrobial activity

Antimicrobial activities of 24 leaf acetone extracts from 12 *M. oleifera* collected during winter and summer were determined against bacteria and fungi, and the results are given as the minimal inhibitory concentration (MIC) (Tables 3 and 4), and total activity (Tables 5 and 6). Antimicrobial activities of extracts are considered significant if the MIC value is 0.10 mg/ml or lower, moderate if 0.10 < MIC ≤ 0.63 mg/ml, and weak if MIC is greater than 0.63 mg/ml (Eloff, 2004; Kuete, 2010). Based on these criteria, all the test samples had significant to weak antimicrobial activities with MICs between 0.04 and 2.50 mg/ml against bacteria and between 0.16 to >2.50 mg/ml against fungi.

There was a much larger variation between antibacterial activities of extracts to bacteria. The variability coefficients for bacteria were 75.2% (average value 0.297 mg/ml and standard deviation 0.395 mg/ml) in winter and 31.3% in summer. The variability coefficients were lower for fungi; 19.2% in winter and 23.1% in summer.

3.2.1. Antifungal activity

Extracts of trees L1, L2, L3, L4, L5, L6, LP1, LP2, and LP3 harvested in winter had moderate antifungal activity against *C. albicans* (ATCC strains) with MIC of 0.63 mg/ml in all cases. Extracts L2, L3 and L4 as well as L6, LP1, LP2, LP3, LP5 and LP6 from trees collected in winter had also moderate antifungal activities against *A. fumigatus* (MICs 0.63 mg/ml) and *C. neoformans* (MICs 0.63 mg/ml), respectively. Such as LP2 (MIC 2.50 mg/ml), others samples collected in winter had weak antifungal activity against *C. albicans* (isolate strains) with MIC 1.25 mg/ml. Samples harvested from summer had moderate antifungal activity against *C. albicans* isolate strains with MIC between 0.16 and 0.31 mg/ml, while some of those samples had moderate to poor activity against the ATCC strains of *Candida* and *C. neoformans* (MICs from 0.63 to >2.50 mg/ml). Apart from L1 (MIC 0.31 mg/ml), L2, L3 and LP6 (MICs 0.63 mg/ml in all cases) with moderate activity, all the samples collected during summer had weak activity against *A. fumigatus* (MICs 1.25–2.50 mg/ml).

3.2.2. Antibacterial activity

The results of antibacterial activities of the 12 *M. oleifera* acetone extracts on four bacteria are presented in Table 4. From samples

Table 3
Minimum inhibitory concentration (MIC) in mg/ml of acetone leaf extracts from 12 *M. oleifera* against fungi. The results are the mean of three replicates and the standard deviation was zero.

Samples[a]	Fungi[b] (winter)									Fungi[b] (summer)								
	C.a		C.A		C.n[c]		A.f		Average	C.a		C.A		C.n[c]		A.f		Average
	16 h	24 h	16 h	24 h	16 h	24 h	24 h	48 h		16 h	24 h	16 h	24 h	16 h	24 h	24 h	36 h	
L1	1.25	1.25	0.63	0.63	1.25	1.25	1.25	1.25	1.10	0.31	0.31	2.5	>2.50	0.63	>2.50	0.31	0.31	0.73
L2	1.25	1.25	0.63	0.63	1.25	1.25	0.63	0.63	0.94	0.31	0.31	0.63	2.50	1.25	1.25	0.63	0.63	0.94
L3	1.25	1.25	0.63	0.63	1.25	1.25	0.63	0.63	0.94	0.31	0.31	0.63	1.25	0.63	1.25	0.63	1.25	0.78
L4	1.25	1.25	0.63	0.63	1.25	1.25	0.63	0.63	0.94	0.31	0.31	0.63	0.63	0.63	0.63	1.25	1.25	0.71
L5	1.25	1.25	0.63	0.63	1.25	1.25	2.50	2.50	1.41	0.31	0.31	1.25	1.25	1.25	1.25	2.50	2.50	1.33
L6	1.25	1.25	0.63	0.63	0.63	0.63	1.25	1.25	0.94	0.31	0.31	0.63	1.25	1.25	1.25	2.50	2.50	1.25
LP1	1.25	1.25	0.63	0.63	0.63	0.63	2.50	2.50	1.25	0.16	0.16	0.63	0.63	1.25	1.25	2.50	2.50	1.14
LP2	2.50	2.50	0.63	0.63	0.63	0.63	1.25	2.50	1.41	0.16	0.31	1.25	1.25	1.25	1.25	1.25	1.25	1.00
Lp3	1.25	1.25	0.63	1.25	0.63	0.63	>2.50	>2.50	1.33	0.16	0.16	0.63	0.63	0.63	0.63	2.50	2.50	0.98
LP4	1.25	1.25	1.25	1.25	1.25	1.25	2.50	>2.50	1.56	0.31	0.31	1.25	2.50	1.25	1.25	2.50	2.50	1.48
LP5	1.25	1.25	0.63	1.25	0.63	0.63	1.25	1.25	1.01	0.16	0.16	0.63	0.63	0.63	0.63	2.50	2.50	0.98
LP6	1.25	1.25	1.25	1.25	0.63	0.63	1.25	1.25	1.10	0.16	0.16	>2.50	>2.50	1.25	1.25	0.63	0.63	1.01
Average	1.35	1.35	0.73	0.84	0.94	0.94	1.42	1.44		0.25	0.26	0.97	1.25	0.99	1.08	1.64	1.69	
Controls[d]																		
Amp B	0.63	0.63	0.32	0.32	0.63	0.63	0.63	0.63	0.55	0.63	0.63	0.32	0.32	0.63	0.63	0.63	0.63	0.55

[a] L = Leaves bearing tree and LP = Leaves and Pods bearing tree.
[b] C.a: *Candida albicans* (isolate), C.A: *Candida albicans* (ATCC strains), C.n: *Cryptococcus neoformans*, A.f: *Aspergillus fumigatus*.
[c] With this microorganism little reaction was observed after 16 h and values were measured after 24 and 36 h.
[d] Amp B: Amphotericin B in μg/ml.

Table 4
Minimum inhibitory concentration (MIC) in mg/ml of acetone leaf extracts from 12 *M. oleifera* against bacteria. The results are the mean of three replicates and the standard deviation was zero.

Samples[a]	Bacteria[b] (winter)									Bacteria[b] (summer)								
	P.a		S.a		E.c		E.f		Average	P.a		S.a		E.c		E.f		Average
	1 h	2 h	1 h	2 h	1 h	2 h	1 h	2 h		1 h	2 h	1 h	2 h	1 h	2 h	1 h	2 h	
L1	0.31	0.31	0.31	0.31	0.31	0.31	0.16	0.16	0.27	0.16	0.16	0.31	0.31	0.31	0.31	0.16	0.16	0.24
L2	0.31	0.31	0.16	0.31	0.16	0.16	0.16	0.16	0.22	0.16	0.16	0.16	0.31	0.31	0.31	0.16	0.16	0.23
L3	0.31	0.31	0.31	0.31	0.16	0.16	**0.08**	**0.08**	0.22	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
L4	0.63	0.63	0.31	0.31	0.16	0.16	0.16	0.16	0.32	0.31	0.31	0.16	0.16	0.63	0.63	0.31	0.31	0.35
L5	0.31	0.31	0.63	0.63	1.25	1.25	0.31	0.31	0.63	0.31	0.31	0.16	0.16	0.16	0.16	**0.08**	**0.08**	0.18
L6	0.31	0.31	0.16	0.16	0.16	0.16	0.16	0.16	0.20	0.31	0.31	0.16	0.16	0.16	0. 16	**0.08**	0.16	0.20
LP1	0.31	0.31	1.25	1.25	2.50	2.50	0.31	0.31	1.10	0.16	0.16	**0.08**	**0.08**	0.31	0.31	**0.08**	**0.08**	0.20
LP2	0.31	0.31	0.16	0.16	**0.04**	**0.04**	0.16	0.16	0.17	0.16	0.16	0.16	0.16	**0.08**	0.16	**0.08**	0.16	0.14
LP3	0.31	0.31	0.16	0.16	0.16	0.16	0.16	0.16	0.20	0.16	0. 16	0.16	0.16	0.16	0.31	0.16	0.16	0.20
LP4	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.31	0.16	0.16	0.16	0.16	**0.08**	**0.08**	0.20
LP5	0.31	0.31	0.63	0.63	0.31	0.31	0.63	0.63	0.47	0.16	0.16	**0.08**	**0.08**	**0.08**	**0.08**	**0.04**	**0.04**	0.09
LP6	0.31	0.31	0.63	0.63	0.63	0.63	0.63	2.50	0.78	0.31	0.31	0.16	0.16	0.16	0.16	0.31	0.31	0.24
Average	0.32	0.32	0.41	0.42	0.50	0.50	0.26	0.41	0.40	0.21	0.23	0.16	0.17	0.22	0.25	0.14	0.16	0.21
Controls[c]																		
Gen	1.56	1.56	0.78	0.78	0.39	0.39	1.56	1.56	1.07	1.56	1.56	0.78	0.78	0.39	0.39	1.56	1.56	1.07

Bold figures indicate MIC <0.1 mg/ml.
[a] L = tree bearing leaves, LP = tree bearing leaves and Pods.
[b] P.a: *Pseudomonas aeruginosa*, S.a: *Staphylococcus aureus*, E.c: *Escherichia coli*, E.f: *Enterococcus faecalis*.
[c] Gen: gentamicin in μg/ml.

harvested in winter: L3 and LP2 had significant activity against *E. faecalis* (MIC 0.08 mg/ml) and *E. coli* (MIC 0.04 mg/ml). Samples L5, LP1 and LP6 had respectively weak activity against *E. coli* (MIC 1.25 mg/ml), *S. aureus* (MIC 1.25 mg/ml) and *E. coli* (MIC 2.50 mg/ml), and *E. faecalis* (MIC 2.50 mg/ml after 2 h incubation), while other samples had moderate activity against the four bacteria (MICs 0.16–0.63 mg/ml).

From samples collected in summer: L5 (MIC 0.08 mg/ml), L6 (MIC 0.08 mg/ml after 1 h incubation), LP1 (MICs 0.08 mg/ml), LP2 (MICs 0.08 mg/ml after 1 h incubation), LP4 (0.08 mg/ml) and LP5 (MICs 0.04–0.08 mg/ml) had significant activity against *E. faecalis* (L5, L6, LP1, LP2, LP4, and LP5), *S. aureus* (LP1, and LP5), and *E. coli* (LP2, and LP5), respectively. Other samples had weak antibacterial activity with MIC ranging from 0.16 to 0.63 mg/ml.

3.3. Antioxidant activity

The antioxidant activities of acetone extracts from 12 *M. oleifera* collected in winter and summer were determined using DPPH method and the results (Table 7) are reported in terms of the concentration of the sample decreasing 50% of free radical scavenging (IC_{50}). All the test samples had radical scavenging activity with the IC_{50} values ranging from 34.72 to 109.62 μg/ml, as compared to the reference standard L-ascorbic acid (IC_{50} 2.41 μg/ml). For samples collected in winter, LP5 (IC_{50} 34.72 μg/ml), LP2 (IC_{50} 35.00 μg/ml), LP6 (IC_{50} 38.60 μg/ml), L5 (IC_{50} 41.42 μg/ml), LP1 (IC_{50} 45.37 μg/ml), L1 (IC_{50} 49.59 μg/ml), L3 (IC_{50} 53.06 μg/ml), and L2 (IC_{50} 58.32 μg/ml) had the highest antioxidant activity followed by LP4, L4, L6, and LP3 with the IC_{50} values of 94.83, 94.97, 77.97 and 105.87 μg/ml, respectively. From samples harvested during summer, extracts such as LP6 (IC_{50} 45.18 μg/ml), L2 (IC_{50} 48.45 μg/ml), LP3 (IC_{50} 51.08 μg/ml), LP2 (IC_{50} 52.54 μg/ml), L5 (IC_{50} 56.73 μg/ml) and LP1 (IC_{50} 61.68 μg/ml) had highest antioxidant activity followed by LP4, L6, L1, L3, L4, and LP5 with the IC_{50} values of 72.83, 74.92, 76.60, 85.71, 93.85, and 109.62 μg/ml, respectively. If more polar extractant than acetone was used, the antioxidant activities of the extracts may have been higher.

4. Discussion

The antifungal activity of the acetone leaf extracts of 12 *M. oleifera* collected in two seasons were generally weak in this study. However, LP1, LP3 and LP6 collected during summer were the most antifungal

Table 5
Total activity in ml/g of acetone leaf extracts from 12 *M. oleifera* against fungi calculated by dividing mass in mg in1 g of extract with their MIC values in mg/ml. This indicated to what volume one g of the extract can be diluted and still inhibits the relevant microorganism.

Samples[a]	Fungi[b] (Winter)									Fungi[b] (Summer)								
	C.a		C.A		C.n[c]		A.f		Average	C.a		C.A		C.n		A.f		Average
	16 h	24 h	16 h	24 h	16 h	24 h	24 h	48 h		16 h	24 h	16 h	24 h	16 h	24 h	24 h	48 h	
L1	17	17	33	33	33	33	17	17	25	86	86	11	5	42	5	86	86	51
L2	50	50	100	100	100	100	100	100	88	77	77	38	10	19	19	38	38	58
L3	48	48	95	95	48	48	95	95	72	83	83	41	20	41	20	41	20	44
L4	45	45	89	89	45	45	89	89	67	56	56	28	28	28	28	14	14	32
L5	40	40	80	80	40	40	20	20	45	73	73	18	18	18	18	9	9	30
L6	31	31	61	61	61	61	31	31	46	58	58	29	14	14	14	7	7	25
LP1	34	34	68	68	68	68	17	17	42	176	176	45	45	22	22	11	11	64
LP2	28	28	112	112	112	112	56	28	74	153	79	20	20	20	20	20	20	44
LP3	43	43	85	43	85	85	11	11	51	173	173	44	44	44	44	11	11	61
LP4	29	29	29	29	29	29	15	7	25	84	84	21	10	21	21	10	10	33
LP5	21	21	43	21	43	43	21	21	29	134	134	34	34	34	34	9	9	52
LP6	19	19	19	19	37	37	19	19	24	169	169	5	5	22	22	43	43	60

[a] L = tree bearing leaves, LP = tree bearing leaves and pods.
[b] C.a: *Candida albicans* (isolate), C.A: *Candida albicans* (ATCC strains), C.n: *Cryptococcus neoformans*, A.f: *Aspergillus fumigatus*.
[c] With this microorganism little reaction was observed after 16 h and values were measured after 24 and 36 h.

Table 6
Total activity in ml/g of acetone leaf extracts from *M. oleifera* calculated by dividing mass in mg in 1 g of extract with their MIC values in mg/ml. This indicated to what volume one g of the extract can be diluted and still inhibits the relevant microorganism.

Samples[a]	Bacteria[b] (winter)									Bacteria[b] (summer)								
	P.a		S.a		E.c		E.f		Average	P.a		S.a		E.c		E.f		Average
	1 h	2 h	1 h	2 h	1 h	2 h	1 h	2 h		1 h	2 h	1 h	2 h	1 h	2 h	1 h	2 h	
L1	67	67	67	67	67	67	130	130	83	166	166	86	86	86	86	166	166	126
L2	**203**	**203**	**393**	**203**	**393**	**393**	**393**	**393**	**321**	149	159	149	77	77	77	149	149	122
L3	194	194	194	194	376	376	751	751	306	160	160	160	160	160	160	160	160	160
L4	89	89	181	181	350	350	350	350	243	56	56	109	109	27	27	56	56	62
L5	163	163	80	80	40	40	163	163	112	73	73	141	141	141	141	281	281	159
L6	125	125	242	242	242	242	242	242	213	58	58	113	113	113	113	226	113	113
LP1	137	137	34	34	17	17	137	137	81	176	176	351	351	91	91	351	351	242
LP2	**228**	**228**	**441**	**441**	**1765**	**1765**	**441**	**441**	**890**	153	153	153	153	306	153	306	153	191
LP3	172	172	333	333	333	333	333	333	293	173	173	173	173	173	79	173	173	161
LP4	227	227	227	227	227	227	227	227	227	163	84	163	163	163	163	326	326	193
LP5	86	86	43	43	86	86	43	43	65	**134**	**134**	**269**	**269**	**269**	**269**	**537**	**537**	**302**
LP6	75	75	37	37	37	37	37	15	44	87	87	169	169	169	169	87	87	128

Bold figures indicate trees with high total activity.
[a] L = tree bearing leaves, LP = tree bearing leaves and pods.
[b] P.a: *Pseudomonas aeruginosa*, S.a: *Staphylococcus aureus*, E.c: *Escherichia coli*, E.f: *Enterococcus faecalis*.

active samples against *C. albicans* (isolate strains) with total activities of 176, 173 and 169 ml/g, respectively. Samples L2, LP2, L3, and L4 from winter as well as LP1, LP3, and LP6 from summer had the highest antifungal activity with average total activities of 88, 74, 72, 67, 64, 61, and 60 ml/g, respectively. We can also notice from the overall result that samples collected in winter had higher antifungal activity compared to those harvested in summer. This corroborates the antioxidant property that is more efficient for samples from winter than those from summer. This may be due to the presence of more phenols than other class of constituents in the plant at that season, to combat the presence of eventual free radicals (Awouafack et al., 2013). The DPPH radical scavenging activities of water leaf extracts of *M. oleifera* collected in India was previously reported and the IC_{50} values (18.15 and 19.12 μg/ml) in two stages of maturity were not different (Sreelatha and Padma, 2009). Similar results were obtained with samples L4 (IC_{50} 94.97 and 93.85 μg/ml) and L6 (IC_{50} 77.97 and 74.92 μg/ml). Antioxidant and antibacterial activities of defatted *M. oleifera* seed flour were recently reported and bound phenolic and free phenolic extracts had IC_{50} values for DPPH radical scavenging activity of 0.9 and 14.9 mg/ml (Singh et al., 2013).

Among all the samples from winter, LP2 had the highest antibacterial activity with the average total activity of 890 ml/g. The total activity is obtained by dividing the mass in g of the extract with MIC value and this indicated to what volume 1 g of the sample can be diluted and still inhibits the growth of the relevant microorganism (Eloff, 2000, 2004). For instance, sample LP2 harvested during winter had a total activity of 1765 ml/g against *E. coli*, this implies that 1 g of LP2 dissolved in 1765 ml will still inhibit the growth of *E. coli*. Most samples harvested in winter had fungicidal and bactericidal effects. Taking in consideration average MICs, sample LP5 collected in summer had good antibacterial activity (average MIC 0.09 mg/ml) but its average total activity was three times lower than that of LP2. Tree LP2 could be an outstanding candidate for treating *E. coli* infections based on its low MIC and high total activity.

Table 7
Antioxidant activities of acetone leaf extracts from 12 *M. oleifera* collected in two seasons (winter and summer).

Samples[a]	IC_{50} (μg/ml)	
	Winter	Summer
L1	49.59 ± 0.20	76.60 ± 0.01
L2	58.32 ± 0.01	48.45 ± 0.01
L3	53.06 ± 0.01	85.71 ± 0.02
L4	94.97 ± 0.01	93.85 ± 0.01
L5	41.42 ± 0.01	56.73 ± 0.04
L6	77.97 ± 0.01	74.92 ± 0.01
LP1	45.37 ± 0.01	61.68 ± 0.02
LP2	35.00 ± 0.01	52.54 ± 0.02
LP3	105.87 ± 0.02	51.08 ± 0.01
LP4	94.83 ± 0.04	72.83 ± 0.02
LP5	34.72 ± 0.18	109.62 ± 0.02
LP6	38.60 ± 0.01	45.18 ± 0.03

[a] L = tree bearing leaves, LP = tree bearing leaves and pods, L-Ascorbic acid (IC_{50} 2.41 ± 0.01) as reference standard.

5. Conclusion

The aim of this study was to determine the variation in antibacterial and antioxidant activity between the different trees. The large variation in antimicrobial and antioxidant activities of 24 acetone leaf extracts of 12 *M. oleifera* trees could make it possible to select trees for cloning and propagation if the antimicrobial activity is stable. If the propagated trees have the same nutritional value as other Moringa trees the antimicrobial activity could be of benefit to communities where these plants are grown as a food source. Isolation of the active compound and propagation studies is currently under way.

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