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An Investigation into the *In Vitro* Antibacterial Activity of *Moringa* Leaf and Seed Extract

Yasser Jabah Alsendi*, Vicki Anderson and Saeid Al Matif

Department of Pharmacy, Liverpool John Moores University, Saudi Arabia

***Corresponding Author:** Yasser Jabah Alsendi, Department of Pharmacy, Liverpool John Moores University, Saudi Arabia.

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Abstract

Aim: Investigate the antibacterial activity of aqueous and alcoholic (ethanol) extracts of *Moringa* leaves and seeds on *Escherichia coli and Staphylococcus aureus* using Kirby-Bauer antimicrobial Disk diffusion susceptibility assays.

Materials and Methods: Water and ethanol extractions of ground *Moringa* seed and ground *Moringa* leaf were prepared and compared against ethanol alone as well as antibiotic treatments in *Kirby-Bauer* assays against *E. coli* and *S. aureus*. Gentamycin was used as a positive control antibiotic against *E. coli* and Ciprofloxacin was used for *S. aureus*.

Key Findings: ANOVA for treatments against *E. coli* and *S. aureus* showed there were statistically significant differences between treatment groups: ($F(5,48) = 120.10$, $p = 0.000$). and ($F(5,48) = 176.69$, $p = 0.000$ respectively. Water extraction of *Moringa* seed showed the highest level of antibacterial activity, and in contrast there was no statistical significant difference between the ethanol extractions of *Moringa* seed and leaf versus ethanol alone.

Conclusion: The results confirm that there are compounds in *Moringa* seed and leaf that have antibacterial activity. It is likely that these compounds are hydrophilic as suggested by the water extractions showing the most inhibition compared to ethanol extractions. Further studies are needed to identify the specific compounds most responsible for the antibacterial activity found which could lead to new antibiotics being developed.

Keywords: *Antibacterial Activity; Moringa Leaf and Seed; Escherichia coli; Staphylococcus aureus*

Abbreviations

MLW: *Moringa* Leaves Water Extract; MSW = *Moringa* Seeds Water Extract; MLE = *Moringa* Leaves Ethanol Extract; MSE = *Moringa* Seeds Ethanol Extract

Background

With new bacterial species being discovered every year [1], and existing bacteria developing antibiotic resistances it becomes increasingly important to have an array of antibiotics to use in our arsenal to treat various bacterial infections and diseases.

Many plants have been traditionally used in medicine and the *Moringa* plant (*Moringa oleifera*) is no exception [2]. The *Moringa* plant is a kind of deciduous tree that grows quite commonly in India and China, with virtually all parts of the plant being edible and exhibiting antibacterial properties [3].

One traditional use of *Moringa* seeds is using the seed cake to purify turbid water by flocculation [4]. The seed cake is a by-product from pressing the seeds for the oil they contain, and the seed cake contains a flocculating dimeric cationic protein known as MOCP or Flo which has been shown to damage the bacterial cell membranes and causing them to fuse [5]. Another molecule that has shown antibacterial activity found from *Moringa* seed is lectin, which is also water-soluble [6]. Lectin shows antibacterial activity against a variety of bacteria, and it has been shown to reduce the growth of *Staphylococcus aureus* (*S. aureus*) in polluted waters.

The *Moringa* leaf powder was used for hand washing as the powder was effective as an anti-septic and detergent when wetted in advance due to phytochemicals in the leaves [7]. The seed press cake was furthermore used as a wastewater conditioner to dewater and dry fecal sludge (due to its already mentioned flocculation property) [8].

Crude extracts of *Moringa* leaf have also been studied for their antibacterial effects against four Gram-positive bacteria including *S. aureus* and two Gram-negative bacteria including *Escherichia coli* (*E. coli*) [9]. This particular study found that ethanol extractions of *Moringa* leaf inhibited the growth of the six bacteria in the study. However another study found the aqueous ethanol extractions of *Moringa* leaf to have effective antibacterial activity against *S. aureus* but not *E. coli* [10]. Chemical analysis of the extracts with antibacterial activity found a mixture of compounds including saponins, alkaloids, polyphenols, anthraquinones, flavonoids, coumarins, tannins, triterpenes, sterols as well as a few secondary metabolites [11].

The current study focuses on *E. coli* and *S. aureus* as they are relatively ubiquitous in or on our bodies. *S. aureus* is also quite common in hospitals*. E. coli* has been well-studied and is in fact a natural inhabitant of the human gut biome and rarely causes disease [12]. However certain strains of E. coli can cause "Traveller's diarrhoea" [13], urinary tract infections (UTIs) [14] and food poisoning amongst other diseases. *E. coli* infections that cause traveller's diarrhoea can be treated with fluoroquinolones or azithromycin but as doctors want to avoid the development of antibacterial resistance, antibiotic treatment is usually avoided and these cases are usually treated by focusing on rehydration to replace fluids and electrolytes.

S. aureus is an opportunistic pathogen that makes up part of the normal microflora on human skin, upper respiratory tract and women's lower reproductive tract [15]. *S. aureus* can cause skin infections, bacterimia, sinusitis and others [16]. These infections are usually treated with β-lactam antibiotics such as penicillin, methicillin and others [16]. With the rise of penicillin-resistant strains of *S. aureus* the alternative β-lactam antibiotics have been relied on more, and then when methicillin-resistance arose with the appearance of MRSA (methicillin-resistant *Staphylococcus aureus*) alternative antibiotics such as clindamycin and linezolid were developed to treat MRSA infections [17].

S. aureus is particularly common in hospitals and very prone to developing antibiotic resistance so it has become increasingly important to find and develop new antibiotics to increase our arsenal against virulent bacteria like *S. aureus*. It has been previously suggested that the *Moringa* leaf extracts can be used to treat various infections alone or combined with antibiotics [11]. This could be a more effective alternative as *Moringa* leaves and seeds have many compounds that show antibacterial activity [18] decreasing the likelihood that *S. aureus* can develop resistances to all these compounds, as well as these compounds could act as organic blueprints in the development of novel antibiotics.

Extracts of the stem bark have been shown to be effective in treating UTIs in one small clinical study, and in another acetone extract of *Moringa* leaves were shown to be effective inhibitors of growth for six UTI-related bacteria including *E. coli* and *S. aureus* [19].

Aim of the Study

This present study aims to provide more data for the *in vitro* antibacterial activity of aqueous and ethanol extract of *Moringa* leaves as well as *Moringa* seeds against *E. coli* and *S. aureus*.

Materials and Methods

Extract preparation for *Moringa* leaves and seeds

Water extraction of leaf and seed

10g of *Moringa* seed was ground and dissolved in 200 ml hot distilled water (60°C - 70°C) then stored for 72 hours in a closed bottle. After which vacuum filtration with Whatman filter was used and the extraction was stored in a bottle at room temperature for further testing. For *Moringa* leaf, 10g of leaf was ground to powder and then the extraction was carried out the same way as the seeds.

The aqueous seed extraction had a final concentration of 0.01775 g/ml and that of the leaf was 0.01615 g/ml.

Ethanol extraction of leaf and seed

The process for both *Moringa* leaves and seeds was the same. The *Moringa* leaves were washed with distilled water and then left to dry at room temperature before being ground up in an electric blender. Then 10g of ground *Moringa* leaf powder was dissolved in 200ml of hot ethanol (60°C - 70°C) then stored for 72 hours in a closed bottle. Finally the solution was filtered using vacuum filtration without a Whatman filter and the extract was stored in a bottle at room temperature. The same was done for 10g of *Moringa* seeds.

The ethanol seed extraction had a final concentration of 0.0558g/ml and that of the leaf was 0.0443g/ml.

Collection and culturing of bacteria

The following Gram-negative and Gram-positive bacteria were obtained from Public Health England:

a) *Escherichia coli* (ATCC 25922)

b) *Staphylococcus aureus* (ATCC 25923).

A nutrient broth (6.5 ml in 500 ml of distilled water) was made and mixed with each bacterium before examination. Then a fluid sample of each bacterium was extracted and placed in a separate container to be incubated for 24 hours in an autoclave at 37ºC.

Assaying for antibacterial activity

Kirby-Bauer test

Three sets of plates containing nutrient agar (14g dissolved in 500 ml distilled water) were inoculated with 0.2 ml of bacteria. Then 6mm discs were soaked in either antibiotics, aqueous leaf extract and aqueous seed extract or antibiotics, ethanol leaf extract, ethanol seed extract and ethanol as shown in figure 1 and 2 respectively. The antibiotic disc was used as a positive control.

The antibiotic discs used in this test were:

- Gentamycin 10µg (G10)(MASTDISCs) for *E. coli*.
- Ciprofloxacin 1µg (C1)(MASTDISCS) for *S. aureus*.

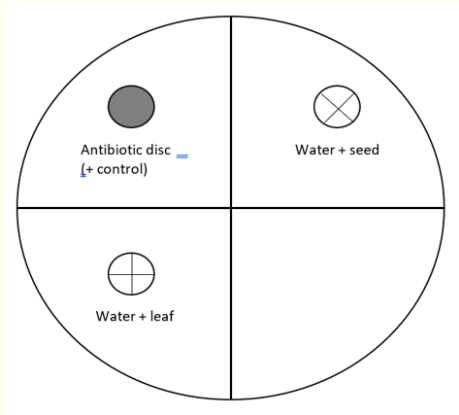

Figure 1: *Arrangement of the 6mm discs of different treatments starting from the top left going clockwise: antibiotic as a positive control (Gentamycin 10µg for E. coli and Ciprofloxacin 1µg for S. aureus), water extraction of moringa seed and water extraction of moringa leaf.*

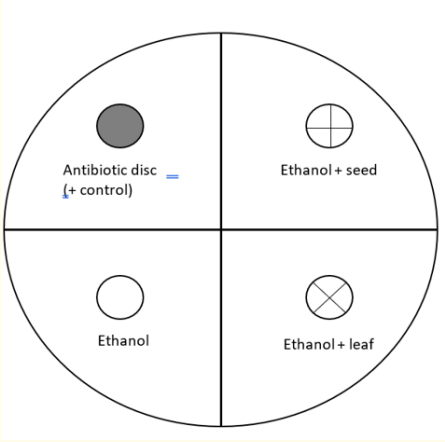

Figure 2: *Arrangement of the 6mm discs of different treatments starting from the top left going clockwise: antibiotic as a positive control (Gentamycin 10µg for E. coli and Ciprofloxacin 1µg for S. aureus), ethanol extract of moringa seed, ethanol extract of moringa leaf, and just ethanol.*

High-performance liquid chromatography

Preparation of ethanol solutions

The ground *Moringa* seed/leaf (0.3g) was immersed in 3 ml of ethanol solvent for 10 minutes to allow sample solutions to develop. The solution was then centrifuged at 4000 rpm/min for 20 minutes, and then the supernatant was transferred to a 10-mL volumetric flask. After carrying out the procedure twice, 0.1% w/v of the sample solution was filtered out using a syringe filter to triplicate vials.

Conditions and equipment

A high-performance liquid chromatography (HPLC) system (6 pc Agilent 1100 Series) was used in this study. The injector consisted of a 10 µL loop, UV detector (UVD 170U) monitoring peaks simultaneously at 280nm, 320nm, and 360nm. A reversed phase RP-C18 analytical column (Agilent 110A, 150 x 4.6 µm, 5 µm) was used with a C18 guard column (Phenomenex, Torrance, CA).

The mobile phase was made of acetonitrile (solvent A) as well as 2% acetic acid in water (v/v) (solvent B). The flow rate was maintained at 0.8 ml/min for a complete run time of 60 min and the gradient program was as follows: 100% B to 0% B in 30 min, 85% B to 50% B in 20 min, 50% B to 0% B in 5 min and finally 0% B to 100% B in 5 min.

Injection volume was 50 µl and all samples were filtered through a 0.45 µm Acrodisc syringe filter (Gelman Laboratory, MI) before injection.

Statistical analyses

Statistical analyses were performed using Minitab® and one-way ANOVA with Tukey's post hoc. A p value < 0.05 was considered statistically significant. Data was expressed as +/- SEM, and comparisons between the control and experimental groups were carried out using Excel®.

Ethics statement

Application Reference Number: 111301-01112019.

The PBS undergraduate research ethics screening process deemed that this research did not require an ethical review.

Key findings

Determination of bacterial inhibition

Kirby-Bauer assay results for *E. coli*

As shown in table 1, the means for the zone of inhibition of 6 treatments were calculated along with the standard error.

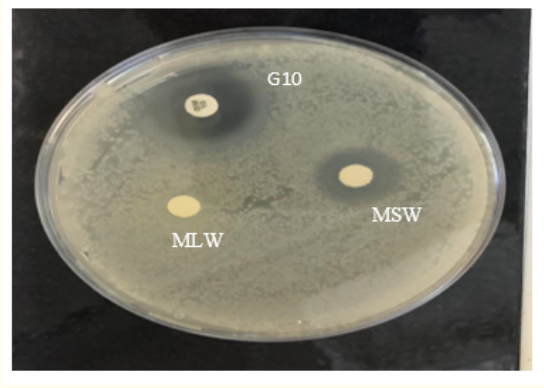

Figure 3: *Picture of petri dish inoculated with E. coli and the zones of inhibition shown for three treatments: 10 µg gentamycin (G10), water extract of moringa seed (MSW), water extract of moringa leaf (MLW).*

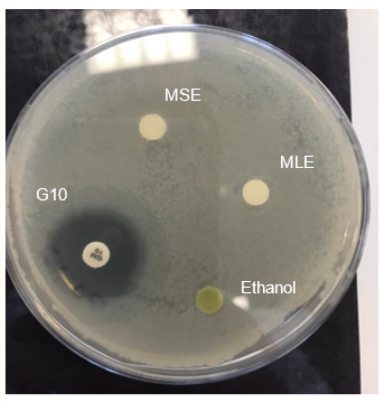

Figure 4: *Picture of petri dish inoculated with E. coli and the zones of inhibition shown for four treatments: 10 µg gentamycin (G10), ethanol extract of moringa seed (MSE), ethanol extract of moringa leaf (MLE), and ethanol.*

Zone of inhibition (mm) for *E. coli*						
	Ethanol	**MLE**	**MSE**	**MLW**	**MSW**	**Gentamycin**
Mean	0.11	0.56	0.89	3.11	8.56	17.22
SEM	0.11	0.24	0.31	0.61	0.69	1.12

Table 1: *The zone of inhibition of six treatments against E. coli.*

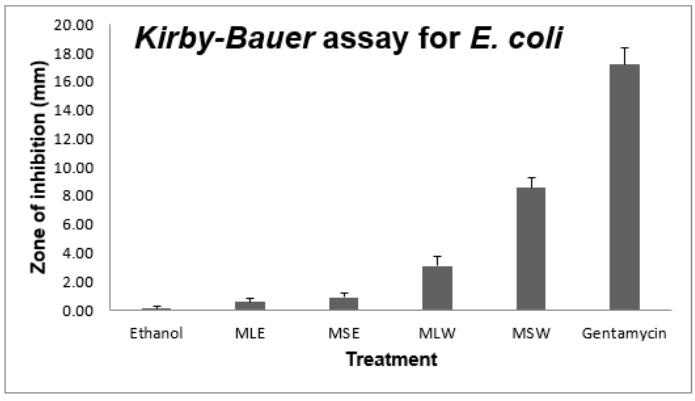

Figure 5: *The mean zones of inhibition against E. coli for Gentamycin, ethanol, as well as the water and ethanol extractions of moringa leaf and seed are displayed along with the standard error.*

Figure 5 shows that all the extractions had some antibacterial activity. The results also showed that the antibacterial activity of aqueous extractions of both *Moringa* seed and leaf was higher compared to the ethanol extraction but to confirm this we carried out a one-way ANOVA (Table 2) followed by a Tukey test (Table 3 and figure 7) to see if there were any significant differences between the treatment groups and which pairs were indeed different.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	5	2035.0	407.007	120.10	0.000
Error	48	162.7	3.389		
Total	53	2197.7			

Table 2: *One-way ANOVA of the zones of inhibition of the six treatment groups against E. coli. There was a statistical significant difference between the groups as determined by one-way ANOVA (F(5,48) = 120.10, p = 0.000).*

Factor	N	Mean	Grouping			
Gentamycin	9	17.22	A			
MSW	9	8.556		B		
MLW	9	3.111			C	
MSE	9	0.889			C	D
MLE	9	0.556			C	D
Ethanol	9	0.111				D

Table 3: *Pairwise results of the Tukey test with 95% confidence limit showing groupings of the six treatments against E. coli. Means that do not share a letter are significantly different.*

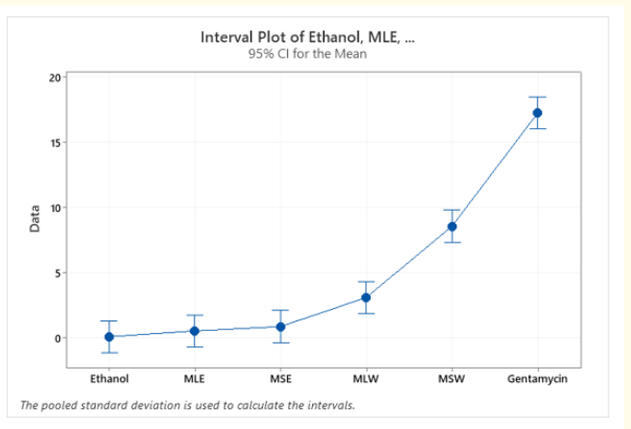

Figure 6: *The interval plot for the six treatment groups against E. coli.*

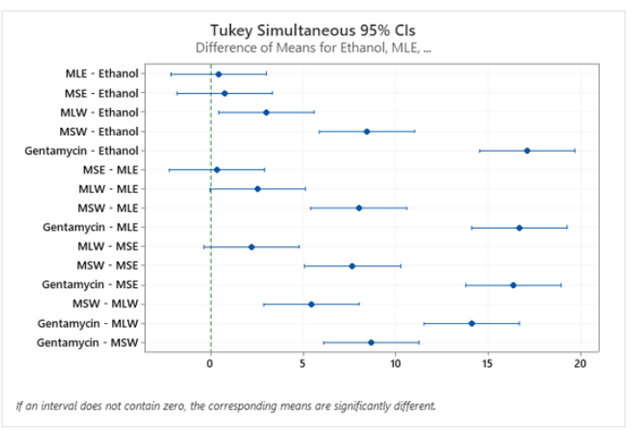

Figure 7: *Boxplot of Tukey test for the six treatment groups again E. coli comparing all treatment pairs.*

The ANOVA showed there were significant statistical differences between the different treatments groups, with the Tukey post-hoc test showing that there in fact 4 groupings that are different:

a) The positive control gentamycin

b) *Moringa* seed water extract

c) The two ethanol extractions and *Moringa* leaf water extract

d) The ethanol extractions with ethanol alone.

From table 1 and figure 5 we can see therefore that MSW had half the antibacterial activity of Gentamycin and more than twice the inhibitory effect of MLW. Furthermore, MLW and MSW showed over 2 and 4 times the antibacterial activity than the ethanol extractions respectively. Finally, as MSE and MLE were not significantly statistically different from treatment with ethanol, it can't be determined if the antibacterial activity was not due to ethanol.

Kirby-Bauer assay results for *S. aureus*

Zone of inhibition (mm) for *S. aureus*						
	Ethanol	**MLE**	**MSE**	**MLW**	**MSW**	**Ciprofloxacin**
Mean	0.00	0.00	2.22	1.89	3.11	18.44
SEM	0.00	0.00	0.28	0.26	0.35	1.19

Table 4: *The zone of inhibition of six treatments against S. aureus.*

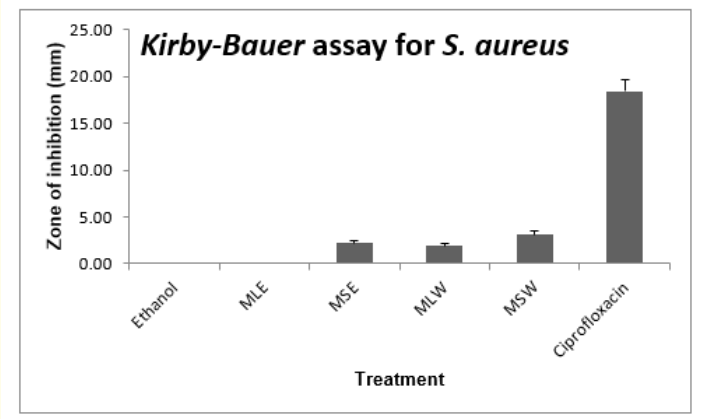

Figure 8: *The mean zones of inhibition against S. aureus for Ciprofloxacin, ethanol, as well as the water and ethanol extractions of moringa leaf and seed are displayed along with the standard error.*

The antibacterial activity of the water and ethanol extractions show a slightly different pattern for *S. aureus* as compared for *E. coli,* with ethanol and MLE not showing any antibacterial activity at all. We carried out ANOVA (Table 5) and a Tukey post-hoc test (Table 6 and figure 10) to get a clearer picture of the results.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	5	2237.3	447.456	176.69	0.000
Error	48	121.6	2.532		
Total	53	2358.8			

Table 5: *One-way ANOVA of the zones of inhibition of the six treatment groups against S. aureus.*

As shown in table 5 there was a statistically significant difference between the treatment groups against *S. aureus* with one-way ANOVA (F (5,48) = 176.69, p = 0.000).

Factor	N	Mean	Grouping		
Ciprofloxacin	9	18.44	A		
MSW	9	3.111		B	
MSE	9	2.222		B	C
MLW	9	1.889		B	C
MLE	9	0.000000			C
Ethanol	9	0.000000			C

Table 6: *Pairwise results of the Tukey test with 95% confidence limit showing groupings of the six treatments against S. aureus.*
Means that do not share a letter are significantly different.

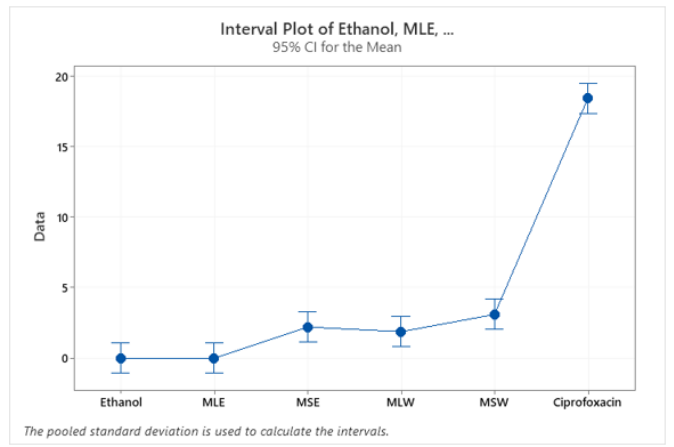

Figure 9: *The interval plot for the six treatment groups against S. aureus.*

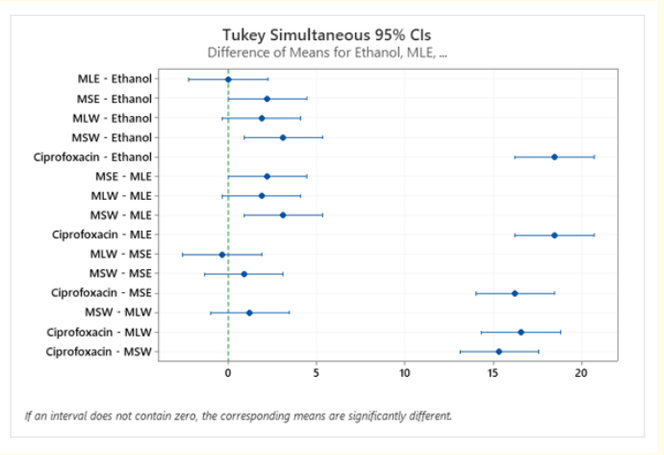

Figure 10: *Boxplot of Tukey test for the six treatment groups again S. aureus comparing all treatment pairs.*

The Tukey test (Table 6) showed that there were 3 groups of treatments that were significantly statistically different:

a) The positive control Ciprofloxacin

b) Both water extractions with ethanol extraction of *Moringa* seed

c) Ethanol, both ethanol extractions, and water extraction of *Moringa* leaf.

Based on the results, MSW also had the highest antibacterial activity out of all the extractions, though this wasn't significantly different from MLW and MSE. Ciprofloxacin was more than 6 times more inhibitory than all the other treatments. Ethanol and MLE showed no antibacterial activity.

HPLC results for methanol and ethanol extractions of *Moringa* seed

Ethanol and methanol extracts were made for *Moringa* seeds and leaves as described in section Preparation of ethanol solutions of this paper, which were separate from the extracts made for the Kirby-Bauer assays.

Signal = 280nm			Signal = 320nm			Signal = 360nm		
Peak #	**Retention time (min)**	**Area %**	**Peak #**	**Retention time (min)**	**Area %**	**Peak #**	**Retention time (min)**	**Area %**
1	1.932	3.4631	1	1.931	5.6785	1	1.933	2.5467
2	2.016	3.937	2	2.014	5.2259	2	2.015	2.5739
3	2.11	0.7713	3	2.109	0.6599	3	2.109	0.3236
4	2.165	0.7062	4	2.167	0.4138	4	2.168	0.2026
5	2.371	2.0334	5	2.331	2.5277	5	2.373	1.2447
6	2.433	0.5133	6	2.43	0.5552	6	2.431	0.2694
7	2.498	0.2423	7	2.578	1.0625	7	2.578	0.4985
8	2.582	0.888	8	2.64	1.1748	8	2.644	0.4927
9	2.643	0.9436	9	2.693	0.8389	9	2.695	0.453
10	2.696	0.8671	10	2.833	0.602	10	2.833	0.2603
11	2.837	0.5597	11	3.034	0.6127	11	3.035	0.2884
12	2.957	0.2675	12	3.118	1.4	12	3.119	0.6674
13	3.035	0.5885	13	3.215	0.4275	13	3.214	0.2116
14	3.122	1.3774	14	34.459	20.9698	14	4.621	0.5342
15	3.219	0.7746	15	35.165	18.357	15	34.458	29.4169

16	20.716	26.8583	16	36.584	9.0278	16	35.162	33.2162
17	34.458	19.8249	17	37.238	14.2535	17	36.586	9.4824
18	35.166	18.5389	18	49.823	1.3651	18	37.238	10.1727
19	36.586	3.9359	19	52.286	1.9214	19	58.794	7.1447
20	37.238	6.2361	20	58.804	12.9261			
21	53.833	0.6615						
22	54.086	1.2102						
23	54.308	0.4669						
24	56.075	1.4436						
25	56.439	0.5579						
26	58.036	2.3328						

Table 7: *Results of HPLC for ethanol extraction of moringa leaf for 3 signals 280nm, 320nm and 360nm with retention times and percentage area under the curve.*

Signal = 280nm			**Signal = 320nm**			**Signal = 360nm**		
Peak #	**Retention time (min)**	**Area %**	**Peak #**	**Retention time (min)**	**Area %**	**Peak #**	**Retention time (min)**	**Area %**
1	1.831	0.6483	1	1.93	25.5745	1	1.934	22.7821
2	1.935	10.7088	2	2.146	2.9194	2	2.146	2.7644
3	2.03	2.1042	3	2.266	2.8337	3	2.266	2.8104
4	2.152	1.7824	4	2.337	2.6766	4	2.337	2.5132
5	2.268	1.4947	5	2.406	4.0875	5	2.406	4.1319
6	2.339	1.5738	6	2.531	1.5728	6	2.53	1.5884
7	2.411	3.1568	7	2.615	3.1696	7	2.617	2.8464
8	2.533	1.1591	8	2.699	2.1614	8	2.7	2.0436
9	2.619	2.6693	9	2.805	1.508	9	2.81	1.4798
10	2.704	2.9798	10	2.945	4.2213	10	2.945	3.7697
11	2.809	2.7241	11	3.024	2.3401	11	3.024	2.2765
12	2.949	2.5758	12	3.107	2.875	12	3.109	2.5976
13	3.026	1.7942	13	34.468	3.6506	13	34.469	15.3923
14	3.125	2.2021	14	35.172	4.8668	14	35.171	17.0435
15	3.206	1.0064	15	37.235	6.1114	15	36.586	2.0209
16	20.687	41.0928	16	49.802	2.4162	16	37.248	6.2013
17	34.468	1.9782	17	58.774	27.0151	17	58.043	2.4082
18	35.158	2.5427				18	58.837	5.3297
19	37.238	2.523						
20	53.828	0.8429						
21	54.321	1.8153						
22	56.07	2.6922						
23	57.33	0.9611						
24	58.023	2.1905						
25	58.818	4.7816						

Table 8: *Results of HPLC for ethanol extraction of moringa seed for 3 signals 280nm, 320nm and 360nm with retention times and percentage area under the curve.*

Signal = 280nm			Signal = 320nm			Signal = 360nm		
Peak #	Retention time (min)	Area %	Peak #	Retention time (min)	Area %	Peak #	Retention time (min)	Area %
1	1.815	3.4365	1	1.803	8.4523	1	1.806	6.6516
2	1.856	4.1414	2	1.947	4.8115	2	1.949	3.1895
3	1.948	7.2153	3	2.137	1.6006	3	2.138	1.3228
4	2.14	1.2004	4	2.239	1.5554	4	2.242	1.2914
5	2.245	0.9663	5	2.322	0.9587	5	2.324	0.9217
6	2.325	0.8707	6	2.583	2.793	6	2.583	2.1209
7	2.471	0.4883	7	2.688	2.2319	7	2.688	1.7354
8	2.587	1.1396	8	2.754	2.7758	8	2.756	2.2411
9	2.69	0.7896	9	2.852	0.9233	9	2.853	0.7926
10	2.759	1.4554	10	34.41	16.297	10	3.196	1.814
11	2.856	0.5744	11	35.132	16.4467	11	34.419	22.1066
12	19.364	5.9267	12	36.589	3.9161	12	35.131	21.2996
13	20.441	40.0694	13	37.241	9.0771	13	36.586	5.1506
14	34.416	7.4798	14	49.823	2.0947	14	37.245	13.6586
15	35.134	8.1205	15	58.79	26.066	15	58.817	15.7036
16	36.588	1.9882						
17	37.242	5.7783						
18	56.083	1.0779						
19	58.04	1.952						
20	58.767	5.3294						

Table 9: *Results of HPLC for water extraction of moringa leaf for 3 signals 280nm, 320nm and 360nm with retention times and percentage area under the curve.*

Signal = 280nm			Signal = 320nm			Signal = 360nm		
Peak #	Retention time (min)	Area %	Peak #	Retention time (min)	Area %	Peak #	Retention time (min)	Area %
1	1.86	6.8784	1	1.806	2.0441	1	1.808	0.768
2	1.949	1.7957	2	1.883	1.4093	2	1.888	0.5713
3	2.104	2.0262	3	2.104	0.6366	3	2.104	0.2439
4	2.215	2.1197	4	2.215	1.2096	4	2.218	0.3907
5	2.444	0.651	5	2.439	0.3386	5	2.294	0.0786
6	2.553	1.1159	6	2.559	0.6674	6	2.435	0.0865
7	2.65	0.548	7	2.653	0.3158	7	2.561	0.2089
8	2.745	0.7965	8	2.745	0.5697	8	2.654	0.1307
9	2.832	0.3391	9	2.83	0.2594	9	2.746	0.2494
10	20.383	11.7203	10	29.957	0.4113	10	2.832	0.1244

11	29.971	2.7184	11	34.411	40.1993	11	29.974	1.6958
12	34.411	31.6513	12	35.131	27.2018	12	30.17	2.0921
13	35.132	23.1466	13	36.591	7.5063	13	34.411	38.5006
14	36.593	5.3483	14	37.241	11.014	14	35.129	28.767
15	37.24	5.338	15	49.822	0.5582	15	36.589	10.9062
16	54.076	0.9857	16	57.616	0.8151	16	37.241	9.853
17	54.288	0.4531	17	58.77	4.8437	17	38.003	2.2447
18	56.082	0.8234				18	57.629	0.3998
19	56.411	0.4689				19	58.079	0.1915
20	58.04	1.0759				20	58.838	0.9477
						21	58.92	1.5493

Table 10: *Results of HPLC for water extraction of moringa seed for 3 signals 280nm, 320nm and 360nm with retention times and percentage area under the curve.*

All extractions had a mixture of 15 - 26 compounds as shown in tables 7-10. Further analysis was not done to determine the nature of these compounds.

Discussion

It comes as no surprise that the *Moringa* leaf and seed extracts displayed antibacterial properties as the *Moringa* plant has been used for centuries as a well-known anti-septic. What is more interesting is that the active compounds that confer this antibacterial property are likely hydrophilic as ethanol extractions of the *Moringa* leaf provided no more inhibition against bacterial growth than ethanol alone and although the seed ethanol extraction showed more inhibition, this wasn't statistically significant, suggesting that whatever compounds were extracted using ethanol were either not antibacterial or in some way affected in the ethanol solution.

We found a similar pattern of antibacterial activity against both *E. coli* and *S. aureus* where the water extractions showed more antibacterial activity than the ethanol extractions, and the *Moringa* seed extractions for both water and ethanol was higher than the *Moringa* leaf extractions as can be seen in table 1 and figure 5 for *E. coli* as well as table 4 and figure 8 for *S. aureus*.

From a biological perspective it makes sense that the seeds contain more antibacterial potency than the leaves as the seeds will find themselves in the soil and therefore are more exposed to various bacteria than the aerial parts of the *Moringa* tree. It is well-known that the bacterial environment around plant roots is very different from the surrounding "bulk soil" and the number of bacteria can be up to 10 times greater than in the bulk soil [20].

The results also showed that the aqueous extractions tended to have greater zones of inhibition than the ethanol extractions. This would suggest that the most potent antibacterial compounds in *Moringa* are hydrophilic and so these should be isolated, purified and further studied in future studies to find new antibiotics to treat *E. coli* and *S. aureus* infections. The aqueous seed extraction was found to be particularly effective against *E. coli*, so this could be a promising place for future studies to develop effective herbal teas made from the *Moringa* plant for common *E. coli* infections without resorting to antibiotics.

For both bacteria, the ethanol extractions were not significantly different to just ethanol. The great variability of results led to these 3 treatments overlapping. We also found no zone of inhibition with ethanol treatment for *S. aureus* as well as MLE which was strange as ethanol does inhibit bacteria growth slightly. It's possible that *S. aureus* could remain viable at the concentration of ethanol used as an-

other study found that *S. aureus* actually remained viable and increased biofilm production in the presence of ethanol [21]. Therefore, in the presence of alcohol *S. aureus* would simply increase biofilm production protecting itself from both the ethanol and other antimicrobial compounds in the *Moringa* leaf ethanol extract.

Our results conflict slightly with previous studies that found *E. coli* to be resistant to both aqueous and ethanol *Moringa* leaf extracts [19], whereas we did observe some antibacterial activity for these treatments. However due to the overlap between the ethanol extractions and ethanol itself, we can't conclude that the extractions had antibacterial activity. But the same study found that leaf extracts were effective against *S. aureus*, though we observed a very weak effect.

Another study found that 3 bioactive compounds extracted from *Moringa* seed had potent antibacterial effects: 4-(α-l-rhamnopyranosyloxy)benzyl isothiocyanate, methyl N-4-(α-l-rhamnopyranosyloxy) benzyl carbamate (both known compounds), and 4-(β-d-glucopyranosyl-1→4-α-l-rhamnopyranosyloxy)-benzyl thiocarboxamide.[18] These compounds should be matched with the retention times we obtained in our HPLC results in future studies and their bactericidal activities further documented. 4-(β-d-glucopyranosyl-1→4-α-l-rhamnopyranosyloxy)-benzyl thiocarboxamide was found to have the most potent bactericidal effect in that study so this could be the reason why we also found the seed extracts to have more antibacterial activity than the leaves.

With new bacteria (and new viruses) coming up every year, we need to continually find and develop new treatments against these pathogen. Plants are a hidden reservoir of potential candidates for new antibiotics not just directly but also as blueprints to develop synthetic compound derivatives to further optimise the compound's effects. Future studies should concentrate on the *Moringa* seed extracts as these contain the most potent antibacterial activity with our HPLC results as a reference to identify new and old compounds for further study.

Conclusion

Moringa seed extracts showed more antibacterial activity than the *Moringa* leaf extracts, with the water extracts being more potent than the ethanol extracts suggesting that certain hydrophilic compounds are the most potent bactericide within the seeds.

Future studies should focus on the *Moringa* seed extracts to isolate the compounds responsible for its antibacterial activity.

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