Review Paper

The antimicrobial properties of *Moringa oleifera* Lam. for water treatment: a systematic review

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Abstract
Plant extracts have been used as alternatives to the conventional chemical water treatment. *Moringa oleifera* Lam. is one of the plants used for this purpose due to its antimicrobial and coagulant properties. However, there is no systematization of *Moringa's* application methodology. Different parts of the plant, extraction methods and concentrations can be applied to remove several pathogens present in contaminated drinking water. In the present work, reported *Moringa* applications with antimicrobial effect were systematically reviewed, in order to identify effective methodology(ies) for water treatment. Forty-nine articles were screened for: (1) part of the plant used, (2) extraction method, (3) extract concentration, (4) targeted pathogens, and (5) inhibition zone obtained. Nine articles complied with these criteria and were carefully analyzed; eight of them reported on leaf extracts and only one on seed extracts. Two approaches were used: analysis by pathogen and overall analysis. A total of eight different extraction methods were reported. Extract concentrations used ranged from 0.02 to 800 mg mL^{-1} and were tested on twenty pathogens. Our analysis revealed that none of such methods is effective against all the tested pathogens. However, leaf extracts obtained with distilled water or with 95% ethanol were the most effective ones for a higher number of pathogens such as *Escherichia coli* and, possibly, *Vibrio cholerae*. Moreover, *Moringa*'s extract concentration of 30 mg mL^{-1} obtained by the 95% ethanol extraction method was the most efficient. Findings suggest an effective procedure to use *Moringa*, reinforcing its importance as an environmentally friendly alternative for water treatment in areas lacking a water supply system.

Keywords Antimicrobial activity · Inhibition zone · Extraction methods · Pathogens · Water purification

Abbreviations

95% EE	95% ethanol extraction
95% PEE	95% petroleum ether extraction
AEE	Absolute ethanolic extraction
CE	Chloroform extraction
CME	Cold methanol extraction
DWE	Distilled water extraction
GB	Guinea-Bissau
MO	*Moringa oleifera*

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1 Introduction

According to the World Health Organization (WHO), contaminated drinking water is estimated to cause 502 000 diarrhoeal deaths each year [1]. "More than 2 billion people lack access to safe drinking water and more than double that number lack access to safe sanitation. With a rapidly growing global population, demand for water is expected to increase by nearly one-third by 2050. Since the 1990s, water pollution has worsened in almost all rivers in Africa, Asia, and Latin America. The deterioration of water quality is expected to further escalate over the next decades and this will increase threats to human health, the environment and sustainable development" [2]. Access to piped water is usually limited in low- and middle-income countries due to the poor performances of supply infrastructures and to the presence of pathogenic microorganisms, even in piped water. WHO and UNICEF are responsible for promoting the monitoring of water quality by the national or local authorities, based on physico-chemical and microbiological parameters [3]. Microbial contamination of groundwater due to sewage outfalls and agricultural runoff can be a serious threat. Globally, the most commonly occurring diseases (and agents) transmitted through drinking of unsafe water are: infectious hepatitis (A, B and C viruses), cholera (*Vibrio cholerae*), bacillary dysentery (*Shigella* spp.), typhoid (*Salmonella enterica*), paratyphoid (*Salmonella paratyphi*), salmonellosis (*Salmonella* spp.), colibacillosis (*Escherichia* coli), giardiasis (*Giardia lamblia*), cryptosporidiosis (*Cryptosporidium* spp.) and amoebiasis (*Entamoeba group*) [4].

According to the United Nations World Water Development (UN-Water), ensuring adequate supplies of safe drinking water is one of the four priorities to reduce waterborne diseases [2]. However, in developing countries, socio-economic problems and political constraints make it difficult to manage this resource in a sustainable way, such as 100% coverage of piped water. To address this issue, it is critical to find innovative alternatives to enable communities to use and treat water in affordable and easy ways [5, 6].

Several works on antimicrobial activity of plants, including *Moringa oleifera* (MO) have been tested as viable alternatives to chemical compounds in the treatment of drinking water and wastewater and other purposes [7–9].

Many studies have shown that the leaf, flower, bark, root, seed and nearly all types of MO tissues exhibit antimicrobial activity against several pathogens such as *Vibrio cholerae* and *Escherichia coli* and also viruses, fungi and parasites [10–12]. However, it is difficult to find in the literature, which methodologies are effective for antimicrobial purposes and for which pathogens. Therefore, the main goal of this study was to review the effective methodology(ies) concerning the application of MO for antimicrobial purposes, focusing on the plant part used, extraction method, extract concentration, pathogens studied and inhibition zone obtained.

2 Methodology

2.1 Search strategy

Three electronic databases (PubMed, ScienceDirect, and Scopus) were searched using combinations of Medical Subject Headings (MeSH) terms and free text words such as: "*Moringa oleifera*" AND "antimicrobial activities" (MeSH).

2.2 Study selection

The review followed the established systematic reviews' methodology (according to PRISMA guidelines). Publications were included in the study when all of the following selection criteria were met: (1) corresponding to research articles i.e. publications structured as Introduction, Material, and Methods, and Results/Discussion, or similar; (2) available as Free Full-Text; (3) written in English or Portuguese; (4) published until the date of the search (31st July 2018); (5) publications' results explicitly reporting the antimicrobial effect of MO, describing the pathogenic targets, the extraction method, concentration of the extract, parts of the plant and the inhibition zone obtained.

2.3 Data synthesis and analysis

Data were extracted from the selected publications into a digital data-extraction form.

Analysis of data considered both the effectiveness and the efficiency of an extract and its method of production. Effectiveness was measured by the number of pathogens against which an extract produced a significant inhibition zone.

2.3.1 Pathogen analysis

Firstly, all the assay was done on the plate and the inhibition zones were registered which mean measurement from the centre of the point of the infection to the edge of the area with no growth that is the radius of inhibition around the point of infection.

Secondly, the most effective and efficient extracts for each target pathogen were analyzed.

Effectiveness was measured by the size of the inhibition zones produced; the more effective extracts produce larger inhibition zones (for a particular pathogen). Efficiency was measured by the coefficient of efficiency, i.e., the ratio between the concentration of the extract and the inhibition zone it produced (assuming a linearity between them).

2.3.2 Overall analysis

Secondly, an overall analysis was performed, exploring effectiveness and efficiency of extracts produced by all screened methods, regardless of the target pathogen. For this overall analysis, an extract was considered effective whenever it produced an inhibition zone equal to or greater than 6.0 mm [13].

3 Results

The preliminary data search (cf. Search strategy) resulted in a total of 49 publications; from these, only 9 addressed the process for antimicrobial extracts according to the established criteria (cf. Study selection) and were analyzed.

All 9 analyzed reports were published after 2010 and also all of them came from a variety of scientific groups such as West Africa, Europe, Asia and Latin America. Eight of them used MO leaf extract, one used seed extract, and none used root or flower extracts. A total of eight different extraction methods were reported: 95% petroleum ether extraction (95% PEE), chloroform extraction (CE), 95% ethanol extraction (95% EE), cold methanol extraction (CME), absolute ethanolic extraction (AEE), distilled water extraction (DWE), hexane, butanol and acetone extractions. The analyzed articles tested extracts of MO leaves and seeds to determine their antimicrobial activity on twenty pathogens: *Aeromonas caviae*, *Bacillus anthracis*, *B. cereus, Enterococcus cloacae, E. faecalis*, *Enterobacter* ssp., *Escherichia coli, Klebsiella pneumoni*ae, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi, Serratia marcescens*, *Shigella* dysenteriae, *Staphylococcus aureus*, *S. epidermidis, Streptococcus pneumoniae, S. pyogenes*, *S. thermophilus, Vibrio cholerae* and *V. parahaemolyticu*s. MO extracts were tested in concentrations ranging from 0.02 to 800 mg mL^{-1}.

3.1 Pathogen analysis

One research article reported antimicrobial activity of MO against *Aeromonas caviae*, with an inhibition zone ranging from 21.2 to 22.3 mm regardless of the extraction method (DWE or 95% EE) and the concentrations used (53 or 111 mg mL^{-1}). The combination of DWE and 53 mg mL^{-1} concentration of leaf extract was the most effective. However, results were very close to those obtained with the extract of the same concentration obtained by 95% EE [14].

According to Adetitun et al. (2013), MO leaf extract showed an antimicrobial effect against *Bacillus anthracis* using three different extraction methods: AEE, CME and 95% EE. The extract obtained through AEE was the most efficient, with an inhibition zone of 4.0 mm at extract concentration of 5 mg mL^{-1}, as well as the most effective, with an inhibition zone of 14.0 mm at extract concentration of 50 mg mL^{-1}. The CME and 95% EE methods were also effective, achieving inhibition zones of 11.0 and 12.0 mm, respectively, at an extract concentration of 75 mg mL^{-1}.

Two research articles showed the antimicrobial effect of MO leaf extract against *Bacillus cereus* using four different extraction methods: DWE, CE, butanol, and acetone. The leaf extract obtained by DWE was the most efficient, with an inhibition zone of 21.8 mm at an extract concentration of 100 mg mL^{-1} [15, 16].

One article evaluated the MO leaf extract antimicrobial activity against *Enterobacter* spp. using several methods: CME, 95% EE, AEE, DWE, 95% PEE, butanol, hexane, and acetone extraction. Two of them were very effective: DWE and 95% EE achieved inhibition zones of 14.5 and 19.0 mm, respectively, at 30 mg mL^{-1} of extract concentration, 95% EE being the most efficient one, with an inhibition zone of 19 mm. There were no positive results from butanol, CME, hexane, acetone, AEE or CE [17].

DWA and 95% EE showed antimicrobial activity of MO leaf against *Enterococcus faecalis*. The extract concentration of 53 mg mL^{-1} obtained by 95% EE was the most efficient one, with an inhibition zone of 17.0 mm. However, results were similar with the extract of the same concentration obtained by DWE, with an inhibition zone of 16.3 mm. There were no significant differences between inhibition zones achieved by extract concentrations of 111 mg mL^{-1} regardless of the extraction method (DWA: 19.4; 95% EE: 17.8 mm) [14].

There was just one study testing MO leaf antimicrobial activity against *Enterococcus cloacae* based on a DWE method at 100 mg mL^{-1} of extract concentration, with a 23.4 mm inhibition zone [18]. Regarding *Escherichia coli*, five different methods were reported and shown to be effective: 95% EE, CME, AEE, DWE, and acetone extraction. The most efficient one was CE: with a leaf extract concentration of 0.02 mg mL^{-1}, it showed an inhibition zone of 9.0 mm [19]. An effective result was obtained with DWE, with 30 mg mL^{-1} of extract concentration corresponding to an inhibition zone of 16.8 mm. However, the most efficient method was 95% EE, with a 21.0 mm of inhibition zone at 30 mg mL^{-1} of extract concentration [17].

The evaluation of MO seed extract is reported by one study, using CME, DWE, and acetone extraction methods

against *Escherichia coli*. CE was the most efficient method, achieving an inhibition zone of 13.3 mm at 50 mg mL^{-1} of extract concentration [20].

One research article showed MO leaf antimicrobial activity against *Klebsiella pneumoniae*. An inhibition zone of 16.0 mm was obtained by the 95% EE method, which demonstrated to be the most efficient one at 5 mg mL^{-1} of extract concentrations. Even so, DWE, AEE, butanol, and acetone extraction were also shown to be effective against this pathogen [13]. DWE, acetone extraction and 95% EE were effective against *Proteus vulgaris,* with inhibition zones ranging from 1.0 to 15.5 mm and extract concentrations of 25–200 mg mL^{-1}. DWE was the most efficient one, at an extract concentration of 100 mg mL^{-1}, with an inhibition zone of 15.5 mm [13].

All methods tested (DWE, 95% EE, CME, butanol and acetone extraction) demonstrated the antimicrobial activity of MO leaf extract against *Pseudomonas aeruginosa*. The CE was the most efficient one, producing an inhibition zone of 10.0 mm at a concentration of 0.02 mg mL^{-1} [19]. An effective result was also obtained with DWE, with an inhibition zone of 12.5 mm achieved by 100 mg mL^{-1} of extract concentration. With the same methodology, other authors used 30 mg mL^{-1} of extract concentration and achieved an inhibition zone of 3.3 mm [19, 21].

Four different extraction methods (CME, DWE, acetone extraction and 95% EE) combined with MO leaf extract at different concentrations (30, 75, 100 and 200 mg mL^{-1}) were effective against *Salmonella typhi*, achieving inhibition zones of 8.0, 13.0, 21.0, 23.5 and 6.6 mm for each concentration, respectively. One article reported that MO seed extract produced by CME, DWE and acetone extraction method was effective against *S. typhi* at a concentration of 50 mg mL^{-1}, with inhibition zones of 15.3, 7.6 and 19.0 mm, respectively [20]. Despite effective results with the seed extract, a study showed that MO leaf extract is more efficient against *S. typhi*: when using 95% EE method at 30 mg mL^{-1} of extract concentration, an inhibition zone of 23.0 mm was obtained [17].

There are not many reports regarding MO antimicrobial activity against *Salmonella enteritidis*, but Abdallah [15] showed that the extract of MO leaves has such antimicrobial properties, based on the acetone extraction method. When a concentration of 200 mg mL^{-1} was used, an inhibition zone of 6.6 mm was obtained. The 95% EE and DWE method had no effect on *S. enteritidis*.

MO leaf extract showed an antimicrobial effect against *Serratia marcescens*, using 95% EE and DWE. For an extract concentration of 30 mg mL^{-1}, the inhibition zones obtained were 11.2 and 17.0 mm respectively. DWE extract was therefore shown to be the most efficient one [17].

Four different extraction methods (95% EE, DWE, CME and acetone extraction) were revealed effective against *Shigella dysenteriae*, the best performance corresponding to 95% EE: with 30 mg mL^{-1} of extract the inhibition zone obtained was 19.0 mm. Acetone extraction and CME also produced effective results but with much higher extract concentrations [17].

Many articles based on different extraction methods such as DWE, butanol, acetone, 95% EE, CE, and CME reported the antimicrobial activity of MO leaf extract against *Staphylococcus aureus*. At extract concentrations ranging from 0.02 to 800 mg mL^{-1}, inhibition zones of 6.0–23.3 mm were obtained. Among the methods tested the CE is the most efficient one, with a 6.0 mm inhibition zone at 0.02 mg mL^{-1} of extract concentration [19]. The other methods were also effective, with 95% EE and DWE presenting inhibition zones of 22.3 and 22.0 mm, respectively, although at a much higher extract concentration, 53 mg mL^{-1} [14].

Abdallah (2016) showed antimicrobial activity of MO leaf against *Staphylococcus epidermidis* testing four extraction methods (DWE, butanol, CE and acetone). Using 200 mg mL^{-1} of extract, CE was the most efficient one, with an inhibition zone of 16.0 mm while with DWE an inhibition zone of 12.3 mm was achieved at the same concentration.

Only one article reported MO leaf antimicrobial activity against *Streptococcus pneumoniae* by using 95% EE. The best performance was at 200 mg mL^{-1} of extract, with an inhibition zone of 4.3 mm. This extract also produced large inhibition zones but at a much higher concentration [22].

Two extraction methods, CE and 95% PEE, were used to demonstrate the antimicrobial activity of MO leaf against *Streptococcus pyogenes* and the results showed that CE is the most effective one at 0.02 mg mL^{-1} of extract concentration, with an inhibition zone of 7 mm. 95% PEE was not effective [19]. The AEE method was the only one associated with MO leaf extract antimicrobial activity against *Streptococcus thermophilus*, presenting inhibition zones of 14.0 and 15.0 mm at extract concentrations of 25 and 75 mg mL^{-1}, respectively [13]. All the other tested methods—95% PEE, CE, DWE, hexane extraction, 95% EE, CME and acetone extraction—showed no antimicrobial activity against *S. thermophilus*, regardless of the extract concentration (5, 25, 50, 75 and 100 mg mL^{-1}).

Nine different methods (CME, 95% EE, AEE, DWE, 95% PEE, CE, hexane, butanol and acetone extraction) were tested to verify the antimicrobial activity of MO leaf against *Vibrio cholerae* but only CME and 95% EE were effective. 95% EE was the most efficient one at 5 and 25 mg mL^{-1} of extract, with inhibition zones of 15.0 and 9.0 mm respectively. For the CME method, the best inhibition zones were 4.0, 5.0 and 6.0 mm at extract concentrations of 5, 50 and 75 mg mL^{-1}, respectively [13].

One research article demonstrated antimicrobial activity of MO leaf extract against *Vibrio parahaemolyticus*, with an inhibition zone of 20.7 mm at 53 mg mL^{-1} of extract using the DWE method. In the same study, a 20.7 mm inhibition zone was also achieved by the same extraction method but using a much higher concentration of extract, 111 mg mL^{-1}. Although DWE was the most efficient one at low concentration, 95% EE also presented effective results with an inhibition zone of 21.9 mm at a concentration of 111 mg mL^{-1} [14].

3.2 Overall analysis

No single MO-based method produced an extract that was effective against all the targeted pathogens.

Only one study tested MO seed extract (at 50 mg mL^{-1}), using the acetone, CME and DWE extraction methods. The most effective result was achieved by acetone extract, that showed effective antimicrobial activity against *Escherichia coli*, *Salmonella typhi* and also *Shigella dysenteriae* with inhibition zones of 13.3, 19.0 and 18.6 mm, respectively [20]. At the same extract concentration (50 mg mL^{-1}) MO leaf extract was not effective against *E. coli* and *S. typhi* for any type of extraction method [13]. The comparison between the seed and leaf extracts of MO can only be made based on the results concerning *E. coli, S. typhi* and *S. dysenteriae*, the pathogens tested with both parts of MO and the same extraction method (DWE), although at different concentrations (50 and 30 mg mL^{-1}). As displayed in Table 1, leaf extract at 30 mg mL^{-1} was more effective (achieving larger inhibition zones), and even more efficient (using a lower extract concentration), than seed extract at 50 mg mL^{-1} [17].

Table 1 Pathogens against which MO seed and leaf extracts at different concentrations and obtained by DWE were tested, and resulting inhibition zones

Parts of *Moringa oleifera*	Pathogen	Concentration (mg mL-1)	Inhibition zone (mm)	References
Seed	*Escherichia coli*	30	8.30	[20]
	Salmonella typhi	30	7.66	
	Shigella dysenteriae	30	7.66	
Leaf	*Escherichia coli*	50	16.80	[17]
	Salmonella typhi	50	8.00	
	Shigella dysenteriae	50	14.90	

Leaf extracts obtained through DWE and 95% EE were effective against the highest number of pathogens (Table 2). MO extract from DWE was effective against fourteen different pathogens: *Aeromonas caviae, Bacillus cereus, Enterococcus faecalis, E. cloacae, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi, Serratia marcescens, Shigella dysenteriae, Staphylococcus aureus, S. epidermidis, Vibrio parahaemolyticus*; it was also the most efficient one against seven pathogens out of those [13, 14, 16]. The 95% EE—MO extract was effective on thirteen pathogens: *Aeromonas caviae, Bacillus cereus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi, Serratia marcescens, Shigella dysenteriae, Staphylococcus aureus, Streptococcus pneumoniae, Vibrio cholerae* and *V. parahaemolyticus* pathogens, being also the most efficient against ten out of those (Table 2) [14–17, 21].

The reported MO leaf extract concentrations ranged from 0.02 to 800 mg mL^{-1} and corresponded to inhibition zones ranging from 2.0 to 25.4 mm. Not all concentrations of MO leaf extracts obtained by a particular method presented effective antimicrobial activity. According to the reviewed articles, for DWE and 95% EE, an extract concentration of 30 mg mL^{-1} was the one effective against a higher number of pathogens. 95% EE-MO extract was effective against six pathogens: *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Serratia marcescens, Shigella dysenteriae* and *Pseudomonas aeruginosa,* while DWE–MO extract at 30 mg mL^{-1} was effective against the same pathogens except for *P. aeruginosa* [17]. Furthermore, the 95% EE-MO extract at 30 mg mL^{-1} presented larger inhibition zones than the DWE- MO extract at the same concentration (Table 3). The analyzed articles also showed that the inhibition zones obtained with extract concentrations over 30 mg mL^{-1} were smaller or similar to those obtained at 30 mg mL^{-1} [13–16, 20]. In fact, MO extracts resulting from DWE and 95% EE at 30 mg mL^{-1} achieved inhibition zones larger than 14.0 mm for the majority of pathogens tested.

However, when using 30 mg mL^{-1} of extract obtained through DWE or 95% EE, no effect was observed on bacteria such as *Aeromonas caviae, Bacillus anthracis, Staphylococcus aureus, Streptococcus pneumoniae,* and *Vibrio parahaemolyticus,* which were tested at concentrations 53, 75, 53, 400, 53 mg mL^{-1}, respectively. *S. aureus* was the only one tested at 30 mg mL^{-1} of extract concentration, with an inhibition zone less than 6.00 mm [13, 14, 21]. At a similar concentration (25 mg mL^{-1}), the MO extract obtained with 95% EE was also effective against *Klebsiella pneumoniae* and *Vibrio cholerae* (Table 2). DWE-extract was not effective for any pathogens in concentrations under 30 mg mL^{-1} [13, 17].

Table 2 Pathogens against which MO leaf extracts at different concentrations, and obtained by 95% Ethanol Extraction method and Distilled Water Extraction method, were effective, and respective inhibition zones

Pathogens	95% ethanol extraction method		Distilled water extraction method		
	Concentration (mg mL^{-1})	Inhibition Zone (mm)	Concentration (mg mL^{-1})	Inhibition Zone (mm)	References
Aeromonas caviae	53	21.20	53[a]	21.40	[14]
Bacillus anthracis	75[a]	11.00	n/a	n/a	[13]
Bacillus cereus	n/a	n/a	100[a]	21.80	[16]
Escherichia coli	30[a]	21.00	30	16.80	[17]
Klebsiella pneumoniae	25[a]	10.00	–	–	[13]
	30[a]	18.00	30	11.86	[17]
Enterococcus faecalis	53[a]	17.00	53	16.10	[14]
E. cloacae	n/a	n/a	100[a]	23.40	[16]
Shigella dysenteriae	30[a]	19.00	30	14.90	[17]
Salmonella typhi	30[a]	23.00	30	8.00	[17]
Serratia marcescens	30	11.16	30[a]	17.00	[17]
Pseudomonas aeruginosa	30[a]	6.30	–	–	[21]
	–	–	100[a]	12.50	[13, 16]
Staphylococcus aureus	53[a]	22.30	53	22.00	[14]
Streptococcus pneumoniae	400[a]	6.30	n/a	n/a	[22]
Vibrio cholerae	5	15.00	–	–	[13]
	25	9.00	–	–	
Vibrio parahaemolyticus	53	17.80	53[a]	20.70	[14]
Proteus vulgaris	–	–	100[a]	15.50	[16]
Staphylococcus epidermidis	n/a	n/a	200[a]	12.30	[15]

IZ Inhibition zone

[a]most efficient extract; n/a was not tested; - for IZ < 6 mm

Table 3 Pathogens against which MO extracts, obtained by 95% Ethanol Extraction method and Distilled Water Extraction method, were effective at a concentration of 30 mg mL^{-1} or higher, and respective inhibition zones

Pathogens	95% ethanol extraction method		Distilled water extraction method		
	Inhibition Zone (mm)		Inhibition Zone (mm)		References
Escherichia coli	21.00[a]	16.00[b]	16.00[a]	6.67[b]	[17, 21]
Klebsiella pneumoniae	19.00[a]	6.30[b]	11.00[a]	15.00[b]	[16, 17, 22]
Shigella dysenteriae	19.00[a]	n/a	14.90[a]	7.66[b]	[17, 20]
Salmonella typhi	23.00[a]	–	8.00[a]	23.50[b]	[13–17]
Serratia marcescens	11.16[a]	n/a	17.00[a]	n/a	[17]
Pseudomonas aeruginosa	6.33[a]	6.67[b]	–	12.50[b]	[16, 21]

IZ Inhibition zone

[a]inhibition zone at 30 mg mL^{-1} of extract; [b] inhibition zone at extract concentration over 30 mg mL^{-1}; - for IZ < 6 mm; n/a was not tested

Finally, the overall analysis showed that 95% EE was the most efficient method for a large group of pathogens and even though some of them are more efficiently inhibited by DWE, 95% EE can still be quite effective in those cases. However, some groups of pathogens (e.g. *Salmonella enterica* and *Streptococcus thermophilus*) were not affected by either method in any of the concentrations tested (5, 25, 50, 75, and 200 mg mL^{-1}) [13, 15]. *Streptococcus pyogenes* was not tested for both methodologies.

4 Discussion

Even though the literature generally reports that almost all plant parts of MO exhibit antibacterial properties, only the evidence regarding MO leaf and seed extracts met our inclusion criteria by describing the microorganisms tested, the extraction methods and concentrations used and the inhibition zones achieved. It is difficult to compare the effects of leaf and seed extracts and to determine which one is the most efficient since the studies on antimicrobial activity of seed extracts are scarce.

Adding to its antimicrobial activity against several groups of microorganisms, MO seed extract also appears

to be a good coagulant that can be applied in the water treatment process involving coagulation, therefore representing a viable alternative to conventional coagulants such as aluminium sulphate, iron salts III or organic polymers [23, 25]. Articles regarding coagulation processes by MO seed powder were not included in the present review because most of them do not quantify the microorganisms removed. However, MO seed powder has also been widely reported to reduce water turbidity and also reduction coliform count which makes the seed powder a good source for water purification [26, 27]. Even though the articles that addressed the used of moringa to treat drinking water none of them quantified and compared that infected and treated water according to European Union standard for a drinking water. Recently and after our revision, Morgan [30] shown that MO powder significantly reduced 87% *E. coli* colonies in contaminated water and also similar result as well have been reported by Vunain [31] by reduction of microbial load through turbidity reduction. In addition, a single 100 mg of MO seed powdered is the quantity required to eradicating 99.9% of the microbial load from 1 L of water [32]. Although water turbidity reduction implies a reduction of pathogens it is not clear whether they remain in the water or are deposited in the sludge resulting from water treatment.

Regarding leaf extracts, the DWE and 95% EE extraction methods were the most effective ones for a higher number of pathogens. DWE was effective for fourteen pathogens (Table 2) but 95% EE was the most efficient one for a higher number of pathogens.

The 30 mg mL^{-1} leaf extracts presented the highest effectiveness in terms of number of inhibited pathogenic species and the size of inhibition zones obtained; however, only two articles tested this concentration. *Vibrio cholerae,* an important pathogen responsible for cholera, was not tested at 30 mg mL^{-1} of 95% EE extract. The available reports indicate that this extract is more effective at 5 mg mL^{-1} than at 25 mg mL^{-1} and ineffective at 50, 75 and 100 mg mL^{-1}, which raises the question of why higher extract concentrations decrease its effectiveness. Confirmation of effectiveness of the 95% EE leaf extract at several concentrations against this pathogen would be very important, namely at 30 mg mL^{-1}.

Since the most effective results at 30 mg mL^{-1} were reported in just one article [17], we can discuss whether these results were explained by the concentration or by other variables that may influence effectiveness [11, 12]. Moreover, other authors used the same concentration against the same pathogens without effective results [21]. In fact, variables such as of the age of leaves and seeds, stirring type and duration, ratio between MO powder and ethanol, distilled water and other solvents, the temperature of evaporation, plant collection conditions including location, season, as well as date and time of day, are also critical for effectiveness [28]. Results concerning *Escherichia coli* [13, 20] reinforce that hypothesis, showing that the same extraction methods or even the same extract concentration lead to completely different results.

These findings suggest that factors other than the extraction method and extract concentration are relevant for the antibacterial effectiveness of MO. To overcome this complexity, the amount of bioactive compounds for the antimicrobial activity should be quantified in the MO extract.

Actually, it is important to remark that all the plants currently studied to determine their antimicrobial activity contain bioactive compounds responsible for antimicrobial activity such as glucosinolates (β-thioglucoside-N-hydroxysulfates), isothiocyanates, organic carbamates, chalcone oxazolidinone hybrids and thiocarbamate; these and the other mentioned variables may alter the amount of bioactive compounds in the extract. This variability could explain why there are no standard procedures to use MO for water treatment although its antimicrobial properties are frequently reported in the literature.

The selection of a systematized methodology for water treatment is not straightforward, since there is not one method that is effective against all the tested pathogens. So, the choice of plant part, extraction method and extract concentration should be based on the most frequent and virulent pathogens in the target areas. Generally, the pathogens responsible for the most frequent and the most severe water-borne diseases are *Escherichia coli, Klebsiella pneumoniae, Enterobacter* spp., *Salmonella typhi* and *Vibrio cholerae.*

DWE and 95% EE MO leaf extracts at 30 mg mL^{-1} would inhibit all those pathogens except *Vibrio cholerae* which, as mentioned before, was not tested at this concentration but is very effectively inhibited at 5 and 25 mg mL^{-1} Since the most effective results from the MO extract were presented in the same paper and no other similar study is available to establish a comparison, the success of these extracts against this pathogen should be expected with caution.

It is worth noting that DWE and 95% EE are chemical processes, which could make them difficult to apply in low-income countries. Moreover, the selection of a methodology for community-based water treatment should also take the following criteria into account: low capital, high efficiency, keep or increase water quality, easy operation, and low maintenance cost and waste production.

DWE and 95% EE methodologies are currently considered clean technologies to extract natural compounds and use them for water treatment processes, replacing the conventional chemical compounds. From the two, 95% EE could be more appropriate to treat water contaminated

by microorganisms such as *Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi* or *Vibrio cholerae,* considering that its efficiency encompasses those pathogens and is much higher than that of DWE, which is less efficient (Table 2). However, DWE is easier to implement and might be more appropriate to treat water contaminated by *Enterococcus cloacae, Pseudomonas aeruginosa, Serratia marcescens* and *Vibrio parahaemolyticus*, since it is more effective against these pathogens than 95% EE. In terms of toxicity, the available literature shows that neither DWE nor 95% EE threaten human health. However, it is much easier to handle distilled water than ethanol. Moreover, DWE can be the most economical methodology for countries with poor resources, and it is environmentally cleaner. Water contaminated with a combination of pathogens could require a different extraction method (tailored procedure).

Nowadays one of the most widely used methods and apparently the most efficient one to extract bioactive compounds from plants is the Supercritical Fluid Extraction method [29]. This method is relatively new and, among the publications dealing with MO leaf extract for antimicrobial activity, it was not possible to find an article that meets our selection criteria (cf. section 2.2). Although this paper focuses on the methodologies previously used, the Supercritical Fluid Extraction should be taken into account for a further study even though it still involves high costs.

5 Conclusions

In conclusion, an antimicrobial effect of MO was clearly shown and can be used as a water treatment strategy. Even though there is not one methodology for all pathogens, leaf extracts obtained from 95% EE and DWE extraction methods, at a concentration of 30 mg mL^{-1}, presented effective results for critical groups of pathogens including the most common ones in contaminated water, such as *Escherichia coli, Klebsiella pneumoniae, Enterobacter ssp.* and, possibly, *Vibrio cholerae*. The MO-based water treatment strategy is cheap and simple and may therefore constitute a community-based water treatment strategy in areas lacking large-scale water treatment or piped water. Given that literature has already shown that leaf and seed extracts of *Moringa* are not toxic to humans, further research should address its implementation in the low- and middle-income countries to treat drinking water, as well as the extraction process for that purpose. In the meanwhile, it is important to engage in experimental studies to validate the effectiveness of the procedures that presented the best results concerning MO antibacterial activity by using the same condition for all methodologies and also the same strains in order to standardize extracts.

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Compliance with ethical standards

Conflict of interest All author declares that they have no conflict of interest.

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