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Research Article

Exploring the Phytochemistry and Antimicrobial Effects of *Moringa oleifera* on Waterborne Bacteria

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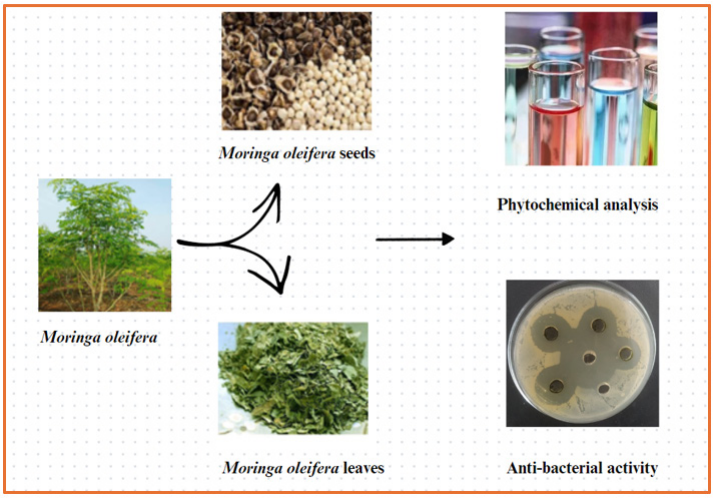
Abstract

Graphical abstract



Many developing countries are facing severe health issues due to invasion of harmful bacteria. The microorganisms that enter into the mouth by the means of feaco-oral route, are causing mortality among children. Moreover, it is estimated that inadequate sanitation, contaminated water, or a shortage of water account for as much as 80% of all diseases and illnesses worldwide. Plants are abundant in secondary metabolites and are employed in the indigenous medical system to treat a variety of diseases. It has been discovered that the parts of the plant of *Moringa oleifera* have amazing ability for enhancing quality of water due to the presence of various phytochemicals in them. In present study, the qualitative analyses of phytochemicals present in *Moringa* seed and leaf extracts was carried out and the extracts were tested for their anti-bacterial activity against various pathogens associated with water-borne diseases. The results acquired from this research revealed that the seeds and leaves of *Moringa* possesses anti-bacterial activity. Therefore, this study looks into the water purifying potential of *Moringa oleifera* as well as its role against a number of bacterial pathogens.

Keywords: *Moringa oleifera*; Phytochemicals; Bacterial; Pathogens; Anti-bacterial

Abbreviations

M. oleifera: *Moringa oleifera; E. coli: Escherichia coli; S. aureus*: *Staphylococcus aureus;* NCCLS: National Committee for Clinical Laboratory Standards

Introduction

The word 'infection' refers to the replication of pathogenic microorganisms in the body of host [2]. Infections like diarrhoea, cholera, typhoid are the most common infections among the population of developing countries which usually occurs due to the ingestion of contaminated food or water [1]. Around the world, a large portion of the population lives in developing nations with poor access to clean drinking water [3]. Microbial contamination of water occurs when animal or human sewage contaminates source water that has not been appropriately treated via filtration, chlorination or other means. In underdeveloped nations, diarrhoea has been linked to almost six million infant deaths annually [4]. Population growth and increased industrialization cause the waterborne infections, one of the problems caused by groundwater contamination [5]. Among various enteric pathogens, *Escherichia coli* and *Staphylococcus aureus* are causal agents of various types of infections [6,7]. The resistance to these bacteria for antibiotics and capacity to build biofilms make the treatment challenging. The inappropriate discharge of untreated effluents from industries, directly into agricultural areas and various water bodies is one of the most important causes of groundwater pollution [8]. Heavy metal buildup in the food chain is ultimately a result of untreated industrial effluents [9]. This information is of great significance to many nations, which has promoted the creation of alternative and practical approaches or technologies to deal with the problem of supply of safe water [10].

Moringa oleifera, also known as 'horseradish tree', is a rapidly emerging and abundantly planted tree in India. Due to abundance of bioactive chemicals found in the *Moringa* plant, every component has been linked to several health and nutritional advantages [11]. Numerous pharmacological characteristics, such as anti-inflammatory, antihypertensive, analgesic, anticancer and antioxidant capabilities, have been supported by prior scientific studies [12,13]. Along with the advantages of nutrition for health and therapeutic value, this plant has been identified as a coagulant in purification of water, having no adverse effects on health, even at significantly higher dosages [14]. Due to the high concentration of bioactive substances found in *Moringa oleifera*, including tannins, flavonoids, alkaloids, polyphenols, isothiocyanates, carotenoids and saponins, the seeds and leaves have also demonstrated notable pharmacological capabilities [15]. *Moringa oleifera* inhibits the growth of different pathogens [16]. In this study, the anti-bacterial activity of seed and leaf extracts of *Moringa oleifera* was checked on waterborne bacteria.

Materials and Methods

Chemicals and reagents

All of the chemicals employed in this study were obtained from Merck and Hi-Media in Mumbai, India; Sigma-Aldrich in Steinheim, Germany; Sigma Chemicals Co. in the United States of America. Every compound was utilized precisely as supplied and was of excellent analytical quality.

Collection of plant seeds and leaves

The seeds (along with their seed coat) and leaves of *Moringa oleifera* plant were collected from the Herbal Garden Neri, Hamirpur (H.P.). After collection, the seeds and leaves of *Moringa oleifera* were pretreated with 5 % sodium hypochlorite solution for 5 min, followed by soaking in distilled water for 2 min. The seeds and leaves were then kept for drying and further grounded into fine powder.

Clinical isolates

The clinical isolates (*Escherichia coli* MTCC No. 46 *and Staphylococcus aureus* MTCC No. 87) were procured from the Department of Biotechnology, Himachal Pradesh University, Summer hill, Shimla.

Extraction of plant seeds and leaves

The powdered sample of seeds and leaves of plant was extracted with n-hexane, petroleum ether, chloroform, ethyl acetate, ethanol, distilled water, methanol and acetone using maceration. In this procedure, 20 g of seed and leaf powder was soaked separately in 200 mL of each of the solvent. They were kept at 37°C at shaking for 3 days to allow the release of active molecules. The extracts were then separated and filtered using Whatman no.1 filter paper. The supernatant of each extract was then kept for evaporation. Further, the extracts were stored at 4°C.

Qualitative phytochemical analysis

Different qualitative tests were performed to check the presence of various phytochemicals in extracts.

Test for presence of tannins

A few drops of 0.1% ferric chloride solution were added to 1 mL of seed and leaf extracts. The formation of blue-black precipitates specified the presence of tannins.

Test for presence of flavonoids

A mixture of 1 mL of concentrated H_2SO_4 and 2 mL of ammonia solution was prepared using 1 mL of each extract. The emergence of a yellow colour indicated the presence of flavonoids.

Test for presence of alkaloids

2 mL of Mayor's reagent was put into 1 mL of each extract. The production of brown or yellow precipitates specified the presence of alkaloids**.**

Test for presence of saponins

Each extract was diluted with 1-2 mL of distilled water, vigorously stirred, and observed for the continuous formation of honey comb foam.

Test for presence of steroids

To 2 mL of anhydrous chloroform, 1 mL of each extract was dissolved. The solution was then thoroughly mixed with concentrated H_2SO_4. Steroids were detected by the creation of a reddish-brown colour ring at interface.

Test for presence of terpenoids

A mixture of 1 mL of each extract, 2 mL of concentrated H_2SO_4, and 1 mL of chloroform was prepared. Terpenoids were present at the interface where a reddish-brown colour appeared.

Anti-bacterial efficacy of extract of *Moringa oleifera* seeds and leaves

Anti-bacterial activity of seed and leaf solvent extracts od *Moringa oleifera* was tested against *Escherichia coli* MTCC No. 46 and *Staphylococcus aureus* MTCC No. 87 by agar well diffusion method according to the National Committee for Clinical Laboratory Standards (NCCLS). *Escherichia coli* MTCC No. 46 and *Staphylococcus aureus* MTCC No. 87 bacterial cultures were spread on agar plates. After the plates had fully dried, six-millimetres wells punchout were made on agar medium and various volumes (20, 30, 40, and 50 µL) of leaf and seed extracts were added to distinct wells. The respective solvents were utilized as negative controls, while the antibiotic (tetracycline) served as positive control. The plates were then incubated for 24 hours at 37°C. Following incubation, the zone of inhibition was measured and compared.

Results and Discussion

Collection of seeds and leaves

The seeds and leaves of *Moringa oleifera* which were shade dried are shown in Figure 1 (a-d). After complete drying, they were converted into fine powder.

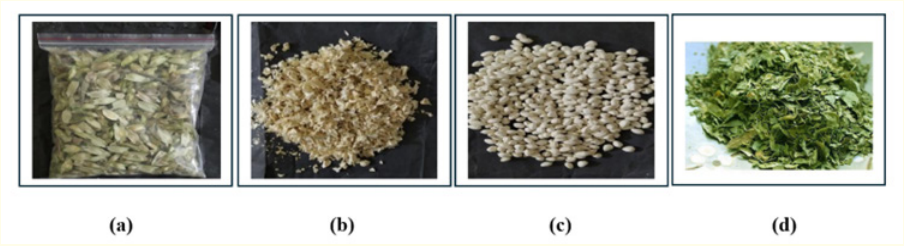


Figure 1: (a) Seeds of *Moringa oleifera* inside the seed coat (b) Coat of *Moringa oleifera* seeds (c) Seeds of *Moringa oleifera* without seed coat and (d) Leaves of *Moringa oleifera.*

Phytochemical analysis

Qualitative analysis

The results of phytochemical analysis of solvent extracts of seed and leaf are shown in Table 1 and Table 2 respectively.

The ethanol, distilled water, methanol and acetone extracts of *Moringa* seed powder indicated the presence of the greatest number of phytochemicals (Table 1). The ethanolic extract indicated the existence of tannins, alkaloids, flavonoids and steroids. Tannins, alkaloids, steroids and terpenoids were present in distilled water extract. All phytochemicals except alkaloids were present in the methanolic extract, with the exception of alkaloids. Alkaloids, flavonoids and tannins were detected in the acetone extract. The distilled water extract of seed indicated the existence of alkaloids, saponins and tannins [17]. According to research in the literature, alkaloids and flavonoids are bioactive compounds present in medicinal plants—have potent antibacterial properties [18-20]. Phytochemical constituents are responsible for pharmaceutical and hazardous effects in plants, according to various studies [21-23].

Extract / Phyto Chemicals	n-Hexane	Petroleum Ether	Ethyl acetate	Chloroform	Ethanol	Distilled Water	Methanol	Acetone
Tannins	-	+	+	+	+	+	+	+
Flavonoids	-	-	-	+	+	-	+	+
Alkaloids	-	-	-	-	+	+	-	+
Saponins	-	-	+	+	-	-	+	-
Steroids	-	+	-	-	+	+	+	-
Terpenoids	-	-	+	-	-	+	+	+

Table 1: The phytochemical examination of solvent-extracted *Moringa oleifera* seed extracts.

Extract / Phyto Chemicals	n-Hexane	Petroleum Ether	Acetone	Ethyl acetate	Ethanol	Distilled Water	Methanol	Chloroform
Tannins	-	-	-	-	+	+	+	+
Flavono0ids	-	-	+	-	+	+	+	+
Alkaloids	-	-	-	-	+	+	-	+
Saponins	-	-	-	+	+	-	+	-
Steroids	-	+	-	-	-	+	+	+
Terpenoids	-	-	+	+	-	+	+	+

Table 2: The preliminary phytochemical analysis of different solvent extracts of *Moringa oleifera* leaves.

Therefore, the existence of various metabolites in seed extracts of *Moringa oleifera* probably make it an effective antibacterial substance.

The ethanol, distilled water, methanol and chloroform extracts of leaves indicated the presence of maximum number of phytochemicals. The ethanolic leaf extract indicated the presence of tannins, flavonoids, alkaloids and saponins. Tannins, flavonoids, alkaloids, steroids and terpenoids were present in distilled water extract. The presence of tannins, flavonoids, saponins, steroids and terpenoids was detected in methanolic extract. The chloroform extract revealed the presence of tannins, flavonoids, alkaloids, steroids and terpenoids. The present research showed the presence of the phytochemicals, which is in agreement with results obtained by previous research [24]. The findings of present study were also supported by a study which revealed that the extracts of *Moringa* leaves showed the presence of tannins, flavonoids, alkaloids and saponins [25].

These results showed that due to these phytochemicals, extracts of seed and leaves of *Moringa oleifera* exhibited strong anti-bacterial activity against various pathogens. Thus, seeds and leaves could act as potential nutrient source for animals and humans [26].

Anti-bacterial activity assay

The microorganisms like *Staphylococcus aureus* and *Escherichia coli* are present in water in very large amount. These are the major causes of various water borne diseases. The seeds and leaves of *Moringa oleifera* play a very important role in inhibiting the growth of these organisms.

Anti-bacterial activity of extracts of *M. oleifera* seeds against *E. coli*

Anti-bacterial activity of seed extracts was studied using agar well diffusion assay. Tetracycline served as a positive control, while the corresponding solvents served as negative controls. All the extracts showed maximum inhibitory zone of 19 mm at volume of 50 µL against *E. coli* (Figure 1 a-d). The inhibitory zone of 14 mm, 15 mm, 17 mm and 19 mm respectively was shown by ethanol extract at volume of 20, 30, 40 and 50 µL as shown in Figure 2 (a). The zone of inhibition of 19 mm at volume of 50 µL but no activity was observed at 20 µL volume by distilled water extract as shown in Figure 2 (b). The methanol extract showed inhibitory zone of 10 mm, 14 mm, 15 mm and 19 mm respectively at volume of 20, 30, 40 and 50 µL as shown in Figure 2 (c). The acetone extract showed zone of inhibition of 10 mm, 15 mm, 15 mm and 19 mm respectively at volume of 20, 30, 40 and 50 µL as shown in Figure 2 (d). Our findings were confirmed by various reports on anti-bacterial activity of seed extracts of *Moringa oleifera*. A significant inhibitory effect against *E. coli* was shown by methanolic and aqueous extracts of *Moringa* seeds [27]. The seed ethanol extract showed the inhibitory zone of 9 mm, 12 mm and 16 mm against *E. coli* at volume of 100 µL, 200 µL and 400 µL respectively but water extract showed no inhibition against *E. coli* [28]. The acetone seed extract showed the zone of inhibition of 19 mm at volume of 50 µL against *E. coli* [29] which is in accordance with the present study.

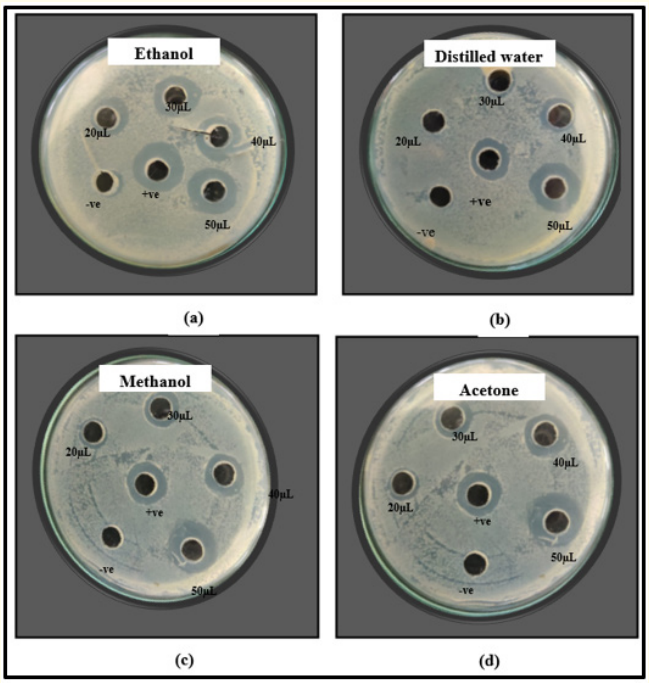


Figure 2: Zone of inhibition shown by (a) ethanol (b) distilled water (c) methanol and (d) acetone extracts of *M. oleifera* seed against *E. coli.*

Maximum zone of inhibition was shown by all four extracts at volume of 50 µL as shown in Table 3.

Extract	Zone of inhibition against *Escherichia coli* (mm)					
	20 µL	30 µL	40 µL	50 µL	Positive	Negative
Ethanol	14	15	17	19	20	0
Distilled water	0	10	13	19	17	0
Methanol	10	14	15	19	17	0
Acetone	10	15	15	19	17	0

Table 3: Anti-bacterial activity of extract of *M. oleifera* seed against *Escherichia coli.*

Anti-bacterial activity of extract of *M. oleifera* seed against *Staphylococcus aureus*

Anti-bacterial activity of seed extracts was studied against *S. aureus*. The ethanol extract showed inhibitory zone of 10 mm, 16 mm, 16 mm and 17 mm respectively at volume of 20, 30, 40 and 50 µL as indicated in Figure 3 (a). The distilled water extract formed inhibitory zone of 10 mm and 11 mm at volume of 40 and 50 µL respectively but no activity was observed at 20 µL and 30 µL volume as shown in Figure 3 (b). The inhibitory zone of 10 mm, 14 mm,

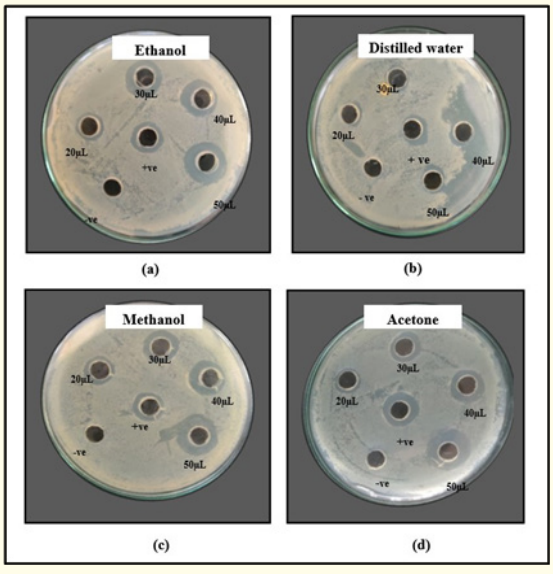


Figure 3: Zone of inhibition shown by (a) ethanol (b) distilled water (c) methanol and (d) acetone extracts of *M. oleifera* seeds against *Staphylococcus aureus.*

16 mm and 20 mm respectively at volume of 20, 30, 40 and 50 µL was shown by methanol extract as indicated in Figure 3 (c). The acetone extract showed inhibition zone of 10 mm, 12 mm, 14 mm and 18 mm respectively at volume of 20, 30, 40 and 50 µL as shown in Figure 3 (d). In a previous study, the ethanol, distilled water and methanol seed extracts showed the zone of inhibition of 22 mm, 17 mm and 27 mm at volume of 100 µL respectively [30,31].

Maximum inhibition zone of 20 mm was shown by methanol extract at volume of 50 µL. All four extracts showed maximum zone of inhibition at volume of 50 µL as shown in Figure 3 and Table 4.

Anti-bacterial activity of extracts of *M. oleifera* leaves against *E. coli*

Anti-bacterial activity of leaves extracts was studied against *E.* coli. No inhibition of *E. coli* was shown by ethanol extract of leaves as shown in Figure 4 (a). The distilled water extract showed zone of inhibition of 20 mm, 27 mm, 27 mm and 35 mm respectively at volume of 20, 30, 40 and 50 µL as shown in Figure 4 (b). The methanol extract showed zone of inhibition of 22 mm, 22 mm, 24 mm and 29 mm respectively at volume of 20, 30, 40 and 50 µL as shown in Figure 4 (c). The chloroform extract showed zone of inhibition of 23 mm, 26 mm, 30 mm and 32 mm respectively at volume of 20, 30, 40 and 50 µL as shown in Figure 4 (d). In a recent study, ethanolic and distilled water leaf extract formed the inhibitory zone of 21 mm and 16.80 mm at 30 µL volume respectively against *E. coli* [32]. The methanolic leaf extract did not form inhibitory zone against *E. coli* and distilled water extract formed inhibitory zone of 9 mm and 10 mm at volume of 20 µL and 30 µL respectively [32]. The chloroform extract showed inhibition zone of 11 mm at 20 µL volume against *E. coli* [33].

Extract	Zone of inhibition against *Staphylococcus aureus* (mm)					
	20 µL	30 µL	40 µL	50 µL	Positive	Negative
Ethanol	10	16	16	17	14	0
Distilled water	0	0	10	11	14	0
Methanol	10	14	16	20	14	0
Acetone	10	12	14	18	17	0

Table 4: Anti-bacterial activity of *M. oleifera* seed extracts against *Staphylococcus aureus.*

The zone of inhibition of 35 mm and 32 mm was shown by distilled water and chloroform extract of leaves at 50 µL volume as shown in Table 5.

Anti-bacterial activity of extracts of *M. oleifera* leaves against *S. aureus*

No inhibition of *S. aureus* was shown by ethanol extract of leaves as shown in Figure 5 (a). The distilled water extract showed inhibitory zone of 16 mm,18 mm, 19 mm and 20 mm respectively at volume of 20, 30, 40 and 50 µL as shown in Figure 5 (b). The methanol extract showed inhibitory zone of 23 mm, 25 mm, 26 mm and 27 mm respectively at volume of 20, 30, 40 and 50 µL as shown in Figure 5 (c). The inhibitory zones of 16 mm, 20 mm, 20 mm and 21 mm respectively at volume of 20, 30, 40 and 50 µL were shown by

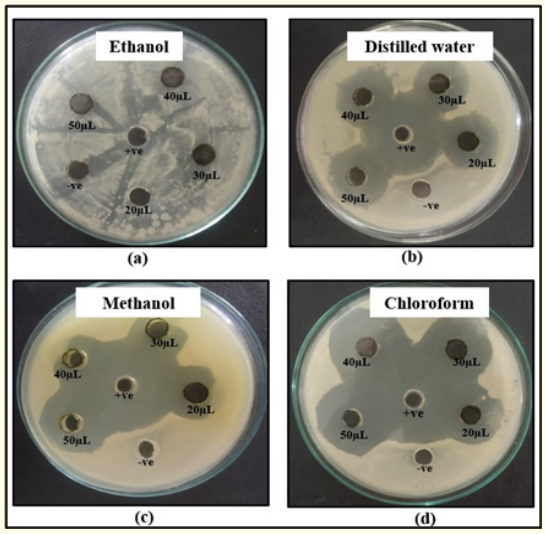


Figure 4: Zone of inhibition shown by (a) ethanol (b) distilled water (c) methanol and (d) chloroform extracts of *M. oleifera* leaves against *E. coli.*

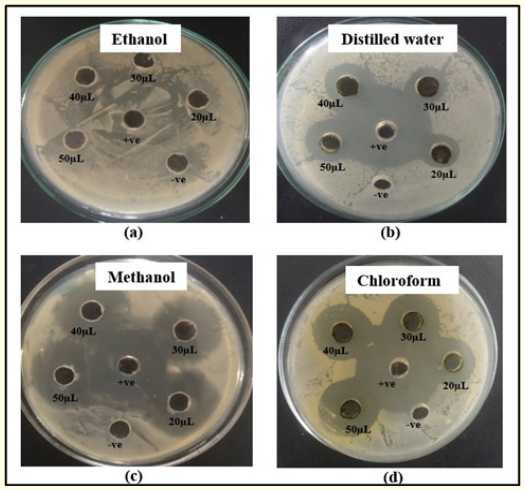


Figure 5: Zone of inhibition shown by (a) ethanol (b) distilled water (c) methanol and (d) chloroform extracts of *M. oleifera* leaves against *S. aureus.*

Extract	Zone of inhibition against *Escherichia coli* (mm)					
	20 µL	30 µL	40 µL	50 µL	Positive	Negative
Ethanol	0	0	0	0	0	0
Distilled water	22	27	27	35	37	0
Methanol	22	22	24	29	34	0
Chloroform	23	26	30	32	40	0

Table 5: Anti-bacterial activity of extracts of *M. oleifera* leaves extracts against *Escherichia coli.*

Extract	Zone of inhibition against *Staphylococcus aureus* (mm)					
	20 µL	30 µL	40 µL	50 µL	Positive	Negative
Ethanol	0	0	0	0	0	0
Distilled water	16	18	19	20	40	0
Methanol	23	25	26	27	40	0
Chloroform	16	20	20	21	32	0

Table 6: Anti-bacterial activity of extracts of *M. oleifera* leaves against *Staphylococcus aureus.*

chloroform extract as shown in Figure 5 (d). However, the ethanolic extract of *M. oleifera* formed the zone of inhibition of 8 mm at 20 µL volume [33]. In another study, the distilled water extract had no zone of inhibition against *S. aureus*, whereas methanolic extract had zones of inhibition of 10 mm, 9 mm, 15 mm, and 17 mm at volumes ranging from 20 to 50 µL [32]. The chloroform extract of *M. oleifera* formed inhibitory zone of 10 mm at 20 µL volume against *S. aureus* [33].

The maximum inhibitory zone of 27 mm was shown by methanol extract of leaves of *M. oleifera* as indicated in Table 6.

Conclusion

The present study focuses on the various phytochemical constituents in *Moringa oleifera* and their anti-bacterial activity. According to our study, the seed and leaf extracts of *Moringa oleifera*

inhibit the growth of various organisms like *E. coli* and *S. aureus.* Extracts of plant seeds and leaves could thus be utilized to treat infectious disorders caused by microorganisms. These extracts are likely to be promising natural antibacterial agents with uses in the management of bacteria that cause disease.

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Competing Interests

The authors declare that there is no competing interest.

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