

ANTIMICROBIAL ACTIVITY OF *MORINGA OLEIFERA* AGAINST MULTIDRUG-RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM RAW MILK

TIRADO-TORRES, D.¹ – CHAN-KEB, C. A.² – PÉREZ-BALÁN, R. A.² – AKE-CANCHÉ, B.² –
GÓMEZ-SOLANO, M. I. – ARAGÓN-GASTÉLUM, J. L.² – GÓMEZ-LÓPEZ, I.³ – AGUIRRE-CRESPO,
F. J.² – LÓPEZ-RAMOS, M. C.² – GUTIÉRREZ-ALCÁNTARA, E. J.*

¹*Departamento de Ingeniería Civil, División de Ingenierías, Campus Guanajuato, Universidad
de Guanajuato, Av. Juárez N° 77, Col. Centro, Guanajuato, Gto., México*

²*Facultad de Ciencias Químico-Biológica, Universidad Autónoma de Campeche Av. Agustín
Melgar S/N, Buena Vista, CP: 24039 Campeche, Cam., México*

³*Centro Nacional de Metrología, Querétaro, México*

*Corresponding author
e-mail: ejgutier@uacam.mx

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Abstract. The objective of the study was to evaluate the antimicrobial activity of aqueous and ethanolic *Moringa oleifera* leaf, stem and seed extracts against multidrug-resistant *Staphylococcus aureus* strains isolated from raw milk in Hidalgo Mexico in 2017. The conventional method was used to identify and isolate *S. aureus*. All isolates were screened for antibiotic sensitivity to 12 antibiotics using the disk-diffusion method, in order to select twenty multidrug-resistant strains. The antimicrobial activity of the *M. oleifera* leaf, stem and seed extracts (aqueous and ethanolic) was tested using the disk-diffusion agar method, with penicillin used as a positive control. Sixty-five *S. aureus* strains were isolated from 56% of the raw milk sample, with an average count of 4.5×10^5 CFU/ml, this is considered as a potential public health risk. All the *S. aureus* strains exhibited resistance to at least two antibiotics. Sixty-five strains exhibited resistance to penicillin and ampicillin. In contrast, all showed sensitivity to ciprofloxacin. Ethanol extract exhibited a higher degree of antimicrobial activity compared to the aqueous extracts and penicillin. This reveals that the leaves, stems and seeds of *M. oleifera* could be an alternative for the control of infections caused by *S. aureus* in humans and cows with mastitis.

Keywords: *foodborne pathogens, natural antimicrobial, bacteria, resistance, antibiotics*

Introduction

Raw milk is an ideal growth medium for different microorganisms, as it is considered a vehicle for *Staphylococcus aureus* (*S. aureus*) infection in humans (Zecconi and Hahn, 2000). This foodborne pathogen is considered as one of the world's leading causes of disease outbreaks related to food consumption and is responsible for a variety of manifestations and diseases (Jamali et al., 2014). Although the precise number of Staphylococcal infections outbreaks is unknown in Mexico, previous studies have indicated that raw milk and dairy products manufactured from raw milk play an important role in outbreaks in humans (Rania et al., 2013; Basanisi et al., 2017). The contamination of milk and milk products with this pathogenic bacteria is mainly caused by the processing and handling of cows with mastitis in unhygienic environments (Thaker et al., 2013).

In Mexico, the production of bovine milk reached 11,707,494 tons in 2016, of which, 14,973 tons was produced in the municipality of Francisco I. Madero (SAGARPA,

2016). As *S. aureus* is capable of acquiring antibiotic resistance determinants, its isolates often exhibit resistance to multiple classes of antimicrobial agents (Rybak and Laplante, 2005). Multiresistant *S. aureus* emerged decades ago due to the widespread and often inappropriate use of antibiotics in livestock (Mehli et al., 2017). The trend of rising antibiotic resistance continues despite the restrictions imposed on its use, both clinically and in food production (EFSA, 2009; NFSA, 2018). Resistant bacteria can be transmitted to humans through food, particularly that of animal origin and/or consumed raw (Phillips et al., 2004), and is a growing public health problem.

Increasing antibiotic resistance in pathogenic bacteria has led to growing demand for alternative safe and natural antimicrobials. The focus is currently on biologically active components isolated from plant species, such as *Moringa oleifera* (*M. oleifera*) (Arora and Onsare, 2014), used in food or herbal medicine, as these may provide a new source of antibacterial compounds (Gutiérrez-Alcántara et al., 2015).

M. oleifera is reported to have an antimicrobial effect on pathogenic bacteria (Brilhante et al., 2015; Peixoto et al., 2011; Viera et al., 2010). The antimicrobial properties of *M. oleifera* have been attributed to different parts of the plant, such as the leaves, seeds, pods and stems (Ferreira et al., 2011; Arora et al., 2013). In addition, studies conducted on this plant have revealed promising anti-inflammatory (Ezeamuzie et al., 1996), pro-coagulant (Nkurunziza et al., 2009), flocculant (in water treatments) (Beltrán-Heredia and Sánchez-Martín, 2009), detoxifying, immune boosting, and anti-parasitic activity (Thilza et al., 2010), for the treatment of diarrhea and skin infections (Farooq et al., 2012). Moreover, it is rich in nutrients and has been used in different products such as oils, foods, condiments and medicine (Viera et al., 2010). However, less extensive research has been conducted on the antimicrobial effects of *M. oleifera* on multidrug resistant pathogenic strains. Currently there are no studies focused on analyzing the effect of the leaf, seed and stems together of *M. oleifera*, previously good results have been observed separately (Brilhante et al., 2015; Lar et al., 2011). The objective of the present study was to evaluate the antimicrobial activity of aqueous and ethanolic *M. oleifera* leaf, stem and seed extracts against multidrug-resistant *S. aureus* strains isolated from raw milk.

Materials and methods

Collection of samples

A total of 100 bulk-tank milk samples (one liter per sample) were collected from between April and August 2017 from 4 farms located in the municipality of Francisco I. Madero, Mexico (Figure 1). One farm was located within the Universidad Politécnica de Francisco y Madero (UPFIM), while the other three farms were located approximately 3.5 km from the university. The four farms (A-B-C-D) had less than 7 Holstein cows each and the age ranged between 2-2.5 years.

The samples were placed in sterilized bags under aseptic conditions and transported in an icebox to the laboratory, where they were then analyzed no more than one hour after their purchase from the dairy farms.

Isolation and detection

The bacteriological method used for identifying and isolating *S. aureus* was performed according to Mehli et al. (2017), in which 10 mL of raw milk was aseptically

removed from each sample, placed in bags containing 90 mL of sterile peptone water (1.0 g bacteriological peptone and 8.5 g L⁻¹ sodium chloride) (Bioxon, the State of Mexico, Mexico), and homogenized manually for 2 min. Ten-fold dilutions were made using sterile peptone water, after which the appropriate dilutions (0.1 mL) were spread on Baird-Parker agar supplemented with 1% egg yolk tellurite emulsion (Bioxon, Estado de Mexico, Mexico). The plates were placed in an incubator (Labtech LIB-150M, USA) for 24-48 h at 37°C.

Suggestive colonies of *S. aureus* (black, shining, and convex, with a 1.0-1.5 mm diameter and surrounded by a clear zone) were selected for seeding in tubes containing Brain Heart Infusion (Bioxon, the State of Mexico, Mexico), which were then incubated for 35°C for 24 h. The presumptive strains of *S. aureus* were confirmed using coagulase, catalase, DNase, acetoin production and maltose fermentation tests (Saka and Terzi, 2018). All strains were also tested for the presence of staphylococcal enterotoxin using a commercial ELISA test kit (3M Tecra Code: FSA1156, New South Wales, Australia). The confirmed *S. aureus* strains were then preserved at 3-5°C for subsequent study.

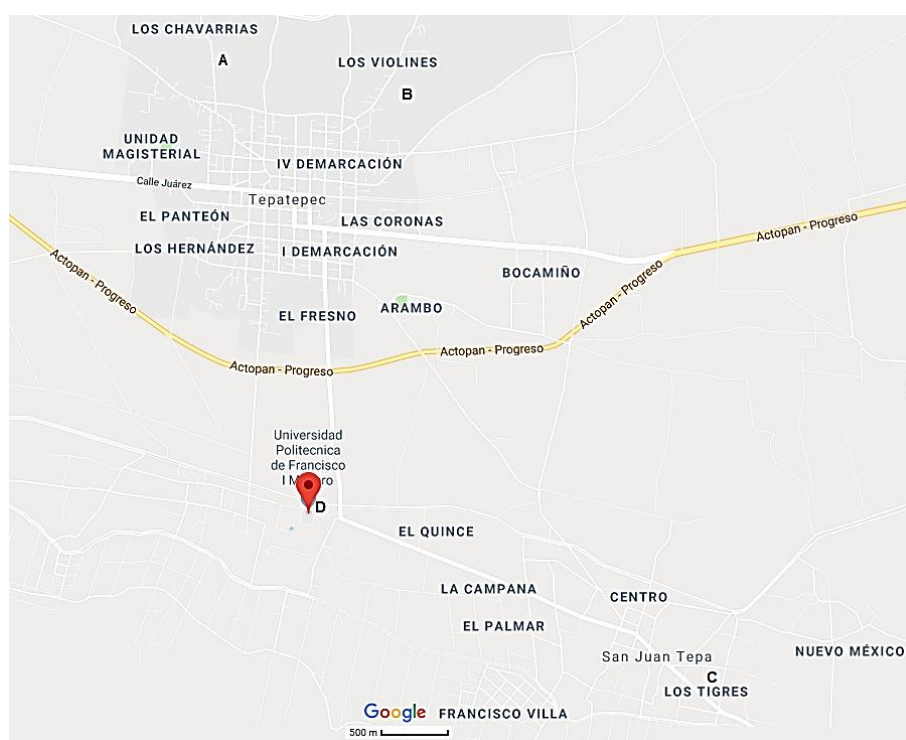


Figure 1. Location of the study areas

Antibiotic susceptibility testing

All *S. aureus* isolates were screened for antibiotic sensitivity to 12 different antibiotics using the disk-diffusion method on Muller-Hinton agar (Oxoid) with commercially available disks (Oxoid) in accordance with the Clinical and Laboratory Standards Institute (CLSI, 2014). The following antimicrobial agents were tested: ampicillin (10µg/mL); cefalotin (30 µg/mL); cefotaxim (30 µg/mL); ciprofloxacin (5 µg/mL); clindamycin (30 µg/mL); dicloxacillin (1 µg/mL); erythromycin (15 µg/mL); gentamicin (10 µg/mL); penicillin (10 IU); tetracycline (30 µg/mL); trimethoprim/sulfamethoxazole (25 µg/mL); and, vancomycin (30 µg/mL). The results

were evaluated after incubation at 35°C for 24 h, while the interpretations of antibiotic resistance were based on CLSI criteria (CLSI, 2014). For quality control, a reference strain of *S. aureus* (ATCC 25923) was used in the study.

Plant material and extract preparation

The extracts were obtained from *M. oleifera* specimens grown in Acapulco, the state of Guerrero, Mexico, and provided by the Universidad Autónoma de Guerrero. The age of the plant was two years.

The fresh leaves, stems and seeds were collected only once in May of 2017, they were cleaned and washed with water and dried in a heated chamber (Cole Parmer 399553-20, I, USA) at 40°C/24 h.

The *M. oleifera* extracts were produced following a previously described method (Gutiérrez-Alcántara et al., 2015; Cruz-Gálvez et al., 2013). Briefly, all the materials were pulverized using an electric blender (Oster BPST02-B00-013, Mexico). About 100 g of the powdered materials (leaves, stems and seeds) were weighed and placed in sterile glass flasks, after which 900 mL of 70% v/v ethanol was added. The flasks were sealed and stored at room temperature for 7 days.

The extracts were filtered through Whatman No. 1 filter paper, with the ethanol extract then evaporated to dryness under reduced pressure using a rotary evaporator (BÜCHI, Vacuum AQ3 176 Controller V-800; Flawil, Switzerland) at 40°C, leaving only the concentrated extract of the constituents of the powdered materials.

To prepare the aqueous extract, 100 g of the powdered materials (leaves, stems and seeds) was placed in a sterile glass flask, to which 900 mL of sterile distilled water was added. This was then heated to boiling point for 10 min and cooled to room temperature. The water was eliminated from the concentrate as described above, with the dried extracts then stored in sterile plastic bags at room temperature until use.

Preparation of the extract solution

A solution was prepared from each aqueous and ethanolic *M. oleifera* extract concentrate (produced from 5 g leaves, stems and seeds), using 100 ml of distilled water (final extract concentration: 50 mg mL⁻¹). *S. aureus* ATCC 25923 strain was used as a quality control.

Inoculum preparation and inoculation. The antibacterial activity of *M. oleifera* leaf, stem and seed extracts was tested using the disk-diffusion agar method, as previously described (Gutiérrez-Alcántara et al., 2016), although with some modifications (it changed the Trypticase Soy Agar agar for a Muller Hinton and penicillin was used as a positive control). Briefly, antibiotic-resistant *S. aureus* strains isolated from raw milk were inoculated in tubes containing 3 mL trypticase soy broth (TSB, Bioxon, the State of Mexico, Mexico) and incubated at 35°C for 24 h. The cultures were washed with sterile isotonic saline solution (0.85% NaCl) via centrifuge at 3500 rpm for 20 min, with the pellets then resuspended in sterile peptone water at approximately 10⁹ CFU/mL. A decimal dilution of these washed cultures was produced using isotonic saline solution to achieve a final approximate concentration of 8 log CFU/mL. A 100 µL suspension was taken from the first dilution of each washed bacterial culture, inoculated on Mueller Hinton plates and extended over the agar (Bioxon, Estado de Mexico, Mexico). Filter paper (Whatman No. 5) disks were placed on the surface of each agar plate. Aliquots (10 µL) of each extract were then placed on each disk (final dose per disc: 1 mg extract), with isotonic saline solution used as a negative control and penicillin as a

positive control. Each test was replicated three times. The plates were incubated for 24 h at 35°C, and then examined for the presence of bacterial inhibition zones around the disk. The diameter (mm) of any resulting inhibition zones was measured and the average diameter values were calculated for each extract.

Statistical analysis

Diameter of *S. aureus* growth was measured and expressed as means of percentage growth inhibition of three replicates. Significant differences were calculated using statistica 8 program (StatSoft, Inc., Tulsa, version 8).

Results and discussion

Prevalence of *S. Aureus*

Sixty-five *S. aureus* strains were identified and isolated from 56% (Table 1) of the raw milk sample, with an average count of 4.5×10^5 CFU/ml. More than one staphylococci colony per plate were evaluated from only eight of the samples. Farm A presented 16 positive samples, while the Farm D presented 11 positive samples (the UPFIM farm).

Table 1. Prevalence of *Salmonella* in the sample of the farms

Farm	Positive samples	Prevalence %
A (n=25)	16	64
B (n=25)	13	52
C (n=25)	16	64
D (n=25)	11	44
Total (n=100)	56	56%

The average *S. aureus* counts obtained in this research exceed the number of bacteria necessary (1.0×10^5 CFU/mL) to produce sufficient enterotoxin in order to induce foodborne intoxication (Cupakova et al., 2012). Moreover, they increase the probability of the production of staphylococcal toxins that are resistant to both the boiling process carried out in homes when raw milk is bought and pasteurization processes (Tebaldi et al., 2008). The isolates obtained in this study should be considered as a potential public health risk, as this pathogen may enter the food chain.

The *S. aureus* frequency observed in the raw milk samples (56%) coincides with the high *S. aureus* frequency reported in raw milk and dairy products in Greece and the USA (57.8% and 62%, respectively) (Papadopoulos et al., 2018; Haran et al., 2012). Furthermore, the results of this study are higher than those reported in Brazil by Fagundes et al. (2010) who observed a 7.3% frequency in raw milk samples, 6.7% in samples taken from individual cows, and 10.8% from bulk tank milk. In Italy, observed a 12.9% frequency in milk samples and dairy products (Basanisi et al., 2016). Riva et al. (2015) found that the prevalence of *S. aureus* was 9.1% in raw milk, while Normanno et al. (2007) reported a 17% contamination rate in milk and dairy products.

It should be noted both that other studies have reported prevalence rates of 66-86.1% (Saka and Terzi, 2018; Rania et al., 2013; Freitas et al., 2015; Rola et al., 2015), and that other authors have also reported the prevalence (13% and 30%) of this pathogen in

pasteurized milk (Akindolire et al., 2015; De Oliveira et al., 2011). The presence of these bacteria, after pasteurization, can be attributed to either the inefficacy of the thermal process or post-process contamination.

S. aureus is usually considered as a major cause of mastitis (Jamali et al., 2015). Certainly, many factors, such as improper bulk tank cleaning, dirty udders, an infected cow, dirty establishments and inappropriate hygiene conditions during milking, storage and manufacturing, are responsible for variations in the prevalence of *S. aureus* in dairy products, including milk (Roberson et al., 1998). For this reason, it is necessary to monitor animals state of health and improve hygienic conditions for milking, washing and the systematic disinfection of plants (Basanisi et al., 2017).

Antimicrobial susceptibility

All the *S. aureus* strains isolated from the raw milk exhibited resistance to at least two antibiotics. Sixty-five strains exhibited resistance to penicillin and ampicillin, followed by dicloxacillin. In contrast, all showed sensitivity to ciprofloxacin, while fifty-nine strains were sensitive to erythromycin (Table 2), fifty-three were sensitive to vancomycin, and fifty-three to tetracycline. The strains showed similar profile resistant to tetracycline and erythromycin, this may be possible because they act on protein synthesis. Ten strains presented intermediate resistance to cefotaxim. Similarly fourteen strains of *S. aureus* presented intermediate resistance to cefalotin and clindamycin despite being from different groups.

Table 2. Counts for *S. aureus* strains exhibiting resistance (R), intermediate resistance (I), or sensitivity (S) to 12 antibiotics

Antibiotic	R	I	S
Ampicillin	65*	0	0
Penicillin	65	0	0
Dicloxacillin	40	20	5
Cefotaxim	32	10	23
Cefalotin	31	14	20
Clindamycin	29	14	22
Gentamicin	28	12	25
Trimethoprim/Sulfamethoxazole	20	15	30
Vancomycin	9	3	53
Tetracycline	8	1	56
Erythromycin	5	1	59
Ciprofloxacin	0	0	65

*strain counts

The results of this study are similar to those obtained from research conducted in China (Yang et al., 2016), in which *S. aureus* strains isolated from bovine mastitis cases were resistant to penicillin (84.09%). In Greece, the most frequently observed resistance was to penicillin (99.3%) (Papadopoulos et al., 2018), while Jamali et al. (2015) observed resistance to penicillin (47.4 %) in strains isolated from raw milk and dairy products. The high percentage of *S. aureus* that is resistant to penicillin could be due to the widespread administration of this antimicrobial to control and treat infections found

in dairy farms (Jamali et al., 2013). Lowy (2003) reported that staphylococcal resistance to penicillin is mediated by β -lactamase, an extracellular enzyme which is synthesized when *S. aureus* is exposed to β -lactam antibiotics.

Resistance to ampicillin was high in all strains (100%), which is in accordance with the natural resistance of *S. aureus* β -lactams induced by exposure to penicillins (Fish et al., 1995). Other studies have shown resistance to ampicillin (Bernardo et al., 2005; Chudobova et al., 2015; Thaker et al., 2013). The results obtained here for ciprofloxacin coincide with a study in which *S. aureus* strains isolated from raw milk and dairy products were sensitive to ciprofloxacin (100%) (Jamali et al., 2015); moreover, Papadopoulos et al. (2018) reported that 97.09% of strains were sensitive to this antibiotic. In addition, strains isolated from clinical cases, raw milk and dairy product in both Mexico and India have been reported to be resistant to ciprofloxacin (Miranda-Novales, 2011; Thaker et al., 2013). Vancomycin-sensitive *S. aureus* has been isolated in Turkey and China (Rağbetli et al., 2016; Yang et al., 2016), while nine strains resistant to vancomycin were found in this study. This resistance is due to the acquisition of the van gene, which is transferred via a plasmid (Bustos-Martínez et al., 2006).

Strains of *S. aureus* that are resistant to erythromycin and tetracyclines but sensitive to gentamycin have been isolated from retail meat in Denmark (Tang et al., 2017), a profile which is different to that observed in the present study for *S. aureus* strains isolated from raw milk samples.

In-vitro* antimicrobial activity of *M. oleifera

The testing, conducted in this study, of the effect of the leaves, stems and seeds of *M. oleifera* on the twenty antibiotic-resistant *S. aureus* strains isolated from raw milk (previously selected for being multiresistant to 12 antibiotics) revealed that all the strains were sensitive to the two extracts (Table 3).

Table 3. Average diameter values of *M. oleifera* extract produced with two solvents versus antibiotic-resistant *S. aureus* strains and control strains

Strains	Zones of inhibition (Diameter in mm)		
	Ethanollic extract	Aqueous extract	Penicillin
1	24 ± 02	23 ± 04	10 ± 0.2
2	25 ± 03	22 ± 04	10 ± 0.2
3	25 ± 02	23 ± 02	11 ± 0.1
4	25 ± 01	23 ± 01	10 ± 0.2
5	25 ± 02	23 ± 08	11 ± 0.2
6	25 ± 02	21 ± 04	11 ± 0.2
7	25 ± 1.0	23 ± 01	11 ± 0.1
8	24 ± 02	23 ± 06	10 ± 0.2
9	25 ± 02	22 ± 04	9 ± 0.2
10	25 ± 02	23 ± 03	9 ± 0.2
11	25 ± 03	23 ± 03	10 ± 0.3
12	25 ± 02	21 ± 01	10 ± 0.2
13	25 ± 03	23 ± 06	10 ± 0.2
14	25 ± 02	23 ± 08	10 ± 0.2
15	24 ± 02	21 ± 06	11 ± 0.2
16	25 ± 02	23 ± 06	11 ± 0.1
17	25 ± 02	23 ± 04	10 ± 0.2
18	25 ± 01	22 ± 02	11 ± 0.2
19	24 ± 02	23 ± 02	10 ± 0.2
20	25 ± 02	21 ± 02	10 ± 0.2
*SA	25 ± 02	22 ± 02	10 ± 0.2

* SA (*S. aureus* ATCC 25923)

The ethanolic extract presented the highest inhibitory effect against isolates from raw milk with halos of inhibition of 24.83 mm (mean). The mean of the halos of inhibition for the aqueous extract was 22.17 mm, while the penicillin presented halos of inhibition of 9.77 mm, and the halos of inhibition for the control strains were similar to the halos for the multiresistant strain. The strains 1, 8 and 18 showed similar diameters with the extracts used, while the control strain showed similar diameters with the strain 2.

In addition, penicillin, ethanol and aqueous extract showed highly significant difference ($p < 0.0001$).

Currently there are no studies focused on analyzing the effect of the leaf, seed and stems together of *M. oleifera*, previously good results have been observed separately (Brilhante et al., 2015; Lar et al., 2011).

The halos of inhibition found in this study (21-25 mm) coincide with a research in which *M. oleifera* seeds were studied for antimicrobial activity against *Bacillus subtilis*, *Salmonella typhimurium*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Vibrio cholerae* and *S. aureus* (Ruttarattanamongkol and Petrasch, 2015). The above mentioned research found that *S. aureus* presented the maximum inhibition zone of 20.67 and 24.67 mm. In contrast Peixoto et al. (2011) observed inhibition halos of up to 35 mm when challenging *S. aureus* with aqueous and ethanolic *M. oleifera* leaf extracts, but they also showed halos de inhibicion de 21-25 mm against *Vibrio parahaemolyticus* and *Enterococcus*. Some authors have attributed the antibacterial effect of the leaves to the presence of saponine, tannic, phenolic and alkaloid phytoconstituents (Doughari et al., 2007).

Working on the same plant species, Bukar et al. (2010) demonstrated the antibacterial activity of ethanolic extract of its seed, via the agar well diffusion method, against *S. aureus*, *Salmonella typhi* and *E. coli*. The activity of the two extracts was compared to a standard antibiotic (penicillin), as this antibiotic showed low efficiency in the previously isolated resistance profiles for the *S. aureus* strains. However, the two *M. oleifera* stem, leaf and seed extracts (ethanolic and aqueous) exhibited higher antibacterial activity compared to penicillin.

The ethanolic extract has been shown to present antimicrobial properties against *S. aureus*, with the findings of this research differing from Brilhante et al. (2015) who reported low antimicrobial effectiveness in ethanol extracts of *M. oleifera* pods, leaves, stems and seeds against *Vibrio spp.* strains, as well as no effect against *E. coli*. Furthermore, the results of the present study concur with those reported previously by other researchers in an evaluation of the antimicrobial effect of *M. oleifera* seed extracts against non-antibiotic resistant *S. aureus* (Viera et al., 2010; Ruttarattanamongkol and Petrasch, 2015).

The aqueous extract used in the present study presented inhibition halos of 22.17 mm, which concurs with the results reported previously by research which evaluated the antimicrobial effect of aqueous *M. oleifera* seed extract against *S. aureus*, *Vibrio cholerae* and *E. coli* isolated from shrimp samples, observing inhibition halos of 19-25 mm (Viera et al., 2010).

While Lar et al. (2011) reported that the aqueous extract of *M. oleifera* seeds had no effect on *E. coli*, *Shigella flexneri* and *S. typhi*, appreciable antimicrobial activity was demonstrated by ethanolic seed extract on the same bacteria.

Similarly Kalpana et al. (2013) found antimicrobial activity using ethanolic extract of leaves of *M. oleifera* against *S. aureus* with diameters of 10-15 mm.

The antimicrobial activity of *M. oleifera* has been attributed to antimicrobial peptides and bioactive compounds (Prasad and Elumalai, 2011). Wang et al. (2016) state that 4-(α -L-rhamnopyranosyloxy) benzyl isothiocyanate, methyl N-4-(α -L-rhamnopyranosyloxy) benzyl carbamate, and 4-(β -D-glucopyranosyl-1 \rightarrow 4- α -L-rhamnopyranosyloxy)-benzyl thiocarboxamide are the three compounds of *M. oleifera* seed that present potent antibacterial activity against some pathogens.

Specifically, the compound 4-(α -L-rhamnopyranosyloxy) benzyl isothiocyanate was found to inhibit the growth of *S. aureus* (Galuppo et al., 2013).

Almost all parts of the *M. oleifera* plant have an antimicrobial effect. In a previous study, Zaffer et al. (2014) found that the aqueous extract of *M. oleifera* bark presented high activity against *S. aureus*, while Devi et al. (2011) demonstrated the antibacterial activity of methanolic *M. oleifera* bark extract against *Bacillus spp.* and *S. aureus*.

In India, Devendra et al. (2011) demonstrated that chloroform *M. oleifera* leaf extract inhibits the growth of *S. aureus* and *Streptococcus pyogenes*, with halos of inhibition of 6.2 and 6.0 mm, these diameters were lowest than ours. Nevertheless, Moyo et al. (2012) used water extract of *M. oleifera* and they did not show any antimicrobial activity against *S. aureus*, this differ with our results. Our findings also differ with that report by Singh et al. (2013), who reported that the ethanolic and aqueous extracts had low activity against the same pathogen.

It has also been shown that the *M. oleifera* flower has an antimicrobial effect against strains of *V. cholerae* and *E. coli* (Brilhante et al., 2015), an antibacterial property of *M. oleifera* flowers that has been attributed to a substance called pterygospermin (Anwar et al., 2007). Many studies have suggested that different crude extracts obtained from different *M. oleifera* tissues present antibacterial activities against both Gram-negative and Gram-positive bacteria (Wang et al., 2016; Bukar et al., 2010; Brilhante et al., 2015; Peixoto et al., 2011). Differences in polarity among the various solvents (methanol, ethanol, chloroform, water, petroleum ether, and ethyl acetate) may be responsible for the differences in the solubility of plant active principles, hence the variation in the degree of antimicrobial activity (Patel et al., 2018).

This study has shown that *S. aureus* strains resistant to multiple antibiotics are commonly found in the raw milk sold in the municipality of Francisco I. Madero, constituting a serious public health risk for the region's population. The most effective antibiotics against *S. aureus* were found to be ciprofloxacin, erythromycin and tetracycline.

The indiscriminate use of antibiotics for prophylactic and other therapeutic purposes could be the reason for the increased antimicrobial resistance of *S. aureus*.

Ethanol extract exhibited a higher degree of antimicrobial activity compared to the aqueous extracts and penicillin. This reveals that the leaves, stems and seeds of *M. oleifera* could be an alternative for the control of infections caused by *S. aureus* in humans and cows with mastitis.

Future studies will need to evaluate the antibacterial effect of *M. oleifera* leaf, stem and seed extract against other multidrug resistant-strains, such as *E.coli*, *Salmonella*, *Shigella*, *Listeria monocytogenes*, and *V. cholerae*.

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