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Article

Antimicrobial activity of plants native to Sonora, Mexico, against pathogenic bacteria isolated from milk from cows diagnosed with mastitis

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Abstract:

Bovine mastitis is a disease caused by pathogenic bacteria that infect the mammary gland of dairy cattle, which generates significant economic losses, in addition, due to the excessive use of antibiotics to treat this disease, microorganisms have created resistance to these drugs, therefore, new alternatives are sought for this purpose. The objective was to evaluate the antimicrobial effect of extracts of plant native to Sonora against pathogenic bacteria isolated from cows diagnosed with mastitis. Seventeen ethanolic extracts were obtained from plants native to Sonora, and their antimicrobial activity was evaluated by the agar diffusion method against seven pathogens isolated from milk from cows with mastitis, using a concentration of 50 mg/ml of each extract. The content of total phenols and flavonoids was determined by spectrophotometry. The results showed that extracts of *Ibervillea sonorae* (wereke, tuber), *Populus alba* (poplar, leaves), *Ambrosia ambrosioides* (chicura, stems), *Krameria sonorae* (cosahui, roots) and *Prosopis velutina* (mesquite, leaves) were effective in eliminating *S. aureus*, *Streptococcus* spp., *E. coli*, *Enterobacter* spp., *Proteus* spp., *Shigella* spp. and *Citrobacter* spp. ($P<0.05$). In addition, extracts high in total phenols and flavonoids (wereke, poplar, chicura, cosahui and mesquite) showed an inverse correlation with respect to pH (r= -0.94, r= -0.92, respectively) ($P<0.05$) and had greater antimicrobial activity against the tested pathogens. Therefore, the extracts of plants from Sonoran could represent an alternative for the control of Gram (+) and Gram (-) pathogens that infect the mammary gland of dairy cattle.

Key words: Mastitis, Pathogens, Antimicrobial, Plant Extracts, Natural Alternative, Phenols, Flavonoids.

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Introduction

Mastitis is the main infectious disease that occurs in dairy cattle. The origin of this disease is multifactorial and may depend on the management, production system and environmental conditions in which the cattle are found, and occurs as a response to the infection of a great

biodiversity of microorganisms, such as mycoplasmas, yeasts, fungi, viruses and bacteria[1], and usually manifests as an inflammation in the mammary gland, which, depending on the severity of the infection, can generate fibrosis, mammary edema, atrophy of mammary tissue, abscesses or gangrene; in addition, it can alter the physical and chemical properties of milk, increasing the number of somatic cells and the microbial load in the milk, which can lower the pH of the milk and alter the taste and smell. Likewise, the milk from cows with mastitis has less lactose, fat and caseins, which decreases its technological properties for the food industry[2,3].

Sometimes mastitis can be detected clinically, that is, when the presence of pus or blood in the mammary gland and milk is physically observed; whereas, on other occasions, mastitis occurs subclinically and is usually more difficult to detect, since inflammation in the udder is not visible and milk shows a normal physical appearance[4,5]. Some of the pathogenic bacteria responsible for this disease are *Staphylococcus aureus*, *Streptococcus agalactiae*, *Corynebacterium bovis* and *Mycoplasma bovis*, which can cause significant damage to the mammary gland, such as lesions, and in more severe cases, they can generate necrosis in the tissue. Generally, infection with these bacteria occurs at the time of milking[3,6], although, on other occasions, infection may also occur through contact with other bacteria present in the environment, such as *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, among others[5]. Although these bacteria are considered pathogenic, they are usually less aggressive at the time of infection, in addition, milk with the presence of these microorganisms cannot be marketed[5,6]. Today, mastitis remains one of the biggest challenges in dairy farms, since it is estimated to represent 70 % of dairy farmers' expenses, which generates annual economic losses of approximately $35 billion dollars worldwide and $2 billion dollars in the United States[7,8,9]. In Mexico, losses of $2 and a half million pesos are estimated, which represents between 20 and 30 % of clinical mastitis, so the losses could still be greater due to the other 70-80 % of animals that present subclinical mastitis[10].

In Sonora, studies of bovine mastitis are very scarce, however, in a dairy farm in Santa Ana, Sonora, the presence of subclinical mastitis was found in 18.3 % of the animals, while the incidence of clinical mastitis was 5.35 %, where the average monthly cost of each animal with mastitis was $185.40 and the total cost was $30,966.34, $12,470.75 (40.3 %) corresponding to subclinical mastitis and $18,459.59 (59.7 %) to clinical mastitis[11]. Today, mastitis is considered the most expensive disease within dairy farms due to the decrease in milk production, waste of contaminated milk, replacement of animals and use of medicines[10]. In this context, the excessive use of therapies with antibiotics to prevent or treat this disease has caused some microorganisms to adapt and acquire resistance to these drugs, for example, *S. aureus* has shown 59.5 % and 49.6 % resistance to penicillin and ampicillin, respectively; while some strains of *Streptococcus* spp. have reported 40 %, 80 % and 73 % resistance against erythromycin, oxytetracycline and penicillin, respectively; in

addition, some strains of *E. coli* have shown 88.24 % resistance against erythromycin, oxytetracycline, penicillin and streptomycin and 70.59 % resistance against gentamicin, therefore, one of the great challenges of the Health Sector is to reduce the use of antibiotics in animals and humans[12-15]. In this context, the use of natural chemical compounds derived from plants to treat diseases in humans and animals has been increasing in recent decades[16,17]. In Mexico, it is estimated that there are around 26,000 species of plants, of which around 4,000 species are used to treat diseases in a traditional way[18,19]. Although it has been reported that some plants native to northwestern Pakistan have been effective in eliminating bacteria associated with bovine mastitis[9], the use of plants from Sonora, Mexico, for this purpose has not been reported. However, their antimicrobial potential against the bacteria of the collection *Helicobacter pylori* ATCC 43504, *Mycobacterium tuberculosis* H37Rv, *Escherichia coli* ATCC 35219 and 25922, *Shigella flexneri* ATCC 12022 and *Salmonella typhimorium* ATCC 14028[19,20,21] has been evidenced. Considering that Sonora has a great biodiversity of native plants, of which around 400 are used by local ethnic groups to treat diseases[20], and that some of these plants have also shown antimicrobial potential, it is interesting to evaluate the antimicrobial effect of extracts of plants native to Sonora against pathogenic bacteria isolated from cows diagnosed with mastitis.

Material and methods

Preparation of ethanolic extracts

The extracts were obtained from 17 plants native to the state of Sonora, Mexico (Table 1), which were harvested in the Botanical Garden of the Department of Agriculture and Livestock (DAG, for its acronym in Spanish) of the University of Sonora (UNISON, for its acronym in Spanish) and identified in the Herbarium of the DAG. Each plant was dehydrated at 34 °C in a hot air oven (Thelco, Precision Science, model 28, USA) and then pulverized in a mill (Pulvex Mini 100, MX) until obtaining a particle size of 100 microns. Subsequently, 100 g of dry matter was placed, and 100 ml of 99 % pure ethanol (Sigma-Aldrich, St. Louis MO) was added in a hermetically sealed glass bottle, which were stored for 5 days in the dark at 25 °C[22]. The extracts were filtered with Whatman No. 41 filter paper and the plant material was dehydrated again. The difference in weight of the plant material before and after its storage was considered as the amount of soluble chemical compounds extracted from plants[23]. The ethanolic extracts were then concentrated in a rotary evaporator (Yamato RE300) at 40 °C and adjusted to 50 mg/ml with a 20 % dimethyl sulfoxide (DMSO) solution. Finally, the extracts were stored in the dark at 4 °C until use.

Table 1: Identification and parts of the plants used in the ethanolic extracts

Key	Common name	Family	Scientific name	Part
E1	Poplar	Salicaceae	*Populus alba*	Leaves
E2	Batamote	Asteraceae	*Baccharis glutinosa*	Stems
E3	Chicura	Asteraceae	*Ambrosia ambrosioides*	Stems
E4	Cosahui	Krameriaceae	*Krameria sonorae*	Root
E5	Guaje	Fabaceae	*Leucaena leucocephala*	Leaves
E6	Guamúchil	Fabaceae	*Pithecellobium dulce*	Bark
E7	Jojoba	Simmondsiaceae	*Simmondsia chinensis*	Leaves
E8	Mesquite	Fabaceae	*Prosopis velutina*	Leaves
E9	Palo verde	Fabaceae	*Parkinsonia microphylla*	Stems and leaves
E10	Palo verde azul	Fabaceae	*Cercidium floridum*	Stems and leaves
E11	Rama blanca	Asteraceae	*Encelia farinosa*	Leaves
E12	Sangregado	Euphorbiaceae	*Jatropha cardiophylla*	Stems
E13	Tepehuaje	Fabaceae	*Lysiloma watsonii*	Leaves
E14	Torote	Burseraceae	*Bursera microphylla*	Leaves
E15	Vinorama	Fabaceae	*Acacia constricta*	Leaves
E16	Wereke	Cucurbitaceae	*Ibervillea sonorae*	Tuber
E17	Zamota	Fabaceae	*Coursetia glandulosa*	Stems

Plants harvested in the Botanical Garden of the DAG of UNISON.

Determination of total phenols

One milligram of extract was used and mixed with 0.5 ml of Folin-Ciocalteu reagent. Then, 10 ml of distilled water and 1 ml of saturated Na_2CO_3 were added and homogenized for 3 min. Finally, the mixture was measured to 25 ml with distilled water and left to stand for 1 h in a place free of light. Absorbance was measured at a wavelength of 750 nm on a spectrophotometer (Spectro Max MD, EU) and total phenol content was expressed as milligrams of gallic acid equivalents per gram of extract[24].

Determination of total flavonoids

Zero point two five milligrams of extract were used and mixed with 5 ml of distilled water. Afterwards, 0.3 ml of a 5 % $NaNO_2$ solution was added, and the mixture was left to stand in the dark for 6 min. Subsequently, 0.6 ml of a 10 % $AlCl_3 \cdot 6H_2O$ solution was added and left to stand until the reaction was complete. Finally, 2 ml of NaOH (1 M) was added and the mixture was measured to 10 ml with distilled water. Absorbance was measured at a wavelength of 510 nm in a spectrophotometer (Spectro Max MD, EU) and the total flavonoid content was expressed as milligrams of quercetin per gram of extract[24].

Place of study and sample collection

The samples were taken from two farms located on the outskirts of the city of Hermosillo, Sonora, Mexico. Site-1 belongs to the ejido La Yesca, located southwest of the city 10 km away, while Site-2 belongs to the ejido Los Bajotes, located northwest of the city 12 km away. The cows were selected according to the technique of the California mastitis test[25]. The samples were collected over a year and taken from the 4 quarts of 15 cows from Site-1 and 15 from Site-2. Prior to sample collection, the teats were immersed in a 1 % iodine-based solution for 30 sec, and subsequently the excess of iodine was removed with a disposable towel. Then 10 ml of milk was collected from each quarter in a sterile bottle with a screw cap and each teat was again immersed in the disinfectant solution. Finally, the samples were transported at 4 °C and processed two hours after their collection[26].

Isolation and identification of bacteria associated with mastitis in milk

For the isolation and identification of the bacteria from the milk from cows diagnosed with mastitis, the methodology reported by Rodríguez and Muñoz[27] was used. The samples were seeded by stria in Columbia agar base added with 5 % of ram's blood (BD Difco, Sparks, MD) and MacConkey agar (BD Difco, Sparks, MD) and incubated at 37 °C for 48 h. Afterwards, a Gram stain, coagulase test, oxidase test (Kovacs reagent) and catalase test (3 % H_2O_2) were performed to differentiate the isolated colonies. The selected colonies were purified three times by subsequent cultures under the conditions stated above. The isolated bacteria were identified by the commercial kits API20E, API Staph and API Strep (BioMérieux, Marcy, France), following the instructions of the manufacturers. The identified bacteria were stored at -80 °C in 80 % glycerol (v/v) until their use.

Antimicrobial activity of ethanolic extracts

Bacteria isolated from milk from cows with mastitis were propagated in the BHI broth culture medium (brain-heart infusion, BD Difco, Sparks, MD). Subsequently, three plates were prepared with BHI agar (brain-heart infusion, BD Difco, Sparks, MD) for each of the pathogenic bacteria and four sterile discs of Whatman No. 41 filter paper of 6 mm in diameter were placed in each plate, where 20 µL of each ethanolic extract (50 mg/ml) were added. Finally, the plates were incubated at 37 °C for 24 h. Halos greater than 3 mm were considered as inhibition according to the criteria used by Heredia-Castro[28].

Statistical analysis

A completely randomized one-way experimental design at 95 % confidence was used, with three repetitions per treatment. The mean comparison test was performed by Tukey-Kramer at a significance level of 0.05 %, and the correlation analysis was performed with 95 % reliability. The statistical software used was NCSS version 11.

Results and discussion

The chemical compounds responsible for the antimicrobial activity of plants are synthesized in the cytoplasm of cells, and within these compounds are flavonoids, which are part of a group of chemical compounds called phenols[29]. Table 2 shows the chemical analyses and yield of ethanolic extracts. The results showed that the pH of the ethanolic extracts varied in a range of 4.35 to 6.22, with extract E5 being the most acidic and E11 being the least acidic ($P<0.05$). Likewise, extract E5 had the highest concentration of total phenols (143.68 ± 0.04 mg) and total flavonoids (95.10 ± 0.05 mg), while extract E11 had the lowest values for total phenols (56.28 ± 0.05 mg) and total flavonoids (30.08 ± 0.90 mg) ($P<0.05$). The pH of the extracts may be due to the acidic nature of the total phenols and flavonoids, or to the presence of other polar compounds such as tannins, benzoic, oleic, stearic and lignoceric acids, among others[30]. In this context, Al-rifai *et al*[24] studied two medicinal plants from Saudi Arabia (*Convolvulus austroaegyptiacus* and *Convolvulus pilosellifolius*) and reported that the total phenol and flavonoid content of the ethanolic extracts was similar to those found in this study. In addition, in the ethanolic extracts of *Vernonia amygdalina* and *Tephrosia purpurea*, the presence of total phenols and flavonoids was also found[31,32], however, Bitchagno *et al*[33] did not find the presence of flavonoids in the ethanolic extract of the fruit of *Tectona grandis*.

This suggests that the chemical compounds present may vary from one part of the plant tissue to another.

Table 2: Chemical analysis and yield of ethanolic extracts

Extract	pH	Total phenols	Total flavonoids	Yield (%)
E1	4.86	130.26 ± 0.05^{d}	89.23 ± 0.08^{d}	6.33
E2	5.22	115.45 ± 0.03^{f}	80.06 ± 0.03^{h}	5.54
E3	5.15	120.33 ± 0.06^{e}	85.09 ± 0.07^{f}	5.45
E4	4.82	135.03 ± 0.06^{c}	91.06 ± 0.02^{c}	4.51
E5	4.35	143.68 ± 0.04^{a}	95.10 ± 0.05^{a}	7.82
E6	5.34	110.03 ± 0.04^{i}	70.06 ± 0.08^{k}	4.02
E7	4.4	140.65 ± 0.07^{b}	93.05 ± 0.07^{b}	8.39
E8	5.34	112.12 ± 0.03^{g}	74.05 ± 0.80^{i}	6.49
E9	5.43	95.23 ± 0.08^{j}	71.05 ± 0.03^{j}	6.72
E10	5.6	85.24 ± 0.06^{k}	70.09 ± 0.04^{k}	6.55
E11	6.22	56.28 ± 0.05^{l}	30.08 ± 0.90^{l}	7.44
E12	5.22	115.02 ± 0.04^{f}	81.18 ± 0.06^{g}	5.23
E13	4.57	130.14 ± 0.09^{d}	93.78 ± 0.05^{b}	8.62
E14	5.3	111.56 ± 0.02^{h}	79.28 ± 0.20^{h}	8.35
E15	5.41	110.09 ± 0.06^{i}	73.29 ± 0.07^{i}	6.65
E16	5.55	109.89 ± 0.07^{i}	70.47 ± 0.80^{k}	9.32
E17	5.11	120.02 ± 0.04^{e}	87.55 ± 0.40^{e}	5.85

Total phenols= mg of gallic acid/g of extract; Total flavonoids= mg of quercetin/g of extract.
^{abcdefghijk} Different literal indicates a significant difference between the data of the same column ($P<0.05$).

On the other hand, the yield of the extracts was variable, with extract E16 showing the highest yield (9.32 %) and extract E6 being the one with the lowest yield (4.02 %). In agreement with this study, variations in the yield of extracts obtained with plants from Pakistan were also reported[34]. In addition, similar results were reported by Mostafa *et al*[35], where *Punica granatum* had the highest yield (9.74 %), while *Cuminum cyminum* had the lowest yield (3.12 %). Likewise, the yield of ethanolic extracts of 49 medicinal plants from Indonesia was evaluated in another study, and it was found that the highest yield was for the fruit of *Salacca zalacca* (77.89 %), while the lowest yield was reported in the root of *Plectranthus scutellarioides* (3.07 %). Additionally, the authors reported that extracts made with leaves showed higher yield compared to extracts where roots or woodier parts of the plants were used[36], which coincides with what was found in this study. This suggests that the amount of ethanol-soluble compounds depends on each plant and the part used.

On the other hand, mastitis is an infectious disease of bacterial origin and is usually very persistent inside dairy farms. Table 3 shows the bacteria identified by biochemical tests and the frequency with which they appear in the milk from cows diagnosed with mastitis. The results showed that, at Site-1, *E. coli* occurred most frequently in 58 of 60 samples analyzed, followed by *S. aureus* with 35, *Proteus* spp. with 6 and *Enterobacter* spp. with 4. On the other hand, at Site-2, *E. coli* was found in 43.8 % of the samples analyzed, followed by *S. aureus* with 32.85 %, *Streptococcus* spp. with 10.95 %, *Shigella* spp. with 8.76 % and *Citrobacter* spp. with 3.65 %. In this study, *E. coli* and *S. aureus* were the most representative pathogens for both herds, since their presence was found in 82.50 % of the total samples analyzed. Other authors have mentioned that bacteria of the genus *Streptococcus*, as well as *E. coli* and *S. aureus*, are common microorganisms in cows diagnosed with mastitis[5,37,38], which coincides with what was found in this research. The microorganisms that cause mastitis varied between Site-1 and Site-2, which suggests that the environment and animal management may influence the biodiversity of the pathogenic microorganisms that cause mastitis[5].

Table 3: Bacteria identified by biochemical tests and frequency of pathogens present in the milk from cows diagnosed with mastitis

Place	Bacteria	Frequency	Percentage
Site-1			
	Staphylococcus aureus	35	33.98
	Escherichia coli	58	56.31
	Proteus spp.	6	5.83
	Enterobacter spp.	4	3.88
Total		103	100.00
Site-2			
	Staphylococcus aureus	45	32.85
	Streptococcus spp.	15	10.95
	Escherichia coli	60	43.8
	Shigella spp.	12	8.76
	Citrobacter spp.	5	3.65
Total		137	100.00

Frequency= number of times the same pathogen occurs in different samples; Percentage= Frequency * 100/Total.

Table 4 shows the antimicrobial activity of ethanolic extracts against pathogenic bacteria isolated from cows diagnosed with mastitis. The results showed that extract E16 presented the highest antimicrobial activity against *S. aureus* (20.50 ± 1.70 mm) (P<0.05), while extracts E9 and E10 had the lowest activity (5.50 ± 0.70 and 5.50 ± 0.70) (P<0.05). On the other hand, extracts E1 and E16 (*Populus alba* and *Ibervillaea sonorae*) showed the highest activity against *E. coli* (13.00 ± 1.51 mm and 13.00 ± 1.40 mm) (P<0.05), while extract E13

had the lowest activity (3.00 ± 0.70 mm) (P<0.05) and extract E10 did not present activity against that pathogen (P>0.05). Likewise, extract E16 had the highest activity against *Enterobacter* spp. (16.00 ± 2.40 mm) (P<0.05), while extract E9 showed the lowest activity (5.50 ± 0.70 mm) (P<0.05) and extracts E2, E10, E15 and E17 had no antimicrobial activity against the same pathogen (P>0.05). Extracts E8 and E16 showed the highest activity against *Proteus* spp. (13.50 ± 1.11 mm and 13.50 ± 2.70 mm) (P<0.05), while extracts E2, E9 and E10 had the lowest antimicrobial activity (5.50 ± 0.60 mm, 5.50 ± 0.70 mm and 5.50 ± 0.70 mm) (P<0.05), and extracts E5 and E13 showed no activity against this pathogen (P>0.05). Similarly, extract E16 presented the highest antimicrobial activity against *Streptococcus* spp., whereas extracts E12 and E17 had the lowest activity (5.00 ± 0.41 mm and 5.00 ± 0.50 mm) (P<0.05), and extracts E5 and E13 were not efficient against this pathogen (P>0.05). On the other hand, extracts E2 and E8 had the highest antimicrobial activity against *Shigella* spp. (15.50 ± 2.32 mm and 16.00 ± 1.40 mm) (P<0.05), while extracts E3, E9 and E14 showed the lowest activity (5.50 ± 0.68 mm, 5.50 ± 0.70 mm and 5.0 ± 1.41 mm) (P<0.05), and extract E15 showed no activity against that pathogen (P>0.05). Finally, extract E16 presented the highest antimicrobial activity against *Citrobacter* spp. (17.00 ± 2.4 mm) (P<0.05), while extract E14 showed the lowest activity (4.5 ± 1.41 mm) (P<0.05), and extracts E2, E9 and E17 were not shown to be effective against this bacterium (P>0.05).

Similar results have been reported in *S. aureus* isolated from cows with mastitis using the extracts of *Piptadenia viridiflora* and *Schinopsis brasiliensis*[39], likewise, it has been reported that the extracts of *Calpurinia aurea*, *Croton macrostachyus* and *Nicotiana tabacum* were efficient in inhibiting the growth of *S. aureus*, which causes mastitis in ruminants[40]. It has also been reported that *S. aureus*, *Staphylococcus epidermidis* and *Streptococcus agalactiae* isolated from cows with mastitis were susceptible to the extract of *Zingiber officinale* Roscoe[41] and the extract of *Sanguisorba officinalis* was efficient in inhibiting the formation of the biofilm of *S. aureus* isolated from cows with mastitis. This is favorable since the biofilm is a protective barrier of the bacteria, and by inhibiting its formation, the bacterium is exposed to the natural protection of the host[42]. Finally, the effect of purified compounds extracted from plants (*trans*-cinnamaldehyde, eugenol, carvacrol and thymol) demonstrated their efficacy by inhibiting the growth of *S. aureus*, *E. coli*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *Streptococcus uberis* isolated from cows with mastitis[43].

Table 4: Antimicrobial activity of ethanolic extracts against pathogenic bacteria isolated from milk from cows with mastitis

EXT	*S. aureus*	*Streptococcus* spp.	*E. coli*	*Enterobacter* spp.	*Proteus* spp.	*Shigella* spp.	*Citrobacter* spp.
E1	9.00 ± 1.21^{e}	9.00 ± 1.41^{d}	13.00 ± 1.51^{a}	10.00 ± 1.21^{e}	8.00 ± 0.92^{f}	8.00 ± 0.61^{d}	10.00 ± 1.22^{c}
E2	6.00 ± 0.75^{gh}	8.50 ± 0.90^{d}	8.00 ± 1.16^{d}	n.p	5.50 ± 0.60^{g}	15.50 ± 2.32^{a}	n.p
E3	11.50 ± 2.16^{cd}	12.50 ± 2.12^{c}	10.50 ± 0.80^{c}	15.00 ± 0.70^{ab}	9.50 ± 1.10^{d}	5.50 ± 0.68^{f}	6.50 ± 0.70^{d}
E4	10.50 ± 1.11^{d}	8.50 ± 2.42^{d}	10.50 ± 1.15^{c}	12.00 ± 2.62^{d}	11.50 ± 1.19^{b}	11.00 ± 1.15^{c}	14.00 ± 2.00^{b}
E5	12.50 ± 2.32^{bc}	n.p	11.00 ± 2.34^{bc}	8.50 ± 0.92^{f}	n.p	12.50 ± 2.15^{b}	5.00 ± 0.12^{e}
E8	12.00 ± 1.31^{b}	12.00 ± 1.81^{c}	12.00 ± 1.33^{ab}	10.50 ± 1.41^{e}	13.50 ± 1.11^{a}	16.00 ± 1.40^{a}	10.00 ± 1.31^{c}
E9	5.50 ± 0.70^{h}	14.50 ± 1.70^{b}	7.00 ± 0.80^{de}	5.50 ± 0.70^{g}	5.50 ± 0.70^{g}	5.50 ± 0.70^{f}	n.p
E10	5.50 ± 0.70^{h}	8.50 ± 1.20^{d}	n.p	n.p	5.50 ± 0.70^{g}	8.00 ± 1.41^{d}	9.00 ± 1.40^{c}
E12	5.00 ± 1.2^{h}	5.00 ± 0.41^{f}	4.50 ± 0.50^{g}	8.50 ± 0.60^{f}	7.00 ± 1.31^{f}	11.50 ± 1.70^{bc}	15.00 ± 2.70^{b}
E13	7.50 ± 1.10^{f}	n.p	3.00 ± 0.70^{h}	13.50 ± 2.70^{c}	n.p	6.50 ± 0.70^{e}	9.50 ± 0.70^{c}
E14	9.00 ± 1.71^{e}	7.00 ± 1.22^{e}	6.0 ± 1.40^{ef}	14.0 ± 1.41^{bc}	10.00 ± 2.22^{c}	5.0 ± 1.41^{f}	4.5 ± 1.41^{f}
E15	8.50 ± 1.12^{f}	15.50 ± 1.62^{b}	10.50 ± 0.70^{c}	n.p	11.50 ± 1.12^{b}	n.p	6.50 ± 0.70^{d}
E16	20.50 ± 1.70^{a}	19.50 ± 1.90^{a}	13.00 ± 1.40^{a}	16.00 ± 2.40^{a}	13.50 ± 2.70^{a}	8.00 ± 1.4^{d}	17.00 ± 2.4^{a}
E17	6.50 ± 0.70^{g}	5.00 ± 0.50^{f}	5.50 ± 0.70^{fg}	n.p	8.50 ± 0.70^{de}	6.50 ± 0.70^{e}	n.p

EXT= extract; (extracts 6,7 y 11 did not present any activity). Results expressed in mm of inhibition halos; Concentration of extracts= 50 mg/ml; n.p.= it did not present activity.

^{abcde} Different literal indicates a significant difference between the data in the same column (P<0.05).

It is interesting to mention that the pH of the extracts showed an inverse correlation with the concentration of total phenols (r= -0.94, P<0.05) and total flavonoids (r= -0.92, P<0.05), that is, the extracts with the lowest pH had higher concentrations of total phenols and flavonoids. In addition, extracts with the highest content of these compounds had greater antimicrobial activity, which suggests that this effect could be associated with the amount of total phenols and flavonoids present in the extracts, since it has been suggested that flavonoids have a hydroxyl (-OH) functional group that increases hydroxylation reactions on the surface of bacteria, altering their functionality and decreasing the thickness of the lipid bilayer, altering

the fluidity of the cell membrane and increasing its permeability, causing the outflow of ions and intracellular proteins, causing the death of bacteria, or they can modify the metabolism of bacteria by altering the synthesis of DNA and proteins, which can cause the death of bacteria[44,45].

Conclusions and implications

The ethanolic extract of *Ibervillea sonorae* (wereke) was the most efficient in eliminating pathogenic bacteria isolated from the milk from cows diagnosed with mastitis. However, extracts of *Populus alba* (poplar), *Ambrosia ambrosioides* (chicura), *Krameria sonorae* (cosahui) and *Prosopis velutina* (mesquite) also exhibited significant antimicrobial activity. In addition, antimicrobial activity was related to the content of total phenols and flavonoids present in the extracts. Therefore, extracts of plant native to Sonora, Mexico, can be considered in *in vivo* tests as an alternative and natural treatment to control infections in the mammary gland caused by different microorganisms in dairy cattle.

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