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Antimicrobial Activity of Plants Used in the Prevention and Control of Bovine Mastitis in Southern Brazil

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SUMMARY. **Based on informal interview, ethnoveterinary information about plants used in the prevention and control of bovine mastitis in Southern Brazil were obtained. *Alternanthera brasiliana* (L.) Ktze. ("penicilina"), *Foeniculum vulgare* Mill ("funcho"), *Aloe arborescens* Mill. ("babosa"), *Achillea millefolium* L. ("mil-em-rama"), *Baccharis trimera* (Less.) DC. ("carqueja"), *Solidago chilensis* Meyer ("lanceta"), *Symphythum officinale* L. ("confrei"), *Sambucus nigra* L. ("sabugueiro"), *Chenopodium ambrosioides* Linn. ("erva Santa Maria"), *Mentha* sp. ("hortelã"), *Ocimum basilicum* L. ("alfavaca"), *Parapiptadenia rigida* (Benth.) Brean ("angico-vermelho"), *Allium sativum* L. (alho), *Cuphea carthagenensis* (Jacq.) Macbr. ("sete-sangrias"), *Sida rhombifolia* L. ("guanxuma"), *Phytolacca dioica* L. ("umbu") and *Solanum mauritianum* Scop ("fumeiro-brabo") were cited as helpful. In order to validate the traditional practice, the decoctions obtained with the plants were analyzed for the *in vitro* antimicrobial activity against *Staphylococcus aureus* and *Salmonella choleraesuis* by the agar dilution method. *Alternanthera brasiliana, Achillea millefolium, Baccharis trimera* and *Solidago chilensis* extracts where active against *S. aureus* while *Symphythum officinale, Sambucus nigra, Mentha* sp., *Ocimum basilicum, Parapiptadenia rigida* and *Cuphea carthagenensis* extracts were active against both microorganisms. For all the cited species, scientific data were reviewed aiming to establish a correlation between popular use and biological properties. The data found in literature for several of these plants could justify the use in the bovine mastitis treatment for antimicrobial, anti-inflammatory and wound healing activities.**

INTRODUCTION

Bovine mastitis, the inflammation of the mammary gland associated with bacterial infection, continues to be the most frequent and costly disease of dairy cattle all over the world [1]. Financial losses due to mastitis occur for both subclinical and clinical stages of the disease and include discarded milk, transient reductions in milk yield and premature culling [2].

Sanitary programs for the prevention and control of the disease recommend the use of antiseptic and disinfectant agents to prevent the occurrence and transmission of the microorganisms using antibiotics only in severe cases [3]. The implantation of environmental sanitary programs is frequently limited by the cost of commercial products, and the development of bacterial resistence to the biocides.

The aim of this work was to collect ethnoveterinary information about plants used in the prevention and control of bovine mastitis in Southern Brazil and to evaluate the antibacterial activity of the plants against *Staphylococcus aureus,* the main causative agent of the disease, and *Salmonella choleraesuis* as a model of Gram-negative microorganism.

MATERIALS AND METHODS

Ethnobotanical assessment

The plants were selected during the devel-

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opement of the project "Rural - RS: validation of agroecological technology for the control of mamitis, endoparasites and ectoparasites using medicinal plants in Rio Grande do Sul, South Brazil" with the participation of the Faculdade de Veterinária da UFRGS and Faculdade de Veterinária da UFPel, Associação Riograndense de Empreendimentos de Assistência Técnica e Extensão Rural (EMATER/RS), Fundação Coordenação de Projetos, Pesquisas e Estudos Tecnológicos (COPPETEC), the rural community "Assentamento São Domingos" (Garruchos municipality); "APLESETE – Associação dos Produtores de Leite de Sete de Setembro" (Sete de Setembro municipality) and the rural communities of agrarian reform: "Assentamento Filhos de Sepé" (Viamão municipality), "Assentamento Lagoa do Junco" (Tapes municipality), "Assentamento Capela" (Nova Santa Rita municipality), "Assentamentos Integração Gaúcha and Padre Josino" (Eldorado do Sul municipality). In order to obtain information about the medicinal plants, a semi-structured interview asking about procedures carried out to prevent and or to treat mastitis was applied to persons considered experts by the people of the communities.

Plant material

Plants were collected between December 2002 and February 2003. The material was carefully dried and powdered. Voucher specimens were deposited in the herbarium of the Universidade Federal do Rio Grande do Sul (ICN).

Antibacterial activity

Plant extracts

Aqueous extracts were obtained boiling (15 min) 100 g of the dried and powdered plant materials with 1.000 mL of distilled water. The volume was completed to 1.000 mL with water in order to compensate the evaporation.

Bacterial strains

Tests were performed against *Staphylococcus aureus* - ATCC 6538 (test culture with 3.1 x 10^9 CFU/mL) and *Salmonella choleraesuis* - ATCC 10708 (test culture with 2.1 x 10^9 CFU/mL). Microorganisms cultures were maintained on Nutrient Agar (Merck®).

Antibacterial activity test

The antibacterial activity was evaluated by serial dilution techniques [4]. Modification was introduced into the original technique by testing the extracts with 8 dilutions (10^{-1} to 10^{-8} CFU/mL) of the bacteria inoculum instead of only one high infecting dose [5]. The results were expressed as "dilution of the inhibited bacteria" and "dilution of the inactive bacteria". The inhibited dilution was determined comparing the turbity of assay tubes containing 5 mL of the plant aqueous extract, 5 mL of culture medium and 0.1 mL of the bacteria in suspension, and a control tube (plant extract and culture medium only). To evaluate the inactivated dilution, an aliquot from the assay tubes where no turbity was observed was transferred to tubes containing 1 mL of brain heart infusion broth (BHI - Merck®) and incubated for 24 h at 37 °C. After the incubation, the inoculum was placed onto a Petri dish with nutrient agar (Nutrient Agar - Merck®) and incubated for 24 h at 37 °C to observe the presence of bacterial growth. The dilutions and verification of the initial infecting dose of the inoculum was done as indicated by Neder [6]. Each experiment was only valued if at the end of the dilution line (10^{-8} CFU/mL) of the initial inoculum there was bacterial growth.

Literature revision

For all the analyzed species, the scientific data were reviewed in the data basis MEDLINE; Science Direct, LILACS, SCIELO and SCIRUS without limiting period. The traditional use was reviewed on recognized ethnobotanical references about Brazilian Flora.

RESULTS AND DISCUSSION

The plants used in South Brazil to prevent and control mastitis are presented in Table 1. Among the plants cited as useful to treat mastitis, several of them are currently used in popular medicine in South Brazil. As examples, *Alternanthera brasiliana* (L.) Kuntze (Amaranthaceae), *Foeniculum vulgare* Mill. (Apiaceae), *Symphytum officinale* L. (Boraginaceae), *Parapiptadenia rigida* (Benth.) Brenan (Fabaceae), *Aloe arborescens* Mill. (Liliaceae) and *Sida rhombifolia* L. (Malvaceae) are used as anti-inflammatory and antibiotic to treat cystitis, throat infection, wounds, ovarium infections, intestinal diseases, diarrhea and general infection [7-13].

The aqueous extracts of the plants were assayed for the antibacterial activity. The results are shown in Table 2.

Among the plants, some of them were previously investigated for the antimicrobial activity.

Alternantera brasiliana

No works about the antibacterial and anti-

Family	Species	Vernacular name	Used part	Way of use
Amarantaceae	*Alternanthera brasiliana* (L.) Ktze.	"penicilina"	aerial parts	oral and topic
Apiaceae	*Foeniculum vulgare* Mill.	"funcho"	aerial parts (with flowers)	oral
Asphodelaceae	*Aloe arborescens* Mill.	"babosa"	leaves	topic
Asteraceae	*Achillea millefolium* L.	"mil-em-rama"	aerial parts	oral
	Baccharis trimera (Less.) DC	"carqueja"	aerial parts (with flowers)	oral and topic
	Solidago chilensis Meyer	"lanceta"	aerial parts	oral
Boraginaceae	*Symphythum officinale* L.	"confrei"	leaves	topic
Caprifoliaceae	*Sambucus nigra* L.	"sabugueiro"	leaves	oral and topic
Chenopodiaceae	*Chenopodium ambrosioides* Linn.	"erva Santa Maria"	aerial parts (with flowers)	oral
Lamiaceae	*Mentha* sp.	"hortelã"	aerial parts (with flowers)	topic
	Ocimum basilicum L.	"alfavaca"	aerial parts	intramammary
Leguminosae	*Parapiptadenia rigida* (Benth.) Brean	"angico vermelho"	bark	intramammary
Liliaceae	*Allium sativum* L.	"alho"	bulb	oral
Lythraceae				
	Cuphea carthagenensis (Jacq.) Macbr.	"sete sangrias" (with flowers)	aerial parts	oral
Malvaceae	*Sida rhombifolia* L.	"guanxúma"	aerial parts (with flowers)	oral
Phytolaccaceae	*Phytolacca dioica* L.	"umbu"	leaves	oral
Solanaceae	*Solanum mauritianum* Scop.	"fumeiro brabo"	leaves	oral

Table 1. Plants used in South Brazil to prevent and control the bovine mastitis.

fungal activities of *A. brasiliana* were found but the extracts of the plant presented inhibitory activity against the herpes simplex virus [14]. The plant was also evaluated for the antinociceptive activity [15]. Another species from this genus showed activity against the respiratory syncytial virus [16].

Foeniculum vulgare

Fennel oil had high effect on *Candida albicans,* and bactericidal action on *Salmonella typhimurium* and *S. dysenteriae* [17]. The essential oil showed activity also against *Escherichia coli, Listeria monocytogenes* and *Staphylococcus aureus* [18].

Aloe arborescens

No works on the antimicrobial activity of this plant were found. Nevertheless, *Aloe vera* inner gel shows antimicrobial effect [19], improves wound healing and inhibits inflammation [20]. These activities are due to the mucilaginous content. As *A. arborescens* is a plant rich in mucilages it can have the same effects.

Achillea millefolium

The *in vitro* antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* (Asteraceae) were investigated. The oil showed antimicrobial activity against *Streptococcus pneumoniae, Clostridium perfringens, Mycobacterium smegmatis, Acinetobacter lwoffii, Candida albicans* and *C. krusei* while water-insoluble parts of the methanolic extracts exhibited slight or no activity [21]. The plant is used in other parts of the world to treat abscesses, wounds, injuries and mastitis of ruminants [22].

Baccharis trimera

Baccharis trimera showed strong anti-inflammatory and analgesic properties in mice which seem to be due, at least partly, to the inhibition of prostaglandin biosynthesis [23]. The aqueous extract demonstrated antimicrobial activity *in vitro* [5].

Plant	Activity	*S. aureus* CFU/mL	*S. chorelaesuis* CFU/mL
A. brasiliana	Inhibition	$3.1x10^6$	n.a
	Inactivation	n.a	n.a
F. vulgare	Inhibition	n.a	n.a
	Inactivation	n.a	n.a
A. arborescens	Inhibition	n.a	n.a
	Inactivation	n.a	n.a
A. millefolium	Inhibition	$3.1x10^6$	n.a
	Inactivation	$3.1x10^6$	n.a
B. trimera	Inhibition	$3.1x10^8$	n.a
	Inactivation	$3.1x10^8$	n.a
S. chilensis	Inhibition	$3.1x10^7$	n.a
	Inactivation	$3.1x10^5$	n.a
S. officinale	Inhibition	$3.1x10^8$	$2.1x10^4$
	Inactivation	n.a	$2.1x10^4$
S. nigra	Inhibition	$3.1x10^8$	$2.1x10^7$
	Inactivation	$3.1x10^7$	$2.1x10^7$
C. ambrosioides	Inhibition	n.a	n.a
	Inactivation	n.a	n.a
Mentha sp.	Inhibition	$3.1x10^4$	$2.1x10^3$
	Inactivation	$3.1x10^4$	n.a
O. basilicum	Inhibition	$3.1x10^3$	$2.1x10^3$
	Inactivation	n.a	0
P. rigida	Inhibition	$3.1x10^8$	$2.1x10^8$
	Inactivation	$3.1x10^8$	$2.1x10^6$
A. sativum	Inhibition	n.a	n.a
	Inactivation	n.a	n.a
A. arborescens	Inhibition	n.a	n.a
	Inactivation	n.a	n.a
C. carthagenensis	Inhibition	$3.1x10^8$	$2.1x10^8$
	Inactivation	$3.1x10^6$	$2.1x10^5$
S. rhombifolia	Inhibition	n.a	n.a
	Inactivation	n.a	n.a
P. dioica	Inhibition	n.a	n.a
	Inactivation	n.a	n.a
S. mauritianum	Inhibition	n.a	n.a
	Inactivation	n.a	n.a

Table 2. Number of colony forming units (CFU) inhibited and or inactivated by the extracts. *S. aureus* ATCC 6.538; *S. choleraesuis* ATCC 10.108; n.a = not active.

Solidago chilensis

Essential oils and ethanolic extracts from the leaves of *Solidago chilensis* were evaluated for anti-*Candida albicans* activity. The essential oil showed activity while the ethanol extract was not effective at any of the concentrations tested [24].

Symphythum officinale

No reports on the antimicrobial activity of the leaves of *S. officinale* were found. Nevertheless, the plant is used in other parts of the world to treat abscesses, wounds, injuries, and for udder edema of ruminants [22].

Sambucus nigra

No data related to mastitis was found in the literature. Nevertheless the plant is known for the antiviral activity. Evidences suggest the components isolated from the plant may have immunomodulatory as well as direct antiviral actions [25].

Chenopodium ambrosioides

Although the principal use of *C. ambrosioides* is as acaricide and nematocide agent, the essential oil extracted from the leaves of this plant was tested for the antifungic activity. The oil completely inhibited the mycelial growth exhibiting a broad fungitoxic spectrum against several species of fungus [26].

Mentha sp.

The essential oils obtained by hydrodistillation from *Mentha longifolia, M. suaveolens* and *M. aquatica,* among other *Mentha* species showed an interesting antibacterial activity, mostly against Gram-positive bacteria *(Bacillus cereus, B. subtilis, Staphylococcus aureus, S. epidermidis* and *Streptococcus faecalis)* [27-29]. The aqueous extract of *M. longifolia* was evaluated for potential antimicrobial activity against methicillin-resistant *Staphylococcus aureus*. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the ethanolic extract of *M. longifolia* were in the range of 3.125 mg/mL and 12.50 mg/mL, respectively [30].

Ocimum basilicum

Basil *(Ocimum basilicum)* is a popular culinary herb, and its essential oils have been used extensively for many years in food products, perfumery, and dental and oral products. Basil essential oils and their principal constituents were found to exhibit antimicrobial activity against a wide range of Gram-negative and Gram-positive bacteria, yeast, and mold [31].

Parapiptadenia rigida

This plant, rich in tannins, showed antibacterial activity against the Gram-positive bacteria, *Staphylococcus aureus, S. epidermidis, Bacillus subtilis* and *Micrococcus luteus* [32].

Allium sativum

Garlic has long been used medicinally, most

recently for its cardiovascular, antineoplastic, and antimicrobial properties [33]. The antimicrobial activity was demonstrated by animal data, epidemiologic studies, uncontrolled clinical trials, *in vitro* studies or indirect evidence [25].

Cuphea carthagenensis

This plant, known in South Brazil as *setesangrias,* has some medicinal purposes and was used in the Paraguay War (1865-1870) to treat diarrhea, probably in cases of cholera, one of the main problems of the war. The plant is still widely used in South Brazil as a panacea. The aqueous extracts (decoction and infusion) demonstrated activity against both Gram-positive *(S. aureus* and *M. luteus)* and Gram-negative *(E. coli, S. typhimurium* and *P. aeruginosa)* microorganisms [34].

Sida rhombifolia

The antibacterial activity of crude extracts from the leaves of *Sida rhombifolia* was investigated. The extract showed weak antibacterial activity against both Gram-positive and Gram-negative test organisms [35].

Phytolacca dioica

No data related to *P. dioica* and bovine mastitis was found in literature. Nevertheless, other species from this genus are claimed to act upon the skin and upon the mammalian glands. Antibacterial and antifungal activities of *P. dodecandra,* traditionally used in the treatment of various skin disorders, have been shown [36,37].

Solanum mauritianum

No assays investigating the antimicrobial activity of this plant was found but its ethanolic extract demonstrated cyclooxygenase inhibitory activity [38]. Thus the claimed effect of the plant in bovine mastitis could be related to the anti-inflammatory activity.

In conclusion, among the seventeen plants used to treat or to prevent bovine mastitis, nine are administered orally, two topically, four oral or topically and two by intramammary via. The aqueous extracts of *Symphythum officinale, Sambucus nigra, Mentha* sp., *Ocimum basilicum, Parapiptadenia rigida* and *Cuphea carthagenensis* were active against *Staphylococcus aureus* and *Salmonella cholleraesuis,* while *Alternanthera brasiliana, Achillea millefolium, Baccharis trimera* and *Solidago chilensis* extracts were active only against Gram positive bacteria. None of the extracts promoted activity against Gram negative bacteria exclusively. Seven extracts were not active against both of the microorganisms evaluated. Nevertheless the popular use of the plants could be related with other activities such as anti-inflammatory and wound healing having a positive effect in some secondary aspects of the bovine mastitis.

Further and more comprehensive studies with medicinal plants used in bovine mastitis are necessary, especially with those plants already used popularly. These plants could be a veritable and cheaper substitute for conventional drugs since they are easily obtainable and the extracts can easily be prepared via a simple process of decoction or infusion.

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