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Patents of plant products applied to bovine mastitis in the INPI

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Abstract

Bovine mastitis is an inflammation of the mammary gland of dairy cattle, and it significantly affects the dairy sector. Its frequent treatment is the use of antibiotics, but they cause several problems, such as the formation of resistant pathogens. Hence, one treatment option is the use of medicinal plant-based products. Brazil is among the largest cattle breeders. It has a rich floristic diversity, and there is widespread knowledge about the therapeutic properties of plants. This study provides a patent map that is derived from Brazil's INPI online database (Brazilian Patent Office). There were 109 patent applications related to mastitis, being 17 patent applications with medicinal plants to treat this disease. Brazil was the largest depositor of patent applications with 37 requests, followed by the United States with 22 applications. The most frequent International Patent Classification was A61K31. Companies are the main applicants, but Science and Technology Institutions showed a significant participation among patent applications that contained medicinal plants in their composition. A patent map is a useful tool for identifying possible technological opportunities.

Keywords: Bovine mastitis. Medicinal plant. Patent. Technological prospecting.

Introduction

Mastitis is an inflammation of the mammary gland resulting from an infection. This condition affects several animals, including cattle, swine, and goats. Damage to the mammary glands allows pathogenic microorganisms to enter or lets existing ones in the animal's microbiota multiply, leading to immune responses and changes in the mammary gland tissue[1].

The dairy sector is directly affected by bovine mastitis. Harmful microorganisms cause physical and chemical changes in milk rooms, impacting milk production and its composition[2].

Bovine mastitis can be divided into two categories based on features of the udder: clinical and subclinical mastitis. In clinical mastitis there are visible changes in the bovine udder, for example edema, erythema and hyperthermia, in addition to the appearance of clots and yellowing of the milk. In contrast, subclinical mastitis has no visible changes, but the increase in somatic cells[3].

Bacteria are a major cause of mastitis, but other microorganisms such as fungi and viruses can also lead to this disease. Due to the various causes of mastitis, treatment can be challenging. Antibiotics are commonly used for treating mastitis. However, many pathogens are not fought by antibiotics due to resistance acquired by them. In addition, using antibiotics in lactating cows necessitates the disposal of milk due to the high risk of drug residues, which pose a threat to public health. Therefore, orthodox treatments offer a lot of risks, such as biotic resistance besides to environmental pollution and intoxication, as a result of which medicinal plant-based products become a promising alternative[4].

The use of plant-based treatments for human diseases is widespread, particularly in developing countries like Brazil. Veterinary phytotherapy is on the rise[5]. Numerous researchers have proven the therapeutic properties of medicinal plants, antibacterial, antifungal, anti-inflammatory, antioxidant activity, among others. Therefore, plants can be utilized for treating bovine mastitis, whether in the form of extracts, essential oils (EO), hydrolate, or oleoresins, etc[5,6].

Brazil has 20% of the world's biodiversity. It has a high potential for the development of such natural products, bioprospecting, however converting this wealth into a product is necessary to encourage research and development, such as financial support from public and private institutions and the collaboration of research institutes and industries[5].

For the protection of herbal products, intellectual property is used, it is the rights granted to a natural or legal person for creations originating from the human intellect. It is divided into industrial property and copyright and related rights. Patents are included in industrial property. A patent is the exclusive right to a creation or a utility model, granted by the State to an individual or legal entity. Depending on national laws, biological materials, and live beings may be patented. In Brazil, plant extracts are not subject to patenting, however, their protection can happen through pharmaceutical and cosmetic compositions[7].

Fostering innovation and creation is crucial for the development of countries, as it is inherent to research and development. In order to develop a new product, it is vital to conduct market analysis and technological mapping. This provides valuable market information, such as the companies investing in research and development, potential market size, and other relevant data. Such information helps guide future decisions and reduces uncertainty[8].

Considering the clinical and economic impact of bovine mastitis, this study aims to survey patent applications in the National Institute of Industrial Property (INPI) database for plant-based products used in treating bovine mastitis.

Material and Methods

The patent applications search was carried out in August until December 2022 using the database of the National Institute of Industrial Property (INPI*, Instituto Nacional da Propriedade Intelectual*), patent databases from Brazil (https://www.gov.br/inpi/pt-br). The methodology of this study consisted of surveying patent applications associated with medicinal plants for the treatment of bovine mastitis. The term *mastite* (mastitis) was used in the abstract field, alone and in conjunction with the word *bovina* (bovine), in Portuguese language, since the database is from Brazil. From the search in which the term “mastitis” was used alone, in the abstract field, data related to International Patent Classifications (CIPs), publication dates, types of applicants for patent applications – companies, individuals’ physicists or science and technology institutions (ICT's) – and the main depositors were organized in graphs.

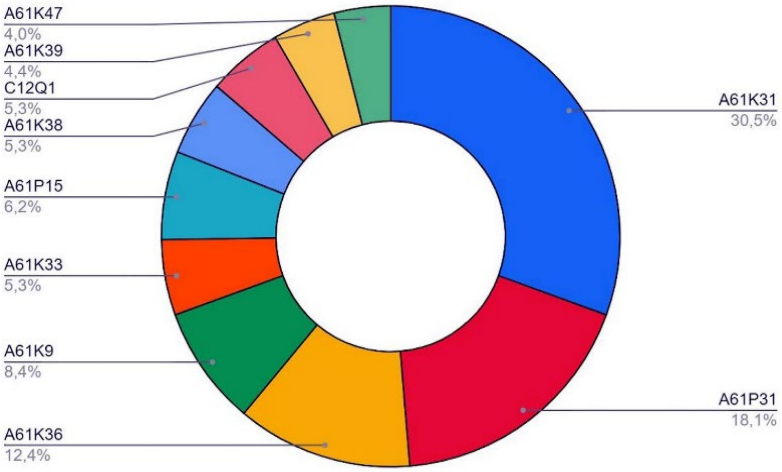
Results and Discussion

The term *mastite* used in the abstract field resulted in 109 patent applications, of these three were duplicates, resulting in 106 patent applications. The combination of the words *mastite* and *bovina* were also applied, acquiring 22 requests. Various products are available for use in dairy farming, including antiseptic formulations for pre-dipping (milking) and/or post-dipping of cow's teats, metallic, alkaline, and acidic solutions for disinfection, pharmaceutical compositions such as antibiotics, synthetic molecules, and essential oils (EO), antigenic molecules for vaccine synthesis, gels, sealants, and products capable of forming a protective layer on the udder, among others.

The extracted patent requests, those that had medicinal plants in their composition, or molecules associated with them, were selected and considered, excluding those that appeared to be duplicates, those with equipment, management systems, formulations only of antibiotics, vaccines and others that did not address the focus of the research.

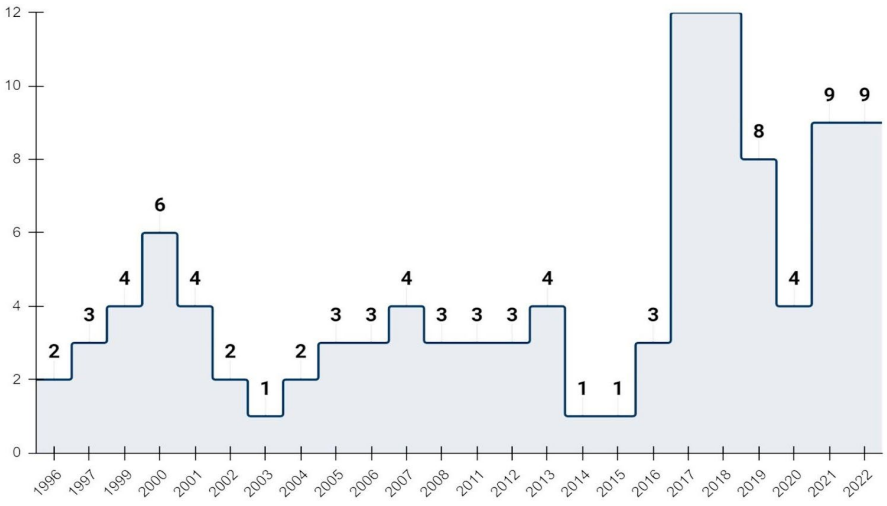
The International Patent Classification (IPC) is shown in **FIGURE 1**. CIP A61k31 was present in 69 patent applications, this one referring to "medicinal preparations containing organic active ingredients". The second CIP is A61P31 which deals with anti-infective preparations. In sequence, the A61k36 classification is assigned to medicinal preparations based on plants, algae, lichens or fungi, including also their derivatives.

FIGURE 1: The top ten IPCs of the 106 INPI patent applications, from August to December 2022.



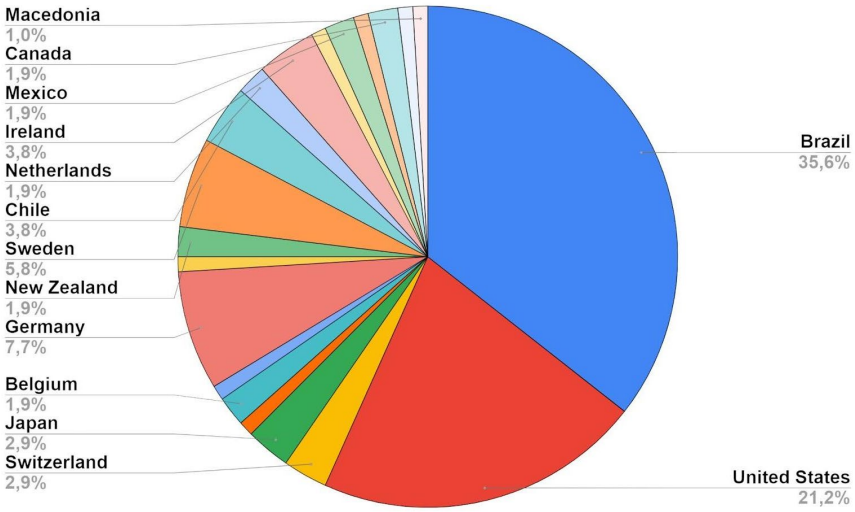
Two patent applications were filed in 1996, both referring to herbal preparations (**FIGURE 2**). And the depositor companies were Valle and Ropapharm. The year 2017 and 2018 showed a greater number of deposits with 12 requests each (**FIGURE 2**).

FIGURE 2: Dates of publication of the 106 INPI patent applications.



The depositor countries are shown in **FIGURE 3**. Brazil was the largest depositor of patent applications with 37 requests, the Science and Technology Institutions (IST) in Brazil deposited 20 requests, individuals 9 and companies 8 patent applications. The second main depositor is the United States with 22. The other requests were from Germany, Sweden, Chile, Ireland, Switzerland and Japan with 8, 6, 4, 4, 3, 3 respectively. Belgium, New Zealand, Netherlands, Mexico and Canada filed 2 requests each.

FIGURE 3: Countries depositors of the 106 INPI patent applications, from August to December 2022.



The types of patent applicants found from the search with the term *mastite* were mostly companies with 60.7% (**FIGURE 4**), followed by IST's and individuals, with 28.3% and 11.3%, respectively. And Pfizer and Bayer were

the main applicants (**FIGURE 5**) with 5 patent applications each, the other applicants were also companies, except for EMBRAPA (*Empresa Brasileira de Pesquisa Agropecuária*), which filed 4 patent applications.

FIGURE 4: Types of depositors of the 106 INPI patent applications, from August to December 2022.

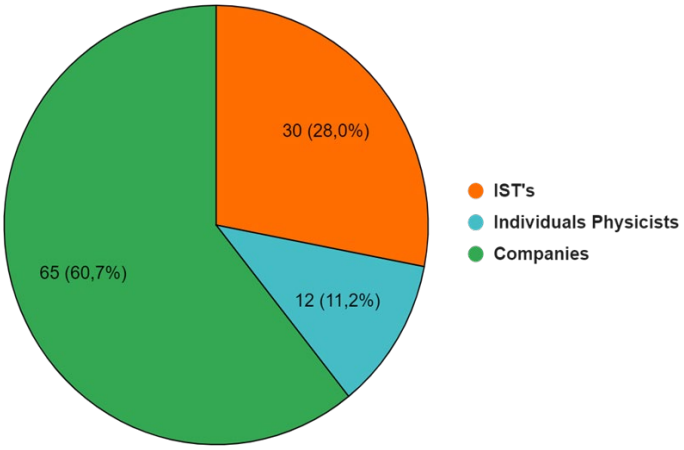
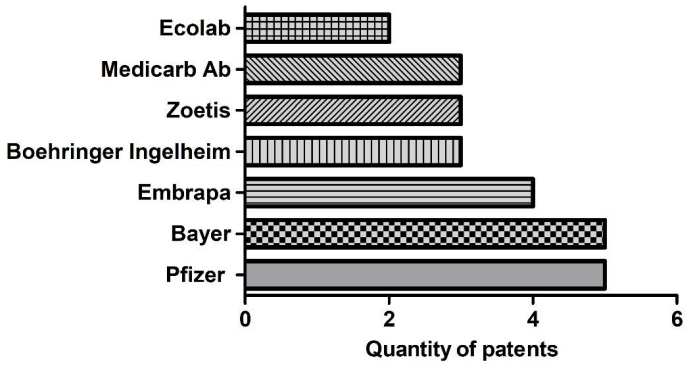
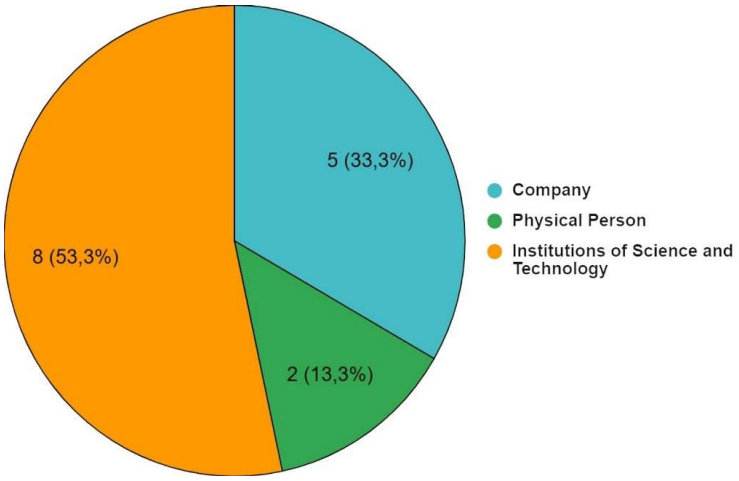


FIGURE 5: Applicants of the 106 INPI patent applications, from August to December 2022.



Among patent applications, 17 contained medicinal plants in their composition, or molecules associated with them. Of there, 9 originate from Institutes of Science and Technology, mainly Brazilian niversities - Federal University of Viçosa with three requests and the remaining six with one each, Pernambuco University, Teaching Association of Ribeirão Preto, University of Cruz Alta Foundation, Federal University of Itajubá, State University of Maringá and Federal Institute of Education Science and Technology of Minas Gerais – approximately 53%, as shown in **FIGURE 6**. Commonly, the initial phases of pharmaceutical research are in research institutions: the discovery of substances, the pharmacological action and pre-clinical tests, that is, the validation of the therapeutic properties of plants. And the large-scale production steps require financial resources from companies.

FIGURE 6: Types of applicants for patent applications with medicinal plants in their composition, INPI database, from August to December 2022.



These 17 requests were extracted and applied in a frame. **TABLE 1** shows the number of patent applications, the product, the applicant and the year of filing. Requests with medicinal plants were present both in the first strategy (*mastite*) and in the second (*mastite* and *bovina*), however these were the same. Tables that are too large for portrait pages should be placed on a separate page in the sequence they appear in the text, in landscape mode, as in the example page below.

TABLE 1: Patent applications with plants found in the technological prospection on the INPI database[1].

Patent Filing Number	Title	Applicants	Date[2]
PI 9608841-9	Composition for both human and veterinary application, and use of the same	Ropapharm	1996
PI 9603224-3	Pharmaceutical composition, method for treating infectious and/or inflammatory processes and use of papain	Vallée S. A.	1996
PI 9812449-8	Pharmaceutical composition based on plant extracts to treat mastitis in domestic animals, and manufacturing process of the pharmaceutical composition to treat mastitis in domestic animals	Laboratório Pisa	1998
PI 0300654-9	Product of plant origin sealant of the teat of lactating animals to prevent the penetration of contaminants and the respective manufacturing process	João Takashi Ohashi	2003
PI 0923252-4 A2	Intramammary nipple sealant	Merial Limited	2009
PI 1005541-0	Herbal pharmaceutical association, herbal pharmaceutical composition, herbal pharmaceutical composition preparation process, and their veterinary uses	Teaching Association of Ribeirão Preto	2010
PI 1103394-0	Dominant compositions based on *macaúba* oil (*Acrocomia aculeata*) and hexane extract of *Salvinia auriculata* roots, product and use for the prevention and/or control of bovine mastitis	Federal University of Viçosa	2011

Patent Filing Number	Title	Applicants	Date[2]
BR 10 2013 018181 1	Process of obtaining a new bactericide from mangabeira latex (*Hancornia speciosa*)	Pernambuco University	2013
BR 10 2013 011129 5	Antiseptic composition for the treatment of bovine mastitis	University of Cruz Alta Foundation	2013
BR 10 2017 020222 4	Pharmaceutical formulations based on essential oils for post-dipping use	Federal University of Viçosa	2017
BR 10 2017 024154 8	Second bovine skin with antimicrobial and biodegradable properties to protect dairy cattle against mastitis	Federal University of Itajubá	2017
BR 20 2018 010159 0	Antiseptic for post-milking application (post-dipping) in cattle for mastitis control	Trio Química Industrial Ltda.	2018
BR 11 2020 015536 5	Use of *Pulsatilla chinensis* extract in the preparation of a drug for the treatment of viral and/or bacterial diseases	Sichuan Inlu Weite Pharmaceutical Technology Co., Ltda.	2019
BR 10 2019 011895 4	Pharmaceutical formulation for the prevention or treatment of bovine mastitis and uses	Federal University of Viçosa	2019
BR 10 2020 018323 0	Medicinal preparation based on essential oils for the treatment, prevention and control of mastitis in domestic animals	Fagner Luiz da Costa Freitas	2020
BR 10 2020 006364 2	Photoactive gels based on safranin O, chlorophyll extract and curcumin in the treatment and prevention of bovine mastitis	State University of Maringá	2020
BR 10 2021 009874 0	Herbal solution for pre and post milking	Federal Institute of Education Science and Technology of Minas Gerais	2022

[1] Data from August to December 2022. [2] Date of deposit.

Plants with therapeutic properties are the main choices for drug development. For the treatment of bovine mastitis, antiseptic products with antimicrobial activities are recurrent, given the cause of the disease, molecules such as menthol, carvacrol, thymol and limonene are examples of substances with such property and mentioned in the INPI patent applicants. These metabolites belong to the class of volatile terpenoids with aromatic characteristics. Comfrey (*Symphytum officinalis* L., Boraginaceae), mint (*Mentha piperita* L., Lamiaceae), papaya (*Carica papaya* L., Caricaceae), *Aloe vera* (L.) Burm.f. (Aloaceae), chamomile (*Matricaria chamomilla* L., Asteraceae) and garlic (*Allium sativum* L., Amaryllidaceae) are some types of plants with this therapeutic potential. Furthermore, these plants' anti-inflammatory and healing properties can help treat mastitis[9].

The company Valle deposited a pharmaceutical compound for the treatment of infections and/or inflammatory processes containing papain and its association with another proteolytic enzyme. The application of papaya in veterinary medicine is often associated with the treatment of verminosis, its latex is widely used in folk medicine due to its ability to eliminate worms, warts and calluses[10]. The other is the patent application entitled. The patent request entitled “Composition for both human and veterinary

application, and use of the same" is a pharmaceutical compound with anti-inflammatory action, consisting of EO from species of Lamiaceae family: *Origanum vulgaris* L., *Thymus vulgaris* L., *T. serpilum*, *T. zugis*, *M. piperita*, *Satureja hortensis* L., *S. montana*, *S. subricata*, *Ocinum gratisimum* L., *Monarda punctata* L., *Mosla japonoica* (Benth. *ex* Oliv.) Maxim., *Salvia officinalis* L., and *Carum corticum* L. (Apiaceae). Apart from treating mastitis, these natural compounds also apply to dermatitis, colibacillosis, and dermatomycosis[11].

A composition for mastitis treatment of bovine, goat and sheep was registered under title "Pharmaceutical composition based on plant extracts to treat mastitis in domestic animals, and manufacturing process of the pharmaceutical composition to treat mastitis in domestic animals". It is composed of *Aloe vera* juice or gel, aqueous extract of *Agave mexicana* Lam. (Agavaceae), EO of *Citrus limon* (L.) Osbeck (Rutaceae) and *Melaleuca alternifolia* Cheel (Myrtaceae), extract of *Symphytum officinalis*, zinc sulfate, sodium salt of ethylenediaminetetraacetic acid, citric acid, ascorbic acid and sodium benzoate. The specific part of the plant used were the leaves of *A. vera* and *Agave atrovirens* Karw., the pericarp of the lemon fruit, the cork of the *M. alternifolia* and the comfrey root. The patent was filed by the Mexican company Laboratorios Pisa[12].

The patent application filed by João Takashi Ohashi deals with a sealant for the teat of lactating animals, called MASTOP, an antiseptic product prepared from rice, corn and manioc starch. It guarantees the safety of the product as it is a topical treatment, claims the absence of absorption route and the non-reaching of blood circulation. It must be used after milking, and is intended for cows, goats and buffaloes[13].

The patent application of the Company Merial Limited refers to a breast sealant intended to treat and prevent mastitis. With a formulation based on plant oils and antacid bismuth subnitrate[14].

The patent application entitled Herbal pharmaceutical association, herbal pharmaceutical composition, herbal pharmaceutical composition preparation process and then veterinary uses (*Associação farmacêutica fitoterápica, composição farmacêutica fitoterápica, processo de preparação de composição farmacêutica fitoterápica e seus usos veterinários*, in Portuguese) is a pharmaceutical mixture formed by EO of *Lippia salviaefolia* Cham. (*syn. Lippia origanoides* Kunth, Verbenaceae) and *L. sidoides* (*syn. L. origanoides*), with the predominant monoterpenes thymol and carvacrol[15].

Patent application number PI 1103394-0 is a medicinal preparation based on *macaúba* (*Acrocomia aculeata* (Jacq.) Lodd. ex Mart., Arecaceae) oil extracted from its almond and *Salvinia auriculata* Aubl. (Salviniaceae) extract, for the development of a household soap, which can be used in the hygiene of the teats and the milker's hand. Its formulation contains 25% *macaúba* oil, 0.5 to 2% *S. auriculata* root extract, 20% EO, 1% to 2% vitamin E, 70% to 80% glycerin. Its purpose is to eliminate the bacteria *S. aureus*, which is one of the main contagious pathogens of subclinical mastitis and chronic infections[4].

The patent application filed by the Federal Rural University of Pernambuco is a method for obtaining a bactericide from the latex of Brazilian *mangabeira* (*Hancornia speciosa* Gomes, Apocynaceae) due to the latex's inhibitory action against *S. aureus*. Aimed specifically for the treatment of mastitis in goats and cows[16].

The antiseptic composition in liquid or gel form based on aqueous extract of *Hedychium coronarium* J.Koenig, Zingiberaceae, was deposited by University of Cruz Alta Foundation, in 2013. *Hedychium coronarium*, also known as *lírio-do-brejo* in Brazil, is native to Asia and has been acclimatized to Brazil. It is commonly used by the Brazilian population to treat infections, sore throats, and rheumatism. *In vitro* and *in vivo* tests were carried out in this invention, both proved the antimicrobial activity of this extract[17].

The patent request “Pharmaceutical formulations based on essential oils for post-dipping use” refers to a mixture for application after milking. It includes EO of cinnamon (*Cinnamomum zeylanicum* Garcin ex Blume, Lauraceae), cilantro (*Coriandrum sativum* L., Apiaceae), oregano (*Origanum vulgare* L., Lamiaceae), clove tree (*Syzygium aromaticum* (L.) Merr. & L.M. Perry, Myrtaceae) and thyme (*T. vulgaris*), pure or in combinations, and to aid in udder tissue regeneration. In this invention, the authors performed numerous experiments to analyze the Minimum Inhibitory Concentration (MIC) of EO in *S. aureus* inoculum. The activity of the 5 volatile oils was considered separately, interacting with each other, and in synergy with the antibiotic cefoperazone, obtaining positive results because all the oils showed antimicrobial action. *Coriandrum sativum* EO alone showed the lowest MIC value. The best results together with cefoperazone were thyme, clove tree and cinnamon. And, the combination of coriander, oregano and cinnamon EO exhibited the greatest bacterial inhibition[18].

The patent application with number BR 102017024154-8 deals with a film-forming solutions composed of poly(vinyl alcohol), arborescent polyglycerol (PGR) and extract of *Dillenia indica* L. (Dilleniaceae). This promotes the formation of a physical barrier in the bovine udder, preventing the entry of harmful microorganisms. Its development takes place through the conjugation of polymers and the extract of *D. indica*. The inventor indicates that PGR is already used in the food industry to decrease the viscosity of chocolate and replace cocoa butter, so the safety of this material is already understood[19].

The company *Trio Química Industrial* patented the composition based on EO that contain phytochemicals, especially thymol and carvacrol, for use after milking. The aqueous solution acts as a barrier to prevent the entry of pathogenic microorganisms and disease-carrying insects. This solution is composed of bi-distilled glycerin, ethoxylated lanolin, both for hydration, thyme EO and *O. vulgare* for its antiseptic actions, xanthan gum (thickener), Brancol (colorant used in the production of cleaning products), methylchloroisothiazolinone (preservative) and distilled water (vehicle)[20].

Patent application number BR 11 2020 015536 5 relates to the use of extracts of *Pulsatilla chinensis* (Bunge) Regel (Ranunculaceae), specifically the saponin B4 compound for the preparation of drugs aimed at viral and/or bacterial diseases. The extract preparation is preferably used in the form of an injection, however it may be in the form of an infusion, powder, lotion or ointment[21].

The named patent application “Pharmaceutical formulation for the prevention or treatment of bovine mastitis and uses” concerns the emollient composition based on *S. officinalis* for pre-dipping and post-dipping. Studies establish the antibacterial property of this plant mainly against *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus cereus* e *Staphylococcus aureus*. Also has antioxidant, antimutagenic and astringent potential. In addition, ethnopharmacological research reports its application for depression, anxiety, dyspepsia, insect bites and others[22].

The State University of Maringá (Paraná, Brazil) produced photoactive gels consisting of chlorophyll extract, curcumin and safranin O (or basic red 2). To make the gel, the chlorophyll extract comes from the New Zealand spinach plant *(Tetragonia tetragonoides* (Pall.) Kuntze, Aizoaceae), the other photosensitive molecules, curcumin and safranin O, were purchased from the Merck company. For its validation, *in vitro* experiments were carried out, in which four species were inactivated – *S. aureus*, *E. coli*, *S. agalactiae* e *C. bovis* – causing bovine mastitis, and *in vivo* observed a reduction in somatic cell counts when used after dipping[3].

The patent whose title is “Medicinal preparation based on essential oils for the treatment, prevention and control of mastitis in domestic animals” belongs to an intramammary product composed of essential oil of rosemary (*Rosmarinus officinalis* L., *syn. Salvia rosmarinus* Spenn, Lamiaceae) and tea tree (*M. alternifolia*). Rosemary essential oil has antimicrobial, immunomodulatory, antioxidant properties, among others, its compounds interact with the bacterial cell membrane, causing lysis or changing cell functioning, while tea tree essential oil also acts on the cell membrane and inhibits cell respiration[23].

The “Phytotherapeutic solution for pre and post milking” is a patent application filed by the Instituto Federal de Educação, Ciência e Tecnologia de Minas Gerais (Brazil), which corresponds to a preparation for topical application, consisting of extract of *Stryphnodendron adstringens* (Mart.) Coville (Fabaceae), *Baccharis crispa* Spreng. (Asteraceae) and *Azadirachta indica* A.Juss oils. These species have already shown antimicrobial properties against *S. aureus*, *Streptococcus agalactiae*, *Streptococcus uberis*, *E. coli* and *Salmonella*, and have anti-inflammatory and healing activities. *Stryphnodendron adstringens* is native to Brazil and is utilized for wound healing, particularly when combined with other plants such as aloe, comfrey, calendula (*Calendula officinalis* L., Asteraceae), and echinacea (*Echinacea* sp., Asteraceae)[10,24-25]. Topical sanitizers are critical points in pre and post-dipping to disinfect cows' teats and reduce environmental contamination associated with bovine mastitis[26].

In dairy cattle, Brazil is the fourth largest producer in the world, between the years 1998 and 2018, losing only to India, the United States and Pakistan. The northeast was the third producer of milk with about 4.3 billion liters of milk, and Pernambuco with 941 million liters of milk. The southern region leads the ranking with 11.5 billion and the southeast region 11.4 billion[2,16].

In this study, Institutes of Science and Technology of Minas Gerais deposited 3 patents, this state is a center in the dairy industry, it is the leader in the acquisition of milk with 25.4%, followed by Paraná and Rio Grande do Sul, with 12.8% and 12.2%, respectively.

Conclusion

Brazil possesses a vast range of flora that can contribute to veterinary medicine, benefit the environment, and promote new products, as well as economic, technological, and scientific development. Since bovine mastitis is a disease that affects several countries, including Brazil, the alternative of applying plants to solve this problem is promising, since they can be combined in several pharmaceutical formulas: sprays, ointments, plasters, extracts and others.

There are a considerable number of patent applications for the treatment of mastitis deposited at the INPI, of the 109 patents, 17 were plant-based pharmaceutical compositions or their phytochemicals with antiseptic and/or immunomodulatory therapeutic potential.

Through this patent mapping, there are several products to treat and prevent bovine mastitis. There are also different species of plants used in the treatment of this disease present in the Brazilian market. These are applied either in synergy with other plants or with other substances with therapeutic potential. Companies were the main depositors of patent applications, but in relation to applications involving phytotherapeutic compositions, Science and Technology Institutions showed a considerable participation. Of the two patent applications from 1996, both relate to phytotherapeutic compositions that use plants in combination – Valle's

is a composition with an enzyme found in papaya, papain with another proteolytic enzyme; and Ropapharm's is a combination of plant derivatives and is not exclusive for the treatment of mastitis. Therefore, it is notable that the use of medicinal plants to treat bovine mastitis has already been studied and accepted, so that they can overcome the problems offered by conventional treatments.

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Conflicts of Interest

The authors declare no conflict of interest.

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Collaborators

Study design: MCA; CPV; RA

Data curation: CSV

Data collect: CSV; GHSC

Data analysis: CSV

Original manuscript writing: CSV; CPV

Review writing and editing: MCA; RA; CPV.

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