

Evaluation of a Nipple Sealer Based on Brad Infusion

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Abstract: This research work was performed to replace the antimicrobials that are usually used in the trading nipples' sealants, changing them by a local herbalist resource, the Brad (*Erodium cicutarium*), as an infusion in the active ingredient and this way detecting new antimicrobial fonts. To evaluate its efficiency, in-vitro analysis was made at school laboratory as well as in the Pharmacognosy Class of the Faculty of Pharmacy and Biochemistry at the Universidad de Buenos Aires, Argentina. Since April 2013 until September 2013, fieldwork was made over bovines at Escuela Agropecuaria Provincial N°1 in Gobernador Gregores, Santa Cruz, Argentina. At in-vitro trials, the results obtained using sealer with brad as an infusion basis, showed a good antiseptic behavior as it reduced in more than 5 logarithms the units maker of colonies, coming from the nipple's skin. At fieldwork, after 21 days of application, it reduced the bacterial load of the nipple's skin in a statistically significant way ($p < 0.001$). A TLC analysis detected that in the brad infusion existed some kinds of flavonoids such as: rutin and other glycosides from quercetine. An HCPL analysis confirmed the presence of phenolic compounds such as quercitine, rutin, chlorogenic acid, isoquercitrine and quercitrine. The cost of the elaborated sealer was up to 30% lower than the trading ones.

Keywords: Nipple sealer, Brad, Antiseptics

1. Introduction

The brad is a vegetal about which the locals say it has curative actions, especially on damaged skin or some digestive diseases. These people use the brad in an infusion to apply it later in different ways. The brad grows almost among Argentina, and in the south Patagonian region, it grows naturally at tableland, next to roads and between rocks. [1] [2] [3].

Among the main reasons why post milking nipples disinfection is performed, are:

- Eliminates bacteria that cause mastitis staying on the skin of the nipple.
- Removes bacteria from swollen nipples.

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→ Improves the quality of the skin of the nipple. [4] [5]

The application of these antimicrobial products for controlling microorganisms producers of breast infections is an usual practice that, because of the indiscriminate use, brings with it some negative consequences such as the generation of resistant bacterial strains. [6] [7].

The residues of milk that leave these commonly used antibiotics should also be considered. [8]

This trial replaces a traditional component of the nipple sealer by an infusion of a resource herbalist of the area: the Brad shepherd.

OBJECTIVES

- Determine the active ingredient of brad.
- Elaborate a nipple sealer based on a brad infusion with a great antiseptic power at in-vitro state and “at field”.

2. Materials and Methods

MATERIALS

This test was performed between March 2012 and August of 2014, at the Escuela Agropecuaria N°1 of Gobernador Gregores, Santa Cruz province, Argentina. Three cows Holando Argentino, with an average age of 6 years, at milking were used. The cows belong to the school. The animals were in production. The test was performed between the 2nd and 5th month of lactation. They were clinically healthy, they gave negative result Protocol of diagnosis of brucellosis (Antigen bufferado in plate) which held the Laboratorio Regional de Sanidad Animal, Concejo Agrario Provincial, Santa Cruz province [9] and the California Mastitis Test (CAM), which is carried out on a monthly basis in the Tambo at school. [10]

METHODOLOGY

Firstly, in-vitro trials were performed and after the evaluation of the antiseptic activity of the product, field trials were carried out as well. In addition, some determinations about the active ingredients of the vegetal were made in the Phamacognosy Class of the Faculty of Pharmacy and Biochemistry at Universidad de Buenos Aires.

The percentage as a summery measure and the variation percentage as a comparison measure were calculated. As a significance statistical test, the χ^2 test was carried on.

Gathered samples of a whole brad specimen were sent to Instituto de Tecnología Agropecuaria (INTA) for a

botanical and taxonomical classification.

With brad, an Infusion based on 10 g of whole plant dried and crushed with mortar and pylon in 250 ml of water, was prepared. This was left to marinate for 12 hours and then boiled for 2 minutes. The obtained infusion was filtered with 4 or 5 fat shapes. It was the same preparation described by local rural residents, who were consulted about how to prepare it and the use that they gave to the plant.

A nipple sealer was developed using the brad infusion and agents of formulation as follow:

Brad Infusion	70 ml
Propilenglicol	20 ml
Carbopol	0,5 g
Glycerin	10 ml
Vitamin A	500.000UI

IN-VITRO TRIALS

Prior sampling

For in-vitro trials, an adaptation of the proposed methodology by the protocol of the National Mastitis Council, (NMC) (EEUU) [11] was made. Samples were taken with three repeats, obtained by application of the Laboratory dish with the culture medium over every nipple's skin of the animals at milking stage, instead of cleaved nipples as proposed by the NMC.

The nutrient agar of plate count (APC) from Laboratorios Britaniawas selected as culture medium.

Inoculum

To get a standard turbidity in the preparation of the inoculum for a 10^8 CFU/ml, a technique described by the Procedures Manual for an antimicrobial sensibility isolated of humans ANLIS-Malbran was used. This worked as an antimicrobial sensibility by diffusion method. [12]

Determination of antimicrobial activity of the brad extract.

20 g of brad's aerial parts were refluxing extracted with 150 ml of MeOH during 2 hours. The solution was filtered hot and then was dried with evaporator. It was dissolved in 5ml of distilled water. Two Laboratory dish piles were prepared. 5laboratory dish with APC and other 5 with APC plus 1ml of brad extract solution before the agar's smelting. [13]

All laboratory dish were sowed with the inoculum prepared with germs of the cows' mammary glands. In every laboratory dish, 10^6 CFU were sowed along with a handle of 0,01ml capacity.

Laboratory dish were incubated at 37°C during 48 hours. In this period, the development of colony forming units (CFU) was registered. The used criteria to determine the antimicrobial activity was the CFU development. Three repeats were made.

Determination of antimicrobial activity of the brad infusion.

ACP culture medium was prepared for 5 laboratory dish. In other group of 5 laboratory dish, the distilled water in the suspension medium was replaced by brad infusion. This was performed to evaluate the antiseptic power.

Those were sowed with the inoculum. Final determinations of CFU were carried out exactly 48 hours after the sowed. Three repeats were made.

DETERMINATIONS CARRIED OUT AT FACULTY OF PHARMACY FROM UNIVERSIDAD DE BUENOS AIRES.

In the Pharmacognosy Class of the Faculty of Pharmacy and Biochemistry at Universidad de Buenos Aires, the following determinations about brad were made:

- Phytochemical groups, which form part of brad, were characterized.
- TLC and HPLC analyzed chromatographic profile in the brad infusion.

To a preliminary evaluation of the phytochemical groups in the aerial parts of brad, a methodology described by Rondina and Coussio (1969), was applied. [14]

TLC analysis

A thin layer chromatography (TLC) was analyzed, in different chromatically systems, silicagel and cellulose over the aerial part of brad and the infusion.

HPLC analysis

The infusion was analyzed by a high performance on liquid phase chromatography (HPLC).

FIELD TRIALS

Sampling was carried out during 21 days, until stabilization on the CFU counting was established. The first sampling was carried out during May 2013 when the cows arrived to the milking room and these we used as "witness samples", i.e. without the nipple sealer application. The second sampling was carried out at the end of

July and August 2013. In these cases, the cows had been routinely treated with the nipple sealer based on brad infusion. During those months the weather was cold and dry.

To develop the field trials, an ARP culture medium was prepared. This filled, antiseptically, some sterile crucible caps of 3,5 cm diameter (9,62 cm² of surface).

Then, these caps were put in Laboratory disso this way they were ready for sampling according as cows arrived to the milking room, i.e. 12 crucible caps with culture medium were prepared per day.

Sampling was carried out applying the crucible caps with culture medium over the extremity of each nipple, with an upward soft pressure. After that, the caps were located inside Laboratory dish and taken to crop oven (37°C) during 48 hours. Following, a CFU counting took place. Finally the four quarters of each bovine (for each day and per week) were averaged.

Costs determination

Costs were pointed in US dollars. The prices of local commercial nipple sealers per liter were researched at veterinaries of Gobernador Gregores.

3. Results and Discussion

BOTANIC TAXONOMICAL CHARACTERIZATION OF BRAD SENT BY LOCAL “INTA”

Family: *Geraniaceae*; Gender: *Erodium*; Specific Epithet: *cicutarium*; Species: *Erodium Citarium* (L.) L’Hér. Ex Aiton. [15]

IN-VITRO TRIALS

The brad concentrate germicide power cut back the increasing CFU number laboratory dish, in 5 log before the inoculum of cow skin germs, using the brad extract and in 4 log with brad infusion usage. (Tables 1 and 2).

Table 1 Brad concentrate germicide power before the inoculum of cow skin germs. (Log CFU average laboratory dish)

	ARP + INOCULUM LOG Σ 5 laboratory dish	ARP + BRAD CONCENTRATE + INOCULUM LOG Σ 5 laboratory dish
DATE 07/05	6	1,3
DATE 08/05	6	1,08
DATE 10/05	6	1,38

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Table 2 CFU number variation in laboratory dish, prepared with brad infusion.

	ARP+INÓCULUM Log Σ 5 laboratory dish	ARP+BRAD INFUSIÓN+ INOCULUM Log Σ 5 laboratory dish
DATE 28/05	6	2
DATE 29/05	6	2
DATE 30/05	6	2

FIELD

TRIAL

Sampling during May 2013 with no sealer application. Diary values obtained from each nipple of a same cow were averaged. After that, a weekly average was made. (Table 3).

Table 3 Witness sampling.

	1° week	2° week	3° week
	CFU/CC	CFU/CC	CFU/CC
COW 53	450 \pm 17	400 \pm 15	420 \pm 15
COW 98	420 \pm 15	400 \pm 14	420 \pm 15
COW 32	400 \pm 14	400 \pm 14	450 \pm 17

(CC) Crucible caps with cultures medium

During July-August 2013, after milking, sampling was carried out with sealer application. Diary values of each nipple of a same cow were averaged. A weekly average was made. (Table 4)

Table 4 Sampling with sealer.

	1° week	2° week	3° week
	CFU/CC	CFU/CC	CFU/CC
COW 53	357 \pm 14	236 \pm 12	166 \pm 10
COW 98	367 \pm 14	287 \pm 12	136 \pm 9
COW 32	357 \pm 15	274 \pm 13	151 \pm 9

(CC) Crucible caps with cultures medium

CFU/CC reduction due to nipple sealer application over the nipple skin, was statistically significant. (P<0,001)

BRAD'S ACTIVE INGREDIENTS RESULTS

According to the results, which were obtained from the phytochemical march carried out on aerial parts of brad, the presence of polyphenolic components (phenolic acids and flavonoids) was determined. Analysis of chromatographic profiles by TLC and HPL, confirmed the presence of such elements.

TLC analysis, in different chromatographic systems, let detect in brad infusion, the presence of flavonoids such as rutin, chlorogenic acid, isoquercitrine, quercitrine and luteoline, as well as other phenolic components, which could not have been identified due to a standards' lack.

COSTS

Elaboration costs at school laboratory casted a 30% reduction compared to the elaborated sealers. (Tables 6 and 7)

Table 6 Market prices of used inputs.

	US\$ COST	AMOUNT NEEDED	US\$
BRAD INFUSION	0	0,07 l	0
GLYCERINE	5,37/l	0,01	0,054
PROPYLENE	9,51/l	0,02	0,19
CARBOPOL	15,85/kg	0,005	0,08
Vit. A (5.000.000 UI)	2,44	500.000 UI	0,244
TOTAL COSTS INPUT / 100 ml			0,57

0,57 US\$ per 100 ml or 5,7 US\$ per liter

Table 7 Local market values of commercial nipple sealer and elaborated sealer cost.

Trademarks	Price at Veterinaries	US\$/l	Difference	Difference
	US\$/l		in US\$	%
"A"	11,59	5,70	5,89	50,80%
"B"	6,34	5,70	0,64	10,12%
Average				30,46%

DISCUSSION

Although, the number of cows, which were analyzed is not enough, it must be taken into account that the school Tambo is practically the only one in Santa Cruz province. The work with animals is reduced to a small number and only with didactic issues. That is why the obtained determinations should be considered as preliminary and should be repeated on a great scale. [16]

These research work results match with other investigations carried out over vegetable species with antiseptic properties, in which it was demonstrated that the “shepherd’s purse” (*Capsella bursa pastoris*) and the “French rosette” (*Tribulus terrestris*) present antimicrobial activity against the *Staphylococcus aureus*, this in cases in which chloroform extract is used, meanwhile the methanolic extract dropped negative results. [17]

As regards its antiseptic mechanism, according to determinations of active ingredients, numerous phytomedicinal products contain quercitine or its glycosides. Flivonoid glycosides are consider as important medicinal products for preventing or treatment of some deaseases. Quercitine and other flavonoids have a wide variety of biological effects, though scientific evidence for prevention or treatment is not enough. [18] [19] [20]

The Food and Drug Administration has not aprove none of this health statements for quercitine. [21]

The International Biochemical and Molecular Biology Union carried out a research about e-coli infection and found the way to prevent it using quercitine in different concentrations. This action can be explained as quercitine can interact with fluoroquinolones (an antibiotic), because quercitine can add to the bacterial gyrase DNA. [22] [23] Studies carried out by the Botanic Class of the Universidad Nacional of Rosario together with the Instituto del Seguro Social de Mexico D.F. showed that the quercitine, quercitrine and morin mixture has a strong inhibitory activity of *Salmonela enteritidis* and *Bacilus cereus* growth in crop states. This effect is enhanced by rutin, getting effects over *Staphilococcus aureus y Escherichia coli*. [24]

These evidences explain, in a way, the brad infusion power to heal skin’s ulcers and wounds as related by locals.

4. Conclusion

From the results analysis, it is reliably concluded that it was possible get a formulation for a post milking nipple sealer with brad infusion as active ingredient, with more than five logarithms reduction at “in-vitro” trials as well as statistically significant reductions for CFU development over the nipple skin ($p < 0,001$), after the nipple sealer application. Flavonoids such as rutin and other quercitrine glycosides were detected in brad infusion. The elaboration cost was a 30% lower than on a trademark product.

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