ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS AGAINST OXACILLIN-RESISTANT *Staphylococcus aureus* STRAINS ISOLATED FROM BOVINE MASTITIS

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Introduction

Bovine mastitis is one of the main economic problems in the milk industry, especially related to pathogenic microorganisms, being *Staphylococcus sp*, one of the principal involved (Diaz, et al., 2010). The prophylactic use of antibiotics and misuse in the prevention of bovine mastitis have generated resistance in microorganisms such as *Sthaphylococcus aureus* against Oxacillin, which makes this disease more difficult to control and treat (Fratini, et al., 2014). In this context, it is necessary to explore new therapeutic alternatives, among them the use of products based on natural plants.

The essential oil of *Thymus vulgary* and thymol, its main compound, showed relevance in the treatment of bovine mastitis (Pozzo, et al., 2011), it can reduce the internalization of *Staphylococcus aureus* in bovine mammary epithelial cells and inhibit the production of nitric oxide (Zhengkai, et al., 2014). Some studies evaluated the morphological changes in *Staphylococcus aureus* Methicillin-resistant of the essential oil of *Cymbopogon citratus* (DC.) composed of citral (74%) and the mixture geranial (40.56%) and neral (33.71%), geraniol (4.64%), finding damages in the cytoplasmic membrane that can be caused by the synergy of the mechanisms of action of the compounds that potentiate the antimicrobial activity (Jareerat Aiemsaard, et al., 2011). Studies show that there is synergy in the antimicrobial activity of medicinal plants (Mundy, et al., 2016)

The objective of this study is to explore the effect of essential oils *Lippia citriodora*, *Lippia alba, Lippia origanoides* y *Thymus vulgaris* by the Kirby-Bauer method, is enhanced against clinical isolates of Oxacillin-resistant *Staphylococcus aureus.*

The size of the sample was used with the GRANMO 7.12 program and the statistical analysis was performed with the IBM SPSS statistical package

Materials and methods

MICROORGANISMS

Fifteen isolates of *S. aureus*, of subclinical and clinical mastitis cases in bovines of the savannah of Bogotá - Colombia, previously characterized in the Laboratory of Microbiology, Faculty of Veterinary Medicine, were studied. By using *Kirby-Bauer,* the method Oxaciline-resistance was evaluated (NCCLS, 2000). Stored in freezing conditions. Thawed and cultured on blood agar incubated at 37 ºC 24 hours to perform the tests.

PREPARATION OF ESSENTIAL OILS

Essential oils were obtained by steam distillation of leaves, flowers, and stems of *Lippia citriodora, Thymus vulgary* (Chocontá – Cundinamarca) and, *Lippia alba* and *Lippia origanoides* (Machetá -Cundinamarca). The characterization of its main significant fractions was carried out by Gas Chromatography-Mass Spectrometry (GC-MS) in the Universidad Industrial de Santander, as shown in Table 1.

Essential oil	Composition (Majority fraction%)
Thymus vulgaris	Thymol (29)
Lippia citriodora	Limonene (38.6), neral (21.6) and geranial (24.8)
Lippia origanoides	Thymol (56) Durenol (21)
Lippia alba	Carvona (41) limonene (38).

Table 1 Relation of essential oils and major fractions-compositions

EVALUATION OF THE ANTIMICROBIAL ACTIVITY

Disk diffusion technique (*Kirby-Bauer*)

For the Kirby-Bauer technique, discs with essential oils and the mixture to be evaluated were prepared by impregnating 7 mm sterile filter paper discs (Iturriaga, et al., 2012) (density 140g/cm2), with 20 µL of oil (Fratini, et al., 2014).

The Muller Hilton plates were inoculated with a solution of Muller Hilton broth at a 0.5 McFarland turbidity scale, the previously prepared disks were placed and incubated for 24 hours at 37 ° C, later, the inhibition zones of the plates were measured in mm.

Additionally, the antibiogram was performed for antibiotics Enrofloxacin, Clindamycin and Chloramphenicol.

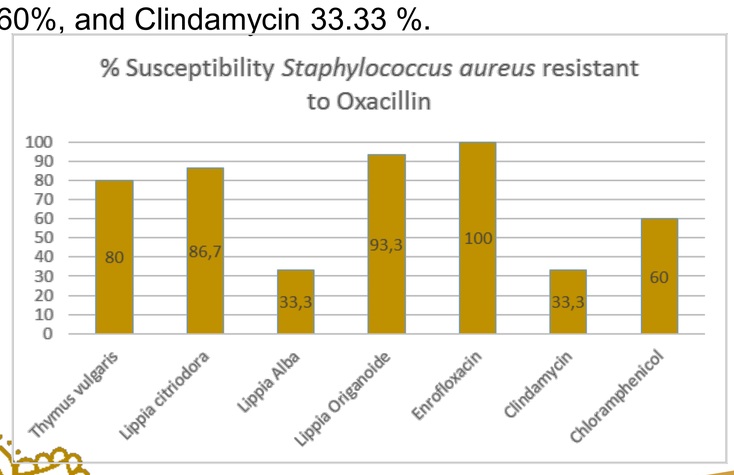
Results

Antimicrobial activity was evaluated from the growth inhibition diameters of the *Staphylococcus aureus*-oxacillin resistant isolates (n = 15), generated by the discs impregnated with essential oils see table 1:

Essential Oil Parameter (mm)	*Thymus vulgaris*	*Lippia citriodora*	*Lippia Alba*	*Lippia Origanoide*	Enrofloxacin	Clindamycin	Chloramphenicol
Mean	23.93 +/- 1.55	24.40 +/- 1.66	21.00 +/- 3.41	31.07 +/- 2.31	26.64 +/- 2.40	22.28 +/- 4.74	21.78 +/- 3.19
Median	24	24	14	40	28	24	21
Minimun	12	18	6	21	21	15	16
Maximun	36	45	45	40	30	30	30

Table 2 Zones of inhibition for essential oils and the mixture (n=15)

The percentage of isolations with an inhibition diameter greater than 21 mm was calculated, finding that only 33.33% of isolates exceeded this value for the AE (for its Spanish acronym). *Lippia alba*, 80% for the AE case. *Thymus vulgary,* 86,67% for the AE. of *Lippia citriodora,* and 93,33% the *Lippia origanoides.* The percentage of isolations with inhibition with antibiotics was: Enrofloxacin 100%, Chloramphenicol 60%, and Clindamycin 33.33 %.



*A sample size of **16** subjects randomly selected will suffice to estimate with a 95% confidence and a precis ion +/- 20 percent units, a population percentage considered to be around 80%*. Granmo 7,12

Conclusions

It is concluded that the essential oils of *Lippia citriodora* (L'He'r), *Lippia origanoides* (Kunth) y *Thymus vulgaris* (L) may be a possible therapeutic alternative against strains of *Staphylococcus aureus* resistant to Oxacillin ($p < 0.05$).

Conflict of interest

The authors declare that they have no conflicts of interest.

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Lippia citriodora (L'He'r)

Lippia alba (MiLL.)

Lippia origanoides (Kunth)

Thymus vulgaris (L)