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EVALUATION OF ANTI-BACTERIAL ACTIVITY OF ETHANOLIC EXTRACTS OF *RUTA CHALEPENSIS*, *HIPPOCRATEA EXCELSA*, *LITSEA GLAUCESCENS* AND *WALTHERIA AMERICANA* AGAINST METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

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Abstract: Antimicrobial resistance is still a problem and therapeutic options are becoming more and more limited. *Staphylococcus aureus* causes skin and soft tissue infections and has a high degree of pathogenicity. The objective of this work was to determine the antibacterial activity of ethanolic extracts of several plants at different concentrations against methicillin-resistant and methicillin--sensitive *Staphylococcus aureus* strains, using the Kirby-Bauer method. The ethanolic extract of Ruda *(Ruta chalepensis)* shows significantly higher antibacterial activity than the extracts of Cancerina (*Hippocratea excelsa*), Laurel *(Litsea glaucescens*) and Tapacola (*Waltheria americana*) alone and in combination. The presence of phenolic compounds, anthocyanins and flavonoids was determined. Rue may contribute to the potential determination of a natural alternative therapy against methicillin-resistant *Staphylococcus aureus*.
Keywords: *Staphylococcus aureus*, resistance, alcoholic extract.

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a microorganism of great medical importance. For many years it has been recognized as one of the main human pathogens. *S. aureus* is part of the family *Micrococcaceae*, genus *Staphylococcus*, which contains more than 30 different species and many of these are microbiota of the skin and mucous membranes in man. It is a Gram-positive, non-motile coccus. It does not form spores and can be found singly, in pairs, in short chains or in clusters. It is a facultative anaerobe, but grows best in aerobic conditions. The organism produces catalase, coagulase and grows rapidly on blood agar. Its colonies measure 1 to 3 mm, produce a typical yellow pigment due to the presence of carotenoids and many strains produce hemolysis at 24-36 hours.[1]

S. aureus is a pathogen that can colonize mucous membranes and skin and cause severe toxin-mediated invasive infections in humans and animals.[2] Its dissemination has increased worldwide in adults and children, with clinical manifestations such as skin and soft tissue infections, pneumonia and bacteremia more pathogenic, associated with high morbidity and mortality rates, which has become a public health problem.[3,4]

Currently, *S. aureus* strains have a wide range of antibiotic resistance and resistant and multidrug-resistant strains can be found. The acquisition of this resistance is mainly due to the horizontal exchange of genes that are carried by mobile genetic elements such as plasmids, transposons (Tn) and insertion sequences (IS). [1]

By the early 1960's the *Staphylococcus* genus had acquired resistance to the vast majority of available antibiotics. Penicillin resistance of 80%-93% or more is now reported in *S. aureus* strains isolated from hospitals and the community. [5,6] Because of penicillin resistance of *S. aureus* strains, penicillinase-stable cephalosporins and semisynthetic penicillins were introduced in the late 1950s. Among these was methicillin, as the antibiotic of choice in the treatment of *S. aureus*. This drug was introduced in Europe in 1959 and a year later the first methicillin-resistant *S. aureus* strain ("methicillin resistant *S. aureus*", MRSA) was detected. Later, in 1963, the first nosocomial outbreak caused by MRSA strains was reported. Since then, multidrug-resistant *S. aureus* strains have been reported worldwide. [5-7]

Resistance is a factor that contributes to the development of complicated skin and soft tissue infections, limits the effect of some antimicrobial agents, requiring the development of new treatments.[8] The use of natural products present in certain medicinal plants are fully adequate to prevent the growth of disease-causing pathogens, particularly some

multidrug-resistant variants.[9] The use of whole plants, barks, roots, leaves, etc., for the treatment of diseases such as respiratory complications has been a common practice in Africa and the Middle East for a long time.[10] The aim of the present work was to evaluate the antibacterial effect of ethanolic extracts of Rue (*Ruta chalepensis*), Cancerina *(Hippocratea excelsa*), Laurel *(Litsea glaucescens*) and Tapacola (*Waltheria americana*) against *S. aureus* (MRSA).

MATERIAL AND METHODS

PLANT MATERIAL AND OBTAINING EXTRACTS

Ruda (stem, flower, leaf), Tapacola (leaves and flower), Cancerina (bark) and Laurel (leaves), acquired in the Sonora market in Mexico City in June 2017, were used for the trials. The plant material was taxonomically identified in the Herbarium FES Iztacala-UNAM, leaving a specimen that was integrated into the ethnobotanical collection of the herbarium. Each botanical material was macerated using 70% ethanol (HYCEL). It was left to rest for 15 days in the absence of light in a cool, dry place, with periodic movements. After this time, the plant material was filtered and removed and the solvent was evaporated under reduced pressure in a Rotaevaporator (Hahn Shin Scientific, HS-2000NS). The extract obtained was placed in an amber flask and stored in conditions at 4°C ± 2°C, until use.

PHYTOCHEMICAL SCREENING

To determine the presence of secondary metabolites present in the extracts, colorimetric tests [anthocyanins-HCl (MEYER); flavonoids-Shinoda method; phenolic compounds-ferric chloride reaction (MEYER)] were performed.[11]

ANTIBACTERIAL ACTIVITY BY DISK DIFFUSION METHOD

Four strains were evaluated, all belonging to *S. aureus*, three[12] of them resistant to methicillin, Ciprofloxacin and Erythromycin identified as 5, 10, 39 and *S. aureus* ATCC 6538, taken from the ceparium of the Microbiology laboratory of the University of Ixtlahuaca C.U.I. Each strain was confirmed for purity (Gram stain), microbial identification (reseeding in selective-differential and biochemical media) and sensitivity profiling with four antibiotics [Cefoxitin 30 µg (BD BBL), Erythromycin 15 µg (BD BBL), Gentamicin 10 µg (BD BBL) and Ciprofloxacin 5 µg (BD BBL)].

From the extract obtained by maceration, different concentrations were prepared with Dimethylsulfoxide (DMSO) (EMSURE ACS), as follows: 1) for Ruda, Cancerina and Tapacola were prepared at 25 mg/mL, 50 mg/mL, 100 mg/mL and 300 mg/mL); 2) for Laurel at 25 mg/mL, 50 mg/mL, 100 mg/mL and 500 mg/mL. The extracts were kept refrigerated at 4°C.

The *S. aureus* strains were seeded by cross streaking, in Trypticasein Soy agar (TSA) (DIBICO), incubating at 37°C for 24 hours, from the seeding isolated colonies were taken, placed in a tube with sterile saline solution, equaling with standard tube 0.5 Mc Farland ($1.5x10^8$ CFU/mL) and where the concentration was then corroborated by obtaining readings between 0.08 and 0.13 absorbance in the spectrophotometer visible range and UV 30% (Velab®) at a wavelength of 625 nm. Each standardized inoculum was sown by closed streak with the help of a sterile swab in a box of Müeller-Hinton agar (DIBICO), then the AA grade discs were placed with sterile forceps and distributed on each petri dish, and 10 µL of the extract to be evaluated were placed on each petri dish.

Each assay was performed in triplicate. The results of antibacterial activity were analyzed based on the statistical program SPSS (19), by

means of an ANOVA test (Analysis of variance), Tukey's comparison of means ($p \leq 0.05$).

Controls used: Negative control: sterile disc with 10 µL of DMSO, sterile discs with nothing, extracts impregnated on sterile discs (each control was placed in a tube with nutrient broth (DIBICO), and incubated at 37°C for 48 h); Positive control: Linezolid 30 µg antibiotic disc (BD BBL).

RESULTS AND DISCUSSION

Traditional Mexican Medicine has played an important role in the treatment of various diseases. Evidently, products of plant origin, particularly dry drugs and extracts, went from occupying a predominant place as first line treatment to a disuse, however, in the last decades they have returned to reach an increasing presence in Medicine. This return has been propitiated by the search for alternatives to natural treatments such as the scientific development of phytomedicines in order to reduce adverse effects, even to zero, added to the resistance generated in recent years and the greater knowledge of the risk-benefit of synthetic drugs.[13]

In this work we used medicinal plants, in particular Ruda, Laurel, Cancerina and Tapacola (acquired in the Sonora market in Mexico City), these are used in different countries, being Mexico an entity of interest in these products for their multiple applications in herbal medicine, for example: Ruda, is attributed antiparasitic, cytotoxic, antiseptic and improvement of digestive and circulation problems, among others[14] ; Cancerina, is very popular in the states of Puebla, Morelos and Guerrero for its antiseptic or curative properties in gastritis with infectious etiology and cancer[15] . As for Laurel, it is an ornamental plant, being a very popular plant in the states of Puebla, Morelos and Guerrero for its antiseptic and curative properties or its common use in traditional cooking as a condiment, in addition, its essential oil has great relevance in the cosmetic industry, mainly as a flavoring agent[16] and its antibacterial activity against different microorganisms, including *S. aureus*[17] has also been demonstrated. Finally, the Tapacola plant has some therapeutic uses, in particular, digestive system ailments and in cases of skin lesions and ulcers.

The taxonomic identification of the botanical material was carried out, in order to know the grouping and classification that allowed us to analyze the plant diversity in a rational and methodical way. At the same time, a specimen was left for collection in the herbarium of the FES Iztacala-UNAM (State of Mexico), which was assigned an internal registry number (see Table 1).

Botanical material		Institution	Registration No.
Common name	Scientific name		
Ruda	*Ruta chalepensis L.*	Herbarium F.E.S-Iztacala (UNAM)	2673
Cancerin	*Hippocratea excelsa* Kunth		2598
Laurel	*Litsea glaucescens H.B.K.*		2597
Tapacola	*Waltheria americana L.*		2674

Table 1. Taxonomic identification of botanical material.

Three strains of *S. aureus* resistant to Methicillin (Cefoxitin), Ciprofloxacin, Erythromycin and Gentamicin, isolated from Public Health institutions[12] were used. Also, a strain of *S. aureus* sensitive to Methicillin. The current interest in the study of this microorganism derives from its high frequency in clinical cases, which is worse, because it presents strains resistant to Methicillin. Consequently, it is one of the main causes of nosocomial infection outbreaks in our country. However, it is not only a national problem, but also extends to other countries. The WHO[18] created a list of three categories according to the urgency in which new antibiotics are needed, where *S. aureus* is within priority 2, classified as high urgency.

Excerpt	Concentration	Mean (Inhibition halo mm) ± Standard deviation	Value of p	
Ruda	50 mg	9.16 ± 1.10*	300 mg vs 50 and 100mg	0.000
	100 mg	13.06 ± 2.46*		0.000
	300 mg	21.08 ± 0.84		
Cancerin	25 mg	7.31 ± 0.27*	300 mg vs. 25, 50 and 100mg	0.000
	50 mg	6.44 ± 0.42*		0.000
	100 mg	10.80 ± 0.26*		0.023
	300 mg	12.55 ± 0.98		
Laurel	25 mg	7.96 ± 0.27*	500 mg vs. 25, 50 and 100mg	0.000
	50 mg	8.90 ± 0.27*		0.000
	100 mg	10.16 ± 0.25*		0.002
	500 mg	12.19 ± 0.65		
Tapacola	50 mg	7.84 ± 0.74*	300 mg vs 50 and 100mg	0.000
	100 mg	10.44 ± 0.60*		0.008
	300 mg	13.57 ± 1.48		
T/R	300 mg/ 300 mg	16.83 ± 0.86*	300 mg Rue vs T/R	0.008
C/R	300 mg/ 300 mg	18.81 ± 0.53	300 mg Rue vs C/R	0.277
R-C-L-T combination	50 mg/25 mg/ 25 mg/ 25 mg/ 100 mg	8.30 ± 0.22*	300 mg Rue vs R-C-L-T	0.000
Control (positive)	Linezolid 30 µg	32.12 ± 0.75*	300 mg Rue vs Control	0.000

Table 2. Antibacterial activity of S. *aureus* strain No. 5 (MRSA) against different plant extracts.

*T/R: Tapacola/Ruda, C/R: Cancerina/Ruda, Combination R-C-L-T: Ruda/Cancerina/Laurel/Tapacola. MRSA: Methicillin Resistant *S. aureus*. *Significant difference (TUKEY, $p \leq 0.05$).

Excerpt	Concentration	Mean (Inhibition halo mm) ± Standard deviation	Value of p	
Ruda	50 mg	8.55 ± 0.84*	100 mg vs. 50 and 300mg	0.000
	100 mg	16.11 ± 1.56		0.999
	300 mg	15.67 ± 1.38		
Cancerin	25 mg	7.62 ± 0.15*	300 mg vs. 25, 50 and 100mg	0.040
	50 mg	8.29 ± 0.15		0.208
	100 mg	8.42 ± 0.61		0.280
	300 mg	10.04 ± 0.85		
Laurel	25 mg	7.33 ± 1.08*	500 mg vs. 25, 50 and 100mg	0.000
	50 mg	9.35 ± 0.28*		0.022
	100 mg	10.32 ± 0.80		0.189
	500 mg	12.18 ± 1.09		
Tapacola	100 mg	9.96 ± 1.20*	300 mg vs 100mg	0.033
	300 mg	12.63 ± 0.40		
T/R	300 mg/ 300 mg	19.01 ± 0.89	Ruda 100 mg vs T/R	0.097
C/R	300 mg/ 300 mg	17.64 ± 1.34	Ruda 100 mg vs C/R	0.664
R-C-L-T combination	50 mg/25 mg/ 25 mg/ 25 mg/ 100 mg	9.35 ± 1.11*	Ruda 100 mg vs R-C-L-T	0.000
Control (positive)	Linezolid 30 µg	32.88 ± 0.76*	Ruda 100 mg vs Control	0.000

Table 3. Antibacterial activity of *S. aureus* strain No. 10 (MRSA) against different plant extracts.

T/R: Tapacola/Ruda, C/R: Cancerina/Ruda, Combination R-C-L-T: Ruda/Cancerina/Laurel and Tapacola. MRSA: Methicillin-resistant *S. aureus*. *Significant difference (TUKEY, $p \leq 0.05$).

Excerpt	Concentration	Mean (Inhibition halo mm) ± Standard deviation	Value of p	
Ruda	50 mg	9.92 ± 0.38*	300 mg vs 50 and 100mg	0.000
	100 mg	16.21 ± 2.46*		0.001
	300 mg	24.46± 0.24		
Cancerin	25 mg	6.96 ± 1.31*	300 mg vs. 25, 50 and 100mg	0.004
	50 mg	7.27 ± 1.01*		0.007
	100 mg	10.53 ± 1.37		0.975
	300 mg	11.28 ± 1.04		
Laurel	25 mg	9.53 ± 0.93	300 mg vs. 25, 50 and 100mg	0.295
	50 mg	9.55 ± 0.92		0.307
	100 mg	10.76 ± 0.16		0.569
	500 mg	11.35 ± 1.04		
Tapacola	50 mg	7.58 ± 0.66*	300 mg vs 50 and 100mg	0.004
	100 mg	8.08 ± 0.27*		0.006
	300 mg	12.94 ± 0.25		
T/R	300 mg/ 300 mg	21.43 ± 2.84	Ruda 300 mg vs T/R	0.405
C/R	300 mg/ 300 mg	22.61 ± 1.22	Ruda 300 mg vs C/R	0.854
R-C-L-T combination	50 mg/25 mg/25 mg/ 25mg/ 100 mg	9.65 ± 0.39*	Ruda 300 mg vs R-C-L-T	0.000
Control (positive)	Linezolid 30 μg	32.00 ± 1.02*	Ruda 300 mg vs Control	0.002

Table 4. Antibacterial activity of *S. aureus* strain No. 39 (MRSA) against different plant extracts.

T/R: Tapacola/Ruda, C/R: Cancerina/Ruda, Combination R-C-L-T: Ruda/Cancerina/Laurel and Tapacola. MRSA: Methicillin-resistant *S. aureus*. *Significant difference (TUKEY, $p \leq 0.05$).

Excerpt	Concentration	Mean (Inhibition halo mm) ± Standard deviation	Value of p	
Ruda	50 mg	9.01 ± 2.28*	300 mg vs 50 and 100mg	0.000
	100 mg	14.93 ± 0.47*		0.020
	300 mg	24.44 ± 3.01		
Cancerin	50 mg	8.62 ± 0.82*	300 mg vs 50 and 100mg	0.000
	100 mg	11.30 ± 0.70*		0.007
	300 mg	14.18 ± 0.43		
Laurel	25 mg	8.43 ± 0.39*	500 mg vs. 25, 50 and 100mg	0.000
	50 mg	8.87 ± 0.65*		0.000
	100 mg	9.99 ± 0.23*		0.001
	500 mg	12.86 ± 0.61		
Tapacola	100 mg	8.33 ± 0.36*	300 mg vs 100mg	0.014
	300 mg	10.64 ± 0.59		
Control (positive)	Linezolid 30 μg	32.70 ± 0.96	300 mg vs Control	0.529

Table 5. Antibacterial activity of *S. aureus* strain ATCC 6538 against different plant extracts.

T/R: Tapacola/Ruda, C/R: Cancerina/Ruda, Combination R-C-L-T: Ruda/Cancerina/Laurel and Tapacola. MRSA: Methicillin-resistant *S. aureus*. *Significant difference (TUKEY, $p \leq 0.05$).

Being important the search for alternative therapies, we evaluated the antibacterial activity of the extracts obtained against different strains of *S. aureus*, obtaining that the ethanolic extract of Ruda *(Ruta chalepensis)* showed the greatest effect of the extracts evaluated, against all the strains used, obtaining inhibition halos from 15.67 ± 1.38 mm to 24.46 ± 0.24 mm, at a concentration of 300 mg/mL, a little below the Linezolid control with an inhibition halo of 32.88 ± 0.76 mm, in spite of being below the control, it allows us to define that it is exerting an action on resistant strains that have generated mechanisms to evade the action of antibiotics and it remains to be verified if the observed action is bactericidal or bacteriostatic (See Tables 2-5).

In contrast, compared to other investigations, the concentrations and inhibition halos vary among authors of some reviews; Rodrigues et al.[19] and Mohammed[20] , found no activity in the extract of Ruda *(Ruta chalepensis*) against *S. aureus*; while Ouerghemmi et al.[21] , evaluated the activity in flowers, leaves and stem at a concentration of 5 mg/disc, finding inhibitions from 15 ± 0.6 mm to 16.3 ± 0.6 mm; Bonjar et al.[22] , evaluated the antibacterial activity at a concentration of 20 mg/mL and obtained an average inhibition halo of 10 mm, the same activity was found by Alzoreky et al.[23] , at a lower concentration of 10 mg/mL, Toribio et al.[24] , obtained a halo of 16 mm from a methanolic extraction of 20 g of dried aerial parts, when compared with what was obtained in this work, it is observed that a similar inhibition (9.92 ± 0.38 mm) is achieved, but at a higher concentration (50 mg/mL). At the same time, the greatest antibacterial effect was 24.46 ± 0.24 mm at a concentration of 300 mg/mL, a result similar to the study carried out by Ivanova[25] , which reports an inhibition halo of 23 mm, but at a lower concentration (0.5 mg/mL).

As for, the extract of Cancerina (*Hippocratea excelsa*), shows greater inhibition at a concentration of 300 mg/mL, the inhibition halos were from 10.04 ± 0.85 mm to 12.55 ± 0.65 mm, although there was very low or no inhibition at the minimum concentration of 25 mg/mL.

Nevertheless, the inhibition halos of the extract of Laurel (*Litsea glaucescens*) corresponded to 7.33 ± 1.08 mm up to 12.86 ± 0.61 mm at a concentration of 500 mg/mL, there is an antibacterial effect, however, it is very little, in contrast to the study reported by Ouibrahim et al.[26] , although it differs from the extraction method, using as final product an essential oil and obtaining inhibition halos from 8.4 to 22.4 mm; however, Millezi et al.[17] , evaluated the Laurel extract against four microorganisms, among them *S. aureus*, of all the concentrations, only the highest concentration of 50% had activity, with an inhibition halo of 8 mm, much lower than that obtained in this research work.

On the other hand, the inhibition halos obtained from Tapacola extract (*Waltheria americana*) ranged from 10.64 ± 0.59 mm to 13.57 ± 1.48 mm at a concentration of 300 mg/mL, however, when comparing the results with what has been reported, there is only information that reports antibacterial activity, but there are no studies to support it, only the work elaborated by Okwute et al.[27] , who worked with a different species (*Waltheria indica*), where he obtained an inhibition halo of 23 ± 0.15 mm at a concentration of 10 mg/mL.

On the other hand, it was decided to make combinations among the extracts, in order to know if there is a synergic effect that potentiates the inhibitory effect. Of all the possible combinations, it was observed that Ruda, at a concentration of 300 mg/mL, presented the greatest inhibitory effect with respect to the other extracts, it was combined with Tapacola and Cancerina, which were the extracts with

the best consecutive antibacterial activity, both at a concentration of 300 mg/mL. Also, the possible toxicity that the components of the plants could present was considered, therefore, it was decided to handle minimum concentrations of the four extracts that had antibacterial activity being 50 mg, 100 mg, 25 mg and 25 mg; for Ruda, Tapacola, Laurel and Cancerina, respectively. The results of the 1:1 combination (300 mg/mL) of Ruda with Tapacola, inhibition halos were obtained from 16.83 ± 0.86 mm to 21.43 ± 2.84 mm, when compared with the individual results of Ruda 24.46 ± 0.24 mm and Tapacola 13.57 ± 1.48 mm, the inhibition halos were lower. Mainly, it is observed that there was a synergistic effect with respect to Tapacola extract alone, on the contrary, Ruda extract at a concentration of 300 mg/mL still had the greatest effect despite the combination (see Table 2-4).

In relation to the combination of the four extracts at lower concentrations, inhibition halos of 8.30 ± 0.22 mm to 9.65 ± 0.39 mm were reached, i.e., a decrease in inhibition can be noted, so there is an antagonistic effect by one or more extracts being in combination, so that, it is not recommended to use these extracts in combination as a possible therapy against *S. aureus.*

The results of the antibacterial activity differ from those of several studies in other countries, partly due to the agro-climatic conditions, the age of the plant, the type of species, the type of plant material used (leaves, flowers, stems) that could generate different compounds, certainly necessary for its development, adaptation and survival, which are a fundamental part of the antibacterial activity.

In the phytochemical tests on the extracts evaluated, the presence of phenolic compounds, flavonoids and anthocyanins was determined (see Table 6), possibly conferring the antibacterial effect. Likewise, the presence of phenolic compounds in Ruda, coincide with what Naveda et al.[14] reported, where they performed a phytochemical march on stems, flowers and leaves, consequently, positive to the phenolic compounds test, perhaps, with the antibacterial effect against *S. aureus*. Likewise, flavonoids derived from phenolic compounds, have in their chemical structure a variable number of phenolic hydroxyl groups, which easily penetrate the bacterial cell membrane, bind and precipitate protoplasmic proteins, denaturing them, that is, act as protoplasmic poisons.[28] Another aspect, it is likely, that the location and number of hydroxyl groups in the phenol group are related to the toxicity of the polyphenols against the microorganism.[29] Also, these flavonoids cause bacterial death by inhibiting the synthesis of ribonucleic acid or deoxyribonucleic acid, because they have a planar structure similar to that of the puric and pyrimidic bases, therefore, they can intercalate forming hydrogen bridges with the bases in the single or double chain and in this way the flavones alter the three-dimensional structure of nucleic acids, preventing their proper *de novo* synthesis, as a result, causing reading errors during transcription.[30]

	Excerpt			
Test	Laurel	Cancerin	Ruda	Tapacola
Anthocyanins	+	+	+	+
Flavonoids (Shinoda)	+	+	+	+
Phenolic compounds (iron chloride)	+	+	+	+

Identification of secondary metabolites of extracts (Laurel, Cancerina, Ruda and Tapacola).

+: Presence (qualitative test).

Finally, it is suggested to carry out more studies on Ruda or Tapacola, since they were the extracts with the best activity that inhibited the growth of *S. aureus* strains resistant to Methicillin, especially in the future to be able to use the metabolites of the plant extracts as a therapeutic alternative.

CONCLUSIONS

The four extracts of the different plants had an inhibitory effect so the hypothesis is finally proved. The extract of rue shows the best antibacterial effect against methicillin-resistant *Staphylococcus aureus* and methicillin-sensitive *Staphylococcus aureus*. The extract of rue (*Ruta chalepensis* L.) according to phytochemical assays contains phenolic compounds to which this antibacterial effect is attributed, due to their different known mechanisms of action.

The result of this research contributes to the potential determination of a natural alternative therapy against Methicillin Resistant *S. aureus* (MRSA).

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REFERENCES

1. Bustos J.A, Hamdan A, Gutiérrez M. *Staphylococcus aureus*: la reemergencia de un patógeno en la comunidad. Rev Biomed. 2006; 17:287-305.

2. Guaca Y, Moncayo J, Santacruz J, Álvarez A. Comparación de métodos fenotípico y genotípico en la detección de *Staphylococcus aureus* meticilino resistente en centros hospitalarios de Pereira. Rev. Méd. Risaralda. 2018;24 (2):85-89.

3. Ortiz A, Fang E, Prokocimer P, Flanaganb S, Zhu X. Surveillance of tedizolid activity and resistance: *In vitro* susceptibility of Gram-positive pathogens collected over 5 years from the United States and Europe. Braz J Infect Dis. 2016; 20:184-92.

4. Ochoa V, Guzmán A, Caicedo Y. Infección por *Staphylococcus aureus* meticilino resistente adquirido en la comunidad. Gastrohnup. 2012; 2:46-57.

5. Velázquez M. Surgimiento y diseminación de Staphylococcus aureus meticilinoresistente. Salud Pública Méx. 2005; 47:381-7.

6. Kanafani Z, Fowler V. Staphylococcus aureus infections: new challenges from an old pathogen. Enferm Infecc Microbiol Clin. 2006; 24:182-93.

7. Acosta G, Rodríguez G, Longoria E, Castro M. Evaluación de cuatro métodos para la detección de *Staphylococcus aureus* meticilino-resistente de muestras clínicas en un hospital regional. Rev Salud Públ Méx. 2012; 54(1): 1-6.

8. Fala L. Sivextro (tedizolid phosphate) approved for the treatment of adults with acute bacterial skin and skinstructure infections. Am Health Drug Benefits. 2015; 8:111-5.

9. Kamboj V. Herbal medicine. Curr Sci. 2000; 78:35–51.

10.Lewis K, and Ausubel F. Prospects of plant derived antibacterials. Nat Biotechnol. 2006; 24: 1504-1507.

11. Marcano D, Hasegawa M. Fitoquimica Orgánica. 3ra. ed. Venezuela: Universidad central de Venezuela Consejo de desarrollo científico y humanístico. 2018.

12. Delgadillo V. Determinación de la sensibilidad antimicrobiana de *Staphylococcus aureus* provenientes de dos instituciones de salud pública del Estado de México [Tesis de pregrado]. Universidad de Ixtlahuaca C.U.I, México; 2016.

13. Prieto S, Garrido G, González J, Molina J. Actualidad de la Medicina Tradicional Herbolaria. Rev. CENIC. Cuba. 2004; 35 (1).

14. Naveda G. Establecimiento de un proceso de obtención de extracto de ruda (*Ruta graveolens*) con alto contenido de polifenoles (Tesis de pregrado) Facultad de Ingeniería Química y Agroindustria, Quito; 2010.

15. García A. Manejo social de la Cancerina (*Hippocratea*), planta medicinal de la selva baja caducifolia en la cuenca del rio papagayo, Guerrero, México (Tesis de postgrado). Institución de enseñanza e investigación en ciencias agrícolas. Texcoco, Estado de México; 2009.

16. Delucchi G, Farina E, Torres S. *Laurus nobilis (Lauraceae*) especie naturalizada en la República Argentina. Scielo. 2007;42: 3-4.

17. MilleziI A, CaixetaI D, RossoniII D, CardosoIII M, PiccoliI R. In vitro antimicrobial properties of plant essential oils *Thymus vulgaris*, *Cymbopogon citratus* and *Laurus nobilis* against five important foodborne pathogens. J Food Sci Technol. 2012; 32 (1): 167-171.

18. Organización Mundial de la Salud, comunicado de prensa: La OMS publica la lista de las bacterias para las que se necesitan urgentemente nuevos antibióticos. Ginebra: OMS; 2017. Recuperado de: http://www.who.int/mediacentre/news/releases/2017/bacteria-antibiotics-needed/es/.

19. Rodrigues J, Melo M, Lima M. In vitro Antimicrobial activity of plant in acute otitis externa, Braz J Otorhinolaryngol. 2008; 74 (1): 118.

20. Mohammed A. In Vitro Antibacterial, Antifungal and Other Medical Properties of Endangered Medicinal Plant Seeds. Pharmacol Pharm. 2017; 8:189-204.

21. Ouerghemmi I, Bettaieb I, Zohra F, Bourgou S, Pistelli L, Ksouri R, Marzouk B, Saidani M. Antioxidant and antimicrobial phenolic compounds from extracts of cultivated and wild-grown Tunisian *Ruta chalepensis*. J Food Drug Anal. 2017; 25:350-359.

22. Bonjar S, Aghighi S, Karimi A. Antibacterial an antifungal survey in plants used in indigenous Herbal-Medicine of south east region of Iran. J Biol Sci. 2004;4(3): 405.

23. Alzoreky N, Nakahara K. Antibacterial activity of extracts from some edible plants commonly consumed in Asia. Int J Food Microbiol. 2003;80(3): 223-230.

24. Toribio M, Oriani S, Toso R, Tortone C, Fernández J. *Staphylococcus aureus* sensible a extractos metanólicos obtenidos de plantas nativas de la Provincia de la Pampa, Argentina. Ciencia Veterinaria. 2009; 11(1):14-18.

25. Ivanova A, Mikhova B, Najdenski H, Tsvetkova I, Kostova I. Antimicrobial and cytotoxic activity of *Ruta graveolens.* Fitoterapia. 2005; 76: 344-347.

26. Ouibrahim A, Tlili-Ait-kaki Y, Bennadja S, Amrouni S, Djahoudi A, Djebar M. Evaluation of antibacterial activity of Laurus nobilis L., Rosmarinus officinalis L. and Ocimum basilicum L. from Northeast of Algeria. Afr. J. Microbiol. Res. 2013;7(42): 4968-4973.

27. Okwute S, Olakunle A, Orishadipe A. Phytochemical Screening and in Vitro Antimicrobial Activity of *Waltheria Indica Linn* Leaf Extracts. BS. 2017; 3(5): 86-93.

28. Puupponen P, Nohynek C, Heinonen M, Hopia A, Oskman M. Antimicrobial properties of phenolic compounds from berries. J Appl Microbiol. 2001; 90(4): 494-507.

29. Barranco, S. Búsqueda de compuestos antimicrobianos en *Heterothecainuloides, Gnaphaliumoxyphyllum, Passifloraincarnata, Rosmarinus officinalis* y *Ruta graveolens*, [Tesis de pregrado]. Universidad de la Américas, Puebla; 2004.

30. Cushnie T, Lamb A. Antimicrobial activity of flavonoids. Int J. Antimicrob agents. 2005; 26(5): 343-356.