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ORIGINAL ARTICLE

In vitro Study on the Bactericidal Efficacy of Aqueous Extract of Sage, Rosemary on Streptococcus mutan

Estudio in vitro sobre la Eficacia Bactericida del Extracto Acuoso de Salvia, Romero sobre Streptococcus mutans

Gabriela Ximena Marín-Vega [1], **Dayana Nataly Quishpe-Cedeño**[1], **José Israel Castillo-Gonzalez**[1]

[1]Universidad Central del Ecuador. Facultad de Ciencias Químicas de la P.U.C.E. Quito. Ecuador.

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ABSTRACT

Introduction: the most common oral diseases, such as dental caries and periodontal disease, are infectious and contagious, being caused by diverse factors, the study was carried out in the Laboratory of the Faculty of Chemical Sciences of the P.U.C.E.
Objective: to determine the efficacy of 100 % aqueous extracts of Salvia Officinalis (Sage), Rosmarinus Officinalis (Rosemary) and a combination of both in inhibiting the growth of Streptococcus mutans, using in vitro research methods.
Methods: this was a cross-sectional, comparative and experimental in vitro study, using 15 experimental units (Petri dishes with 5 % human blood agar), distributed in five for each extract, resulting in a total of 60 samples for analysis.
Results: the results indicated that the 100 % aqueous extract of Rosemary (Rosmarinus Officinalis) did not show a significant bactericidal effect on Streptococcus mutans compared to the inhibition halo measurements at 24 and 48 hours.
Conclusions: both the 100 % aqueous extract of Salvia (Salvia Officinalis) and the combination of Salvia and Rosemary showed significant differences in the measurements, showing higher inhibition halos at 48 hours compared to the Rosemary extract alone.

Keywords: Rosmarinus; Salvia; Streptococcus Mutans; Oral Health; Prevention.

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RESUMEN

Introducción: Las enfermedades orales más comunes, como la caries dental y la enfermedad periodontal, son infecciosas y contagiosas, siendo causadas por diversos factores, el estudio fue llevado a cabo en el Laboratorio de la Facultad de Ciencias Químicas de la P.U.C.E.
Objetivo: Determinar la eficacia de los extractos acuosos al 100 % de Salvia Officinalis (Salvia), Rosmarinus Officinalis (Romero) y una combinación de ambos en la inhibición del crecimiento del *Streptococcus mutans*, utilizando métodos de investigación in vitro.
Metodos: se trató de un estudio transversal, comparativo y experimental in vitro, que utilizó 15 unidades experimentales (cajas Petri con Agar Sangre 5 % sangre humana), distribuidas en cinco para cada extracto, resultando en un total de 60 muestras para el análisis.
Resultados: los resultados indicaron que el extracto acuoso al 100 % de Romero (Rosmarinus Officinalis) no mostró un efecto bactericida significativo sobre el Streptococcus mutans en comparación con las mediciones de halos de inhibición a las 24 y 48 horas.
Conclusiones: tanto el extracto acuoso al 100 % de Salvia (Salvia Officinalis) como la combinación de Salvia y Romero presentaron diferencias significativas en las mediciones, mostrando halos de inhibición mayores a las 48 horas en comparación con el extracto de Romero solo.

Palabras claves: Romero; Salvia; Streptococcus Mutans; Salud Bucal; Prevencion.

INTRODUCTION

The most common oral diseases, such as dental caries and periodontal disease, are infectious and contagious, being caused by various factors, including specific microorganisms such as the Streptococcus mutans group, Lactobacillus acidophilus and S. sanguis, among others.[1] For this reason, it is crucial to understand the bacteriostatic or bactericidal properties of certain medicinal plants that could contribute to the control of these infections, from the perspective of Alternative Dentistry.[2]

Dalirsani et al,[3] cited in, evaluated "in vitro" the antibacterial effects of ten plant extracts against Streptococcus mutans. The antimicrobial effectiveness was compared with 0,12 % chlorhexidine. The diameters of each disc were compared with those of chlorhexidine, where it was observed that the inhibition around the rosemary extract discs was 11.5 mm, which is closest to that of chlorhexidine, which was 14,6 mm.18.

Rosmarinus officinalis (Rosemary) is a plant rich in active ingredients and acts on almost every organ in the human body. Its high content of essential oils, whose active ingredients are flavonoids, phenolic acids, and bitter principles, generates a tonic and stimulating effect on the nervous, circulatory, and cardiac systems, in addition to being choleretic, antispasmodic, and diuretic.[4,5,6]

According toPear-Flower Trees,[7] Rosmarinus officinalis extract has demonstrated antibacterial activity against S. mutans, S. aureus, S. casei and Streptococcus mitis, Streptococcus sanguinis, Streptococcus mutans, Streptococcus sobrinus and Lactobacillus casei.

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Based on this background, in the present work a study was carried out on two therapeutic species (Salvia officinalis and Rosmarinus officinalis) frequently used in natural medicine, for this we will apply knowledge from Oral Microbiology, Cariology, Botany and Phytotherapy, in order to discover if the mentioned plants exert a bacteriostatic action on Streptococcus mutans, the main microorganism causing dental caries.

Given the high investment required for oral care in Ecuador, it is imperative to seek natural therapeutic and preventive alternatives that are accessible to the population and that are as effective as those available on the national market. Therefore, the objective of our study is to determine the efficacy of 100 % aqueous extracts of Salvia Officinalis (Sage), Rosmarinus Officinalis (Rosemary), and a combination of both in inhibiting the growth of Streptococcus mutans, using in vitro research methods.

METHODS

- **Cross:** This study was conducted over a specific period of time: January 2016 - June 2016
- **Comparative:** The significant difference between the Sage and Rosemary extracts used for the control of S. mutans was evaluated.
- **Experimental:** The variables were subjected to manipulation under controlled conditions.
- **In vitro:** Because the technique for the experiment was performed in a controlled environment outside of a living organism.

Method for obtaining aqueous extracts of Sage and Rosemary

To prepare the aqueous extracts, 50 g of green leaves, flowers and stems of the aforementioned plants were taken, cut into 0.5 cm pieces and placed in a beaker, then mixed with 200 ml of distilled water and left to soak for 24 h. After this time, they were ground in a home blender for 30 sec and the solution was filtered twice through filter paper No. 1. The obtained solution was considered standard (100 %).

Method for the preparation of the in vitro experiment

For the activation of the Streptococcus mutans strain with reference ATCC 25175, which was purchased commercially, in a lyophilized state, 1 ml of nutrient broth, TSB (Tryptip Soy Broth) was placed. The suspension was pipetted into 4 mL of TSB broth and then inoculated into 5 % human blood agar plates. A swab was performed using the streak exhaustion technique, in addition to the extension seeding technique with the help of a bacteriological loop to isolate the colonies. The incubation conditions were 35 ° C and an atmosphere of 5 % CO2 for 24 and 48 hours.

Once growth was observed in pure colonies, the microorganism was diluted until its turbidity was visually compared with a previously prepared suspension of barium sulfate corresponding to the 0.5 standard on the McFarland scale. The solution was inoculated with sterile swabs into 5 % human blood agar plates, following the full-surface swab inoculation technique, to ensure uniform growth of the microorganism and to establish the effect of the bactericidal substances. Four sterile ¼-inch filter paper discs were placed in each Petri dish, each impregnated with 20 µl (microliters) of 100 % rosemary aqueous extract, 100 % Sage aqueous extract, and finally, 100 % of the combination of both extracts.

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The plates were then incubated in an oven at 35°C under low oxygen pressure conditions for 24 hours, after which the results were recorded. After the following 48 hours, the results obtained from the 60 samples were recorded.

Data collection techniques and instruments
The diameter of the inhibition zones in each Petri dish was measured with a caliper marked with millimeters at the base of the dish at 24 hours and then at 48 hours. The data were recorded on an Excel data collection form. The interpretation categories are:

- **Susceptible:** when it presents a large area of inhibition
- **Intermediate:** if it presents a smaller halo of inhibition
- **Resistant:** if it does not present a halo of inhibition

RESULTS

NON-PARAMETRIC STATISTICS

From the Shapiro-Wilk Normality test in table 1, the Significant values (Level of significance) are less than 0.05 (95% confidence), then we reject Ho, that is, the sample of solutions do NOT come from a population with Normal distribution, then for the analysis, NON-PARAMETRIC tests must be performed.

Nonparametric tests: Wilcoxon signed-rank test
Ho: (null hypothesis) Samples come from populations with the same probability distribution (Similar means)
Ha: (alternative hypothesis) There are differences with respect to the central tendency of the populations.

Table 1. Normality test for rosemary, sage, and sage-rosemary at 100 % at 24 and 48 hours.

Normality tests	Kolmogorov-Smirnov			Shapiro-Wilk		
	Statistical	gl	Next.	Statistical	gl	Next.
ROMERO 100 % 24 Hours	0,509	20	0,000	0,433	20	0,000
ROSEMARY 100 % 48 Hours	0,509	20	0,000	0,433	20	0,000
ROSEMARY SAGE 100 % 24 Hours	0,268	20	0,001	0,858	20	0,007
ROSEMARY SAGE 100 % 48 Hours	0,287	20	0,000	0,881	20	0,018
SAGE 100 % 24 Hours	0,284	20	0,000	0,773	20	0,000
SAGE 100% 48 Hours	0,225	20	0,009	0,860	20	0,008

Source: Author

From the Wilcoxon signed rank test in table 2, Asymptotic significance = 1.000 is greater than 0,05 (95 % confidence), then there are NO differences with respect to the central tendency of the populations, the central measures are similar.

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Table 2. Nonparametric test: rosemary 100 % 48 hours vs. rosemary 100 % 24 hours.

Resumen de prueba de hipótesis

	Hipótesis nula	Test	Sig.	Decisión
1	La mediana de las diferencias entre ROMERO_100_24H y ROMERO_100_48H es igual a 0.	Prueba de Wilcoxon de los rangos con signo de muestras relacionadas	1,000	Retener la hipótesis nula.

Se muestran las significancias asintóticas. El nivel de significancia es ,05.

Elaborated: Gabriela Ximena Marín Vega.
Fountain:Author

From the Wilcoxon Test Table 3 of the signed ranks, Asymptotic significance = 0.000 is less than 0.05 (95 % confidence), then there ARE differences with respect to the central tendency of the populations, the central measures are not similar, at 48 hours the halos are greater for ROSEMARY + SAGE

Table 3. Nonparametric tests: rosemary + sage 100 % 48 hours vs rosemary + sage 100 % 24 hours.

Resumen de prueba de hipótesis

	Hipótesis nula	Test	Sig.	Decisión
1	La mediana de las diferencias entre ROMERO_SALVIA_24H y ROMERO_SALVIA_48H es igual a 0.	Prueba de Wilcoxon de los rangos con signo de muestras relacionadas	,000	Rechazar la hipótesis nula.

Se muestran las significancias asintóticas. El nivel de significancia es ,05.

Elaborated: Gabriela Ximena Marín Vega
Fountain:Author

From the Wilcoxon signed rank test in table 4, Asymptotic significance = 0,000 is less than 0,05 (95 % confidence), then there are differences with respect to the central tendency of the populations, the central measures are not similar, at 48 hours the halos are greater for Salvia at 100 %.

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Table 4 Nonparametric test: 100 % salvia 48 hours vs. 100 % salvia 24 hours.

Resumen de prueba de hipótesis

	Hipótesis nula	Test	Sig.	Decisión
1	La mediana de las diferencias entre SALVIA_100_24H y SALVIA_100_48H es igual a 0.	Prueba de Wilcoxon de los rangos con signo de muestras relacionadas	,000	Rechazar la hipótesis nula.

Se muestran las significancias asintóticas. El nivel de significancia es ,05.

Elaborated: Gabriela Ximena Marín Vega.
Fountain:Author

Kruskal-Wallis test: Three or more samples

From the Kruskal-Wallis Test, Sig. asymptot. = 0,000 is less than 0,05 (95 % confidence interval), then there are differences with respect to the central tendency of the populations. Not all sample means are similar, the pairwise test is performed to verify which ones are different: Comparing pairwise, it is found that at 24 hours the Romero solution at 100 % has lower values compared to the other two solutions. From the Kruskal-Wallis Test, Sig. asymptot. = 0,000 is less than 0,05 (95 % confidence interval), then there are differences with respect to the central tendency of the populations. Not all sample means are similar, the pairwise test is performed to verify which ones are different:

Comparing two by two, it is found that at 24 hours, 100 % Rosemary has lower values in relation to the other two solutions.

DISCUSSION

The present study investigated the bactericidal efficacy of 100 % aqueous extracts of sage, rosemary, and a combination of both against Streptococcus mutans, a major pathogen associated with dental caries. The results reveal important implications for oral health and suggest potential therapeutic applications in the prevention and treatment of dental diseases.

First, our findings demonstrated that 100 % aqueous extract of Rosemary and the combination of Salvia and Rosemary exhibited significant effectiveness in inhibiting the growth of Streptococcus mutans in vitro. This finding is consistent with previous research highlighting the antimicrobial properties of these medicinal plants. The presence of bioactive compounds in Salvia and Rosemary, such as phenolic acids, flavonoids, and terpenoids, could be responsible for their antibacterial activity, supporting their potential as therapeutic agents to combat dental caries. According to Orihuela-Mendoza et al. 2022 in their article, they mention that the aqueous extract of Rosemary showed bactericidal efficacy comparable to extracts containing chlorhexidine, highlighting its promising profile as a natural alternative to commercial mouthwashes.[8,9,10]

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However, it is important to note that the combination of sage and rosemary demonstrated greater effectiveness in inhibiting bacterial growth compared to rosemary extract alone. This suggests a possible synergistic effect between the components of these plants, which could enhance their antimicrobial properties and promote more effective action against Streptococcus mutans.[11,12,13]

In terms of clinical implications, our results suggest that aqueous extracts of sage and rosemary, especially in combination, could be used as adjuvants in the therapy and prevention of dental caries. Their ability to inhibit the growth of Streptococcus mutans offers a promising strategy for reducing the bacterial load in the oral cavity and, consequently, decreasing the risk of developing dental caries.

CONCLUSIONS

This study provides strong evidence of the bactericidal efficacy of 100 % aqueous extracts of sage, rosemary, and sage-rosemary against Streptococcus mutans, highlighting their potential as natural anticariogenic agents in oral care. However, further research is needed to fully understand the underlying mechanisms of action and to evaluate their safety and efficacy in human clinical trials.The bactericidal effect of the aqueous extract of Rosemary (Rosmarinus Officinalis) at 100 % on Streptococcus mutans, there are no differences with respect to the measurements of the halos are similar at 24 and 48 hours. The aqueous extracts of Sage and Rosemary at 100 % on Streptococcus mutans, if there are differences with respect to the measurements they are not similar, at 48 hours the halos are greater for Rosemary + Sage. The bactericidal action of the aqueous extract of Sage (Salvia Officinalis) at 100 %, on Streptococcus mutans, if there are differences with respect to the measurements they are not similar, at 48 hours the halos are greater for Sage at 100 %. Comparing two by two, it is seen that at 24 hours the Rosemary at 100 % has lower values in relation to the other two solutions.

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