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Original Article

Growth inhibitory activity of Streptococcus mutans due to the action of essential oil of Peppermint

Actividad inhibitoria del crecimiento de Streptococcus mutans por acción de aceite esencial de la menta

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Abstract

Objective: to verify the inhibitory effect of the essential oil of peppermint at 25, 50, 75 and 100% concentration at 24, 48 and 72 hours on the Streptococcus mutans strain, considering 0.12 chlorhexidine as a positive control. % and as a negative control, distilled water. **Methodology:** experimental in vitro, descriptive, and longitudinal study. 60 white paper discs were analyzed, divided into six groups of 10 discs each group. The white filter paper disks were dipped in each concentration and placed in the Petri dishes inoculated with Streptococcus mutans. By means of the preparation of the essential oil by steam entrainment process, the peppermint oil was obtained, the inhibition halo was measured to verify the inhibitory efficacy on Streptococcus mutans at 24, 48 and 72 hours, respectively. These data were collected in an Excel matrix and exported to the SPSS program, for the different statistical analyzes. **Results:** it was observed that both for the Kolmogorov test with Lilliefors correction (n <50) or with the Shapiro Wilks test, the data referring to the inhibition halo were adjusted to the normal distribution (p <0.05) in all the groups, even in some groups the values were constant (mint the 4 concentrations and both negative and positive control). In the two tests it was shown that the research hypothesis is fulfilled. That there is a high level of inhibition of the essential oil of peppermint in the four concentrations and at the three times it is possible to have an inhibitory effect, between very sensitive and supersensitive. **Conclusions:** peppermint oil is very effective in inhibiting Streptococcus mutans against 0. 12% chlorhexidine. However, research must continue to learn about the chromatographic characteristics of masses and gases and to detail which component is the one that inhibits the bacteria. In addition, it is proposed to investigate in detail alternative drugs available to the population.

Keywords: Microbial Sensitivity Tests, Mentha, Streptococcus mutans.

Resumen

Objetivo: comprobar el efecto inhibitorio del aceite esencial de la menta al 25, 50, 75 y 100 % de concentración a las 24, 48 y 72 horas sobre la cepa de Streptococcus mutans, teniendo en cuenta como control positivo la clorhexidina al 0,12 % y como control negativo, el agua destilada. **Metodología:** estudio experimental in vitro, descriptivo y longitudinal. Se analizaron 60 discos de papel blanco, divididos en seis grupos de 10 discos cada grupo. Se sumergieron los discos de papel filtro blancos, en cada concentración y se colocaron en las cajas Petri inoculadas con Streptococcus mutans. Mediante la elaboración del aceite esencial por proceso de arrastre de vapor se obtuvo el aceite de la menta, se midió el halo de inhibición para comprobar la eficacia inhibitoria sobre el Streptococcus mutans a las 24, 48 y 72 horas, respectivamente. Estos datos se recolectaron en una matriz de Excel y se exportó al programa SPSS, para los diferentes análisis estadísticos. Resultados: se observó que tanto para la prueba de Kolmogorov con corrección de Lilliefors (n <50) o con la prueba de Shapiro Wilks, los datos referidos al halo de inhibición se ajustaron a la distribución normal (p<0,05) en todos los grupos, incluso en algunos grupos los valores fueron constantes (menta las 4 concentraciones y control tanto negativo como positivo). En las dos pruebas se demostró que se cumple la hipótesis de investigación. Que existe un alto nivel de inhibición del aceite esencial de la menta en las cuatro concentraciones y a los tres tiempos se logra tener un efecto inhibitorio, entre muy sensible y supersensible. **Conclusiones:** el aceite de la menta es muy efectivo para inhibir al Streptococcus mutans frente a la clorhexidina al 0,12 %. Sin embargo, se debe continuar la investigación, para conocer sobre las características cromatográficas de masas y gases y detallar qué componente es el que inhibe a la bacteria. Además, se plantea investigar a detalle medicamentos alternativos al alcance de la población.

Palabras clave: Pruebas de Sensibilidad Microbiana, Mentha, Streptococcus mutans.

Introduction

Streptococcus mutans stands out as the most frequently isolated species in dental plaque. The production of biofilm, known as dental plaque, is a harmful action of Streptococcus mutans on the tooth surface. Consequently, it is recognized as the most significant microorganism in the onset of caries.[1].

It is detailed that the essential oil of plants inhibits Candida albicans among other bacteria that affect humans, especially those that act in the mouth. Increasing the percentage of concentration of the essential oil there would be a greater inhibition.[2] Indeed, it is unknown in what percentage the essential oil of peppermint would inhibit Streptococcus mutans.

In 2012 Villota[3] affirmed that essential oils have been considered fusions with highly complex, volatile components, composed of hydrocarbons and oxygenated compounds such as alcohols [4,] aldehydes, ketones, esters, and acids. While chamba[5] defined that essential oils were complex mixtures of organic compounds, which came from the secondary metabolism of plants.

On the other hand, Usano[6] He asserted that essential oils come from the same chemical family, terpenoids; These have the property of granting multiple pleasant aromas to the oils and are usually found in the bark of the leaves, reaching 2% essential oils, and the fruits, up to 5% essential oils.

According to Palacios[7] At present, approximately 3,000 essential oils are known, of which only 10% are really of commercial interest. Essential oils were used as raw material in the pharmaceutical, food, cosmetic and herbal industry. Although in the pharmaceutical field, terpene-free oils are demanded, because only the pharmacological active principles of plants have been needed[7].

Essential oils: Plants with essential oils are located mainly in the Labiadas (mint, lavender, thyme, lavender, rosemary) and umbelliferous (anise, fennel) families [8]. Mint is a plant of the genus of the Lamiaceae family, it is used as a condiment, to make infusions, also to prepare or accompany alcoholic beverages, also for other types of beverages, as is the case in countries such as Ecuador, Peru, and Bolivia, where it is used to prepare medicinal water [2,9]. In these countries, mint is economically important to produce resins, tannins, and edibles, in many cases they were also cultivated as ornamentals and in pre-Columbian times it also had some aboriginal importance as a medicinal herb[10].

At present, it has been confirmed that plants have antimicrobial, anti-inflammatory, antifungal, antipyretic, decongestant, analgesic, healing purgative properties and they have even been used to treat dental pain; many of these properties are described by empirical healers, not by the scientific method. For this reason, investigations directed towards the elimination or reduction of Streptococcus mutans have been outlined, in fact the use of antimicrobials reappears as one of the components for its control [10,11].

Indeed, it has been shown that when measuring the antifungal inhibition halo of lemon verbena essential oil, it reached a level higher than expected [5]. For this reason, the interest in delving into deepening these studies from the scientific dimension[12].

Materials and methods

Experimental, descriptive and longitudinal study, the population consisted of 60 white paper discs, divided into six groups of 10 discs each group. The white filter paper discs were immersed, in each concentration and placed in the Petri dishes inoculated with Streptococcus mutans. By means of the preparation of the essential oil by steam entrainment process, the peppermint oil was obtained, the inhibition halo was measured to verify the inhibitory efficacy on Streptococcus mutans at 24, 48 and 72 hours, respectively. These data were collected in an Excel matrix and exported to the SPSS program, for the different statistical analyzes. It is worth mentioning that the formula was used for hypothesis contrast studies, aimed at the comparison between two means or proportions.

Steam distillation:

This method has been the most used and the most effective to extract essential oils, in which high temperatures were applied to process the plant[13,] there was a greater control of the distillation rate; This method is usually the most recommended, but it should be mentioned that it is not the only one [10-14].

This process is identified by coupling the plant material and this in turn releases the essence, to later condense. One of the characteristics of essential oils is that they are not soluble in water, which makes it easier for them to be carried away by a stream of water vapor. [15-16].

Experimental research (in vitro)

To measure the inhibitory effect, the Streptococcus mutans strain ATCC 25864 was used; said strain was inoculated in the Petri dishes prepared with lamb blood agar. The discs were placed and subsequently the inhibition halos of each of the discs were measured at the three established times.

Population and sample size

In this study, a simple random sampling was considered, since a Usano model was chosen [6]. In this context, the procedure was followed, in which three kilograms of mint leaf were collected, selected from the middle of the plant towards the top, in such a way that the best green and fresh leaves were guaranteed. It was transferred to the laboratory in a plastic cooler with ice at a temperature of 19 degrees centigrade. Once in the laboratory, it was sectioned into small fragments of 2 cm in diameter, then the essential oil was obtained. Once the oil is obtained, subjected to heating for 30 minutes at 45 ° C temperature to concentrate the essential oil; filtered and then allowed to stand. The dilutions corresponding to 25%, 50%, 75% and 100% concentration were carried out.

Once the oil was obtained, the experiment was carried out, dividing into six groups, each group of 10 discs each, in such a way that the white filter paper discs were immersed in each concentration and placed in the Petri dishes that were inoculated. with Streptococcus mutans, then the halos of each of the discs were measured at 24 hours, 48 hours and 72 hours.

- *The first group of 25%;*
- *the second group of 50%;*
- *the third group 75%;*
- *the fourth group 100%;*
- *the fifth group with the positive control with 0.12% chlorhexidine15 and sixth negative control group with distilled water.*

A sensitivity assessment was also performed, considering the following scale: null <8, sensitive 9 to 12, very sensitive 12-18 and highly sensitive> 18.

Selection of filter paper discs

60 sterile filter paper discs of 6 mm in diameter were used, which were manipulated through sterile forceps and those that showed signs of deterioration were discarded.

Subsequently, sterile filter paper discs of ¼ inch were placed in each Petri dish; prior to being impregnated in 25, 50, 75 and 100% peppermint essential oil solutions each; with the help of a 20 ul (microliter) calibrated pipette and sterile disposable tips that were discarded for each concentration. The same procedure was carried out for the positive control and for the negative control.

Results

It was observed that both with the Kolmogorov test with Lilliefors correction (n <50) or with the Shapiro Wilks test, the data referring to the inhibition halo were adjusted to the normal distribution (p <0.05) in all groups. , even in some groups the values were constant (mint the 4 concentrations and both negative and positive control), so it was necessary to use non-parametric statistics for the inferential analysis (Table 1).

Since a significant difference is considered when the significance p <0.05, it can be inferred that the 50% mint does not differ in its results with the 75% mint, but it does present a difference with respect to the 100% mint and to the positive control. The 75% peppermint differs in its inhibitory capacity from the 100% peppermint and the positive control. The 100% peppermint differs significantly from the positive control. Likewise, it is evident that, despite the numerical differences, this difference is not significant when varying the time (for the same concentration). That is, the inhibitory capacity can be considered stable from 50% concentration (Table 2).

Table 1. Results of the normality test

GROUP	Kolmogorov-Smirnova			Shapiro-Wilk		
	Statistical	Gl	S.I.G.	Statistical	Gl	S.I.G.
Mint 25% (24 h)	0.021	10	0.000	0.021	10	0.011
Mint 25% (48 h)	0.021	10	0.000	0.021	10	0.011
Mint 25% (72 h)	0.021	10	0.000	0.021	10	0.012
Mint 50% (24 h)	0.021	10	0.000	0.010	10	0.000
Mint 50% (48 h)	0.021	10	0.000	0.030	10	0.000
Mint 50% (72 h)	0.021	10	0.000	0.040	10	0.000
Mint 75% (24 h)	0.012	10	0.000	0.009	10	0.000
Mint 75% (48 h)	0.024	10	0.004	0.024	10	0.012
Mint 75% (72 h)	0.024	10	0.004	0.024	10	0.012
Mint 100% (24 h)	0.015	10	0.091	0.020	10	0.025
Mint 100% (48 h)	0.012	10	0.002	0.021	10	0.025
Mint 100% (72 h)	0.012	10	0.002	0.021	10	0.025
Chlorhexidine 0.12% (24 h)	0.010	10	0.001	0.011	10	0.002
Chlorhexidine 0.12% (48 h)	0.010	10	0.001	0.011	10	0.002
Chlorhexidine 0.12% (72 h)	0.010	10	0.001	0.011	10	0.002
Distilled water (24 h)	0.000	10	0.000	0.000	10	0.000
Distilled water (48 h)	0.000	10	0.000	0.000	10	0.000
Distilled water (72 h)	0.000	10	0.000	0.000	10	0.000

Table 2. U Mann Whitney test results

	Mint 50% (48 h)	Mint 50% (72 h)	Mint 75% (24 h)	Mint 75% (48 h)	Mint 75% (72 h)	Mint 100% (24 h)	Mint 100% (48 h)	Mint 100% (72 h)	Chlorhexidine 0.12% (24 h)	Chlorhexidine 0.12% (48 h)	Chlorhexidine 0.12% (72 h)
Mint 50% (24 h)	0.07	0.18	0.06	0.07	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Mint 50% (48 h)		0.18	0.04	0.08	0.06	0.00	0.00	0.00	0.00	0.00	0.00
Mint 50% (72 h)			0.02	0.07	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Mint 75% (24 h)				0.08	0.07	0.00	0.02	0.02	0.00	0.00	0.00
Mint 75% (48 h)					0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mint 75% (72 h)						0.00	0.00	0.00	0.00	0.00	0.00
Mint 100% (24 h)							0.37	0.27	0.00	0.00	0.00
Mint 100% (48 h)								0.02	0.00	0.00	0.00
Mint 100% (72 h)									0.00	0.00	0.00
Chlorhexidine 0.12% (24 h)										0.00	0.00
Chlorhexidine 0.12% (48h)											0.00
Chlorhexidine 0.12% (72h)											

There is a high level of inhibition of the essential oil of peppermint in the four concentrations and at three times it is possible to have an inhibitory effect, between very sensitive and supersensitive (Table 3).

although not on Streptococcus mutans, but in the same way using essential oil. In such a way that the inhibitory effect of peppermint oil against S. mutans turns out to be very effective, compared both with the different authors and with the positive control.

Table 3. Sensitivity to Streptococcus mutans by group (%)

Group	ASSESSMENT			
	Null	Sensitive	Very sensible	Extremely sensitive
Mint 50% (24 h)	80.0%			
Mint 50% (48 h)	80.0%			
Mint 50% (72 h)	80.0%			
Mint 75% (24 h)	50.0%	40.0%		
Mint 75% (48 h)	50.0%	40.0%		
Mint 75% (72 h)	50.0%	40.0%		
Mint 100% (24 h)	10%	20.0%		08.0%
Mint 100% (48 h)	10.0%	20.0%		08.0%
Mint 100% (72 h)	10.0%	20.0%		08.0%
Chlorhexidine 0.12% (24h)		20.0%	10.0%	
Chlorhexidine 0.12% (48h)		20.0%	10.0%	
Chlorhexidine 0.12% (72h)		20.0%	10.0%	

Discussion

In the present investigation, it was observed that both for the Kolmogorov test with Lilliefors correction

(n <50) or with the Shapiro Wilks test it was shown that the research hypothesis is fulfilled. Indeed, a high level of inhibition of Streptococcus mutans by the essential oil of peppermint is evidenced in the four concentrations. In fact, at three times an inhibitory effect was observed, between very sensitive and supersensitive.

Based on statistical evidence, it was determined that the inhibitory capacity of mint essential oil at 100% is important against Streptococcus mutans, greater than the positive control. Solano in 2016[17], showed in its results a maximum of 14 mm of the oily extract of rosemary, on the Streptococcus mutans, on the other hand, the peppermint oil reaches 20 mm at 48 hours. In the same way, Segura obtained 7.32 cm of inhibition at 48 hours and at 80% concentration.[2]

In addition, Villota and Usano [3-6] obtained sensitive results, which reached 13 and 15 cm halo respectively,

Statistical tests demonstrate the inhibitory power of peppermint essential oil. Above all, it exceeds 0.12% chlorhexidine in inhibition time, since it only inhibits up to 48 hours, and the essential oil of peppermint manages to maintain its inhibitory power up to 72 hours. On the other hand, it would be very interesting to also verify the inhibitory effect of the lemon verbena oil after 72 hours[5].

In our study, the fact that it reaches 19 and 20 cm halo, at concentrations of 75 and 100%, at 48 and 72 hours, allows us to confirm the inhibitory effect, both with normality tests and with practical evidence of inhibitory halos. Streptococcus mutans strains are sensitive from 50% concentrations. In the same way, at concentrations of 75 and 100%, the latter two are so effective that it even exceeds the inhibition measure of the positive control, which is 0.12% chlorhexidine.

Conclusions

It is concluded that peppermint oil is very effective in inhibiting Streptococcus mutans against 0.12% chlorhexidine. However, future research should investigate in detail the chromatographic characteristics of masses and gases, to specify which component is the one that inhibits the bacteria. For this reason, it is proposed to investigate in detail alternative drugs available to the population.

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