

Antibacterial Activity of Homeopathic Medications *Lycopodium clavatum* and *Arsenicum album* Against Periodontal Bacteria

Evaluación antibacteriana de los medicamentos homeopáticos sobre bacterias periodontales

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ABSTRACT

There are several controversies regarding the efficacy of homeopathic substances; however, these remedies are used in many countries for the treatment of various pathological conditions. The purpose of this study was to evaluate the *in vitro* antibacterial activity of two homeopathic tinctures *Arsenicum album* (mineral extract) and *Lycopodium clavatum* (plant extract) on the periodontal bacteria *Actinomyces israelii*, *Streptococcus sanguinis*, *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* (*P. gingivalis*). Materials and methods: Equal numbers of bacteria were seeded on agar plates containing enriched media with the homeopathic solutions at 1dH and 1cH dilutions. After 7 days of incubation under anaerobic conditions, colony forming units (CFUs) were counted. The antibacterial effect was calculated based on the total number of CFUs observed on non-tincture containing agar, and on the tincture containing plates. Results: No visible growth of any of the strains was observed on the plates containing *Arsenicum album* at any of the dilutions tested. In contrast, when *Lycopodium clavatum* at 1cH dilution was tested, only *P. gingivalis* was susceptible to this compound. Conclusions: The results suggest that the mineral extract tincture had a greater antibacterial activity than the plant extract tincture, also *Lycopodium clavatum* preparation could be an effective inhibitor of periodontal pathogens bacteria such as *P. gingivalis*.

KEYWORDS

Homeopathy; Plant extracts; Antibacterial activity; Periodontal bacteria;
Arsenicum album; *Lycopodium clavatum*.

RESUMEN

Las medicinas homeopáticas se encuentran rodeadas de controversias, principalmente por la falta de investigaciones científicas y modelos clínicos que demuestren su eficacia, sin embargo, estos remedios son utilizados en muchos países para el tratamiento de diversos padecimientos. El objetivo de esta investigación fue evaluar la actividad antimicrobiana *in vitro* de dos tinturas homeopáticas, el *Arsenicum album* (tintura de origen vegetal), y el *Lycopodium clavatum* (tintura de origen mineral) sobre las bacterias periodontales *Actinomyces israelii*, *Streptococcus sanguinis*, *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans* y *Porphyromonas gingivalis*. Materiales y métodos: cantidades iguales de bacterias se sembraron en platos con agar y medio enriquecido con las tinturas homeopáticas, a dos diferentes diluciones 1dH y 1cH. Posteriormente, las unidades formadoras de colonias (CFU) se contaron después de 7 días de incubación bajo condiciones de anaerobiosis, tanto en los platos que contenían las tinturas, como en los platos control. Resultados: Para todas las cepas bacterianas utilizadas en el presente estudio, los platos con *Arsenicum album*, y *Lycopodium clavatum* a una dilución 1dH no mostraron crecimiento visible de CFU. En el caso de la tintura de *Lycopodium clavatum*, solamente la cepa de *P. gingivalis* mostró inhibición a una dilución de 1cH, por el contrario, el *Arsenicum album* mostró una inhibición de todas las cepas utilizadas a la misma dilución. Conclusiones: Los resultados del presente estudio sugieren que la tintura de origen mineral tiene una mayor actividad antibacteriana, comparada con la tintura de origen vegetal. Además, la tintura de *Lycopodium clavatum* puede considerarse como un inhibidor efectivo para bacterias periodontopatógenas como la *P. gingivalis*. Se necesita un mayor número de estudios *in vitro* e *in vivo* para validar estos resultados.

PALABRAS CLAVE

Homeopatía; Extractos de plantas; Actividad antibacteriana; Bacterias periodontales;
Arsenicum album; *Lycopodium clavatum*.

INTRODUCTION

Periodontitis is a chronic destructive inflammatory disease involving Gram-negative bacteria, and the inflammatory response from the host; this chronic and inflammatory condition threatens the integrity of the tooth-supporting tissues, collectively known as the periodontium, they include the gingiva, the periodontal ligament and the alveolar bone (1). The severe form of this disease affects approximately 10-15% of adults worldwide, and if the disease is left untreated, periodontitis may not only cause tooth loss, but can also affect systemic health by increasing the patient's risk for diabetes mellitus, pneumonia, heart disease and preterm birth (2,3).

It has been shown that there are specific associations (complexes) among bacteria in dental biofilms, these microbial complexes represent the sequential colonization and the composition of the most important biofilm formed in the oral cavity; the subgingival dental plaque. Some bacterial strains, mainly belonging to the genus *Actinomyces* (blue complex) and *Streptococcus* (yellow complex) have been identified as early colonizers of the dental surface. The second group of bacteria that functions as a bridge between the early and late colonizers are formed by species belonging to the green, purple, and orange complexes (i.e. genus *Fusobacterium*, *Prevotella*, *Capnocytophaga sputigena* and *Eikenella corrodens*). Finally, the third group of species that appear at late stages

in biofilm development and that are considered actual periodontal pathogens are species of the red complex; *Porphyromonas gingivalis* (*P. gingivalis*), *Tannerella forsythia* and *Treponema denticola* (*T. denticola*) (1,4).

From a clinical point of view, *Actinomyces israelii* (*A. israelii*) and *Streptococcus* (*S. sanguinis*) have been considered beneficial colonizers of the dental plaque. On the other hand, *P. gingivalis* has been associated with periodontal infections, and a higher proportion of this strain has been found in sites with gingival bleeding, inflammation, and suppuration on individuals with advanced periodontitis (4). Also, *Prevotella intermedia* (*P. intermedia*) is closely related to inflammatory conditions of the oral cavity, and the presence of *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) has been related to specific periodontal infections (1).

In the field of dentistry, homeopathy has been used as an adjuvant to conventional therapy, in different presentations, such as, liquids, drops, tablets, pills, granules, or creams. Even more, preparations derived from natural products of plants and mineral substances are currently employed for pathological conditions of the oral cavity, such as periodontitis and dental abscess.

However, there is insufficient information on the antibacterial properties of these products against pathogens responsible for dental infections. Also, the majority of published literature regarding homeopathy has been conducted in the context of toxicology and the immune response under allergic conditions (5).

Among homeopathic remedies available, *Arsenicum album* and *Lycopodium clavatum*, have been used to treat oral abscesses and gingivitis

(6,7), but their activity against bacteria of clinical relevance in dentistry has not been fully explored. Additionally, reports regarding the antimicrobial properties of such remedies are scarce, although they are prescribed for representative symptoms of bacterial related diseases such as gingivitis and periodontitis (6,8).

On the other hand, drug-resistant bacterial strains have been creating serious treatment issues; therefore, the search for new antimicrobial substances effective against resistant pathogenic microorganisms to conventional antibiotic treatments is necessary. Natural resources have been exploited in the last years, and plant compounds could be an alternative source of new or at least coadjuvant treatment (9).

The objective of this investigation was to determine the in vitro antimicrobial efficiency of *Arsenicum album* and *Lycopodium clavatum*, against five important periodontal bacteria stains associated with different periodontal health status.

MATERIALS AND METHODS

BACTERIAL STRAINS

Five bacterial strains from the American Type Cell Culture Collection were used in this study and are listed in Table 1. All strains were grown on HK enriched agar plates, Brain Heart Infusion Agar (26 g/L; BBL, Becton-Dickinson and Co., Sparks, MD) Trypticase Soy Agar (20 g/L; BBL, Becton, Dickinson and Co., Sparks, MD), Yeast extract (10 g/L; BBL, Becton, Dickinson and Co., Sparks, MD), supplemented with 5 µg/mL of Hemin (Sigma-Aldrich, St. Louis, MO), 0.3 µg/mL of Menadione (Sigma-Aldrich, St. Louis, MO), and 5% defibrinated sheep blood (Microlab, Mexico City)], at 35°C under anaerobic conditions (80% N, 10% CO₂ and 10%

N2). The bacterial strains were incubated for five days, and pure cultures of each strain were used in the experiments.

ASSESSMENT OF THE ANTIMICROBIAL ACTIVITY

For the antimicrobial analysis, HK enriched agar plates were prepared by adding dilutions of the mother tinctures of *Arsenicum album* or *Lycopodium clavatum*, to obtain decimal (1dH) and centesimal (1cH) dilutions of each tincture in the agar plates.

The growth from 5 day-cultures of each strain was harvested and placed in individual sterile Eppendorf tubes, containing 900 µL of enriched broth (EB) prepared with Brain heart broth (26 g/L), Trypticase soy broth (20 g/L), Yeast extract (10 g/L), supplemented with Hemin (5 µg/mL), and Menadione (0.3 µg/mL). Each tube was adjusted to have an optical density of 1 at 600 nm in a spectrophotometer. Five serial dilutions of each strain were done, and 100 µL of each dilution were plated on the HK enriched agar plates with or without the homeopathic solutions.

The homeopathic medications of *Arsenicum album* and *Lycopodium clavatum* from Similia laboratories (Similia, México, D.F.) were used. The 1cH and 1dH dilutions were prepared according to Mexican Pharmacopoeia (10, 11). Positive control

plates were prepared by adding 32 µg/mL of tetracycline, and negative control plates were the HK agar plates only. Another control was included by making HK enriched agar plates added with 1cH and 1dH dilutions of the same alcohol (87°) used to prepare the mother tinctures.

Plates were incubated at 35°C under anaerobic conditions for seven days. After incubation, the number of Colony Forming Units (CFUs) was counted using the colony counter aCOlyte 3 HD (Synbiosis, USA), accordingly to the manufacture's instruction. All experiments were performed in triplicate, with three replicates each time.

The antibacterial effectiveness of the homeopathic tinctures was expressed as the inhibition ratio according to the following equation:

$$\text{Inhibition Ratio (\%)} = \left(\frac{A1 - A2}{A1} \right) \times 100$$

Where,

A1=total number of CFUs on the plates without the homeopathic remedies (negative control).

A2=total number of CFUs in the presence of a bacterial growth inhibitor; diluted tincture, antibiotic (positive control), or the diluted alcohol containing plates.

Table 1. Reference strains used for bacterial cultures.

Species	Strain*
<i>Actinomyces israelii</i>	12102
<i>Aggregatibacter actinomycetemcomitans</i> serotype b	43718
<i>Porphyromonas gingivalis</i>	33277
<i>Prevotella intermedia</i>	25611
<i>Streptococcus sanguinis</i>	10556

*American Type Culture Collection, Rockville, MD.

DATA ANALYSIS

Data are presented as the mean \pm standard error of the mean (SEM), and as the percentage of inhibition. Significant differences in the number of CFU's in each type of media were calculated using the Student's t-test, and significant differences between groups were determined using Bonferroni's Modification of Student t-test.

RESULTS

ANTIMICROBIAL EFFECT ON THE TOTAL NUMBER OF COLONY FORMING UNITS (CFU'S)

The results revealed that the tinctures from *Arsenicum album* and *Lycopodium clavatum* are potent antimicrobials against five bacterial strains from the microbiota of the oral cavity associated with different periodontal health status. The total number of CFU's and the percentage of inhibition or periodontal bacteria using two different dilutions (1dH and 1cH) of the tinctures are presented in Tables 2 and 3.

The plates contained the *Arsenicum album* and *Lycopodium clavatum* tinctures showed significant activity against the bacterial growth at 1dH, and 1cH dilution in comparison with the plates contained the alcohol at 87° alone ($p < 0.05$). The *Arsenicum album* tincture, exhibited a strong antimicrobial effect (100% of growth inhibition) similar to tetracycline; a common antibiotic used for the treatment of periodontitis ($p < 0.05$; Table 3). Based on the results of the present study the antibacterial action of the tincture from *Arsenicum album* was more pronounced in all the bacterial strains used, compared with the tincture from *Lycopodium clavatum* (Table 2).

According to the manufacturer, the tinctures are ethanol extracts prepared with 87° alcohol. With the purpose of comparison, in the present study, the 87° alcohol alone was also tested on the agar plates. The 87° alcohol alone showed more than 90% inhibition of the CFU's at 1dH for the Gram-negative bacteria, *A. actinomycetemcomitans* serotype b (96.99%), *P. gingivalis* (91.54%), and *P. intermedia* (95.54%). It also showed good inhibition of CFU's for the Gram-positive, *S. sanguinis* (84.96%), and *A. israelii* (80.36%) (Table 2 and 3). However, for the 1cH dilution, less than the 9% of the growth inhibition was observed in all the strains tested, confirming the antibacterial properties of the homeopathic tinctures used in the present study.

ARSENICUM ALBUM TINCTURE

This tincture showed a 100% of inhibition of the CFU's of all the Gram-negative and Gram-positive periodontal bacteria tested, regardless the dilution used, and its activity was identical to the tetracycline used as positive control. It appears that the antibacterial action of the mineral origin extracts, i.e. *Arsenicum album*, could be much more efficient than plant origin extracts like *Lycopodium clavatum*.

LYCOPODIUM CLAVATUM TINCTURE

In the case of the *Lycopodium clavatum* tincture, the 1dH dilution showed strong activity (100% of inhibition) on the Gram-negative strains; *A. actinomycetemcomitans* serotype b, *P. gingivalis*, and *P. intermedia*. Additionally, an excellent inhibitory effect was observed against the Gram-positive *S. sanguinis* and *A. israelii* (100 and 99.17% of inhibition; Table 3).

Table 2. Antimicrobial activity (inhibition ratio) of *A. album* and *L. clavatum* against oral microorganisms.

Bacterial strain	Percentage of inhibition						
	Tetracycline	Alcohol		<i>A. album</i>		<i>L. clavatum</i>	
		1 dH	1 cH	1 dH	1 cH	1 dH	1 cH
<i>A. israelii</i>	100*	80.36*	3.96	100*††	100*†	99.17*††	85.97*†
<i>A. actinomycetemcomitans</i>	100*	96.99*	8.65	100*††	100*†	100*††	58.65*†
<i>P. gingivalis</i>	100*	91.54*	1.65	100*††	100*†	100*††	27.98*†
<i>P. intermedia</i>	100*	95.54*	0.2	100*††	100*†	100*††	37.4*†
<i>S. sanguinis</i>	100*	84.96*	8.65	100*††	100*†	100*††	58.65*†

dH: decimal dilution, cH: centesimal dilution.

* P<0.05 comparing tincture with non-tincture containing plates.

† P<0.05 and †† P<0.01 comparing alcohol containing at 1cH and 1dH dilutions against tincture containing plates.

Table 3. Comparative bacterial growth inhibition of homeopathic tinctures and alcohol tested.

Bacterial strain	Percentage of inhibition						
	Tetracycline	Alcohol		<i>A. album</i>		<i>L. clavatum</i>	
		1 dH	1 cH	1 dH	1 cH	1 dH	1 cH
<i>A. israelii</i>	+++	-++	---	+++	+++	+++	-++
<i>A. actinomycetemcomitans</i>	+++	+++	---	+++	+++	+++	---
<i>P. gingivalis</i>	+++	+++	---	+++	+++	+++	---
<i>P. intermedia</i>	+++	+++	---	+++	+++	+++	---
<i>S. sanguinis</i>	+++	-++	---	+++	+++	+++	---

+++ , 100-90% strong inhibition

-++ , 60-89% moderate inhibition

--+ , 30-50% weak inhibition

--- , <29% negative inhibition

DISCUSSION

Studies with a similar approach have focused on the antimicrobial effects of plant extracts on periodontal bacteria. For example, Lauk *et al.*(12) evaluated the antimicrobial effect of *Althaea officinalis*, *Arnica montana*, *Calendula officinalis*, *Hamamelis virginiana*, *Illicium verum* and *Melissa officinalis* against 18 bacterial strains isolated from the periodontal pocket's gingival crevicular fluid of periodontal patients. Their results included five strains of *P. gingivalis* and three strains of *Actinomyces odontolyticus*, and showed a good antimicrobial activity of the extracts of *Arnica*

montana, and *Hamamelis virginiana*, which have been already studied for their use in dentistry (12,13). In the current study, we observed a strong *in vitro* antimicrobial activity for the tinctures of *Arsenicum album* and *Lycopodium clavatum*, and the results suggested that the compounds could be a potential target for drug development.

Arsenicum album tincture is obtained from dilutions of Arsenic trioxide (As₂O₃) in a potentiation process to the point in which it is supposed to lose its toxic properties. Many skeptics of homeopathy dismiss its credibility, mostly because the homeopathic remedies are

made by repeated dilutions that lead them to contained extremely low or undetectable amounts of the active substance, therefore, at such small concentrations of the compounds, their effects must be small and weak (14).

However, the effect that homeopathic preparations of *Arsenicum album* showed against intoxication has been well documented (15). On this regard, various studies have suggested that low molecular dose effects appear to be opposite to those caused by high molecular doses (16).

As for the results presented in this study, they showed that dilution of the *Arsenicum album* tincture does not cause a significant change in its antimicrobial activity *in vitro*. Recent physicochemical studies of Chikramane et al. detected nanoparticles of the starting raw material (metals) by transmission electron microscopy in extremely high dilutions prepared with traditional homeopathic manufacturing (17), and our results appear to be consistent with their observations.

It is worth to mention that in the case of *Lycopodium clavatum* tincture, although the inhibition ratio was lower compared to those of the antibiotic tetracycline, the inhibition of the CFU's for the tincture confirmed its antibacterial activity. These results are of interest since they have been obtained with a dilution of the tincture and such dilutions could be considered to have a good potency level.

The *Lycopodium clavatum* is deemed to be a montane plant in the Neotropics, with high alkaloid content, used in folk medicine for the treatment of conditions related to the central nervous system and for motor disorders. Their properties have been extensively reviewed, and it is well accepted that the alkaloids of the plant are responsible for the anticholinesterase and antioxidant activities

in diseases such as Alzheimer (18). However, the results of the present study regarding the antimicrobial effects against the periodontal bacteria, suggest that more studies to evaluate the antimicrobial properties of the plant should be carried out.

CONCLUSIONS

In the present study, we found that both of the compounds used could be effective inhibitors of periodontal pathogens like *P. gingivalis*. However, the mineral extract tincture *Arsenicum album* was more active in the bacterial growth inhibition than the plant extract tincture *Lycopodium clavatum*. Additionally, our results indicate that high potencies were less effective than low potencies, at least with the conditions tested.

The results of the present study showed that is worthy to investigate the potential of other therapies or new compounds that could effectively treat infectious diseases like periodontal infections, and such studies represent a contribution to reduce antibiotic resistance.

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CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

COMPLIANCES WITH ETHICS GUIDELINES

This article does not contain any studies with human or animal subjects performed by any of the authors.

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DISCLAIMER

The content of the manuscript is solely responsibility of the authors and does not necessarily represents the official views of the National Autonomous University of Mexico.

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