ORIGINAL RESEARCH

Antibacterial Efficacy of *Matricaria recutita* Essential Oil against *Porphyromonas gingivalis* and *Prevotella intermedia*: *In Vitro* Study

Román Mendoza[1], Daniel Alvitez-Temoche[2], Lucy Chiong[3], Herbert Silva[4], Franco Mauricio[5], Frank Mayta-Tovalino[6]

Abstract

Aim: To evaluate *in vitro* the antibacterial efficacy of *Matricaria recutita* (chamomile) essential oil at 50 and 75% against *Porphyromonas gingivalis* ATCC 33277 and *Prevotella intermedia* ATCC 25611 at 24 and 48 hours.

Material and methods: The sample consisted of 80 discs and Mueller–Hinton Agar, the medium chosen for the culture. To determine the bacterial sensitivity, discs were placed in each Petri dish with concentrations of essential oil at 50 and 75%, distilled water and 0.12% chlorhexidine. Subsequently, the inhibition halos were measured in millimeters at 24 and 48 hours after culture, with the Kirby–Bauer disk diffusion method.

Results: In groups treated with *Porphyromonas gingivalis*, measurements at 24 and 48 hours yielded 22.14 ± 2.61 and 22.63 ± 2.67 mm for 0.12% chlorhexidine, 18.90 ± 0.41 and 19.22 ± 0.54 mm for 75% essential oil, and 15.55 ± 0.45 and 15.77 ± 0.46 mm for 50% essential oil, respectively. No statistically significant differences were observed among the groups ($p > 0.05$).

Conclusion: No significant differences were found between the antibacterial efficacy of 0.12% chlorhexidine and 50 and 75% essential oil of *Matricaria recutita* on *Porphyromonas gingivalis* and *Prevotella intermedia* at 24 and 48 hours.

Clinical significance: The study demonstrates that essential oil derived from *Matricaria recutita* may effectively combat bacteria associated with periodontal disease. This discovery has the potential to impact dental practice by introducing a natural treatment option. Further research is warranted to fully elucidate the clinical significance and potential applications of this finding.

Keywords: Antibacterial efficacy, *Matricaria recutita*, *Porphyromonas gingivalis*, *Prevotella intermedia*.

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Introduction

Oral health problems are conditioned by individual factors, such as biological and genetic factors, but also by other factors, such as cultural, social, economic, environmental, and geographic factors.[1,2] Rural populations suffer greater difficulty in accessing oral health services that provide adequate oral health education to strengthen prevention and facilitate timely treatment, with negative consequences for the oral health of these populations.[3]

Many factors have contributed to the increase of bacterial strains that are multiresistant to antibiotics, either due to prolonged or inadequate use of these drugs, which generates a serious clinical and public health problem.[4] This makes it necessary to adequately plan alternative modalities to combat the various infections of the oral cavity, together with modern medicine, in a safe, effective, and economical manner, especially in populations of developing countries.[5,6]

Medicinal plants play a prominent role in the health and well-being of many populations, particularly in Peruvian communities, where thousands of plant species are utilized for food, construction, handicrafts, and their therapeutic or toxic properties. With the advent of industrial advancements, it is now possible to extract compounds from these plants for the development of effective medications for various ailments.[7]

One of the main oral diseases is periodontitis, of multifactorial origin, associated with dysbiotic plaque biofilm, whose main characteristics are to be a chronic inflammatory disease and to produce the progressive destruction of the supporting tissues of the teeth.[1] It is now widely accepted that there are many groups of bacteria involved in the development of periodontal disease. The interplay between bacterial aggression and host defense mechanisms significantly influences the development of periodontal damage,[8,9] as noted in the literature. Gram-negative bacilli, including *Actinobacillus actinomycetemcomitans*, *Tannerella forsythia*, *Prevotella intermedia*, *Fusobacterium*, and *Porphyromonas gingivalis*, are among the primary microorganisms implicated in periodontal disease. The etiology requires complex interactions between these microorganisms to establish themselves and create their niches in the oral cavity.[9,10] *P. gingivalis* seems to play a very important role in the etiology, pathogenesis, and progression of inflammatory processes of periodontitis, being able to make the transition from harmless colonizers of the subgingival environment to virulent pathogens.[10] *P. intermedia* is one of the main pathogens

[1–5]Academic Department, Faculty of Dentistry, Universidad Nacional Federico Villarreal, Lima, Peru

[6]Postgraduate Department, Change Research Working Group, Faculty of Health Sciences, Universidad Científica del Sur, Lima, Peru

Corresponding Author: Frank Mayta-Tovalino, Postgraduate Department, Universidad Cientifica del Sur, Lima, Peru, Phone: +51 1 317-1000, e-mail: fmaytat@cientifica.edu.pe

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in cases of periodontitis, inducing the destruction of periodontal tissue, being more prevalent in patients with diabetes.[11]

Matricaria recutita is a vegetable of European origin, but it is produced in an important way in the continents of America and Africa.[12] Clinical studies showed antimicrobial and anti-inflammatory properties of this plant, when used as a mouthwash, with an efficacy like 0.12% chlorhexidine in the treatment of patients with gingival bleeding, as well as significant improvement in periodontal health parameters in patients with periodontitis.[13,14] *Matricaria recutita* presents chamazulene, α-bisabolone, α-farensen, and β-farensen as important volatile components, while among the non-volatile components, flavonoids are the most important for their anticarcinogenic effect since they present oxygen radical scavenging activity. They also present other antioxidants, such as phenolic compounds, chlorophylls, and liposoluble carotenoids.[15]

The need to delve deeper into this subject is the lack of information on the antibacterial properties of the essential oil of *Matricaria recutita*. Therefore, it is important to evaluate the antibacterial potential of chamomile essential oil against various pathogenic bacteria. Thus, the aim of the study was to evaluate the antibacterial efficacy of *Matricaria recutita* essential oil (MREO) against the strains of *Porphyromonas gingivalis* ATCC 33277 and *Prevotella intermedia* ATCC 25611.

Materials and Methods

Study Design

This *in vitro* experimental study was conducted in 2022 at the Microbiology Laboratory of the Faculty of Dentistry at *Universidad Nacional Federico Villarreal* in Lima, Peru.

Sampling Method and Selection Criteria

The study utilized a sample of $n = 80$ Whatman paper disks (Merck KgaA; Darmstadt, Germany), divided among eight groups. The sample size was calculated using the mean comparison formula in the Stata 15® program (College Station, Texas 77845 USA), with an Alpha of 0.05 and a Beta of 0.8. The disks were embedded with (MREO), 0.12% chlorhexidine, and 0.9% sodium chloride solution (NaCl 0.9%), and divided into eight groups based on inclusion and exclusion criteria.

Criteria for Inclusion and Exclusion

Inclusion criteria were Petri dishes containing strains of *Porphyromonas gingivalis* or *Prevotella intermedia* that had been inoculated, and MREO with concentrations of 50% or 75%. Exclusion criteria were Petri dishes containing contaminated or altered strains of the same bacterias essential oils not classified under the *Matricaria recutita* species, and MREO with concentrations other than 50% or 75%.

Allocation

The groups were allocated as follows: Group I–IV were inoculated with *Porphyromonas gingivalis* and treated with 50% MREO, 75% MREO, 0.12% chlorhexidine, or NaCl 0.9%, respectively. Group V–VIII were inoculated with *Prevotella intermedia* and treated with the same agents. Group VIII: NaCl 0.9% inoculated with *Prevotella intermedia.*

Botanical Classification

The plant samples were submitted to the "Museo de Historia Natural de la Universidad Nacional Mayor de San Marcos" for verifying taxonomic classification. The voucher number for the classification was 069-2022-USM. The plant was classified as *Matricaria recutita L.*, belonging to the order *Asterales*, family *Asteraceae*, genus *Matricaria*, and species *Matricaria recutita L.*

Preparation of Essential Oil

The plant specimens were transported to the Central Laboratory Clinibroq EIRL, where 2000 gm of raw material was obtained, identified, and verified for any signs of alteration. The specimens were stored in Kraft paper bags to prevent environmental influence on their properties. They were then washed, dried at room temperature, crushed, and subjected to water vapor distillation to extract the essential oil. The essential oil was prepared by hydrodistilling sterilized specimens using a Clevenger type equipment for four hours in 500 mL of water. The yield of MREO was approximately 1.0%, and it was stored at 4°C until use at concentrations of 50 and 75%.

Microbiological Seeding and Measurement

Bacterial strains were obtained from *Laboratio GenLab* (Lima-Peru). *Porphyromonas gingivalis* ATCC 33277 and *Prevotella intermedia* ATCC 25611 were seeded on Petri dishes prepared with Mueller–Hinton agar and incubated at 37°C in an anaerobic environment. Four disks per Petri plate were prepared, treated with either 75% MREO, 50% MREO, 0.12% chlorhexidine, or NaCl 0.9%. Measurements were taken at 24 and 48 hours intervals using the Kirby-Bauer disk diffusion method. A Mitutoyo 500-196 Digital Vernier Caliper from Japan was used to measure the diffusion halos in millimeters. A total of forty Petri dishes, each containing two disks embedded with the experimental substances, were used for the measurements.

Statistical Analysis

The process of collecting data involved the use of an Excel database, while Stata 15® software [located in College Station, Texas, USA] was utilized for conducting statistical analysis. Normality of data distribution was evaluated through the Shapiro-Wilk test. In order to compare groups, the Kruskal–Wallis test and Mann–Whitney *U* test were employed for inferential analysis, with a significance level set at $p < 0.05$.

Results

At the 24-hour measurement, the greatest antimicrobial activity against *Porphyromonas gingivalis* was observed in the 0.12% chlorhexidine group, with a mean inhibition zone of 22.14 ± 2.61 mm. The 75% concentration of MREO produced a mean inhibition zone of 18.90 ± 0.41 mm, while the 50% concentration yielded a mean inhibition zone of 15.55 ± 0.45 mm (Table 1).

At the 48-hour measurement, the greatest antimicrobial activity against *Porphyromonas gingivalis* was observed in the 0.12% chlorhexidine group, with a mean inhibition zone of 22.63 ± 2.67 mm. The 75% concentration of MREO produced a mean inhibition zone of 19.22 ± 0.54 mm, while the 50% concentration yielded a mean inhibition zone of 15.77 ± 0.46 mm (Table 1).

In the evaluation against *Prevotella intermedia*, the greatest antimicrobial activity at the 24-hour measurement was observed in the 0.12% chlorhexidine group, with a mean inhibition zone of 21.69 ± 0.63 mm. The 75% concentration of MREO produced a mean inhibition zone of 15.88 ± 0.3 mm, while the 50% concentration yielded a mean inhibition zone of 10.27 ± 0.18 mm (Table 2).

Table 1: Comparison of 24-hour and 48-hour measurements against *Porphyromonas gingivalis*

Product	*Concentration (%)*	*Time*	*Mean*	*SD*	*Min*	*Max*	*p**	*p***
Matricaria recutita essential oil	50	24 hours	15.55	0.45	14.96	16.29	0.547	0.435
		48 hours	15.77	0.46	15.17	16.67	0.678	
	75	24 hours	18.90	0.41	18.07	19.75	0.084	0.028
		48 hours	19.22	0.54	18.19	20.3	0.361	
Chlorhexidine	0.12	24 hours	22.14	2.61	19.97	29.14	0.001	0.353
		48 hours	22.63	2.67	20.41	29.75	0.001	

All groups were measured in mm. *Shapiro–Wilk test $p > 0.05$ for statistical significance, **Mann–Withney's *U* test $p < 0.05$ for statistical significance

Table 2: Comparison of measurements at 24 and 48 hours against *Prevotella intermedia*

Product	*Concentration (%)*	*Time*	*Mean*	*SD*	*Min*	*Max*	*p**	*p***
Matricaria recutita essential oil	50	24 hours	10.27	0.18	9.98	10.56	0.369	0.005
		48 hours	10.49	0.17	10.31	10.78	0.158	
	75	24 hours	15.88	0.29	15.05	16.10	0.001	0.003
		48 hours	16.10	0.33	15.25	16.51	0.002	
Clorhexidine	0.12	24 hours	21.69	0.63	20.23	22.15	0.002	0.052
		48 hours	22.07	0.69	20.49	22.71	0.017	

All groups were measured in mm. *Shapiro-Wilk test. $p > 0.05$ for statistical significance, **Mann–Whitney's *U* test. $p < 0.05$ for statistical significance

Table 3: Comparison of the effect of chlorhexidine 0.12% and *Matricaria recutita* essential oil 50 and 75%

			Time		
Microorganism	*Product*	*Concentration (%)*	*24 hours*	*48 hours*	*p**
Porphyromonas gingivalis	*Matricaria recutita* essential oil	50	15.55 ± 0.45	15.77 ± 0.46	
		75	18.9 ± 0.41	19.22 ± 0.54	>0.05
	Chlorhexidine	0.12	22.14 ± 2.61	22.63 ± 2.67	
Prevotella intermedia	*Matricaria recutita* essential oil	50	10.27 ± 0.18	10.49 ± 0.17	
		75	15.88 ± 0.29	16.1 ± 0.33	>0.05
	Chlorhexidine	0.12	21.69 ± 0.63	22.07 ± 0.7	

*Kruskall–Wallis test. $p < 0.05$ for statistical significance

At the 48-hour measurement against *Prevotella intermedia*, the greatest antimicrobial activity was observed in the 0.12% chlorhexidine group, with a mean inhibition zone of 22.07 ± 0.69 mm. The 75% concentration of MREO produced a mean inhibition zone of 16.10 ± 0.33 mm, while the 50% concentration yielded a mean inhibition zone of 10.49 ± 0.17 mm (Table 2).

The Shapiro–Wilk test revealed that data from the groups treated with either MREO oil at a concentration of 75% or with chlorhexidine at a concentration of 0.12% did not follow a normal distribution at either time point against either bacterial species ($p < 0.05$) (Tables 1 and 2).

For the inferential analysis, the Kruskal–Wallis test was used to compare the antibacterial effects of 0.12% chlorhexidine and 50 and 75% MREO oil at 24 and 48 hours against *Porphyromonas gingivalis* and *Prevotella intermedia*. No statistically significant differences were found between the groups ($p > 0.05$) (Table 3).

According to the results of this study, there were no significant differences in the inhibition zones of 50% MREO against *Porphyromonas gingivalis* and *Prevotella intermedia* at 24 and 48 hours. However, when the concentration of MREO was increased to 75%, significant differences were observed in the inhibition zones at both time points. Interestingly, there were no significant differences found between the antibacterial effects of 0.12% chlorhexidine and either concentration of MREO both bacterial species at either time point.

Discussion

Matricaria recutita is a plant of the Asteraceae family whose active principle (apigenin, terpenoids, flavonoids) contribute to its anti-inflammatory and antimicrobiological activities. Since ancient times, it has been used to cure certain diseases. It is generally used in the form of infusions, oils, etc. The recommended dosage will depend on each systemic condition. Although it is necessary to do more research on the safety of this natural remedy. On the contrary, chlorhexidine is a very effective medicine to reduce oral bacteria, however, it can cause side effects, such as stains and taste alteration. Therefore, the search for new products to combat pathogenic flora in the oral cavity should continue.[10]

Matricaria recutita essential oil can be used as an effective adjunct during non-surgical periodontal therapy for chronic periodontitis, with clinical results similar to the gold standard of chlorhexidine, with significant improvement in periodontal pocket probing depth, bleeding rate, clinical adherence level, plaque index, and gingival index.[14] The present study determined the antibacterial efficacy of different concentrations of MERO on strains of *Porphyromonas gingivalis* and *Prevotella intermedia*.

The results of this study are consistent with those of a randomized controlled trial by Batista et al., which demonstrated that mouth rinses containing an ethanolic extract of *Matricaria recutita* were as effective as 0.12% chlorhexidine solution in reducing

gingival bleeding rates in individuals with chronic gingivitis and periodontitis.[13] Similarly, they are compatible with the research results of Ahmad et al. who found inhibition halos of 20 ± 1.20 with ethanolic extract of *Matricaria aurea* on *Porphyromonas gingivalis*.[16] In the present study, in the 48-hour measurement of activity on *Porphyromonas gingivalis*, significant differences [$p = 0.028$] were found only in the group of essential oil of *Matricaria recutita* at a concentration of 75%, it was 19.22 ± 0.54 mm.

The antimicrobial activity with MREO on *Prevotella intermedia* at a concentration of 75%, which was 15.88 ± 0.29 mm, followed by chlorhexidine at 0.12% which was 21.69 ± 0.63 mm, while with MREO at a concentration of 50% was 10.27 ± 0.18. However, the results of the present study are compatible with those of the research conducted by Al Habashneh et al. who evaluated a toothpaste containing natural compounds, such as ratanhia tincture (1.25%), chamomile tincture (1.25%), and myrrh tincture (0.62%) where it was found that a significant decrease in *Prevotella intermedia* count ($p < 0.05$) occurred after its use for four weeks.[17] Similarly, a clinical trial showed the satisfactory effect of the use of *Matricaria recutita* rinses in the reduction of gingival inflammation, compared with another plant such as *Schinus terebinthifolius* and chlorhexidine, having the greatest effect in reducing the plaque index.[18]

In the present study, in the groups of *Porphyromonas gingivalis*, significantly higher values were found in the 48-hour measurement with respect to that performed at 24 hours ($p = 0.028$). In the groups of *Prevotella intermedia*, significantly higher values were found in the 48-hour measurement with respect to that performed at 24 hours ($p = 0.003$).

One of the main challenges encountered during this research was the limited availability of literature on the properties of the plants under study in Peru, which hindered the comparison with previous studies. Moreover, strict monitoring of Petri dishes was essential to prevent contamination during seeding. Despite these challenges, the study was successfully conducted.

The findings of this study shed light on the potential benefits of using plants found in Peru against *Porphyromonas gingivalis* and *Prevotella intermedia* strains, which are critical factors in the development of periodontitis.[19] Compared with antibacterial drugs manufactured by the pharmaceutical industry, *Matricaria recutita* shows promise due to its potential for producing fewer long-term adverse effects, especially when used as a component of toothpastes or mouthwashes.[13,14]

Further research is needed to confirm these findings and to explore the potential long-term benefits of using *Matricaria recutita* as a component of toothpastes or mouthwashes. Future studies could also investigate the optimal concentration and delivery method of MREO for maximum efficacy against *Porphyromonas gingivalis* and *Prevotella intermedia*.

Conclusion

Within the limitations of this study, it was found that there were no significant differences between the inhibition zones measured at 24 and 48 hours for 50% MREO against *Porphyromonas gingivalis* and *Prevotella intermedia*. However, significant differences were observed between the inhibition zones measured at 24 and 48 hours for 75% MREO against both bacterial species. No significant differences were found between the antibacterial effects of 0.12% chlorhexidine, 50 and 75% MREO against *Porphyromonas gingivalis* and *Prevotella intermedia* at either time point.

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