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Original Research Article

Formulation and evaluation of herbal gel containing ethanolic extract of *Momordica charantia* against socransky's periodontal pathogens of the oral cavity-an *in vitro* study

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ABSTRACT

Background: Periodontitis is a multifaceted disease initiated by periodontal pathogens which when left untreated results in inflammation of the supporting tissues of the teeth, resorption of alveolar bone, loss of the periodontal ligament attachment, periodontal pocket formation and/or recession of the gingiva. Hence, the current study aimed at evaluating the efficacy of *Momordica charantia* leaves extract on Socransky's periodontal pathogens and formulating a herbal gel for subgingival local drug delivery for the potential treatment of moderate periodontitis.
Methods: *M. charantia* leaves were authenticated from a recognized taxonomist. They were coarsely powdered following which an ethanol-based extract preparation was done. The extract obtained was then assessed for minimum inhibitory concentration, minimum bactericidal concentration followed by gel formulation which was then subjected to time kill assay and zone of inhibition on periodontal pathogens, the efficacy of which was comparatively evaluated against 0.12% chlorhexidine (CHX) gluconate. Kruskal-Wallis Test was employed with the statistical significance set at $p \leq 0.05$.
Results: The concentration of *M. charantia* ethanolic extract against periodontal pathogens was determined to be 1 gm/100 ml and a statistically significant difference was found in the effectiveness between the gel so formulated and CHX.
Conclusion: The antibacterial activity was evident in the ethanolic extract of *M. charantia* which when used to formulate a herbal gel against anaerobic periodontal pathogens demonstrated efficacy comparable to that of CHX.

Keywords: Antimicrobial activity, Herbal gel, *Momordica charantia*, Periodontal pathogens

INTRODUCTION

The condition that affects the tissue around the tooth structure is referred to as periodontitis. Both systemic and local etiological factors can affect the condition. When left untreated, periodontitis causes inflammation in the tissues that support the teeth, resorption of alveolar bone, loss of attachment of the periodontal ligament, and the development of periodontal pockets and/or recession of the gingiva. Prompt treatment of the disease is crucial since it leads to pre-mature loss of tooth while also impacting patient's overall health. The bacterial association with dental plaque is commonly acknowledged as the aetiology of inflammatory periodontal disorders. The S3 level clinical practice guidelines of the European federation of periodontology state that subgingival debridement, systemic or locally given antimicrobials, and adjuvant treatments (chemical, host-modulating, physical) are necessary for the elimination of the subgingival biofilm and calculus. Despite the positive outcomes, mechanical

debridement on its own has been found to leave behind a considerable number of harmful bacteria in places that are rather unreachable.[1]

The key objective of a microbiological approach to periodontal therapy is to suppress particular pathogenic bacteria and allow for the subsequent recolonization of microbiota that is compatible with health. Research has shown that antimicrobial agents are useful as adjuncts in the treatment of periodontal disease, so researchers have looked for multiple methods to deliver these antimicrobials into periodontal pockets. Localized administration of chemotherapeutic agents may have benefits such as treating specific disease sites, requiring relatively small amounts of the drug to produce a high concentration in the periodontal pocket with little side effects, and reducing the likelihood of the drug causing resistant bacterial strains in other parts of the body. It also lowers the possibility of patient compliance issues.[2]

Dental practitioners have been using chlorhexidine (CHX), a medication that is frequently prescribed for treating of oral health issues. There is no question about its effectiveness in controlling plaque, and it has been in use for almost thirty years. However, its usefulness for the prevention of oral health diseases seems to be controversial due to its many ill effects, which include irritation, dry mouth, taste disturbances and teeth staining. Herbal products are a valuable alternative to CHX because they have medicinal properties and have no undesirable effects.[3]

The dependence on natural products, especially herbs, has not changed despite numerous advancements in pharmacology and synthetic organic chemistry. Approximately 80% of the global population still relies on medicinal plants for their basic medical needs.[4] *M. charantia*, sometimes referred to as bitter melon, balsam pear, or karela, is a tropical and subtropical plant that is mostly found in South America, Asia, and India. It is a member of the Cucurbitaceae family of medicinal plants.

It has a wide range of applications, including being an antioxidant, antidiabetic, helminthic and anticancerigenous. The phytochemical prospection of the dried and fresh leaf extracts revealed the existence of several secondary metabolite groups with antibacterial activity, such as tannins, alkaloids, and flavonoids. The primary ingredients are alpha momorcharin and trans-nerolidol, both of which have bactericidal and anti-microbial properties. Different leaf extracts (water, ethanol, and methanol) have shown antibacterial activity against *E. coli*, Staphylococcus, Pseudomonas, Streptobacillus, and Streptococcus *in vitro*.[5,6]

There was an acute paucity of literature reporting the antibacterial efficacy of *Momordica charantia* against periodontal infections. Therefore, evaluating *M. charantia's* antibacterial capabilities against selective periodontopathic bacteria is the need of the hour. Therefore, this study's goal was to determine whether *M. charantia's* herbal gel formulation had the same potential antibacterial activity against periodontal pathogens as CHX gluconate for the potential treatment of moderate periodontitis.

METHODS

Study design

The present study was an *in vitro* study, conducted according to the guidelines of good laboratory practice from December, 2022 to December, 2023 in the Department of Periodontics, KAHER's KLE Vishwanath Katti Institute of Dental Sciences. The study was approved by the Institutional Research and Ethics Committee of KAHER's KLE Vishwanath Katti Institute of Dental Sciences.

Preparing the extract

The Indian Council of Medical Research, Belagavi (ICMR) certified the herb, *M. charantia*, which was purchased from the market. After cleaning, washing and air drying the *M. charantia* leaves for three to four weeks the dried leaves were ground into a coarse powder using a mechanical grinder and sieved using a screen with 40 no. mesh. After which the powder was subjected to cold maceration with ethanol and water to formulate the extract. In a conical flask, 10 g of powdered dried *M. charantia* leaves were macerated in 100 ml of ethanol at room temperature for 24 hours with occasional shaking. After 24 hours, the mixture was treated using a simple filtration technique. To get a concentrated extract, the solvent was further evaporated using an electric water bath.

Preparing the inoculum

The inoculum was prepared by selecting standard strains of *Porphyromonas gingivalis*-ATCC33277, *Aggregatibacter actinomycetemcomitans*-ATCC29523, and *Tannerella forsythia*-ATCC43037 that had the same morphological type from an agar culture plate. The grown bacteria were then transferred into a tube containing 4-5 ml of brain heart infusion (BHI) broth after each colony was scooped with a sterile loop. For 48 hours, the broth culture was incubated at 37°C until it reached the 0.5 McFarland standards for turbidity.

Determining the minimum inhibitory concentration (MIC)

Ten wells were chosen for extract and triplicates out of 96 well culture plates. In designated wells, an equivalent volume of microbiological media (100 µl) was introduced. Furthermore, 100 µl of extract was added. Serially diluting the extract to the necessary concentrations up to the tenth well after adding it to the first well. As a result, the concentration dropped by 50% in the wells that followed. Each of the ten wells received 10µl of the inoculum, which

was then incubated for two days in the McIntosh and Fildes anaerobic jar. Resazurin dye was introduced after 48 hours, and all colours except the positive control were watched to change from blue/violet to slightly pink/magenta. The MIC value was determined by measuring the concentration at which a blue to pink colour shift was seen. With regard to the three periodontal pathogens, this process was repeated.

Determining the minimum bactericidal concentration (MBC)

Nichrome loop of 2-4 diameter was used for streaking the sample. Prior to streaking, loop was sterilized in Bunsen burner making it red hot and cooled by touching an uninoculated part of the medium. The test tubes containing the samples were blended with pipette followed by taking a one full loop of culture, streaked in zig-zag fashion on BHI agar plates.

The MBC-streaked plates were kept in incubator for 16-18 hours and temperature was set as 37°C. After incubation period, plates were observed for bacterial growth. Bactericidal values were defined as the concentration at which there was no sign of bacterial growth.

Preparation of herbal gel

To prepare the *M. charantia* gel, 1 g/100 ml of extract was utilized, based on MIC and MBC values. A predetermined quantity of gelling polymers i.e., hydroxypropyl cellulose and carbopol 934 were dissolved in fifty ml of distilled water and slowly stirred for three hours using a magnetic stirrer. The mixture was then left for twenty-four hours to fully hydrate. Weighed amounts of sodium saccharin and menthol were dissolved in thirty ml of distilled water. To achieve a uniform dispersion, the extract was combined with carbopol gel and stirred at 500 rpm for 30 minutes using a magnetic stirrer. The produced preservative mixture was added to the above prepared mixture and stirred continuously for 30 minutes at 100 rpm using a magnetic stirrer. Drop by drop, triethanolamine was added to the gel to get its pH to 7.5 and the final weight of the gel was adjusted to 100 gm using distilled. The gel was transferred to room temperature, airtight container. Over the course of six months, this gel remained stable (Table 1).

Performing time kill assay

The Time Kill Assay involved calculating the bacterial strains' vitality while they were exposed to antimicrobial agents at various intervals. In separate test tubes, 1 ml of prepared Brain Heart Infusion broth was mixed with 1 mg of *Momordica charantia* gel and 1 mg of chlorhexidine gel to create a solution of test chemicals. As a negative control, 2 cc of aseptic broth was taken. The growth rate of the bacterial strains was counted at various time intervals, beginning at 0, 2, 4, 6, 8, and 24 hours, in order to create the time-kill curve.

Determining zone of inhibition

In a conical flask, 100 ml of distilled water and 5.2 gram of Brain Heart Infusion Agar were combined to formulate the microbiological medium. After being autoclaved for 15 to 20 minutes, the solution was transferred into glass plates. The test bacteria, *P. gingivalis*, *A. actinomycetemcomitans* and *T. forsythia* were inoculated into agar plates using a standardized inoculum.

Aseptically, two 6 to 8 mm diameter wells spaced equally apart were punched, and then the test compounds-Chlorhexidine gel and *Momordica charantia* gel-were added to the wells and incubated for 24 hours. On the agar plate, a zone of inhibition developed, and its dimensions were noted using a uniform scale.

Performing cytotoxicity assay

This colorimetric assay gauges how much mitochondrial succinate dehydrogenase reduces yellow 3-(4, 5dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). L929 mouse fibroblasts were seeded, and the results were counted. They were placed in a CO2 incubator and cultured for 24 hours at 370C with 5% CO2.

Following a 24-hour period, 100 microliters of test compounds were introduced into the wells, and the mixture was incubated for another 24 hours at 370°C with 5% CO2. The wavelength at which the optical density (OD) was measured was 570 nm.

Evaluation of the prepared gel

The prepared gel was assessed for its physiochemical properties and results were tabulated in Table 2.

Color

A visual inspection was used to verify the formulation's color.

Consistency

Applying the formulation to skin allowed for the evaluation of its consistency.

Odor

By combining the gel with water and observing the smell, the formulations' odors were evaluated.

pH measurement

A digital pH meter was used to measure the pH of the gel formulation. One gram of gel was dissolved in ten millilitres of distilled water and left for two hours. Subsequently, the glass electrode was fully submerged in the gel system to measure the pH of the formulation.

Homogeneity

After the gel was placed inside the container, it was examined visually to ensure that it was homogenous and free from any aggregates.

Viscosity: At 25°C, the viscosity of the prepared gel was measured using a Brookfield viscometer equipped with spindle number 1. The gel was rotated at three different speeds: 0.3, 0.6, and 1.5 rpm. The dial reading that corresponded to each speed was recorded. The produced gels' viscosity was then calculated by multiplying the dial reading by a factor listed in the Brookfield viscometer catalogue.

Spread ability

Spreadability is measured in terms of the number of seconds it takes for two slides to separate from a gel layer positioned in between them when a specific stress is applied. The spread ability is better if it takes less time to separate two slides. It was calculated by using the formula:

S = M×L/T
M = weight tied to upper slide
L = length of glass slides
T = time taken to separate the slides

Extrudability

A standard capped collapsible aluminium tube was filled with the prepared gel and sealed by crimping the end. After the filled tube's weight was determined, it was clamped and placed between two glass slides. After covering the slides with a 500 g weight, the cap was taken off to allow extrusion. Weighing was done on the collected amount of extruded gel.

Statistical analysis

IBM-SPSS® Statistics-Version 21 (IBM Corp., Released 2012, IBM SPSS Statistics for Windows, Version 32.0, Armonk, NY, USA: IBM Corp.) was used to evaluate the data that had been collected and entered into Microsoft Excel. The significance between the developed formulation and CHX for three distinct organisms was tested using the Kruskal-Walli's test. For the test, $p<0.05$ was used as the statistical significance level.

RESULTS

Based on the MIC and MBC values of Momordica charantia extract (10 mg/ml), 1 gm of Momordica charantia extract was used to formulate 1% (w/v) herbal gel. (Figure 1, Figure 2a, Figure 2b and Figure 2c).

Time kill assay

CHX and the *M. charantia* gel formulation exhibited a nearly identical killing profile. After 4 hours, the bacterial density in both cases decreased quickly and significantly. The *M. charantia* herbal gel showed comparable bactericidal efficacy after 7 hours of treatment. With both the CHX and *M. charantia* gel formulation, the growth decreased to around ~1 log 10 at 24 hours (Table 3).

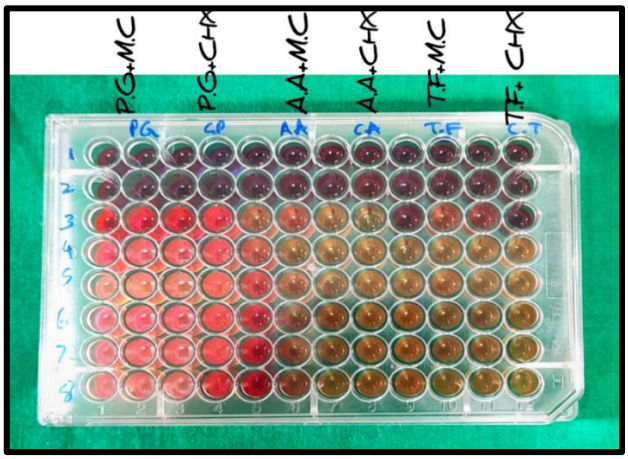

Figure 1: Photograph showing minimum inhibitory concentration (MIC) for *Momordica charantia* gel and Chlorhexidine against (a) *Porphryomonas gingivalis*, (b) *Aggregatibacter actinomycetemcomitans* (c) *Tanerella forsythia.*

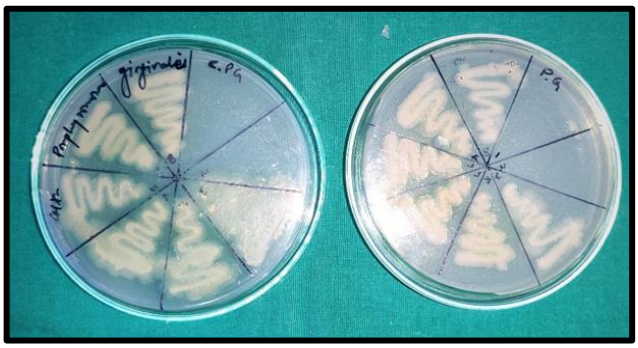

Figure 2a: Photograph showing minimum bactericidal concentration (MBC) for *M. charantia* gel and Chlorhexidine against *Porphryomonas gingivalis*.

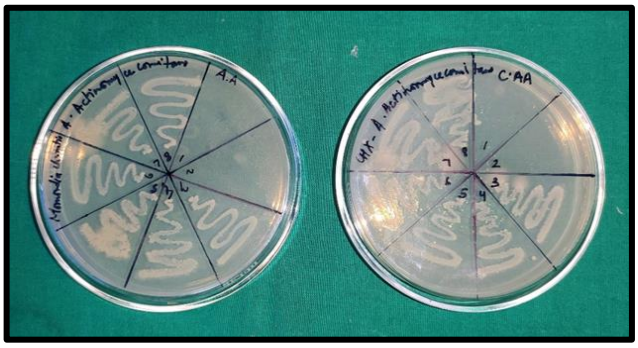

Figure 2b: Photograph showing minimum bactericidal concentration (MBC) for *M. charantia* gel and Chlorhexidine against *Aggregatibacter actinomycetemcomitans*.

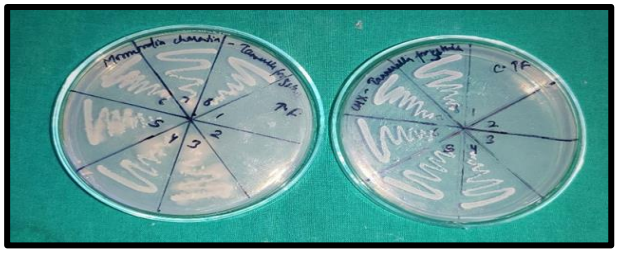

Figure 2c: Photograph showing minimum bactericidal concentration (MBC) for *M. charantia* gel and chlorhexidine against *Tanerella forsythia*.

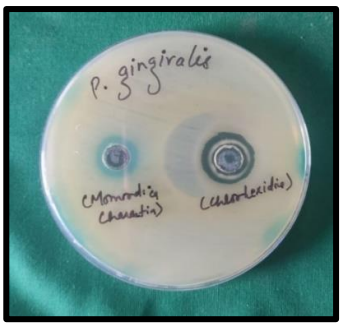

Figure 3a: Photograph showing zone of inhibition against *Porphryomonas gingivalis* after 48 hours of incubation for *Momordica charantia* gel and Chlorhexidine.

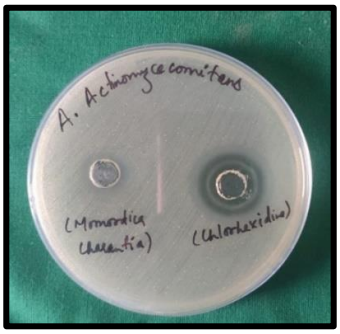

Figure 3b: Photograph showing zone of inhibition against *Aggregatibacter actinomycetemcomitans* after 48 hours of incubation for *Momordica charantia* gel and Chlorhexidine.

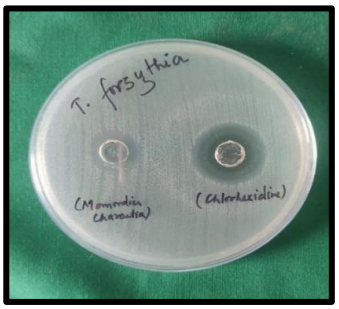

Figure 3c: Photograph showing zone of inhibition against *Tanerella forsythia* after 48 hours of incubation for *Momordica charantia* gel and Chlorhexidine.

Zone of inhibition

Compared to Chlorhexidine at 18 mm, the zone of inhibition against *A. actinomycetemcomitans* with respect to *M. charantia* gel formulation was 14 mm. In comparison to CHX, the herbal gel exhibited a reduced zone of inhibition for *P. gingivalis*.

Herbal gel at 13 mm and CHX at 17 mm against *P. gingivalis* established the zone of inhibition. When *T. forsythia* was incubated for 48 hours, the zone of inhibition for CHX was 20 mm, which was greater than the zone of inhibition for herbal gel, which was 16 mm. Figures 3a, 3b, and 3c depict the zone of inhibition of *M. charantia* gel formulation and 0.12% Chlorhexidine against periodontal pathogens by well diffusion method.

Cytotoxicity assay

Cytotoxic evaluation by MTT Assay concluded that the viability of L929 mouse fibroblast was observed to be 97.82% for Chlorhexidine gel, 104.03% for *M. charantia* gel, which indicates that both gels were biocompatible.

Table 1: Composition of gel formulation.

S. no	Material	Function	Formulation (w/w)
1	Herbal extract	Antibacterial	1% w/w
2	Carbopol 934	Gelling polymer	1% w/w
3	Hydroxypropyl cellulose	Gelling polymer	1% w/w
4	Triethanolamine	Viscosity enhancer and pH adjuster	0.5% w/w
5	Sodium saccharin	Sweetening agent	1% w/w
6	Menthol	Cooling agent	12.5% w/w
7	Ethanol (97%)	Solubilizer, Preservative, Cosolvent	100 ml
8	Distilled water		q. s

Table 2: Physiochemical properties of *M. charantia* gel.

Color	**Pale yellow**
Consistency	Good
Odor	Characteristic
Ph	6.9
Homogeneity	Good
Viscosity	4700 cps
Spreadibility	24.51 gm.cm/sec
Extrudability	87 %

Table 3: Antibacterial susceptibility with respect to time kill assay scores for both the groups.

Porphyromonas gingivalis						
Gels	**0 hour**	**2 hours**	**4 hours**	**6 hours**	**8 hours**	**24 hours**
0.12% Chlorhexidine	0.11	0.11	0.10	0.04	0.02	0.01
Momordica Charantia	0.11	0.10	0.10	0.05	0.03	0.01
Aggregatibacter actinomycetemcomitans						
Gels	**0 hour**	**2 hours**	**4 hours**	**6 hours**	**8 hours**	**24 hours**
0.12% Chlorhexidine	0.11	0.11	0.10	0.05	0.02	0.01
Momordica Charantia	0.11	0.10	0.09	0.06	0.02	0.02
Tannerella forsythia						
Gels	**0 hr**	**2 hours**	**4 hours**	**6 hours**	**8 hours**	**24 hours**
0.12% Chlorhexidine	0.11	0.11	0.07	0.06	0.02	0.01
Momordica Charantia	0.11	0.11	0.08	0.06	0.02	0.01

DISCUSSION

India is home to exquisite biodiversity, including an array of widely accessible medicinal plants and seeds. Antimicrobial action has been reported for a few extracts, particularly against bacterial infections. One such herb that is primarily found in the Indian subcontinent is *M. charantia*. The goal of the current study was to evaluate its extract's antibacterial activity to formulate a gel from the same, against prevalent periodontopathic bacteria. Chemical prospection of *M. charantia's* fresh leaf extracts and fractions has revealed the presence of several classes of secondary metabolites, such as flavonoids (anticarcinogenic, antioxidant, moluscicidal, and antiviral) and tannins (antimicrobial, antiviral, moluscicidal, and anti-tumoral) that are known to be therapeutic in nature.[7,8]

Fresh leaf extract significantly reduced the growth of all tested strains in an *in vitro* study by Costa et al, assessing the antibacterial activity of *M. charantia* extracts and fractions.[9] This effect may have been related to the extracts' chemical composition and their capacity to permeate lipidical layers. Consequently, there is a clear gap in the literature about the uses of *M. charantia* in dentistry, despite the fact that a small number of studies-including those conducted in India-have already documented the antibacterial activity of the plant. The greatest inhibitory effect for T. forsythia was observed when evaluating the effectiveness of *M. charantia* against periodontal bacteria. This analysis led to the conclusion that at the highest concentration, growth was completely inhibited and that the inhibition zones were consistently variable and had risen greatly with the concentration of *M. charantia* formulation. *M. charantia* exhibits an inhibitory effect on certain bacteria that is similar to that of antibiotics. The effective substance to be purified from this plant could be used for developing new, effective antibiotics. The *M. charantia* formulation has shown to be an effective antimicrobial agent against periodontal infections, as depicted in the current investigation. In congruence to findings by Kumar et al and Grover et al, where distinct ethanol, methanol and water-based extracts of the leaves demonstrated *in vitro* antibacterial activities against Staphylococcus, *E. coli*, Pseudomonas, Streptobacillus, Salmonella and Streptococcus, *M. charantia* demonstrated significant activity against *E. coli* in a study also conducted by Costa et al. According to the data currently available from this investigation, *M. charantia* extract significantly inhibits the growth of anaerobic microbes with the resultant effect being equivalent or lesser than that by CHX.[10,11]

M. charantia, in contrast to CHX, is a natural product that has no known side effects. Its religious importance and native origin give it a greater likelihood of being embraced by people.

These outcomes suggest that *M. charantia* may be used in oral hygiene products to lower the prevalence of microorganisms in the oral cavity. Periodontopathic bacteria are primarily thought to be responsible for the onset and progression of periodontitis, despite the fact that the condition itself is complex. For an extended period, the combination of mechanical debridement and chemical therapeutic agents has been regarded as the preferred therapy option with superior outcomes.[12,13] Often used therapeutic agents, such as chemically produced CHX, are thought to be the gold standard when used in conjunction with mechanical debridement to manage plaque. On the other hand, prolonged usage of CHX has been linked to a few negative effects. These factors necessitated the development of an equally potent substitute that helps mitigate all of the negative effects associated with the current medications.[14,15]

Due to its many therapeutic benefits and lesser side effects, herbal medicine is an emerging option that many researchers believe to be the most acceptable form of therapy. Researchers have grown more engaged in the various applications of plants and the compounds they contain that give them their medical and therapeutic qualities.[16] Because this research was conducted *in vitro*, the antibacterial effectiveness of the *M. charantia* extract and its herbal gel formulation is only partially demonstrated. One of the study's shortcomings is that it only used three organisms however periodontal infection is a complex ailment which stems from varied aetiology

associated with several periodontopathic microorganisms. Nonetheless, the current study's selection of periodontal bacteria was based on their prevalence. Future clinical trials will be conducted to determine its cytotoxicity against periodontal tissues and to demonstrate how it affects different microbes and clinical metrics like the decrease in pocket probing depth.

CONCLUSION

Considering that medications derived from natural materials are generally safe and affordable, it becomes crucial to identify plants that have therapeutic qualities and to develop strategies for doing so. The herbal gel formulation of *Momordica charantia* leaf extract demonstrated a significant antimicrobial activity against the periodontopathic bacteria which was comparable to that of chlorhexidine. Given its long-term use, cost effectiveness, and low side effect profile, the formulation presents a novel herbal alternative for the treatment of periodontitis.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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