iJRASET
International Journal For Research in Applied Science and Engineering Technology



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 9 **Issue:** XI **Month of publication:** November 2021

DOI: https://doi.org/10.22214/ijraset.2021.38960

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.429
Volume 9 Issue XI Nov 2021- Available at www.ijraset.com

An In-Vitro Study Comparing the Effects of Citrus Lemon Extract on Porphyromonas Gingivalis, Aggregatibacter Actinomycetemcomitans, and Prevotella Intermedia

Dr. Shivani Vansia
Bachelor of Dental Surgery, Vaidik Dental College & Research Centre, Daman, India

Abstract: ***Plant extracts and phytochemicals having antimicrobial characteristics could be immensely beneficial in medicinal treatments. The present study deals with the effect of Citrus lemon extract over Periodontal pathogens which are primary etiologic factors for periodontal diseases. The current study took into account periodontal pathogens such as Prevotella Intermedia, Aggregatibacter Actinomycetemcomitans, and Porphyromonas Gingivalis. Chlorhexidine is a gold standard antimicrobial agent with a wide antibacterial activity that is commonly used for chemical plaque management. When used for an extended period, however, chlorhexidine is known to stain. As a result, the alternatives are to be explored such as herbal-based agents that can be used regularly. Using the microdilution process and the culture method, the antibacterial effect of citrus lemon extract against periodontal pathogens was assessed using the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and zone of inhibition (ZOI). According to the findings of this study, citrus lemon extract can be used as a natural supplement for treatment purposes.***
Keywords: Antimicrobial, Citrus lemon Extract, Chlorhexidine, Periodontal Pathogens.

I. INTRODUCTION

Ethnomedicine is a complex multi-disciplinary system that incorporates the use of plants, spirituality, and the natural environment and has been used to heal people for generations. Plants have been used for medicinal purposes since the Vedic period. However, herbal medicines were replaced by synthetic medicines a few decades ago due to their rapid effect. Allopathic medicine's side effects are reversing the global trend toward green medicine. Plants have long been thought to have therapeutic properties. Since prehistory, people on all continents have used poultices and ingested infusions of hundreds, if not thousands, of indigenous plants. Medicinal plants, according to the World Health Organization (WHO), would be the best source of a range of pharmaceuticals, thus they should be studied to learn about their qualities, safety, and efficacy in the quest for new antimicrobial chemicals. India is the world's largest producer of medicinal herbs, earning it the nickname "Botanical Garden of the World" [1-3].

Citrus lemon is the botanical name for lemon, a sweet, juicy fruit that belongs to the Rutaceae family. Citrus lemon is one of the most significant and commonly grown fruit crops, with an estimated global production of 120 million tons. In tropical and subtropical climes, lemon trees are commonly planted for their sweet juice and medicinal benefits. Citrus lemon peel is used to treat a variety of diseases, including colic, upset stomach, cancer, diuretic, carminative, immuno-enhancing, stomachic, and tonic to the digestive system, immune system, and skin. It's also used to treat and prevent vitamin deficiencies, as well as colds, flu, and scurvy, as well as viral and bacterial infections. The antibacterial properties of lemon-peel have been documented in the literature [4, 5].

Using the disk diffusion, Dubey et al. demonstrated that extract from Lemon peels have significant antibacterial activity (against Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Shigella flexineri, Bacillus subtilis, and Escherichia coli). Lemon peel extract was found to be beneficial against Klebsiella pneumonia by Chabuck et al. [6].

Periodontal disease is one of the most prevalent dental health issues that people face. Periodontopathogens have been identified in a variety of oral bacteria. Because of their extensive range of virulence factors and interactions with a wide variety of Gram-negative and Gram-positive bacteria, Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, and Prevotella intermedia are particularly implicated in the development of periodontal diseases. These microorganisms are frequently seen in increased numbers in periodontal pockets with active attachment loss, and they may form complicated ecological connections among numerous oral bacteria, control the host immune system, and influence the treatment outcome [7-9].

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Contrary to Citrus lemon, Chlorhexidine, a cationic bisbiguanide with broad antibacterial action, low mammalian toxicity, and a high affinity for attaching to skin and mucous membranes, is an antimicrobial agent. It is active against gram-positive and gram-negative bacteria, yeasts, dermatophytes, and a few lipophilic viruses, among others. It exhibits antibacterial activity that is membrane-active and affects the inner cytoplasmic membrane. For more than two decades, the dental profession has relied on chlorhexidine as the major agent for chemical plaque control. Chlorhexidine, on the other hand, is known to stain when applied for a prolonged period. As a result, alternatives such as herbal-based medications that can be utilized regularly must be investigated. Hence, the current study focuses on the use of citrus lemon extract [10-12].

II. METHODOLOGY

In the current study, the MIC, MBC, and ZOI of the citrus lemon extract on P. gingivalis, A. actinomycetemcomitans, and P. intermedia were evaluated in vitro, and the results were compared to chlorhexidine. For Minimum Inhibitory Concentration (MIC), three groups of organisms were tested on ten different concentrations of citrus lemon extract. The concentrations that tested positive for MIC were then tested for Minimum Concentrator, Bactericidal Concentration, and Bactericidal Concentration (MBC). Moreover, The Zone of Inhibition (ZOI) was also investigated in the positive concentrations in MBC. All these procedures are explained in this section.

A. Minimum Inhibitory Concentration (MIC)

The antimicrobial agent's stock solution is made by combining 100 g of lemon peel extract with 1 millilitre of thioglycolate (TG) broth medium (µg/1ml). 9 dilutions of the drug were generated using the microdilution method using 365 µl of TG broth medium for the MIC. The initial tube was filled with 20 µl of medication from the stock solution. 180µ l of TG broth was added to each of the next nine tubes independently for dilutions. 180 µl was added to the first tube, which was already filled with 180 µl of TG broth. 180 ml was transferred from the 10-1 diluted tube to the second tube to make a 10-2 dilution. For each drug, the serial dilution was performed up to a 10-9 dilution. 10 µl of the needed organisms' stock cultures (P.G, A.A, and P.I) were added to 2 ml of TG broth. 180 µl of the same culture suspension was added to each serially diluted tube, and tubes were airtightly sealed and incubated for more than 48 hours in an anaerobic chamber. The MIC of medicine is defined as the lowest concentration of the drug in a tube that does not produce turbidity.

B. Minimum Bactericidal Concentration (MBC)

A pure culture of a specific microbe is grown overnight, then diluted to a concentration of 1 x 10^5 to 1 x 10^6 CFU/ml in growth-supporting broth (usually Mueller Hinton Broth). The antimicrobial test material is diluted to a stock concentration of about 100 times the estimated MIC. In test tubes, further 1:1 dilution is created. The required microorganism is injected in equal amounts in all dilutions of the test product(s). Every test microorganism has a positive and negative control tube or well to indicate appropriate microbial growth throughout the length of the incubation time and media sterility, respectively. At a suitable time and temperature, the incubation of the tubes was completed. The growth of the microorganisms is represented by turbidity; however, the MIC is the lowest concentration at which no growth is visibly observed. When compared to the MIC dilution, the MBC is the lowest concentration that exhibits a predetermined reduction (such as 99.9 percent) in CFU/ml.

C. Zone of Inhibition (ZOI)

P. Gingivalis, A. Actinomycetemcomitans, and P. Intermedia (overnight cultures grown at 37°C on nutrient agar) were inoculated onto nutrient agar plates by rubbing sterile cotton swabs dipped in bacterial suspensions over the entire surface of the plate. Six of these sets were established. Following inoculation, five-10 mm diameter wells were cut into the surface of each agar plate with a sterile cork borer. Positive concentrations of lemon peel extract from the minimum bactericidal concentration were added to wells in different plates containing the two bacteria mentioned above, as well as one for each set of bacteria distilled water. The plates will be incubated for 24 hours at 37°C. All the plates' zones of inhibition will be measured with a Digital Vernier Caliper. The zones of inhibition's mean scores were calculated and statistically analyzed. All these lemon-peel extract concentrations were prepared and compared to chlorhexidine mouthwash.

III. RESULT AND DISCUSSION

In Minimum Inhibitory Concentration, for citrus lemon extract, as demonstrated in Figure 1, the sensitivity of P. Gingivalis was observed at 100 µg/ml and 50 µg/ml. While the sensitivity of P. Intermedia and A. Actinomycetemcomitans was observed at 100 µg/ml. In chlorhexidine, the sensitivity of P. Gingivalis was observed from 0.2 µg/ml to 100 µg/ml. Whereas, the sensitivity of P. Intermedia and A. Actinomycetemcomitans (In chlorhexidine) was observed 12.5 µg/ml to 100 µg/ml (Figure 1).

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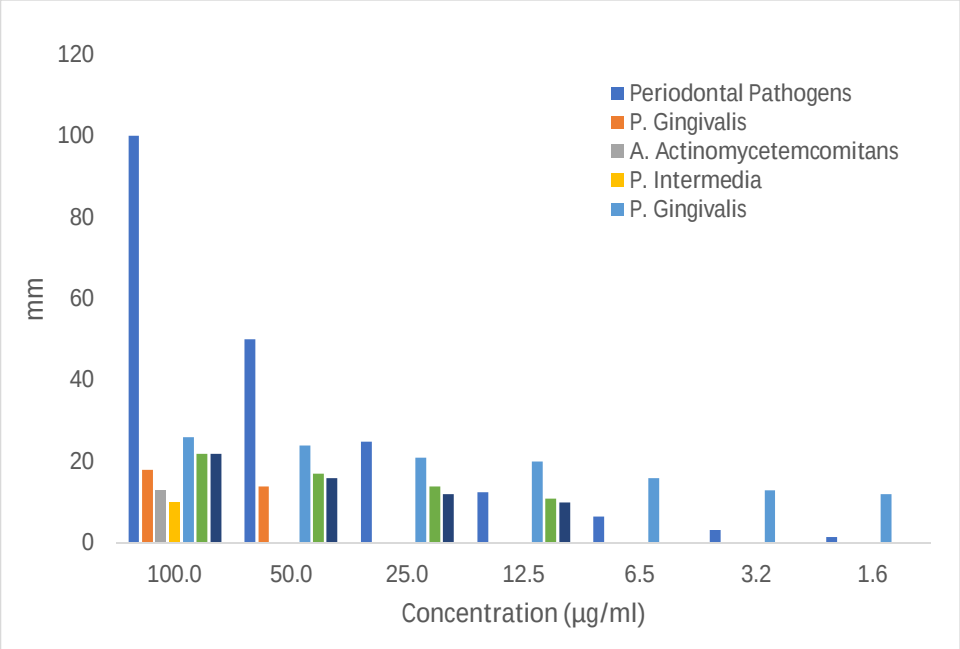


Figure 1: Comparison of Zone of Inhibition

In Minimum Bactericidal Concentration, the bacterial activity of P. Gingivalis for Chlorhexidine was observed at 0.2, 0.4, and µg/ml as depicted in Figure 3. The measurement for Zone of Inhibition, for citrus lemon extract, apparently from Table 1 and Figure 4, the P. Gingivalis showed 18 mm at 100% and 14 mm at 50%. Whereas for chlorhexidine, 26 mm at 100%, 24 mm at 50%, 21 mm at 25%, 20 mm at 12.5%, 16 mm at 6.50%, 13 mm at 3.20%, and 12 mm at 1.6% (Figure 5). For A. Actinomycetemcomitans, the measurement for ZOI for citrus lemon extract showed 13 mm at 100% (Figure 4). Whereas for chlorhexidine, the measurement was 22 mm at 100%, 17 mm at 50%, 14 mm at 25%, and 11 mm at 12.5%. For P. Gingivalis, the measurement for ZOI for citrus lemon extract showed 10 mm at 100% (Figure 4). Whereas, for chlorhexidine, the measurement was 22 mm at 100%, 16 mm at 50%, 12 mm at 25%, and 10 mm at 12.5%

Table 1: Zone of Inhibition

Substance	Periodontal Pathogens	Concentration (µg/ml)						
		100	50	25	12.5	6.50	3.20	1.6
Citrus lemon extract	P. Gingivalis	18 mm	14 mm	-	-	-	-	-
	A. Actinomycetemcomitans	13 mm	-	-	-	-	-	-
	P. Intermedia	10 mm	-	-	-	-	-	-
Chlorhexidine	P. Gingivalis	26 mm	24 mm	21 mm	20 mm	16 mm	13 mm	12 mm
	A. Actinomycetemcomitans	22 mm	17 mm	14 mm	11 mm	-	-	-
	P. Intermedia	22 mm	16 mm	12 mm	10 mm	-	-	-

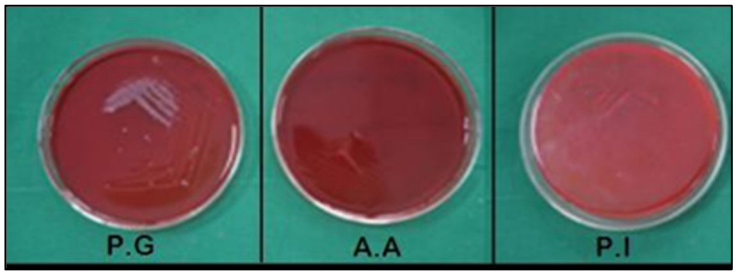


Figure 2: MBC Citrus Lemon Extract

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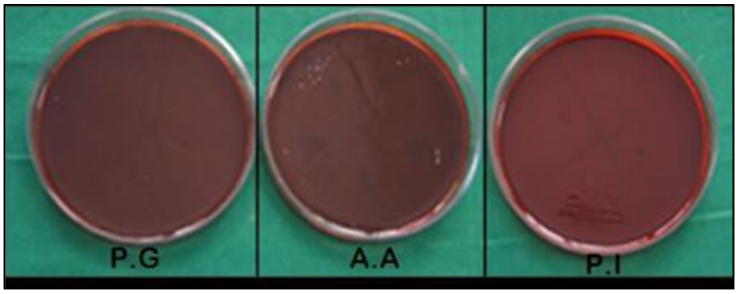


Figure 3: MBC Chlorhexidine

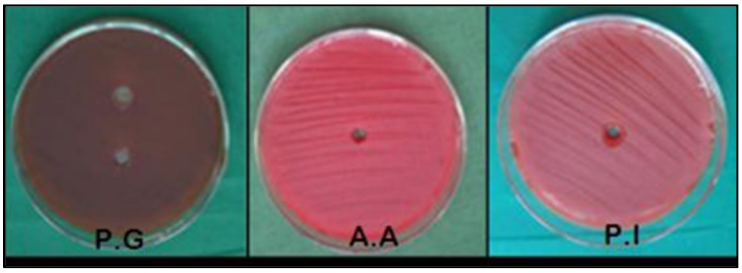


Figure 4: ZOI Citrus Lemon Extract

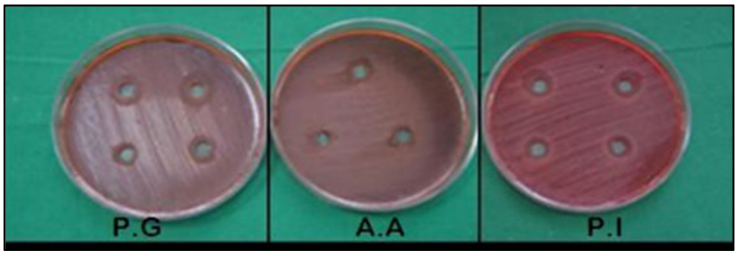


Figure 5: ZOI Chlorhexidine

IV. CONCLUSION

Anti-plaque agents based on the use of broad-spectrum antimicrobials such as chlorhexidine, quaternary ammonium compounds, and antibiotics have resulted in the development of antimicrobial resistance, significant side effects, and the emergence of uncommon infections as a result of their improper use. There is enough scientific data to demonstrate that the antibacterial efficacy of lemon fruit is influenced by its nature. To ensure their activity and efficacy, several therapeutic plants listed in Ayurveda still need to testify according to modern benchmarks. In this study, a pure citrus lemon extract was compared to the standard Chlorhexidine. All concentrations were examined for Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and Zone of Inhibition (ZOI) against periodontal pathogens, ranging from 0.2 percent to the purest form, 100 percent. The results reveal that the purest form of the extract is effective against the periodontal pathogens named P. gingivalis, A. actinomycetemcomitans, and P. intermedia, with chlorhexidine retaining the gold standard. The future scope of the study lies in in-vivo because in in-vitro, there is a limitation for clinical studies.

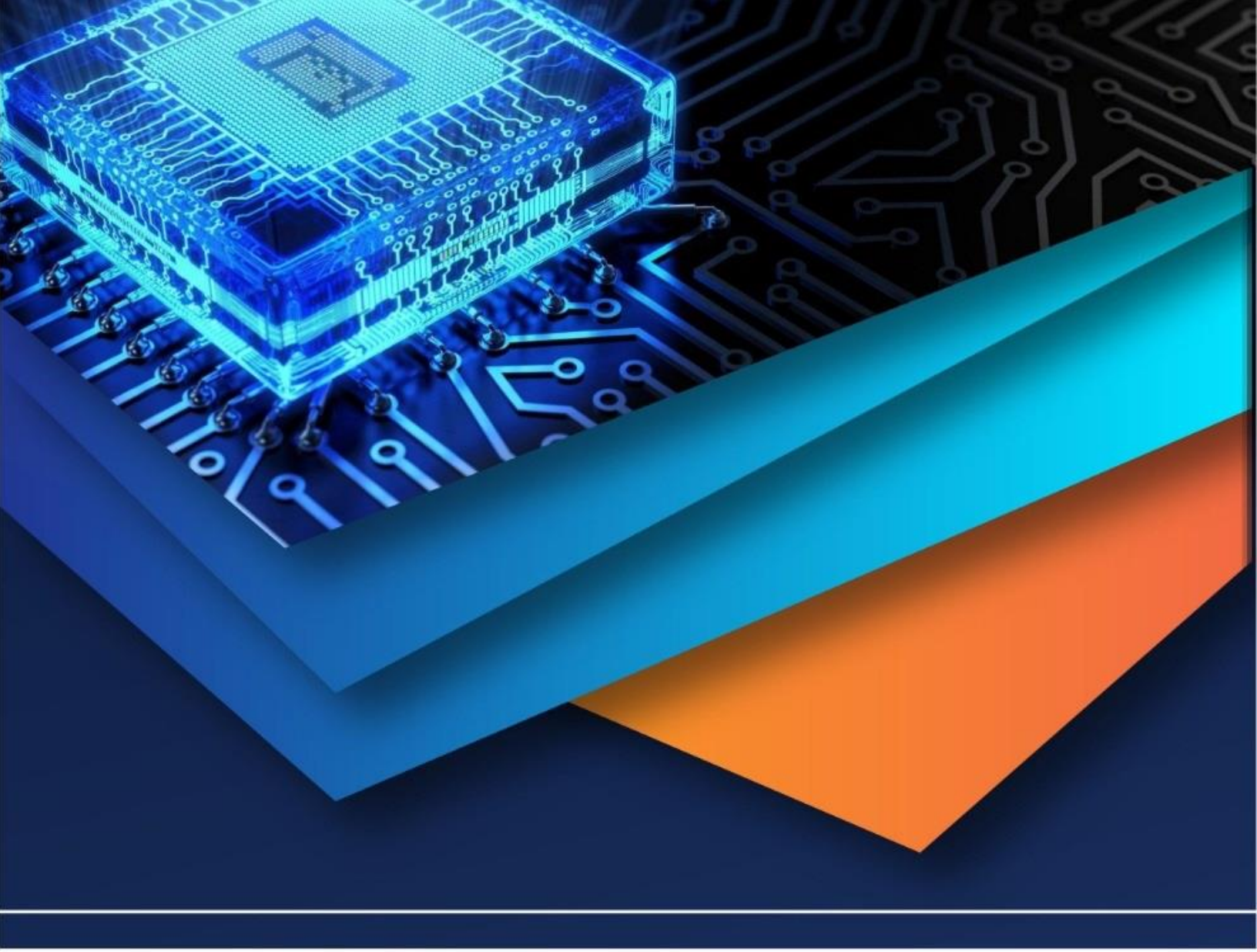
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