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Antimicrobial Efficiency of Different Tea Extract (*Camellia sinensis*) against Periodontal Pathogens

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Abstract

Periodontopathogens are a major contributor to the development of periodontal disease, which has multifactorial aetiology. Patients with periodontitis typically have *Prevotella intermedia*, *Aggrecatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Fusobacterium* species that are commonly found in gingival pockets and subgingival plaques. Different antimicrobial agents have been tried to prevent these microorganisms. As a treatment, herbal medicine made from various plants has been employed. Among these, tea leaf extract demonstrated potential antibacterial activity against infections. The study was designed to assess the antibacterial activity of several varieties of tea extract against periodontal microorganisms. *A. actinomycetemcomitans* and *P. gingivalis*, were grown and maintained in their respective agars for this in-vitro experimental study. Three different concentrations of 250 mg/ml aqueous, 250 mg/ml ethanolic, and 250 mg/ml methanolic solutions were prepared from Japanese green tea, Oolong Chinese tea, and Sabah black tea. The antibacterial activity of tea extracts was assessed by using the Kirby-Bauer disc diffusion method. 1% DMSO and 0.2% chlorhexidine were used as negative and positive control groups respectively. After an incubation of 24 hours at 37 °C, the inhibition zone was measured in millimetres. Japanese green tea at its aqueous and methanolic concentrations exhibited significantly higher mean inhibition zones of 12.38 and 13.56 mm respectively, against *P. gingivalis* at $p<0.001$ compared to *A. actinomycetemcomitans.* Whereas ethanolic extract of green tea showed better inhibition of 15.28 mm against *A. actinomycetemcomitans* at $p<0.001$ compared to *P. gingivalis.* Japanese green tea revealed better antibacterial activity at all three different concentrations compared to other two types of tea.

Keywords: Antimicrobial, *A. actinomycetemcomitans*, *P. Gingivalis*, Tea extracts

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1. Introduction

The oral cavity has several characteristics that make it a unique habitat for a variety of microorganisms. It supports different kinds of microorganisms with its optimal environment [1]. Although the oral cavity has a diverse microbial population, whenever there is an imbalance in this microbial community, that, consequently, leads to oral disorders like dental caries and periodontal diseases, which promote the growth of potentially harmful organisms [2]. It has been demonstrated that the loss of periodontal tissue has a strong correlation with an alteration of the bacterial population in the gingival sulcus from facultative Gram-negative to Gram-positive, organisms [3]. The entire tooth-supporting tissue is affected by a chronic polymicrobial infectious disease called periodontitis. The disease subsequently results in the loss of teeth due to the formation of gingival pockets, breakdown of the alveolar bone, and degradation of the periodontal ligaments. which may have an adverse effect on overall quality of life, including one's aesthetics and mastication [4] [5]. Periodontopathogens are a major contributor to the development of periodontal disease, which has a multifactorial etiology. Patients with periodontitis typically have *Prevotella intermedia*, *Aggrecatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Fusobacterium* species that are commonly found in their gingival pockets and subgingival plaques [6]. *A.actinomycetemcomitans* and *P. gingivalis* are the major periodontal pathogens involved in the process of periodontitis. *A. actinomycetemcomitans* is a facultative gram-negative, anaerobic bacillus. It was formerly believed to be the only bacterium responsible for localized aggressive periodontitis, but now it is recognised as one of the major pathogens causing this aggressive type of periodontal disease [7]. *P. gingivalis* is another main etiological agent in the pathophysiology and development of the inflammatory

processes of periodontal disease. It is an obligately anaerobic, gram-negative rod and as the dental plaque's secondary colonizer, it frequently adheres to main colonizers like *P. intermedia* and *S. gordonii* [3]. Even though mouthwashes are effective aids in managing the oral microbiota in periodontitis, it was found difficult to prevent and treat periodontitis [8]. Antimicrobial agents for gingivitis patients have been shown to offer the best possible care by decreasing the periodontal pocket's depth and lowering the number of periodontal bacteria [9]. However, long-term use of antimicrobials, including chlorhexidine, has been linked to unpleasant negative effects including burning sensations, ulceration of the oral mucosa, bad taste, staining of the teeth, and restorative materials [10]. Additional chemicals with better antimicrobial activity and lower toxicity are needed to be used as mouthwashes, considering the negative effects and possible drawbacks of these agents. The antibacterial properties of medicinal plants and their extracts have drawn a lot of attention and can be implemented as effective substitutes for synthetic chemical agents.

Herbal medications made from diverse plant components are becoming more popular due to their low frequency of serious side effects, stated effectiveness, and low cost. Among them, tea is one of the most well-liked medicinal plants and the second-most consumed beverage in many countries. It also holds important cultural and economic values [11]. Tea leaves can be prepared into three distinct types of tea: unfermented green tea, fermented black tea, and semi-fermented oolong tea [12]. Although green tea is more prevalent among Asians, black tea is known in Western nations, and oolong tea is considered in the Chinese province of Fujian [13]. These teas have well-established anti-inflammatory, antibacterial, anticancer, and antioxidant effects. They have also been demonstrated to reduce the risk of cancer and heart diseases, and obesity, due to their capacity as antioxidants [14] [15]. In consideration of their efficacy, the current study's aim was to assess the antibacterial activity of several varieties of tea extract against periodontal microorganisms.

2. Materials and methods

The current *in vitro* experimental study was carried out at Faculty of Dentistry, SEGi University. It is part of an internal collaboration with Malaysia's Universiti Teknologi MARA (SEGiEC/StR/FOD/41/2021-2022). The G Power software was used to calculate the sample size. The sample size was estimated using a 95% confidence interval and an 80% study power.

2.1. Preparation of microorganisms

Aggregatibacter actinomycetemcomitans DSM 11122 and *Porphyromonas gingivalis* DSM 20709 bacterial strains were purchased from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany). *A. actinomycetemcomitans* was subcultured on Brain Heart Infusion Medium and *P. gingivalis* on Brain Heart Infusion Medium supplemented with 5 mg/L vitamin K, 5 mg/L haemin and 0.5 g/L cysteine. At anaerobic conditions (80% N_2, 10% H_2, 10% CO_2) for 48 hours at 37°C, both were incubated. From an overnight culture, three to five colonies were suspended in saline (0.85% sodium chloride) and calibrated to the turbidity of a McFarland 0.5 standard before the experiment. The disc diffusion experiment was carried out using this standard bacterial suspension.

2.2 Preparation of different concentrations of tea extracts

Four types of tea were procured for the experiment from organic tea outlets in Malaysia. Japanese green tea from Shizuoka, Japan (Group 1); Oolong Chinese tea from Taiwan (Group 2) and Malaysian Sabah black tea (Group 3). For the experiment, three distinct concentrations of each variety of tea were prepared. concentration: 1–250 mg/ml aqueous solution, concentration: 2–250 mg/ml ethanolic solution, and concentration. 3–250 mg/ml methanolic solution.

2.2.1. Aqueous extract

The procedure described by Voina et al. (2020) was used to for the preparation of tea extract [16]. 200 ml of distilled water was boiled, and 20g of tea powder was added into it. It required ten minutes for the entire mixture to brew. Sterile Whatman No. 1 filter paper was used for filtration of the solution to obtain the final extract. On a rotary evaporator, the infusion was concentrated to a fifth of its original volume before being frozen and lyophilized. The lyophilized infusion was redissolved in water to make a stock solution of 250 mg/ml aqueous extracts.

2.2.2. Ethanol and Methanolic extracts

A method described by Troung et al. was used to prepare the extracts [17]. 200 mL each of ethanol (95%) and methanol (95%) were used to soak 20 g of tea powder. The combinations were kept at room temperature for 24 hours in a properly sealed conical flask, shielded from sunlight, and thoroughly stirred with sterile glass rods several times per day. Filtration was performed on the resultant mixtures using Whatman No. 1 filter sheets. To eliminate the ethanol, the extracted liquids were rotary evaporated. The semisolid extracts obtained from rotary evaporation were stored in a freezer at -80°C for a night and freeze dried at -60°C for 24 hours. At 4°C in a refrigerator the extracts were then stored in a sealed container until their next usage. A standard solution containing 250 mg/mL of the extract was obtained by re-mixing it in DMSO (dimethyl sulfoxide) with final concentration of DMSO not more than 1%.

2.3. Disc diffusion method

The procedure given by Kirby-Bauer was employed to assess the antimicrobial effects of tea extracts using disc diffusion technique [18]. A 20 µl of standard solution tea extracts (250mg/mL) were loaded on to the blank antimicrobial susceptibility discs (Oxoid ™) of 6 mm in diameter. As a positive control, the gold standard drug 0.2% chlorhexidine (CHX), was employed. A disc containing 1% DMSO was utilised as a negative control. A sterile cotton swab dipped in the standard bacterial suspension (0.5 McFarland) was used to equally spread the suspension throughout the surface of their respective agar. The discs containing different concentrations of tea extracts were applied on the surface of the agar plates together with negative and positive controls. Plates were incubated at anaerobic conditions (80% N_2, 10% H_2, 10% CO_2) for 48 hours at 37°C. Following incubation, a digital calliper determined the zone of inhibition's diameter in millimetres.

2.4. Statistical Analysis

To conduct statistical analyses, Statistical Package for Social Sciences (SPSS) for Windows, Version 22.0, was released in 2013. Armonk, NY: IBM Corp. was used. For different organisms, the Zone of Inhibition (ZOI) is calculated in terms of the mean as well as the standard deviation for each group in the descriptive analysis. The mean zone of inhibition among *A. actinomycetemcomitans* and *P. gingivalis* at various concentrations of each group was compared by using a student-paired t-test. The significance level was set at $P<0.05$.

3. Results and Discussions

The mean ZOI in 250 mg/ml of aqueous Solution for *P. gingivalis* in Group 1 was significantly higher (12.38 ± 0.12) as compared to *A. actinomycetemcomitans* (9.43 ± 0.17) and significant difference at $p<0.001$. Similarly, the mean ZOI for *P. gingivalis* in Group 2 was significantly higher (10.71 ± 0.23) as compared to *A. actinomycetemcomitans* (9.43 ± 0.17) and significant difference seen at $p<0.001$. Lastly, the mean ZOI for *P. gingivalis* in Group 3 was significantly higher (10.10 ± 0.25) as compared to no expression for *A. actinomycetemcomitans* (0.00 ± 0.00) and statistically significant difference at $p<0.001$ [Table 1, graph 1, and figures 1 and 2]. The mean ZOI in 250 mg/ml of ethanolic Solution for *A. actinomycetemcomitans* in Group 1 was significantly higher (15.28 ± 0.16) as compared to *P. gingivalis* (11.43 ± 0.17) and showed significant difference at $p<0.001$. Similarly, the mean ZOI for *A. actinomycetemcomitans* in Group 2 was significantly higher (14.59 ± 0.15) as compared to *P. gingivalis* [9.21 ± 0.12]. and significant difference at $p<0.001$. The mean ZOI for *A. actinomycetemcomitans* in Group 3 was significantly higher (13.52 ± 0.07) as compared to *P. gingivalis* (10.31 ± 0.37) and statistically significant difference at $p<0.001$ [Table 2, graph 2, and figures 1 and 2]. The mean ZOI in 250 mg/ml of Methanolic Solution for *P. gingivalis* in Group 1 was significantly higher (13.56 ± 0.07) as compared to *A. actinomycetemcomitans* (11.42 ± 0.16) and significant difference at $p<0.001$. Contrastingly, the mean ZOI for mean ZOI for *A. actinomycetemcomitans* in Group 2 was significantly higher (12.20 ± 0.10) as compared to *P. gingivalis* (9.52 ± 0.12) and showed significant difference at $p<0.001$. Similarly, the mean ZOI for *A. actinomycetemcomitans* in Group 3 was significantly higher (12.34 ± 0.12) as compared to *P. gingivalis* (10.56 ± 0.32) and significant difference at $p<0.001$ [Table 3, graph 3, and figures 1 and 2].

The present study demonstrated the effectiveness of three different tea forms against *A. actinomycetemcomitans* and *P. gingivalis.* Better antibacterial effects against *P. gingivalis* were seen with the aqueous extract of green tea with inhibition zone of 12.38 mm. Green tea contains Phenolic acids, Gallic acid, and flavonoids. Green tea polyphenols consist of significant catechins such as epigallocatechin (EGC), epicatechin EG, and EGC gallate (EGCG). The study evaluated the total amount of catechins in different commercial teas in which green tea showed more amount next to oolong tea, black tea, and Pu-Erh tea [19]. The proven antibacterial properties of green tea are mainly due to EGCG, a particularly significant catechin in green tea. The cytotoxic effects of *A. actinomycetemcomitans*–lipopolysaccharide on each cell that are the endotoxins and have a high potential to destruct tissues and cells of the host were greatly decreased by ECG and EGCG [20] [21]. *P. gingivalis*, on the other hand, is an important microbe at the start of periodontitis and produces enzymes that break down proteins. Tea-derived polyphenols limit the formation of proteases in *P. gingivalis*, hence reducing its activity [22]. In our study, green tea ethanolic extract showed better inhibition of 15.28 mm for *A. actinomycetemcomitans* as compared to *P. gingivalis* (11.46 mm). Similar findings were found in the study in which ethanolic green tea extract had a greater inhibitory effect on *A. actinomycetemcomitans* than the green tea aqueous extract at its different concentrations [23]. However, the aqueous extract of green tea was effective against clinically isolated A. *actinomycetemcomitans, P. intermedia, P. gingivalis,* and *S. mutans.* The inhibitory action of green tea is attributed to the fact that EGCG causes membrane disruption and inhibition of DNA gyrase of bacteria to prevent supercoiling of DNA. The toxic metabolites that cause the progression of periodontitis and gingival tissue destruction such as alkaline phosphatase, protein tyrosine phosphatase, and collagenase are neutralized by EGCG [24].

In contrast to the present study results, another study demonstrated the presence of a greater concentration of EGCG in alcoholic green tea extract and was effective against *P. gingivalis* compared to aqueous green tea extract [25]. Catechin extract of green tea showed a greater inhibitory effect at its different concentrations against *P. gingivalis* [26], and against *Porphyromonas gingivalis* and *Prevotella spp*. at its minimal inhibitory concentration of 1.0 mg/ml [27]. The reason for this is mostly based on the attachment of polyphenols to *P. gingivalis* fimbriae. *P. gingivalis* r-gingipain, and K-gingipain were inhibited. Green tea catechins inhibit the enzymatic activity of *P. gingivalis* in a way equivalent to chlorhexidine and antibacterial drugs [28]. Green tea also showed effective results against *P. gingivalis*, and *S. mutans* in saliva when used as a mouth rinse [29].

In our study results, green tea was more effective as compared to oolong and black teas. This could be due to the more catechin in green tea than that in oolong and black teas. This variation results from the fermentation process that causes tea leaves to oxidise. The chemical structures of both black and green teas differ despite the presence of equal quantities of flavonoids. The production of black tea in the fermentation process involves the conversion of these flavonoids and catechins into arubigins and aflavins [30].
In contrast to this, a study with black tea extract with its theophylline derivatives reported antibacterial effects on *P. gingivalis, A. actinomycetemcomitans, P. intermedia,* and *F. nucleatum* [31]. The study in which, both aqueous and methanolic extracts of black tea and green tea showed better antibacterial effects on *A. actinomycetemcomitans, P. gingivalis*, and *P. intermedia* [8]. It was stated that crude aqueous or alcohol extractions are usually used for preliminary testing of plants for potential antibacterial properties. The active organic compounds from plants that have higher antimicrobial activity are mainly by ethanolic and methanolic extractions.

Table 1: Comparison of mean ZOI in 250 mg/ml of Aqueous Solution between *A. actinomycetemcomitans* and *P. gingivalis* using Student Paired t Test in each group.

Groups	Organism	N	Mean	SD	Mean Diff	p-value
Group 1	*A. actinomycetemcomitans*	9	9.43	0.17	-2.95	<0.001*
	P. gingivalis	9	12.38	0.12		
Group 2	*A. actinomycetemcomitans*	9	9.43	0.17	-1.28	<0.001*
	P. gingivalis	9	10.71	0.23		
Group 3	*A. actinomycetemcomitans*	9	0.00	0.00	-10.10	<0.001*
	P. gingivalis	9	10.10	0.25		

Table 2: Comparison of mean ZOI in 250 mg/ml of Ethanolic Solution between *A. actinomycetemcomitans* and *P. gingivalis* using Student Paired t Test in each group.

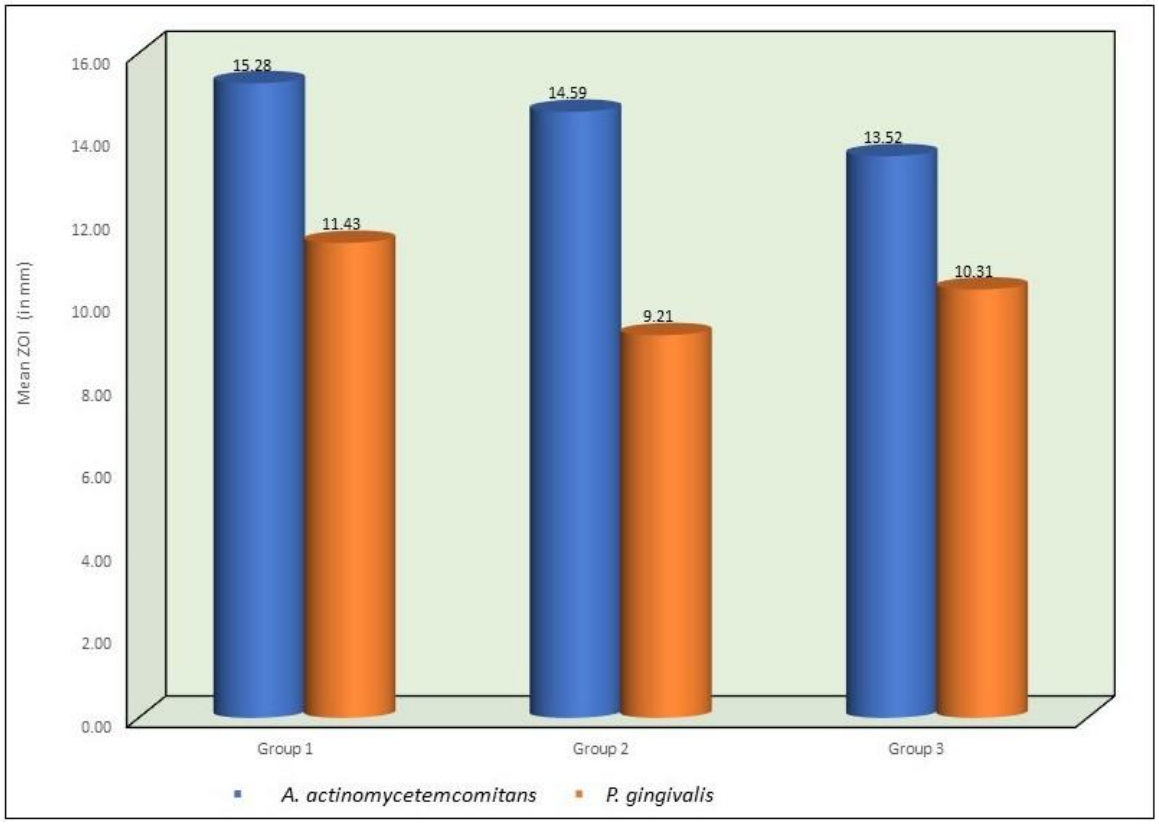
Groups	Organism	N	Mean	SD	Mean Diff	p-value
Group 1	*A. actinomycetemcomitans*	9	15.28	0.16	3.85	<0.001*
	P. gingivalis	9	11.43	0.17		
Group 2	*A. actinomycetemcomitans*	9	14.59	0.15	5.38	<0.001*
	P. gingivalis	9	9.21	0.12		
Group 3	*A. actinomycetemcomitans*	9	13.52	0.07	3.21	<0.001*
	P. gingivalis	9	10.31	0.37		

Table 3: Comparison of mean ZOI in 250 mg/ml of Methanolic Solution between *A. actinomycetemcomitans* and *P. gingivalis* using Student Paired t Test in each group.

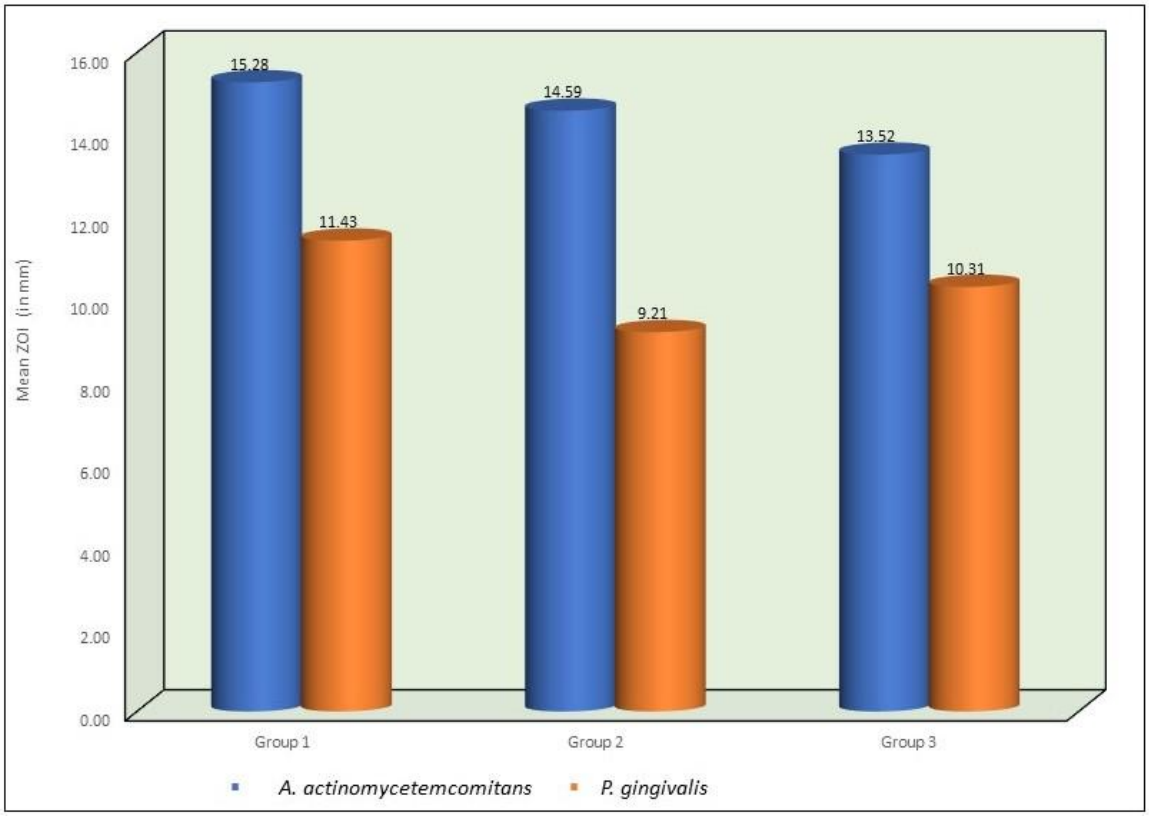
Groups	Organism	N	Mean	SD	Mean Diff	p-value
Group 1	*A. actinomycetemcomitans*	9	11.42	0.16	-2.13	<0.001*
	P. gingivalis	9	13.56	0.07		
Group 2	*A. actinomycetemcomitans*	9	12.20	0.10	2.68	<0.001*
	P. gingivalis	9	9.52	0.12		
Group 3	*A. actinomycetemcomitans*	9	12.34	0.12	1.79	<0.001*
	P. gingivalis	9	10.56	0.32		

* - Statistically Significant

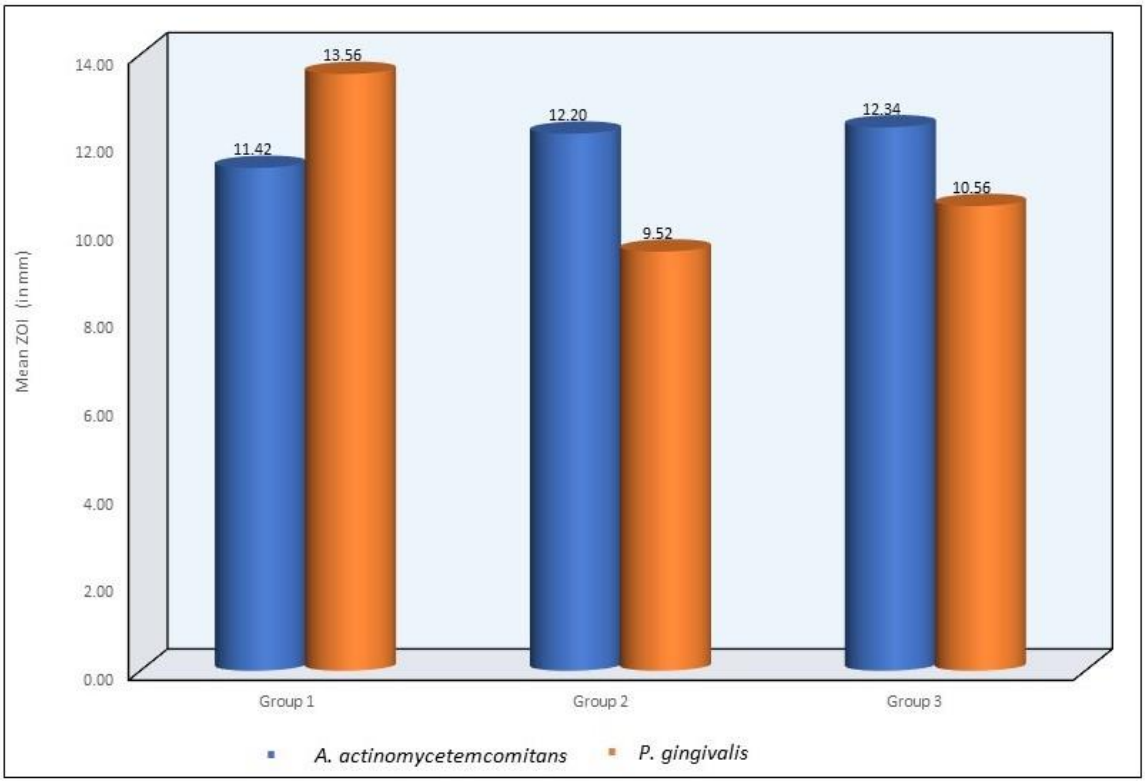
Note: Group 1 – Japanese Tea, Group 2 – Oolong Chinese Tea & Group 3 – Malaysian Sabah Black Tea



Graph no. 1: Mean ZOI in 250 mg/ml of Aqueous Solution between *A. actinomycetemcomitans* and *P. gingivalis* in each group.



Graph no. 2: Mean ZOI in 250 mg/ml of Ethanolic Solution between *A. actinomycetemcomitans* and *P. gingivalis* in each group.



Graph no. 3: Mean ZOI in 250 mg/ml of Methanolic Solution between *A. actinomycetemcomitans* and *P. gingivalis* in each group

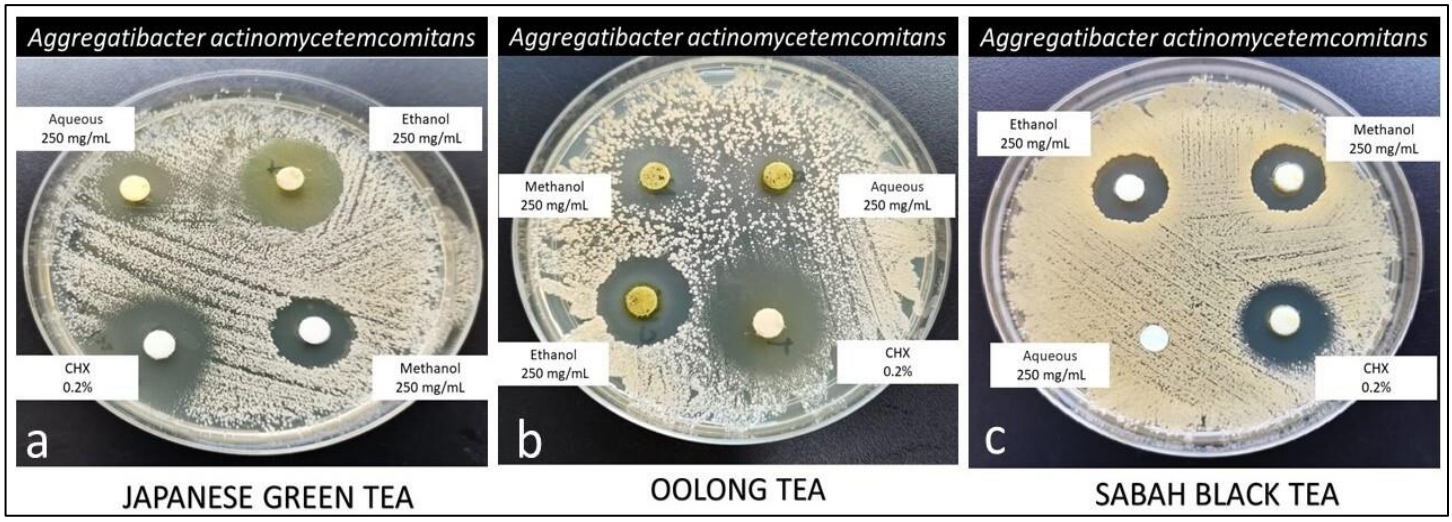


Figure 1: (a, b, and c). The antibacterial activity of Japanese green tea, Chinese oolong tea and Malaysian Sabah black tea against *A. actinomycetemcomitans.*

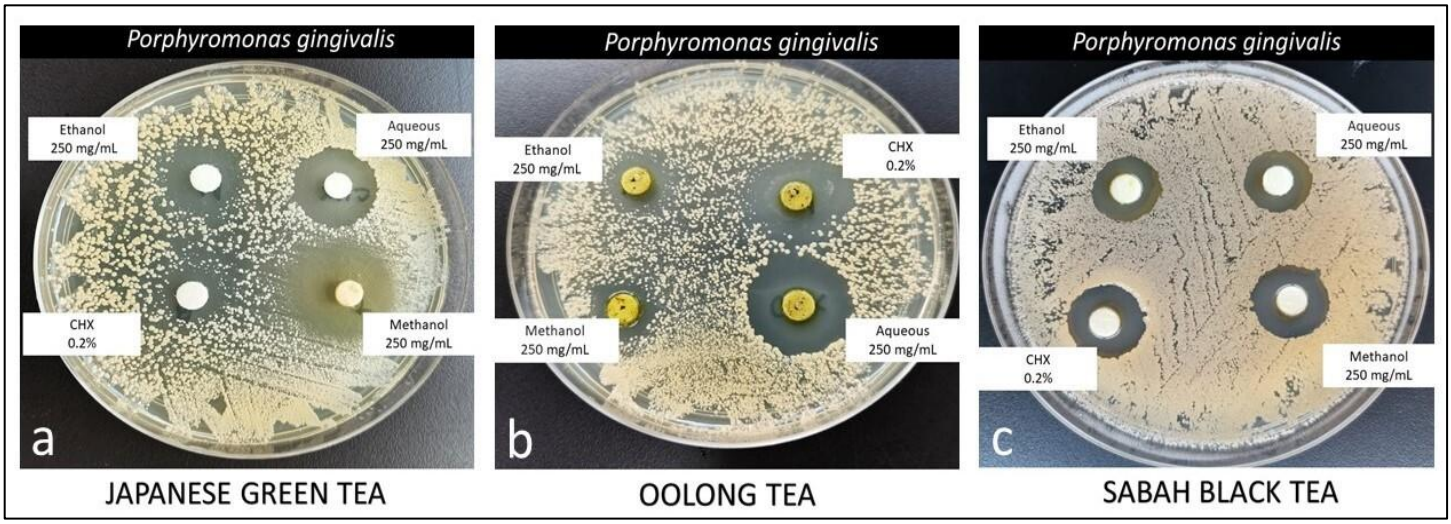


Figure 2: (a, b, and c). The antibacterial activity of Japanese green tea, Chinese oolong tea and Malaysian Sabah black tea against *P. gingivalis*.

On the other hand, 0.2% chlorhexidine was used in the current study as a positive control. It showed a significantly higher mean ZOI of 18.4 mm as compared to all other tea extracts against both *A. actinomycetumcomitans*, as it has been proven to be an antimicrobial drug against various organisms and is used as a mouth rinse.

Limitations of the study

The investigation was limited by the lack of an evaluation of the antibacterial properties of these tea extracts at their minimal inhibitory and minimal bacterial concentrations. The study will be extended for the same. Some notable variations found in *in vitro* studies might not always turn into noticeable alterations in *in vivo* as the host immunity is complex in nature. It is advised to assess the varying impacts of different tea types when consumed at various amounts and temperatures on oral health. Additionally, more clinical comparison research on the biological characteristics of distinct *Camellia sinensis* species from various non-geographical places and seasons will be needed.

Clinical significance: In addition to its numerous health advantages, green tea has been revealed to have antimicrobial effects that make it a viable alternative to other black teas. By combining the polyphenols found in green tea into oral health products, oral tissue health may be effectively sustained. To ascertain whether there are any synergistic effects on different periodontal pathogens, future clinical research should also consider evaluating the antibacterial effects of various concentrations of these tea extracts combined with other antimicrobial drugs. The results of these planned studies could ensue possible development of new and more, natural remedies that could be useful in preventing periodontal diseases with minimal side effects. Furthermore, these formulations could be implemented in addition to currently marketed drugs.

4. Conclusions

According to the obtained results on the Japanese green tea at all three different concentrations of 250 mg/mL aqueous, methanolic and ethanolic solution demonstrated superior antibacterial activity against *A. actinomycetemcomitans*, and *P. gingivalis* when compared to the other two types of tea.

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Conflict of Interest
No conflict of interest.

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References

[1] N. B. Parahitiyawa, C. Scully, W.K. Leung, W.C Yam, L.J Jin, and L.P Samaranayake. (2010). Exploring the oral bacterial flora: current status and future directions. Oral Diseases. 16(2):136-145.
[2] E.A. Eloe-Fadrosh, D.A. Rasko. (2013). The human microbiome: from symbiosis to pathogenesis. Annual review of medicine. 64:145-163.
[3] K.Y. How, K.P. Song, and K.G. Chan. (2016). *Porphyromonas gingivalis*: An Overview of

Periodontopathic Pathogen below the Gum Line. Frontiers in microbiology.7:53.

[4] M.S. Tonetti, S. Jepsen, L. Jin, and J. Otomo-Corgel. (2017). Impact of the global burden of periodontal diseases on health, nutrition, and wellbeing of mankind: A call for global action. Journal of clinical periodontology. 44(5): 456–462.

[5] I. Reynold's, and B. Duane. (2018). Periodontal disease has an impact on patients' quality of life. Evidence-based dentistry. 19(1):14–15.

[6] J. Slots. (2005). Subgingival microflora and periodontal disease. Journal of clinical periodontology. 6(5): 351–382.

[7] D.H. Fine, A.G. Patil, and S.K. Velusamy. (2019). *Aggregatibacter actinomycetemcomitans* (Aa) Under the Radar: Myths and Misunderstandings of Aa and Its Role in Aggressive Periodontitis. Frontiers in immunology.10:728.

[8] M. Niakan, N. J. Naderi, H. Jamshidian, F. Jafariazad. (2017). Antibacterial Effect of Iranian Green Tea and Black Tea against *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis* and *Prevotella Intermedia*. Medical Laboratory Journal. 11(4):13-18.

[9] E. Barca, E. Cifcibasi, and S. Cintan. (2015). Adjunctive use of antibiotics in periodontal therapy. Journal of Istanbul University Faculty of Dentistry. 49(3):55-62.

[10] S.R. Parwani, R.N. Parwani, P.J. Chitnis, H.P Dadlani, and S.V. Prasad. (2013). Comparative evaluation of anti-plaque efficacy of herbal and 0.2% Chlorhexidine gluconate mouthwash in a 4-day plaque re-growth study. Journal of Indian Society of Periodontology. 17(1): 72-77.

[11] B. Tahani, and R. Sabzian. (2018). Effect of *Camellia sinensis* plant on decreasing the level of halitosis: A systematic review. Dental research Journal. 15(6):379-384.

[12] D.E. George, R. Shetty, P.J. Shetty, and L.A. Gomes. (2017). An In vitro Study to Compare the Effect of Different Types of Tea with Chlorhexidine on Streptococcus mutans. Journal of Clinical and Diagnostic Research. 11(9): ZC05-ZC07.

[13] S. Wang, T. Zeng, S. Zhao, Y. Zhu, C. Feng, J. Zhan, et al. (2022). Multifunctional health-promoting effects of oolong tea and its products. Food Science and Human Wellness. 11(3):512-523.

[14] T. Vyas, R. Nagi, A. Bhatia, and S.K. Bains. (2021). Therapeutic effects of green tea as an antioxidant on oral health-A review. Journal of family medicine and primary care. 10(11):3998-4001.

[15] A.M. Teixeira, and C. Sousa. (2021). A Review on the Biological Activity of Camellia Species. Molecules. 26(8):2178.

[16] C. Voina, A. Delean, A. Muresan, M. Valeanu, A.M. Moldovan, V. Popescu, et al. (2020). Antimicrobial Activity and the Effect of Green Tea Experimental Gels on Teeth Surfaces. Coatings. 10(6):537.

[17] D.H. Truong, D.H. Nguyen, N.T.A. Ta, A.V Bui, T.H. Do, and H.C. Nguyen. (2019). Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of severinia buxifolia. Journal of Food Quality. 1:1-9.

[18] A.W. Bauer, W.M. Kirby, J.C. Sherris, and M. Turck. (1996). Antibiotic susceptibility testing by a standardised single disk method. American Journal of Clinical Pathology. 45(4):493-496.

[19] Y.S. Lin, Y.J. Tsai, J.S. Tsay, and J.K. Lin. (2003). Factors affecting the levels of tea polyphenols and caffeine in tea leaves. Journal of Agricultural and Food Chemistry. 51:1864–1873.

[20] M. Saito, O. Tsuzukibashi, N. Shinozaki-Kuwahara, T. Kobayashi, and K. Takada. (2012). Anticytotoxic Effect of Green Tea Catechin on Lipopolysaccharide from *Aggregatibacter actinomycetemcomitans*. International Journal of Oral-Medical Sciences. 11(3): 202-206.

[21] B.J. Shenker, D. Besack, T. McKay, L. Pankoski, A. Zekavat, and D.R. Demuth. (2004). *Actinobacillus actinomycetemcomitans* cytolethal distending toxin (Cdt): evidence that the holotoxin is composed of three subunits: CdtA, CdtB, and CdtC. Journal of immunology. 172(1):410-417.

[22] D. Grenier, and V.D. La. (2011). Proteases of *Porphyromonas gingivalis* as important virulence factors in periodontal disease and potential targets for plant-derived compounds: a review article. Current drug targets. 12(3):322-331.

[23] M.J. Mageed, S. S. Saliem, and W.M.A. Alwatar. (2015). Antibacterial effects of green tea extracts on *Aggregatibacter actinomycetemcomitans* (In-Vitro study). Journal of Baghdad College of Dentistry. 27(3):102-108.

[24] A. Araghizadeh, J. Kohanteb, and M. M. Fani. (2013). Inhibitory Activity of Green Tea (Camellia sinensis) Extract on Some Clinically Isolated Cariogenic and Periodontopathic Bacteria. Medical principles and practice: international journal of the Kuwait University, Health Science Centre. 22(4): 368–372.

[25] M.J. Mageed, and S. S. S. Juma. (2015). Antimicrobial effects of green tea extracts on *Porphyromonas Gingivalis* (in vitro study). Journal of Dental and Medical Sciences (IOSR-JDMS). 14(10): 33-39.

[26] F. Sartini, H. Handayani, and D.D. Putri. (2021). Effectiveness of catechin extract of green tea (camellia sinensis) on *porphyromonas gingivalis.* Journal of Dentomaxillofacial Science. 6(1): 27-30.

[27] M. Hirasawa, K. Takada, M. Makimura, and S. Otake. (2002). Improvement of periodontal status by green tea catechin using a local delivery system: a clinical pilot study. Journal of periodontal research. 37(6): 433–438.

[28] B. Venkateswara, K. Sirisha, and V. K. Chava. (2011). Green tea extract for periodontal health. Journal of Indian Society of Periodontology. 15(1): 18–22.

[29] M. Juliawati, M. Juslily, A.G. Soulissa, A.S. Widyarman, and E. Munadziroh. (2020). Effectiveness of brewed green tea and mouthwash containing green tea extract against *Streptococcus mutans* and *Porphyromonas gingivalis* in Saliva. Scientific Dental Journal. 4(3):101-104.

[30] P. Subramaniam, U. Eswara, and K.R.M. Reddy. (2012). Effect of different types of tea on *Streptococcus mutans*: an in vitro study. Indian journal of dental research. 23(1):43-48.

[31] T.B.L. Bedran, M-P. Morin, D.P. Spolidorio, and D. Grenier. (2015). Black Tea Extract and Its Theaflavin Derivatives Inhibit the Growth of Periodontopathogens and Modulate Interleukin-8 and β-Defensin Secretion in Oral Epithelial Cells. PLoS ONE. 10(11): e0143158.