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The Role of Green Tea Extract on Inhibiting *Porphyromonas gingivalis* as a Major Periodontitis Pathogen: *In Vitro* Study

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ABSTRACT

Porphyromonas gingivalis is one of the anaerobic bacteria that are found in patients with periodontitis. Green tea catechins have antibacterial activity against several oral pathogenic bacteria. This study aimed to determine the ability of the green tea extract fraction to inhibit the growth of *P. gingivalis*. The green tea powder was extracted with hexane by maceration, then the insoluble fraction of the hexane was extracted using high-pressure extraction with water as a solvent ratio of 1:10. Antibacterial activity test was carried out by agar dilution method and agar diffusion disk using Mueller-Hinton Agar (MHA) media. The results showed that green tea extract had antibacterial activity against *P. gingivalis* with an inhibitory value of at least 0.5% (5mg / ml) and an average diameter of the zone of inhibition at a concentration of 4 mg/disc of 16.16 mm. This study concludes that green tea extract can be made in a dosage form to prevent periodontitis.

Keywords: Green tea, Periodontitis, *Porphyromonas gingivalis*.

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INTRODUCTION

Patients with periodontal disease (periodontitis and gingivitis) increase with age. Periodontitis generally affects almost all ages and is not affected in high or low-income countries.[1] Periodontitis can be associated with various systemic diseases, including those related to cardiovascular, respiratory, musculoskeletal, reproductive system, and even oral microbial dysbiosis.[2] Bacteria that are considered as the main factor of periodontitis are *P. gingivalis*. *Porphyromonas gingivalis* is an anaerobic gram-negative bacteria, which is also a normal flora in the oral cavity, which is found mostly in the area of gingival ulcers, subgingival plaques, tongue, tonsils and is a biofilm-forming, in excess amounts can cause periodontitis.[3]

One of the causes of pathogenic bacteria being resistant to antibiotics is that they form a biofilm with other bacteria in that location during their growth. Biofilms are collections of one or more types of microorganisms that can grow on various surfaces. bacteria that form on the surface of teeth is one common example of biofilm dental plaque.[4]

Some bioactive compounds in plants are known to have antibiofilm activity against pathogenic bacteria, including the essential oil and phenolic compounds.[5,6] One product of the tea plant (*Camellia sinensis L.)* is a green tea known to have a high phenolic content, especially catechins. Previous research showed the concentration of green tea phenolics extracted with 80% ethanol is 219.6 mGAE /g extract. Green tea extract also has antibacterial activity and inhibit *Streptococcus mutans* biofilm.[7-9] This study aimed to determine green tea extract's ability to inhibit the growth potential *P. gingivalis* prepared in dosage forms of periodontitis disease prevention.

MATERIALS AND METHODS

Material, Chemical, and Reagent

The green is obtained from one of the products circulating in Makassar, Indonesia. Mueller-Hinton Agar Media, Brain Heart Infusion Broth Media, McFarland 0.5, tannic acid obtained from Merck, Germany. Gallic acid and Epigallocatechin Gallate (EGCG) are obtained from Sigma-Aldrich, disc antibiotics (Oxoid), and other ingredients.

Preparation of Green Tea Extraction

Extraction was carried out according to the previous research method with slight modifications.[10] The green tea is coarsely powdered with a mesh 18 sieve, then extracted by the maceration method with the hexane solvent ratio of 1:10. The hexane-insoluble green tea is then extracted using distilled water under high pressure. The water extract obtained was freeze-dried.

Determination of Total Polyphenol and Tannin Content

Total polyphenol content was determined according to the spectrophotometric method using Folin ciocalteu reagent with standard solutions of gallic acid and EGCG, and total tannin tests used the Follin Dennis method with tannic acid standards according to previous research methods.[10]

Determination of Minimal Inhibitory Concentration by Using Agar Dilution Method

Determination of the Minimal Inhibitory Concentration (MIC) was conducted by dilution so that according to prior research methods with some modifications.[11] The green tea lyophilisate was made of double dilutions so that the final concentration in the Mueller-Hinton Agar medium was sterile 0.125% - 2% w/v. After the media solidified 10 µl of *P. gingivalis* bacterial suspension equivalent to 0.5%, McFarland was scratched on the media's surface. Subsequently incubated in an anaerobic state for 2 days at 37^{o} C. The *Inhibitory Concentration is calculated as the lowest concentration of the test sample that can inhibit P. gingivalis.*

Determination of the Inhibition Zone Diameter Using the Agar Disc Diffusion Method

Lyophilisate antibacterial testing of green tea extract was carried out using the agar diffusion disc method, according to Achmad et al. [12], with slight modifications. Each sterile petridish was poured with 10 ml of sterile MHA media. After solidifying 10 µl of the test bacteria, which is

equivalent to 0.5% McFarland, spread on MHA media. The lyophilisate of green tea extract was dissolved in Dimethylsulfoxide (DMSO) with concentrations of two, namely: 5%, 10%, 20%, and 40%. Each disc was dripped with 10 µl of the test solution and placed on the media. Incubation under the anaerobic condition for 1-2 days at 37^{o} C. After the incubation period, the diameter of the inhibition zone formed was measured.

RESULTS

Extraction and Determination of Total Phenolic and Tannin Content

The extraction results are in the form of water extract lyophilisate with total polyphenol, and tannin content can be seen in table 1. Based on the table, it can be seen that phenolic in the form of tannins is dominant compared to other phenolics, namely the tannin content of 26.36% from the extract or about 77.22% when calculated on the total phenolic content.

Determination of MIC against *P. gingivalis* bacteria using the Agar dilution method (Fournier-Larente et al., 2016)

Make stock concentrations of green tea extract in multiples of 2, namely 1.25% to 20%. Every 1 ml of the sample solution is mixed in 9 ml of Mueller-Hinton Agar media until the media's concentration is 0.125% to 4%. After solidifying the test bacteria are scratched and incubated for 24-48 hours, the results can be seen in Figure 1. After an incubation period of 2x24 hours at a concentration of 0.5% to 20%, there was no visible growth of bacteria where the bacterial streak was carried out. Whereas at a concentration of 0.125% and 0.25%, there was a growth of *P. gingivalis* bacteria, so based on the data above, it can be concluded that the Minimal Inhibitory Concentration (MIC) of green tea extract to inhibit *P. gingivalis* bacteria is 0.5%.

Determination of the diameter of the inhibition zone using the agar disk diffusion method

The results of determining the inhibition zone's diameter from the green tea extract can be seen in Figures 2 and 2. There is a clear zone around the disc containing the test sample, both in amoxicillin as a positive control and from several green tea extract concentrations. This shows that green tea extract can inhibit the growth of *P. gingivalis*.

DISCUSSION

The green tea used is one of the local green tea products circulating in Indonesia. The chlorophyll of green tea powder is partially removed with the solvent hexane to remove the non-polar components. The aim is to optimize the extraction of the green tea catechin components, which are generally more polar. The green tea powder is then extracted using high-pressure extraction with distilled water as the extracting liquid and dried using the freeze-drying principle.[10] The total phenolic content and total tannin content obtained were lower than previous studies using the same method.[10] This is probably because the green tea product used is different, and the ratio of green tea to water used is different. Research conducted by Anita et al. found that the total phenolic content in the form of tannins (condensed tannins) was higher than flavonoids. Catechins in the form of tannins in green tea include epigallocatechin gallate (EGCG), epicatechin gallate (ECG), and epigallocatechin (EGC).[13,14]

Research has been carried out that the MIC of green tea extract against *P. gingivalis* was 250-1000 µg / ml (0.025-1.1%), depending on the bacterial strain.[15] This difference could be due to differences in the green tea extraction method and the bacterial strains used. One method to determine the antibacterial activity of plant extracts apart from determining the minimum inhibitory concentration is the inhibition zone diameter by diffusion method.[16] Green tea extract was able to inhibit the growth of P. gingivalis, previous studies support this but using different extraction methods and different types of tea.[15] The inhibitory effect of green tea extract with the majority of EGCG depends on the dose given to oral epithelial cells with several inhibitory mechanisms, including inhibiting some of the expression of *P. gingivalis* genes (fimA, hagA, hagB) involved in colonization of the host, genes involved in tissue destruction (rgpA, kgp). Also, green tea extract and its EGCG compounds can increase the expression of the htrA protein stress gene (stress protein htrA gene).[15]

CONCLUSION

Green tea extract can inhibit the growth *P. gingivalis* and likely made in the dosage form of prevention of periodontitis.

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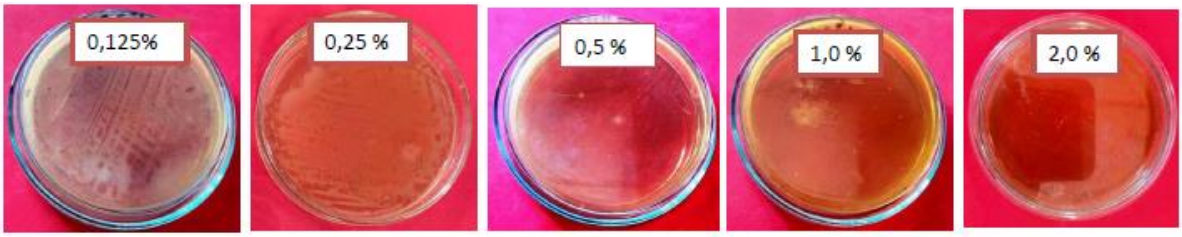

Figure 1: Minimal Inhibitory Concentration (MIC) test results with the agar dilution method after 2x24 hours incubation under anaerobic conditions

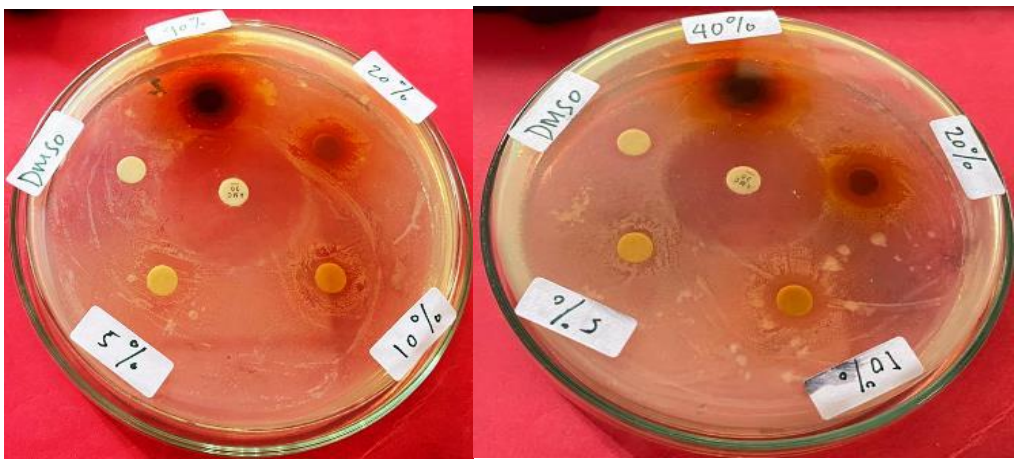

Figure 2: Antibacterial activity test results by diffusion method on MHA media after incubation under anaerobic conditions for 24-48 hours at 37° C

Table 1: Total phenolic content (TPC) and total tannin content (TTC) of Lyophilisate Green tea extract

Sample	TPC based on gallic acid (%)	TPC based on EGCG (%)	TTC Based on Tannic acid (%)
Lyophilisate Green tea extract	34.15 %	41.49 %	26.37 %

Table 2: The diameter of the inhibition zone of green tea extract against *P. gingivalis*

Green tea concentration (%)	Inhibition zone diameter ± Standard deviation (mm)
5	7.67 ± 0.67
10	9.50 ± 0.70
20	12.77 ± 0.47
40	16.17 ± 0.76