ORIGINAL ARTICLE

Phytochemical properties and antibacterial activity of green tea leaf extract from gunung gambir jember against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans:* an experimental study

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ABSTRACT

Introduction: Green tea leaves are abundant in bioactive components, including catechins, flavonoids, and polyphenols. These compounds are known for their antibacterial, anti-inflammatory, and antioxidant properties. In addition, green tea also contains minerals, such as Calcium, Phosphor, Potassium, Magnesium, and Sulphur. However, the precise mineral content and anti-bacterial properties of green tea against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans from Gunung* (Mount) Gambir in Jember Regency are currently unknown. This study aimed to analyze the phytochemical composition and antibacterial activity of green tea leaf extract from *Gunung* Gambir, Jember against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. **Methods:** The bioactive constituents of green tea leaf extract were identified using a UV-Vis spectrophotometer to measure its total flavonoid content (TFC) and total phenolic compounds (TPC). Mineral identification was performed using flame atomic absorption spectrometry. The disc diffusion method was used to assess antibacterial activity by quantifying the diameter of the inhibition zone in cultures of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* treated with green tea leaf extract at concentrations of 3.125%, 6.25%, 12.5%, 25%, 50%, and 100%. Anaerobic conditions were created by incubating the Petri dishes in a desiccator for 24 hours at 37°C. One-Way Anova test was used to analyze the data, and differences were considered statistically significant at $p<0.05$. Subsequently, a LSD test was carried out ($p<0,05$). **Results:** The TPC value was 1.607±0.742 mg GAE/g, while the TFC value was 99.146 ± 0.307 mg GAE/g. Calcium appeared to be the most abundant mineral in the tea extract, measured at 31.65 ppm. The inhibitory power of green tea leaves extract against both bacteria was not significantly different ($p>0.05$). **Conclusion:** Green tea leaves extract from *Gunung* Gambir Jember Regency in Indonesia is rich in phenolic compounds, flavonoids, and certain minerals, such us Phosphor, Calium, Magnesium, Sulphur and Calcium. These constituents are known to possess biological and antibacterial activity against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*.

KEYWORDS

Antibacterial, flavonoid, green tea extract, minerals, phenol, Jember

INTRODUCTION

Green tea, derived from the *Camellia sinensis* plant, is widely consumed by Indonesians. It is abundantly available, easily accessible, and affordable.[1,2] Green tea is an unfermented tea containing natural substances in its fresh leaves, exhibiting antioxidant and anti-inflammatory properties.[3] Oxidative stress, which can cause tissue degeneration and disease development, such as acute and chronic inflammation, often occurs during wound healing.[4,5]

Green tea contains various components with antioxidant properties, including minerals, vitamins, and polyphenols (particularly catechins). These polyphenol concentrations are higher in green tea than in black tea. Flavonoids are the most significant type of polyphenols in green tea, with catechins constituting 30–40% of the water-soluble solids.[6] Catechins exhibits numerous biological activities including antimutagenic, antidiabetic, anti-inflammatory, antibacterial, and antiviral characteristics.

Numerous in vitro and animal studies have evaluated the antioxidant properties found in green tea, focusing on the effectiveness of catechins, particularly epigallocatechin gallate (EGCG). EGCG in green tea contains, amongst others, antioxidant, antidiabetic, anti-inflammatory, antibacterial, anti-acne, and bleaching properties.[7,8] In addition, EGCG also offers important moisturizing, soothing, and easy-to-use advantages.[9]

Currently, approximately 75% of Indonesians suffer from oral inflammatory conditions, including pulpitis, gingivitis, and periodontitis.[10] Historically, the management of oral inflammation has relied on the use of synthetic or artificial substances such as corticosteroids, antibiotics, and analgesics. However, these substances potentially have the risk of side effects, including autoimmune reactions, allergies, nausea, dizziness, and drug resistance.[3]

Inflammation in the oral cavity, characterized by the presence of pathogenic bacteria that trigger an inflammatory response, can manifest as gingivitis and periodontitis. Bacterial biofilm, or dental plaque, play a crucial role in the development of periodontal infection.[11,12] Niloofar *et al*. (2012) has shown that gargling with green tea solution may decrease gum inflammation associated with periodontal disease.[13]

Porphyromonas gingivalis and *Aggregatibacter actinomycetemcomitans* are gram-negative bacteria that contribute to periodontal inflammation by producing exotoxins and endotoxins, leading to destruction of epithelium and collagen fibers. Additionally, they impair cell growth inhibition by damaging the DNA of host cells. Tea extract, particularly green tea extract, has demonstrated significant antibacterial properties against these harmful bacteria, suggesting its potential as a natural remedy for oral health.[1,14,15]

The novelty of this research lies in exploring the potential of locally sourced tea leaf extract from Mount Gambir Jember as a natural remedy for oral cavity inflammation. This study aimed to analyze the phytochemical composition and antibacterial activity of green tea leaf extract from Gunung Gambir Jember against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*.

METHODS

Commercial green tea leaves, sourced from the tea plantation owned by PTPN XII in *Gunung* Gambir, Jember, East Java, Indonesia, were extracted and used for this study. The green tea extract was prepared using maceration technique. 238 grams of dried green tea leaves were ground, sieved through an 80-mesh sieve, yielding *simplicia* powder that was then macerated in 96% ethanol with counterclockwise stirring.

The maceration mixture was filtered through a glass funnel lined with filter paper. The filtrate was then evaporated using a rotary evaporator at 180 rpm and 50°C for 3 hours, yielding 169 grams of thick extract. Subsequently, this extract

was diluted with sterile distilled water to obtain concentrations of 3.125%, 6.25%, 12.5%, 25%, 50%, and 100%.

The total phenolic content (TPC) of samples was quantified using spectrophotometric techniques, with some certain adjustments. Overall, a mixture of 0.5 mL of methanol extract, 2.5 mL of 10% Folin-Ciocalteu reagent, and 2.5 mL of 7.5% 2CO3 were incubated at 45°C for 45 minutes, with an accuracy of ± 1°C (MYQ engineering Pvt. Ltd). Replication was performed three times to determine the absorbance at 765 nm for each sample using a UV-Vis spectrophotometer (LT-2203, Labtronics, India; wavelength range: 190–1100 nm). The TPC was expressed in milligrams gallic acid equivalent per gram of (mg GAE/g) extract and calculated using a standard gallic acid curve.[14]

Total flavonoid content (TFC) was quantified utilizing a modified aluminum chloride colorimetric assay. A mixture of 0.2 ml of 2% $AlCl_3$ (w/v), 2 ml of methanol extract, and 0.2 ml of 5% $NaNO_2$ (w/v) was incubated for 5 minutes. Subsequently, 2 ml of 1 N NaOH was added, resulting in a total volume of 5 ml. Utilizing a UV-Vis spectrophotometer, the absorption at 510 nm was determined after 15 min at ambient temperature. TFC was expressed in mg quercetin equivalent per gram of extract (mg QE/g) and calculated using a standard quercetin curve.[16]

Sodium was identified through distillation, while phosphate and sulfur were detected using spectrophotometry. Potassium, magnesium, and calcium were analyzed by flame atomic absorption spectroscopy (F-AAS). 0.25% tea extract was allocated to the gastrointestinal savings. Then, 1 gr mixture was added to 3 ml concentrated H_2SO_4 with charcoal, and incubated overnight, followed by 4 h at 350°C to allow complete crushing, which was indicated by the emission of white smoke and transparent extracts.

After cooling the material to ambient temperature, 20 ml of distilled water was added. The mixture was then transferred to a distillation apparatus. To facilitate the distillation process 10 ml of 40% NaOH, 10 ml of 1% H_3BO_3, and 6 drops of Conway instructions were added. The distillation conduit was completely submerged in liquid for 4 minutes. The distillate, which had turned green and had a volume of 100-125 ml, was titrated with a standard 0.05 N H_2SO_4 until the solution turned mauve.

Twenty-one grams of Mueller-Hinton agar (MHA) powder were dissolved in 100 ml of Erlenmeyer solution containing sterile sodium and with a rod or spatula. The MHA solution was then brought to a boil and homogenized in a water bath. After sanitizing MHA media in an autoclave at 121°C for 20 minutes, a 4 mm thick media was poured onto each petri dish. Subsequently, the sterilized MHA media was incubated for 24 hour at 37°C to ensure its integrity and prevent contamination.[17]

The disc diffusion method (Kirby-Bauer) was used for antibacterial test in this study Suspensions of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* was streaked in a zig-zag pattern onto the surface of Mueller-Hinton Agar (MHA) media using a cotton swab. Subsequently, green tea extract was applied to sterile paper discs using micropipettes at concentrations of 3.125%, 6.25%, 12.5%, 25%, 50%, and 100%. A positive control was prepared using 0.2% chlorhexidine, while a negative control was prepared using sterile water.

The petri dish was then sealed and placed in an inverted position to prevent water evaporation, which could affect bacterial growth. Anaerobic conditions were created by placing the petri dish in a desiccator for 24h at 37°C. The restricted growth of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* was observed as a clear zone surrounding the paper disc. The diameter of the clear zone was measured to calculate the circular inhibition zone, while for the elliptical inhibition zone the long and short diameters were added together and divided by two.[18]

The One-Way Anova test was conducted to determine whether there were significant differences in the means among the test groups. A follow-up analysis was performed using the Post Hoc Least Significant Difference (LSD) test to identify specific differences between the groups ($p<0,05$).

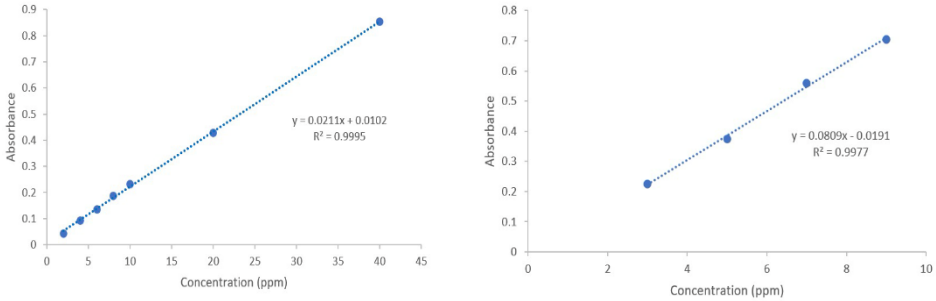
RESULTS

The total polyphenol content (TPC) and total flavonoid content (TFC) of green tea extracts from Gunung Gambir Jember are presented in Table 1. The standard curves used to calculate TPC and TFC are shown in Figure 1.

Table 1. Total phenolic content and total flavonoid content of green tea extracts from Gunung Gambir Jember

Green tea, region	TPC (mg GAE/g extract)	TFC (mg GAE/g extract)
Gunung Gambir Jember Regency	1.607±0.742	99.146±0.307

Data are mean±SD of experiments with different green tea extracts.



(A) (B)

Figure 1. Calibration curve for total phenolic content (A) and total flavonoid content (B)

The P, K, Mg, S, and Ca mineral content of green tea extracts from Gunung Gambir, Jember, is presented in Table 2.

Table 2. Mineral content (P, K, Mg, S, Ca) of green tea extracts from Gunung Gambir Jember

N	P	K	Mg	S	Ca
2.95	0.19	0.58	0.03	0.03	31.65

The mineral content of dry green tea extract was analyzed after digestion in HNO_3 and $HCLO_4$. The green tea leaf extract from *Gunung* Gambir contains phosphorus, kalium, magnesium, sulphur, and calcium. Table 3 shows dose-dependent antibacterial activity data for green tea leaf extracts from *Gunung* Gambir Jember. Antibacterial activity against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* was observed at concentrations of 0, 3.125%, 6.25%, 12.5% 25%, 50%, and 100% green tea extract; lower concentrations were not tested. The highest inhibitory effect on both bacterial strains was observed at the 100% green tea leaf extract concentration.

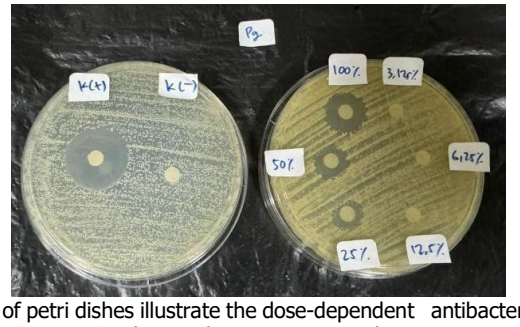


Figure 1. Photographs of petri dishes illustrate the dose-dependent antibacterial activity of green tea extract from *Gunung* Gambir Jember against *Porphyromonas gingivalis*.. K (+), positive control; K (-), negative control; 3.125%, 6.25%, 12.5%, 25%, 50%, 100% are the green tea extract concentrations.

Figure 2. Photographs of petri dishes illustrate the dose-dependent antibacterial activity of green tea extract from Gunung Gambir, Jember, against *Aggregatibacter actinomycetemcomitans*. K (+), positive control; K (-), negative control; 3.125%, 6.25%, 12.5%, 25%, 50%, 100% are the green tea extract concentrations.

Table 3. Dose-dependent antibacterial activity by green tea extracts from *Gunung* Gambir, Jember

Bacteria	Green tea extract (%)						
	0	**3.125**	**6.25**	**12.5**	**25**	**50**	**100**
A. actinomycetemcomitans	0	0	0	0	10.18±0.11^{ns}	12.30±0.1^{ns}	16.40±0.3^{ns}
P. gingivalis	0	0	0	0	10.17±0.16^{ns}	12.27±0.4^{ns}	16.45±0.7^{ns}

ns: not significant

Figures 1 and 2 illustrate the inhibition zones demonstrating the dose-dependent antibacterial activity of green tea extract from *Gunung* Gambir Jember against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* growth. Antibacterial activity was measured as the diameter of the inhibitory zone in millimeters (mm). Green tea extract at a concentration of 100% exhibited the greatest inhibitory effect on both *A. actinomycetemcomitans* (16.40±0.27) and *P. gingivalis* (16.45±0.70). The inhibitory effect of green tea leaves extract against both bacteria was similar or not significantly different ($p>0.05$).

DISCUSSION

Green tea is classified into several categories depending on the maturity of the newly harvested tea leaves. Each variety possesses distinct quality attributes, particularly in relation to fragrance, flavour, and colour amalgamation, all of which significantly influence consumer preferences. However, the maturity level of various tea shoots has a significant impact on the secondary metabolites found in green tea, thereby affecting its biochemical characteristics, such as TPC, TFC, and the bioactivity.

Table 1 illustrates that the total phenolic content (TPC) of the green tea extract from *Gunung* Gambir Jember Regency was approximately 1.607 mg GAE/g extract. Other studies have reported a TPC of green tea leaf extract from Lawang Malang of approximately 1.60 mg GAE/10 mg.[19] Another study found that the TPC of tea products from PTPN XII plants in Jember Regency was around 386,495 mg GAE/g extract. This indicates that there is a large variation in TPC among green tea from different locations.

The TPC of the green tea extract from *Gunung* Gambir region is similar to other regions, except for the PTPN XII plantation area where the TPC is significantly higher compared to those grown in Gambir plantations. Moreover, the variations in polyphenol levels in green tea could be due to the variations in the cultivars (organic or conventional agriculture), and growing conditions (soil composition, altitude, climate as well as processing conditions.[20]

According to Table 1, the TFC (Total Flavonoid Content) of the green tea extract from *Gunung* Gambir Jember Regency was approximately 99,146mg GAE/g extract. Another study reported a TFC of tea products from Makassar, which was approximately 2.77 mg QE/g extract.[21] The disparity in TFC values between these two regions can be attributed to variations in plant cultivation sites, resulting in distinct environmental conditions that affect the production of secondary metabolites in the green tea leaves.

Phenols and flavonoids are the primary bioactive constituents obtained from green tea leaves. Phenols possess a distinctive and potent ability to prevent oxidation.[22] Flavonoids, which belong to the category of phenolic chemicals, possess a range of biological functions, including antioxidant, antiviral, and antidiabetic properties.[23] Both phenols and flavonoids are receiving growing attention in medicinal science and serve as crucial indicators for assessing the quality and therapeutic efficacy of traditional medicines. Hence, the identification of these substances in green tea is crucial.[24]

The approximate mineral composition of tea is 5-7% as elements such as potassium (K), calcium (Ca), phosphorus (P), and magnesium (Mg), as well as small quantities of manganese (Mn), zinc (Zn), and copper (Cu). The amount of Ca ranges between 515.6 to 522.1 mg/L.[25,26] Table 2 shows that green tea extract contains various minerals, including phosphate, potassium, magnesium, sulfur, and calcium, which calcium exhibiting the highest mineral content, at approximately 31.85 ppm. The use of fresh tea leaves green tea processing may be the main reason for the higher calcium content.[25] Calcium is a crucial macromineral that plays a vital role in bone and tooth development. It aids in blood clotting and nerves and brain activation. It also acts as an enzyme activator, stimulates heart muscles function, and prevents the body from absorbing radioactive chemicals.[27]

The National Health Service (NHS) states that the recommended daily intake of calcium is approximately 700 to 800 mg. Moringa leaves contain potassium as one of the macrominerals, ranking second with a concentration of 0.58 ppm. Potassium is involved in various physiological processes in the body, including heart rate regulation, water balance maintenance, nerve signals transmission, acid-base balance maintenance, facilitation of chemical reactions, muscle contractions regulation, insulin release control from the pancreas, and cell membrane permeability maintenance.

According to the NHS, the recommended daily intake of potassium is approximately 2000 mg. The tea leaf extract contains a phosphorus concentration of 0.19 ppm. Phosphorus plays a crucial role in various body functions, including bone and tooth formation, metabolic processes, muscle contraction, nerve activity, formation of phosphatides in blood plasma, acid-base balance maintenance, hormonal activity regulation, and the effectiveness of certain vitamins. Magnesium and sulfur concentrations were found to be the lowest, i.e., around 0.03 ppm.[26]

Based on research results, the growth of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* was inhibited to a similar extent by green tea extract. This is in accordance with research conducted by Araghizadeh, which demonstrated that green tea extract exhibited strong antibacterial activity against *S. mutans, A. actinomycetemcomitans, P. gingivalis, and P. intermedia* and therefore may be used in mouthwashes or dentifrices for prevention of dental caries and periodontal diseases.[28] Statistical indicated no significant difference in the antibacterial activity of green tea leaf extract against *P. gingivalis* and *A. actinomycetemcomitans* (P>0,05).

These findings were most likely due to *P. gingivalis and A. actinomycetemcomitans* being Gram-negative bacteria with an outer membrane that polyphenols can penetrate to mediate cell response. Polyphenols acts as a toxin in the protoplasm, causing porin molecules to break down. Damage to the porin allows polyphenolic compounds to permeate the bacterial cell wall, lowering permeability and potentially slowing bacterial growth or killing the bacteria.[29] Flavonoids have three antimicrobial effects: inhibiting the nucleic acid synthesis, disrupting cytoplasmic membrane function, and interfering with energy metabolism by preventing bacteria from using oxygen, thereby disrupting macromolecular formation and bacterial metabolite absorption.[30]

The present study serves as a preliminary investigation into the potential therapeutic properties of tea from *Gunung* Gambir in the oral cavity. However, further research is required to corroborate these findings. This includes conducting anti-inflammatory testing using various inflammatory parameters in both laboratory (in vitro) and living organisms (in vivo) settings, as well as performing wound-healing tests.

CONCLUSION

Green tea leaves extract from *Gunung* Gambir, Jember Regency, Indonesia, contains high concentrations of phenolic compounds and flavonoids, as well as certain minerals, such us phosphor, kalium, magnesium, sulfur and Calcium that are known for their biological and antibacterial activity against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. Green tea extract from *Gunung* Gambir, Jember Regency, may be a promising treatment option for diseases in the oral cavity. The implications of this study contribute to the discovery of alternative antibacterial therapy derived from herbal materials originating from the local area of Jember.

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