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# The effectiveness of Edel varieties cacao leaf extract as an antibacterial against *Staphylococcus aureus* and *Porphyromonas gingivalis: an experimental study*

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## ABSTRACT

**Introduction:** *Staphylococcus aureus* (*S. aureus*) and *Porphyromonas gingivalis* (*P. gingivalis*) are the two main bacteria that cause dental and oral disease. Cacao leaves have been reported to have antibacterial properties. However, the active compounds in cacao leaves vary depending on the age of the leaves and the place where these plants grow. This research aims to analyze the active ingredients and antibacterial effectiveness of the Edel varieties cacao leaves against *S. aureus* and *P. gingivalis*. **Methods:** This is an experimental study. The Cacao leaves from PTPN 12 Plantation Banjarsari Jember were extracted by the maceration technique in concentrations of 25, 50, 75%, and 100%. The phytochemical test was then made using the Thin Layer Chromatography (TLC). The antibacterial activity of Cacao leaf extract against *S. aureus* ATCC 25923 and *P. gingivalis* ATCC 33277 was examined using the Disc Diffusion technique on Mueller Hinton Agar (MHA) media. **Results**: The Cacao leaf extract of the Edel varieties from Jember contained flavonoids, saponins, and tannins but did not contain alkaloids. In all concentrations tested, the Cacao leafmextract of the Edel varieties from Jember showed the inhibition zones of *S. aureus* and *P. gingivalis*. The concentration of 100% showed the greatest antibacterial activity (The highest antibacterial activity was observed at a concentration of 100%), which was moderate against *S. aureus* (10.98 mm ± 0,93) and strong against *P. gingivalis* (11.54 mm ± 0,41). There was a significant difference among concentrations in both the *P.gingivalis* and *S.aureus* bacterial groups ($p < 0.001$). At the same concentration, there was no significant difference between *P. gingivalis* and *S. aureus* ($p > 0.001$). **Conclusion**: Cacao leaf extract of the Edel varieties contains flavonoids, saponins and tannins and has antibacterial activity against *S. aureus* and *P. gingivalis*.

**KEYWORDS**
cacao leaves extract, Edel varieties, antibacterial, *S. aureus*, *P. gingivalis*

## INTRODUCTION

Oral health remains a problem around the world. Dental and oral health problems are encountered by almost half of the world's population, and gum (periodontal) disease is the 11th most common (prevalent) worldwide.[1] Indonesia's main dental and oral health problems are caries and periodontal diseases. Indonesian Basic Health Research results found that 57.6% of Indonesia's population experienced dental and oral health problems, with cavities being the most significant at 45.3% and gum swelling at 14%.[2] Dental caries is a dental problem characterized by the demineralization of teeth by plaque bacteria. Caries can induce pulp inflammation and progress to the periapical tissue, causing a dentoalveolar abscess.[3]

Periodontal disease is a teeth-supporting tissue disease caused by plaque bacteria and is characterized by gingival inflammation, periodontal pocket formations, and alveolar bone resorption.[4] *Staphylococcus aureus* (*S.aureus*) and *Porphyromonas gingivalis* (*P.gingivalis*) are the primary oral bacteria pathogens that cause dental and oral diseases. *S. aureus* is a bacteria that plays a role in dentoalveolar abscesses, angular cheilitis, parotitis, mucositis, and dry mouth.[5] *P. gingivalis* is the main bacteria that causes chronic periodontitis.[4]

One of the treatments for dental and oral diseases aimed at controlling pathogenic bacteria is administering antibiotics. However, inappropriate or irrational use of antibiotics, and increasing access to antibiotics freely without a prescription has resulted in the acceleration of antibiotic-resistance.[6] It was reported that an oral bacterium is resistant to methicillin,[7] vancomycin, linezolid, macrolides, lincosamides, including clindamycin antibiotics.[8] To minimize the drug resistance, it is necessary to seek alternative materials by utilizing plants in the surrounding environment.

Cacao is one of the most essential foreign exchange-generating commodities in Indonesia. Edel, a fine-type cacao, is the best quality cacao in the world. The Edel cacao costs two to three times higher than other types of cacao. This type of cacao is well-developed in Jember ,the second-largest cacao plantation-producing district in East Java.[9] Several studies have demonstrated the health benefits of cacao. It is reported that cacao may prevent or treat allergies, anxiety, inflammatory conditions, hyperglycemia, insulin resistance, and cancer. [10] Cacao beans[11] and cacao bean husk[12] have antibacterial against *S. mutans*. Cacao bean has been reported to inhibit the growth and adherence of *P. gingivalis*.[13] Meanwhile, cacao leaves have been reported to have antibacterial properties against *S. mutans*[14] and *S. aureus* bacteria at concentrations of 10-30%[15] and 50-100 µl.[16]

No research has been reported on the antibacterial activity against *S. aureus* and *P. gingivalis* of cacao leaves of the Edel varieties from Jember. According to Azzah & Ade,[17] the antibacterial activity of cacao leaves was reported to be greater when compared to cacao pod parts. The content of active compounds in leaves varies depending on the age of the plant, the age of the leaves, and the place where these plants grow.[18] This study aims to analyzed the antibacterial effectiveness of the active ingredients of the Edel varieties of cacao leaves against *S. aureus* and *P. gingivalis*.

## METHODS

This research is an experimental laboratory in vitro study on cacao leaf extract of the Edel varieties, which are well-grown in Jember. The leaves were taken from the Cacao plant Banjarsari PTPN 12 Jember plantation. They have been identified as Cacao plants by The Integrated Agricultural Development of Agricultural Polytechnic of Jember University. The Cacao leaves of the Edel varieties were extracted using the maceration technique. The leaves were washed and air dried at room temperature for 3 days. They were then cut into pieces, dried in an oven at 40°C for 2 days, blended into a powder, and sifted. A total of 500 grams of cacao leaves powder produced from 2 kg of cacao leaves.[Two kilograms of cacao leaves were used to produce 500 grams of ground cacao leaves]. Cacao leaf powder, extracted by maceration technique[11], was dissolved in 96% ethanol at a ratio of 1:5 for 3 days at room temperature. During the maceration process, stirring was repeated every 12 hours for 5 minutes. The maceration results were filtered using filter paper, and the filtrate was evaporated in a 180 rpm rotary evaporator at $50^0$C for 3 hours to obtain cacao leaf extract. The extract was then diluted to 25%, 50%, 75%, and 100% with sterile distilled water.

The extract of cacao leaves of the Edel varieties was examined for its phytochemical ingredient by Thin Layer Chromatography (TLC) analysis, including alkaloid, flavonoid, saponin, and tannin test. For the alkaloid test, the TLC analysis was carried out using the Dragendorff reagent on the stationary phase of silica gel F254 and the mobile phase of Ethyl acetate: methanol:water (9:2:2). The alkaloid test was positive if an orange colour appeared. [Orange color indicated a positive alkaloid test result]. For the flavonoid test, the stationary phase was silica gel F254, and the mobile phase was butanol:glacial acetic acid:water (4:1:5) with ammonia vapor reagent. The result was indicated by yellow coloration. For the saponin test, the stationary phase was silica gel F254, and the mobile phase was n-hexane:ethyl acetate (4:1) with anisaldehyde sulfuric acid. The saponin test was positive if a red-purple coloration appeared. The tannin test performed on the stationary phase was the silica gel F254, and the mobile phase was toluene:acetone:formic acid (6:6:1) with FeCl3 reagent. The alkaloid test was positive if a black colour appeared.

The antibacterial activity of cacao leaf extract was tested using the Disc Diffusion technique on the Mueller Hington Agar (MHA) media. With three streaking movements, the bacterial suspension of *S. aureus* ATCC 25923 and *P. gingivalis* ATCC 33277 that had been adjusted for turbidity with the McFarland 0.5 absorbance scale was inoculated into each MHA medium. The paper discs were then dipped in cacao leaves to extract concentrations of 25, 50, 75, and 100% and placed in the test medium. Chloramphenicol was used as a positive control for *S. aureus*, metronidazole for *P gingivalis* and as a negative control, aquadest was used. After that, the test medium was covered, put in a desiccator, and incubated at 37°C for 24 hours. This treatment was carried out on fourtest media in each test group. Using a vernier calliper, the antibacterial activity was determined by measuring the inhibition zone, which was formed around the disc paper. Each test media was measured three times, and the average was obtained. The results of the inhibition measurements were categorized according to the Davis & Stout[19] category, such as no inhibition (0), weak (diameter < 5mm), moderate (5-10 mm), strong (11-20 mm), and very strong (20-30 mm).

The Shapiro-Wilk normality test and Lavene's homogeneity test were used to examine the data results. Furthermore, the one-way ANOVA test was carried out to determine differences among groups and continued with the LSD post hoc test using SPSS Statistics 25.

## RESULTS

The results of phytochemical ingredients in cacao leaf extract using TLC analysis found that the cacao leaf extract of the Edel varieties from Jember showed positive results for flavonoids, saponins, and tannins but negative results for alkaloid compounds (Table 1).

**Table 1.** The active compounds of cacao leaf extract of Edel varieties from Jember

| Class of the compounds | Results | TLC analysis (precipitate formed) |
|---|---|---|
| Flavonoid | + | Yellow |
| Saponin | + | Red-purple |
| Tanin | + | Black |
| Alkaloid | - | No Orange |

The results of the antibacterial activity of cacao leaf extract against *S. aureus* showed that there was a clear zone around the paper discs that were given cacao leaf extract at all concentrations, which was an inhibition zone formed around the paper discs in the form of round or oval clear areas. This shows that cacao leaves inhibit the growth of *S. aureus*. They exhibited the greatest inhibition zone against *S. aureus* at an exact concentration of 100%, with an average of 10.975 mm, and the smallest one at a concentration of 25%, with an average of 8.2 mm. The antibacterial activity of cacao leaf extract against *S.aureus* was categorized as moderate (Table 2).

**Table 2.** Antibacterial activity of cacao leaf extract of the Edel varieties from Jember against *S. aureus*

| Groups Extract concentration | $\bar{x}$±SD (mm) | Category | *p-value* |
|---|---|---|---|
| 25% | 8.20 ± 0.35[a] | Moderate | |
| 50% | 9.48 ± 0.36[b] | Moderate | |
| 75% | 10.5 ± 0.37[c] | Moderate | 0,001* |
| 100% | 10.98 ± 0.93[d] | Moderate | |
| Positive control | 22.90 ± 1.27 | Very Strong | |
| Negative control | 0 | None | |

*significant at α=0,05 (One Way Anova)
[a,b,c,d] the same superscript shows no significant difference (post hoc LSD)

The results of the antibacterial activity of cacao leaf extract against *P. gingivalis* in this study also showed that the growth of *P. gingivalis* bacteria was inhibited by the extract at all concentrations, as indicated by the formation of a clear zone around the disc paper. The inhibition zone formed around the disc paper was a round or oval, clear area. The measurements of the inhibition zone demonstrated that the average diameter of the inhibition zone of cacao leaf extract against *P. gingivalis* was obtained. The largest inhibition zone seen at the concentration of 100% was 11.54 mm, and the smallest, at a concentration of 25%, was 8.36 mm. The antibacterial activity of cacao leaves at a concentration of 100% extract against *P. gingivalis* is in the strong category (Table 3).

**Table 3.** Antibacterial activities of the Edel varieties of cacao leaf extract from Jember against *P. gingivalis*

| Groups Extract concentration | $\bar{x}$±SD (mm) | Category | *p-value* |
|---|---|---|---|
| 25% | 8.36 ± 0.35[a] | Moderate | |
| 50% | 9.56 ± 0.17[b] | Moderate | |
| 75% | 10.47 ± 0.20[c] | Moderate | 0,001* |
| 100% | 11.54 ± 0.41[d] | Strong | |
| Positive control | 33.06 ± 1.37 | Very Strong | |
| Negative control | 0 | None | |

*significant at α=0,05 (One Way Anova)
[a,b,c,d] the same superscript shows no significant difference (post hoc LSD)

The results of the one-way ANOVA test showed a significant difference ($p<0.05$), and the results of the LSD post hoc test showed that there were significant differences between concentrations in both the *P. gingivalis* and *S.aureus* bacterial groups ($p<0.05$ ). At the same concentration, the antibacterial activity of Cacao leaves extract was no significant difference against *P. gingivalis* and *S. aureus* ($p>0.05$).

## DISCUSSION

*S.aureus* and *P.gingivalis* are the main oral bacteria pathogens that cause dental and oral diseases.s Therefore,control of these two types of bacteria is necessary to administer and prevent the severity of the disease. This research is the first study to show that cacao leaf extract of the Edel varieties from Jember has antibacterial activity against *S. aureus* and *P. gingivalis*. This study's results align with research by Mandhaki et al.[15], which proved the ability of cacao leaf extract from Tulungagung to inhibit the growth of *S. aureus* at lower concentrations than those used in this study. It also corresponds with the study of Singh et al.[16] who reported that cacao leaf extracted from India was proven to have an inhibitory effect on the growth of *S. aureus*. In this study, the inhibition shown by cacao leaf extract of the Edel varieties from Jember against *S. aureus* increased as the concentration of the extract increased, but remained moderate, at all concentrations tested.

So far, no studies have reported the antibacterial activity of cacao leaf extract against *P. gingivalis*. However, parts of cacao tree that have been reported to have antibacterial activity against

*P. gingivalis* are the cacao bean[13,20] and cacao shell.[21] The results of this study proved that the cacao leaf extract of the Edel varieties from Jember has an antibacterial activity on *P. gingivalis*. This study provided evidence that the leaves of cacao plants also have antibacterial activity against *P. gingivalis*. The antibacterial activity enhancement depends on the concentration of the cacao leaf extract. At a concentration of 100 percent, the inhibitory effect of the cacao leaf extract was strong, while at a lower concentration, it was moderate. The inhibition against *S. aureus* and *P. gingivalis* bacteria shown in this study was presumably due to active ingredients. This study found that the Edel varieties cacao leaves extracted from Jember contained flavonoids, saponins, and tannins, but did not contain alkaloids. This result is in line with the study of Singh et al.,[16] who found that cacao leaves from India contained flavonoids, saponins and tannins and did not contain alkaloids. Research on the ethanol fraction of cacao leaves from Tulungagung also contained flavonoids, saponins and tannins.[15] In contrast to this study, in studies by Mandhaki et al.[15], no alkaloid content was examined, so it could not be determined whether the cacao leaves extract from Tulungagung contained alkaloids. [In contrast, the study by Mandki et al. did not examine the alkaloid content in the cacao leaf extract from Tulungagung, so its alkaloid content could not be determined]

The results of this study, however, were slightly different from the opinion of Parbuntari et al.[17] who stated that apart from polyphenolic flavonoids, terpenoid, and saponins, cacao leaves also contain alkaloids. The cacao plant growth environment may result in these differences. In addition, the part of the leaf used can also affect its content [composition]. The compounds in cacao leaves vary depending on the age of the plant, the age of the leaves, and the place where these plants grow.[18] In this study, older leaves of cacao trees, number 5 to 8 from the shoots, were studied. The old leaves were reported to contain more polyphenol than young leaves.

The flavonoid, saponin, and tannin compositions in the cacao leaf extract of the Edel varieties from Jember are assumed to act as antibacterials against *S. aureus* and *P. gingivalis*. Flavonoids, saponins and tannins are active plant ingredients with pharmacological effects[22,23], including antibacterial.[24-26] Several studies have proven the antibacterial abilities of flavonoids, saponins and tannins. The flavonoid and tannin compounds from guava leaf extract have strong antibacterial activity against *S. aureus*.[26] The flavonoid compounds from *Epimedium* species plant extract have been proven to inhibit the growth of *P. gingivalis*.[25] Meanwhile, the saponin compounds from *Quinoa husks* have been reported to have antibacterial effect against halitosis-causing bacteria including *P. gingivalis*.[24] In addition, the tannins from *Anthemis praecox Link*, which is a type of small flowering plant, have an antibacterial effect on *S. aureus*.[22] The antibacterial activity of flavonoids from guava leaves (*Anacardium occidentale L*) extract against *S. aureus* is higher than tannins.[26] In this study, the antibacterial activity of cacao leaf extract of Edel varieties from Jember had the same antibacterial activity against *S. aureus* as against *P. gingivalis,* but it is not yet known which active compounds have the most dominant [potent?] antibacterial effect .

Flavonoids can cause bacterial lysis by denaturing proteins, damaging bacterial cell membranes, and also inhibiting bacterial metabolism.[27,28] Saponins can damage the permeability of bacterial membranes by reducing the surface tension of the bacterial cell wall and resulting in cell death.[29] Tannins can inhibit attachment on the cell of the bacterial surface by inactivating enzymes and microbial cell adhesion, and interfering with protein transport in the inner layer of cells. In addition, tannins inhibit the absorption of sugars and amino acids so that bacteria do not have an energy source.[30] In this research, the percentage of flavonoids, saponins, and tannins in cacao leaf extract of Edel varieties from Jember and the mechanism of action of these active compounds are still unknown; therefore, further research is needed to verify them. The concentration of cacao leaf extract can affect the active compound content, causing differences in antibacterial activity.

This study has proven that the cacao leaves of the Edel varieties from Jember have antibacterial abilities against two main pathogenic bacteria in the oral cavity. It can provide opportunities for the utilization of the cacao leaves of the Edel varieties from Jember to be developed as an antibacterial agent in the oral or dental treatment. However, this research has not investigated the lowest concentration of cacao leaves needed to inhibit or prevent the growth of those bacteria. Further research is needed to study the minimal inhibitory concentration and bacterial concentration, the biocompatibility, and toxicity of cacao leaves extract of the Edel varieties from Jember. The results of this study can add value to the benefits of the cacao leaves of the Edel varieties from Jember.

## CONCLUSION

Cacao leaf extract of the Edel varieties from Jember contains flavonoids, saponins and tannins. The antibacterial activity of Cacao leaf extract of the Edel varieties against *S. aureus* was similar to that of *P. gingivalis*.

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