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Inhibitory Effect of Boesenbergia rotunda Rhizome Ethanol Extract on Porphyromonas gingivalis Lipopolysaccharide

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Abstract: Periodontal disease is an inflammatory condition that damages periodontal supporting tissues and is closely associated with microbial biofilm. Porphyromonas gingivalis is a major periodontal pathogen, and its lipopolysaccharide (LPS) contributes to host inflammatory responses and tissue destruction. This study aimed to determine the inhibitory effect of Boesenbergia rotunda rhizome ethanol extract on P. gingivalis LPS. A laboratory experimental study with a posttest-only control design was conducted in vitro. P. gingivalis ATCC 33277 was treated with B. rotunda extract at 10, 20, and 40 µg/mL, chlorhexidine 0.2% as the positive control, and distilled water as the negative control. LPS concentration was measured using ELISA and analyzed with Shapiro-Wilk, Kruskal-Wallis, and Mann-Whitney tests. The lowest LPS concentration was observed in the 40 µg/mL extract group, with a mean of 2.33 ± 1.46 ng/mL. The Kruskal-Wallis test showed a significant difference among groups ($p = 0.001$). B. rotunda rhizome ethanol extract inhibited P. gingivalis LPS, with 40 µg/mL showing the strongest effect.

Keywords: Boesenbergia Rotunda, ELISA, Lipopolysaccharide, Periodontal Disease, Porphyromonas Gingivalis.

INTRODUCTION

Periodontal disease is an inflammatory disorder affecting the supporting tissues of the teeth, including the gingiva, periodontal ligament, cementum, and alveolar bone. The condition is mainly initiated by microorganisms in subgingival biofilm and may progress to irreversible tissue destruction when oral hygiene and preventive care are inadequate. In Indonesia, national health data indicate that periodontitis remains a substantial oral-health problem, making the development of safe preventive and adjunctive agents relevant for public health practice (Aliyah et al., 2022; Kementerian Kesehatan Republik Indonesia, 2018; Rohmawati & Santik, 2019).

Clinically, periodontal disease is not merely a local gingival disorder but a biological process involving a complex interaction among microbial biofilm, host immune response, and tissue susceptibility. When subgingival biofilm is uncontrolled, the microbial community can

shift from a relatively balanced ecosystem to a dysbiotic community dominated by anaerobic pathogens. This shift prolongs inflammation, increases periodontal pocket depth, and raises the risk of clinical attachment loss. Therefore, periodontal control should not only reduce plaque mechanically but also suppress bacterial virulence factors that stimulate inflammatory damage (Aliyah et al., 2022; Rohmawati & Santik, 2019).

Porphyromonas gingivalis is one of the major pathogens associated with periodontitis. It belongs to the red-complex bacteria together with *Treponema denticola* and *Tannerella forsythia* and is often linked with initiation and progression of periodontal destruction. This bacterium can penetrate the epithelial barrier, modulate host immunity, and produce virulence factors such as fimbriae, gingipains, capsules, outer membrane vesicles, and lipopolysaccharide (LPS) (Aleksijević et al., 2022; Takeuchi et al., 2022; Xu et al., 2020). LPS, as an outer-membrane component of Gram-negative bacteria, is recognized by innate immunity and can trigger mediator activation that contributes to periodontal tissue damage. The biological activity of *P. gingivalis* LPS is strongly influenced by lipid A structural variation, which can alter the intensity of the host inflammatory response (Maldonado et al., 2016)

The use of natural products has become an important research direction because many medicinal plants contain bioactive compounds with antibacterial, antioxidant, and anti-inflammatory properties. *Boesenbergia rotunda*, locally known as temu kunci or fingerroot, is a Zingiberaceae plant used as food and in traditional medicine. Its rhizome contains essential oils, flavonoids, saponins, phenolic compounds, panduratin A, pinostrobin, pinocembrin, and cardamonin, which support broad biological activity (Atun & Handayani, 2017; Kautsari et al., 2021; Mukti & Andriani, 2021; Silalahi, 2017; Widyantari & Sari, 2022). These compounds may disrupt microbial membranes, reduce biofilm formation, inhibit enzymatic activity, and reduce oxidative stress, making *B. rotunda* a promising candidate for studies involving periodontal pathogens.

Previous studies have reported that natural substances and oral-care adjuncts can inhibit *P. gingivalis*, supporting the possibility of alternative antimicrobial approaches in dentistry (Aliyani et al., 2023; Chen et al., 2023; Ramadhani et al., 2022). Related studies on *B. rotunda* have shown antimicrobial and oral antipathogenic activity, including inhibitory effects against *Streptococcus mutans* and *Candida albicans* biofilm formation (Han et al., 2024; Kanchanapiboon et al., 2020; Mahmudah & Atun, 2017; Sanguansermsri et al., 2024). However, studies specifically evaluating the inhibitory effect of *B. rotunda* rhizome ethanol extract on *P. gingivalis* LPS remain limited. This study was therefore conducted to determine the inhibitory effect of *B. rotunda* rhizome ethanol extract on *P. gingivalis* LPS as a basis for developing natural adjunctive agents for periodontal disease prevention.

METHOD

This research was a laboratory experimental study conducted *in vitro* using a *posttest-only control design*. The study measured the ability of *Boesenbergia rotunda* rhizome ethanol extract to inhibit LPS produced by *Porphyromonas gingivalis*. The extraction process was carried out at the Balai Pengujian Standar Instrumen Tanaman Rempah, Obat dan Aromatik, while the ELISA examination was performed at the Microbiology Center of Research and Education Laboratory, Faculty of Dentistry, Universitas Trisakti, from September to December 2024.

The samples consisted of *B. rotunda* rhizome ethanol extract at concentrations of 10, 20, and 40 µg/mL and *P. gingivalis* ATCC 33277. Five groups were used: chlorhexidine 0.2% as the positive control, distilled water as the negative control, and three extract-treatment groups. Replication was determined using the Federer formula, resulting in at least five repetitions for each group. The three concentrations were selected to observe a gradual

concentration response, while the positive and negative controls provided comparative reference conditions.

Table 1. Research variables and measurements

Variable	Operational Definition	Scale
B. rotunda rhizome ethanol extract	Rhizome extract obtained by 96% ethanol maceration and prepared at concentrations of 10, 20, and 40 µg/mL.	Nominal
P. gingivalis LPS production	LPS concentration produced by P. gingivalis after treatment and measured using ELISA.	Ratio

Source: Research data

The rhizome was extracted by maceration using 96% ethanol. The material was washed, drained, sliced to a thickness of 2-3 mm, crushed, and soaked in ethanol at a ratio of 1:5 for 2-3 days. The filtrate was separated and evaporated using a rotary evaporator until a concentrated extract was obtained. Maceration was selected because the method is simple and suitable for obtaining polar and semi-polar compounds, while extraction time, solvent type, and separation technique may influence extract yield and phytochemical composition (Handoyo, 2020; Mawarda et al., 2020; Wijaya et al., 2024).

The test material was prepared by placing 10 mg of extract into a tube, adding distilled water, homogenizing with a vortex, centrifuging, and filtering with a 0.22 µm syringe filter. *P. gingivalis* was cultured in *Brain Heart Infusion Broth* and incubated anaerobically at 37 °C for 24 hours. After treatment and incubation, the supernatant was collected and analyzed using a Human LPS ELISA kit. Standard, blank, and sample solutions were placed into a 96-well plate according to the kit procedure, followed by incubation, washing, biotinylated antibody, Streptavidin-HRP, TMB substrate, and stop reagent. Optical density was read using a microplate reader. ELISA was used because it provides sensitive and quantitative detection based on antigen-antibody interaction and standard-curve calculation (Rohima & Nurminabari, 2018; Santosa, 2020; Visakhadevi et al., 2023).

Data were analyzed using the Shapiro-Wilk test for normality. Because some data were not normally distributed, differences among groups were analyzed using the Kruskal-Wallis test and followed by the Mann-Whitney post hoc test. Ethical clearance was obtained from the Ethics Committee of the Faculty of Dentistry, Universitas Trisakti, with number 762A/S1/KEPK/FKG/8/2024.

RESULTS AND DISCUSSION

The ELISA results were obtained as optical density values and converted into LPS concentrations based on the standard curve. The standard curve produced the equation y = 17.774x1.7759 with a coefficient of determination (R2) of 0.962. This R2 value, which is close to 1, indicates a strong relationship between optical density and LPS concentration, so the converted sample values could be compared quantitatively across treatment groups.

Table 2. Mean ELISA results in each treatment group

Treatment Group	Mean OD	LPS Concentration (ng/mL)
Positive control (chlorhexidine 0.2%)	0.358	3.12 ± 2.04
Negative control (distilled water)	0.713	9.85 ± 2.35
Extract 40 µg/mL	0.306	2.33 ± 1.46
Extract 20 µg/mL	0.367	3.24 ± 1.99
Extract 10 µg/mL	0.569	6.76 ± 1.76

Source: Research data

Table 2 shows that the negative control had the highest mean LPS concentration, whereas the 40 µg/mL extract group had the lowest mean concentration. The negative control produced 9.85 ± 2.35 ng/mL, while the 40 µg/mL group produced 2.33 ± 1.46 ng/mL. The 20 µg/mL group also showed a low LPS concentration of 3.24 ± 1.99 ng/mL, close to the chlorhexidine 0.2% group. This pattern indicates that increasing extract concentration was followed by a decrease in the measured LPS concentration.

The decrease in LPS concentration suggests that the active compounds in *B. rotunda* may affect bacterial stability or processes related to the release of outer-membrane components. Because LPS is located in the outer membrane of Gram-negative bacteria, disruption of membrane integrity may reduce the amount of LPS detected in the culture supernatant. The effect may also reflect reduced bacterial metabolic activity or impaired expression of virulence-related components. Therefore, the finding should be interpreted not only as an antibacterial effect but also as a possible antivirulence effect against *P. gingivalis*.

The 40 µg/mL concentration produced the lowest LPS level, indicating that this concentration may provide sufficient bioactive exposure to produce a stronger biological pressure on *P. gingivalis*. As the extract was a crude ethanol extract, the observed effect was probably caused by the combined action of several compounds, including flavonoids, phenolics, saponins, and chalcone derivatives, rather than a single isolated molecule. This interpretation is consistent with the phytochemical profile of *B. rotunda* and the broad pharmacological activity reported for the plant (Atun & Handayani, 2017; Mukti & Andriani, 2021; Rohima & Nurminabari, 2018; Silalahi, 2017)

The statistical analysis supported the descriptive pattern. The Shapiro-Wilk test indicated that several groups were not normally distributed; therefore, Kruskal-Wallis analysis was used. The Kruskal-Wallis test showed a significant difference among treatment groups ($p = 0.001$). This result means that treatment with *B. rotunda* rhizome ethanol extract affected LPS concentration in the experimental model. The use of a nonparametric analysis was appropriate because the sample size per group was limited and the distribution assumptions were not uniformly met.

Table 3. Normality and Kruskal-Wallis test results

Analysis	Group / Variable	p-value
Shapiro-Wilk	Chlorhexidine 0.2%	0.155*
Shapiro-Wilk	Distilled water	0.047
Shapiro-Wilk	Extract 40 µg/mL	0.040
Shapiro-Wilk	Extract 20 µg/mL	0.207*
Shapiro-Wilk	Extract 10 µg/mL	0.046
Kruskal-Wallis	Inhibition of P. gingivalis LPS	0.001

Source: Research data; *normally distributed data ($p > 0.05$)

The Mann-Whitney post hoc test showed significant differences between the negative control and the 40 µg/mL and 20 µg/mL extract groups. Significant differences also appeared between the higher extract concentrations and the 10 µg/mL group. These results strengthen

the interpretation that the extract response varied by concentration and that 20 and 40 µg/mL were more effective than 10 µg/mL in reducing *P. gingivalis* LPS under the tested conditions.

Table 4. Summary of Mann-Whitney post hoc test

Treatment Pair	p-value	Interpretation
Positive control vs negative control	0.009	Significant difference
Positive control vs extract 10 µg/mL	0.009	Significant difference
Negative control vs extract 40 µg/mL	0.009	Significant difference
Negative control vs extract 20 µg/mL	0.009	Significant difference
Extract 40 µg/mL vs extract 10 µg/mL	0.009	Significant difference
Extract 20 µg/mL vs extract 10 µg/mL	0.009	Significant difference

Source: Research data

The inhibitory activity can be related to flavonoids, phenolic compounds, and saponins contained in *B. rotunda*. Flavonoids have been associated with antibacterial mechanisms such as disruption of bacterial membranes, inhibition of nucleic acid synthesis, interference with energy metabolism, and changes in membrane permeability (Khoirunnisa & Sumiwi, 2019). Saponins can interact with membrane lipid components and may contribute to membrane disruption because of their amphiphilic properties (Purnamaningsih et al., 2017). When these mechanisms occur in Gram-negative bacteria, disturbance of the outer membrane can logically influence LPS release or detection.

The present finding is also consistent with previous reports on the antimicrobial potential of *B. rotunda*. (Mahmudah & Atun, 2017) reported that *B. rotunda* ethanol extract inhibited *Streptococcus mutans*, while (Kanchanapiboon et al., 2020) showed that *B. rotunda* extract could inhibit *Candida albicans* biofilm formation through pinostrobin and pinocembrin. More recent studies also reported antimicrobial, antioxidant, cytotoxic, oral antipathogenic, and antiviral properties of *B. rotunda* rhizome extract and panduratin A (Han et al., 2024; Sanguansermsri et al., 2024). By focusing on *P. gingivalis* LPS, the present study extends the evidence from general antimicrobial activity to a specific virulence-related component of a periodontal pathogen.

In the periodontal context, suppressing LPS is biologically important because LPS can stimulate immune cells and periodontal tissue cells to produce cytokines, chemokines, prostaglandins, and matrix-degrading enzymes. Persistent exposure to LPS may therefore prolong inflammation and promote periodontal destruction. Periodontal inflammation has also been discussed in relation to systemic conditions through chronic inflammatory pathways and possible bacterial dissemination (Nurfaizah & Adam, 2023; Santoso, 2019; Singhrao et al., 2015). Thus, a natural extract capable of reducing *P. gingivalis* LPS may have value as an adjunctive preventive candidate, although clinical relevance still requires further verification.

Although the results were promising, several limitations should be acknowledged. This study used only three extract concentrations, so the optimum inhibitory range has not been established. The study also did not compare different solvents or extraction methods, even though solvent polarity and extraction technique can influence yield and compound composition (Azmir & Wijayanti, 2022; Mawarda et al., 2020). In addition, this research used an *in vitro* planktonic bacterial model, while periodontal pathogens in the oral cavity are commonly organized in complex biofilms. Future studies should evaluate cytotoxicity, biofilm models, active-compound standardization, formulation stability, and possible clinical application.

Overall, the results demonstrate that *B. rotunda* rhizome ethanol extract can inhibit *P. gingivalis* LPS in a concentration-related manner. Chlorhexidine remains a widely used antimicrobial control in dentistry, and other adjunctive approaches such as xylitol and natural agents have been evaluated against *P. gingivalis* (Chen et al., 2023). The current findings

support further investigation of *B. rotunda* as a natural adjunctive material in periodontal disease prevention, especially through the reduction of virulence-associated inflammatory stimulation.

CONCLUSION

Boesenbergia rotunda rhizome ethanol extract inhibited *Porphyromonas gingivalis* lipopolysaccharide based on ELISA measurement. The 40 µg/mL concentration showed the strongest inhibitory effect, with the lowest mean LPS concentration of 2.33 ± 1.46 ng/mL. Kruskal-Wallis analysis showed a significant difference among treatment groups, and the Mann-Whitney post hoc test showed significant differences between the negative control and the 40 µg/mL and 20 µg/mL extract groups. These findings indicate that *B. rotunda* has potential as a natural adjunctive candidate for periodontal disease prevention by reducing a virulence factor of *P. gingivalis*. Further research should include wider concentration ranges, solvent comparison, toxicity testing, biofilm models, active-compound standardization, and formulation development before clinical application can be considered.

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