# Oral antipathogenic and antiviral properties of *Boesenbergia rotunda* (L.) Mansf. Extracts with panduratin A as active compound

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## ABSTRACT

This study investigated the anti-oral pathogen and anti-human coronavirus properties of *Boesenbergia rotunda*, a traditional Thai medicinal plant used for treating oral diseases. *Boesenbergia rotunda* Crude extracts (CBR) and *B. rotunda* fractionated extracts (FBR) of *B. rotunda* were obtained and analyzed. Panduratin A, present in both CBR and FBR, was found to play a key role in their antimicrobial efficacy. Both the extracts of *B. rotunda* exhibited significant antimicrobial activity against *Streptococcus mutans*, *Streptococcus pyogenes*, *Streptococcus sobrinus,* and *Candida albicans* with minimum inhibitory concentration and minimum bactericidal concentration ratios demonstrating their effectiveness. Furthermore, both extracts showed a potent and direct inhibitory effect against human coronavirus 229E, with nearly 99.99% efficacy and a 4-log reduction in viral activity at a concentration of 1,600 µg/ml within 30 minutes. This study demonstrates the potential of *B. rotunda* as an antiviral and antipathogenic agent, with panduratin A as a bioactive compound. FBR is more potent than CBR against oral pathogens. These findings suggest that *B. rotunda* is a promising herbal medicine for treating oral diseases and viral infections.

## INTRODUCTION

*Boesenbergia rotunda* (L.) Mansf, also known as lesser galangal or fingerroot, is a plant native to Southeast Asia that has been traditionally used for medicinal purposes in Thai traditional medicine, including the treatment of oral diseases. It belongs to the family *Zingiberaceae* and is commonly found in Thailand, Indonesia, and Laos. In addition to treating oral diseases, *B. rotunda* has been used to treat various ailments such as inflammation, gastrointestinal disorders, and infections. The rhizomes of *B. rotunda* are often used as decoctions or infusions in traditional medicines [1].

Oral diseases, such as dental caries and periodontitis, represent significant global health challenges, arising from a complex interplay of bacterial and fungal elements within the oral biofilm. While *Streptococcus mutans* and *Candida albicans* are key contributors, the ecological plaque hypothesis suggests that these diseases result from broader microbial dysbiosis rather than the actions of a single pathogen. *Streptococcus mutans*, known for its acidogenic capacity, plays a central role in tooth decay by producing acids and glucans, which not only lead to enamel demineralization but also facilitate an environment that favors cariogenic species over noncariogenic commensal microbes. In addition, *S. mutans* interacts synergistically with *C. albicans*, a fungus commonly present in the oral cavity, contributing to the complexity of cariogenic biofilms [2].

Panduratin A and its derivatives are prenylated flavonoids from *B. pandurata* that exhibit a broad range of biological activities, including strong antibacterial, anti-inflammatory, and anti-cancer activities [3]. Among fingerroot's

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primary ingredients, panduratin A exhibits antiviral activity against HIV-1 and dengue virus [4]. The essential oil from *B. rotunda*, as demonstrated in recent research, exhibits potent antibacterial activity against key oral pathogens, a discovery that significantly enhances its profile as an effective agent in oral healthcare [5]. However, there have been limited scientific investigations into the pharmacological properties of *B. rotunda*, particularly concerning its potential use in the treatment of oral and respiratory tract infectious diseases.

Although the human coronavirus primarily causes respiratory infections, it has been detected in droplets emitted from the oral cavity of infected individuals, from where it can be potentially transmitted [6]. Therefore, investigating the potential anti-viral and antimicrobial activity of *B. rotunda* against common oral pathogens is crucial to reducing the risk of transmission of SARS-CoV2, which may coexist in the oral cavity.

This study aimed to evaluate the antimicrobial and antiviral activities of the ethanolic extract of *B. rotunda* rhizomes and its active component, panduratin A, against oral pathogens such as *S. mutans*, *Streptococcus pyogenes*, *Streptococcus sobrinus, Candida albicans,* and human coronavirus 229E. In addition, this study aimed to assess the cytotoxicity of the extract and determine its antioxidant activity, as well as to identify and quantify its chemical composition to provide a comprehensive evaluation of the therapeutic potential of *B. rotunda* in treating oral and respiratory tract infections.

## MATERIAL AND METHODS

### Preparation of plant extracts

*Boesenbergia rotunda* rhizomes were sourced from suppliers in Chiangmai, Thailand (specimen voucher no. MJU 2102-005), and verified by the Maejo University Herbarium before extraction.

Rhizomes were washed thoroughly with tap water, chopped into small pieces, and dried in an oven at 50°C for 3 days. The plant material was macerated in 95% ethanol at room temperature with agitation at 100 rpm for 72 hours. The extract was filtered through Whatman No. 1 filter paper under vacuum and then concentrated by removing the solvent using a rotary evaporator and freeze drying [7]. The obtained extracts were stored at 4°C until use.

### Column chromatography and thin-layer chromatography (TLC)-bioautography

Column chromatography and TLC-bioautography were used to identify the active compounds in the *B. rotunda* extract that exhibited antibacterial activity as previously described [8]. The plant extract (25 g) was fractionated using column chromatography. Each elution (700 ml) was performed using the following nine mobile phase steps: 1) hexane, 2 to 6) hexane: ethyl acetate (9:1, 4:1, 3:2, 2:3, and 1:4, respectively), 7) ethyl acetate, 8) ethyl acetate: methanol (1:1), and 9) methanol. The fractionated extracts were collected, concentrated under reduced pressure, and freeze dried to obtain dry extracts. The yields of the crude and fractionated extracts were determined by weighing the dried extracts and determining their chemical composition patterns using TLC.

The procedures of TLC-bioautography were performed as described previously with modifications [9]. Two silica gel plates of Silica Gel 60 F254 (MERCK, Germany) were prepared and subjected to identical conditions. One μl of the *B. rotunda* extract was applied to the TLC plates. AR-grade hexane (Fisher Scientific, UK), ethyl acetate (RCI Labscan Ltd., Thailand), and dichloromethane (RCI Labscan Ltd.) were used for TLC separation. The extract was separated using hexane: ethyl acetate: dichloromethane (11:8:1) mobile phase. Separation was performed in a saturated chamber. The TLC plates were dried in a fume hood for 15 minutes to remove the solvent. The compounds separated on the first TLC plate (I) were UV-visualised (Upland, USA, 254 nm), spots were pencil-marked, and retention values ($R_f$) were measured. A second TLC plate (II) was overlaid with molten brain heart infusion agar, seeded with bacteria (0.5 McFarland), and incubated in a moist chamber at 37°C for 24 hours and then sprayed with 1% thiazolyl blue tetrazolium bromide (AppliChem, Spain), before incubation at room temperature for 30 minutes. The clear spots appearing on the purple background suggested the presence of an antibacterial agent. Fractions with the same TLC bioautographic patterns were pooled.

### High-performance liquid chromatography (HPLC) for panduratin A analysis

The presence of panduratin A was determined by the Thailand Institute of Scientific and Technology Research using HPLC following the method described previously [10].

### Antimicrobial activity

The oral pathogens used for testing were *S. mutans* DMST 18777, *S. pyogenes* DMST 30563*, S. sobrinus* DMST 35719, and *C. albicans* DMST 5815 (obtained from the Department of Medical Sciences, Ministry of Public Health, Thailand). The broth dilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), as described previously [11]. Briefly, the extract was prepared at concentrations from 0.06 to 128 mg/ml in 10% dimethyl sulfoxide (DMSO). The plates were incubated at 37°C for 24 hours in an anaerobic jar for bacteria and under aerobic conditions for *C. albicans*.

### Virus inhibition assay

The antiviral activity of an extract was determined by the Tropical Medicine Diagnostic Reference Laboratory, Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University, Thailand, using a virus inhibition assay in Vero cells with a 50% tissue culture infectious dose ($TCID_{50}$) assay, in accordance with ASTM E1052-20 [12]. Briefly, the human coronavirus 229E (ATCC® VR-740™) was multiplied by propagating Vero cells and stored at −80°C. The extract was prepared at a concentration of 1,600 μg/ml and was incubated with $1 \times 10^5$ $TCID_{50}$/ml of human coronavirus at 37°C for 30 minutes.

### Cytotoxicity assay

The cytotoxicity of the extracts was determined by the School of Science, King Mongkut's Institute of Technology Ladkrabang, Thailand, using the MTT assay on Vero cells,

following the method as previously described [13]. Briefly, the Vero cells were grown in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum and 100 U/ml penicillin-streptomycin at 37°C in a humidified atmosphere containing 5% $CO_2$. Cells were seeded in 96-well plates at a density of $1 \times 10^4$ cells/well and incubated for 24 hours. The cells were then treated with the extract at concentrations of 125, 250, 500, 1,000, and 2,000 µg/ml for 24 hours. After treatment,
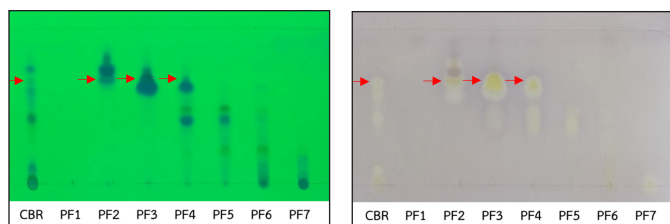
the cells were incubated with MTT (5 mg/ml) for 4 hours at 37°C. The formazan crystals were dissolved in DMSO and the absorbance at 570 nm was measured using a microplate reader. The 50% cytotoxic concentration ($CC_{50}$) causing visible morphological changes in 50% of the Vero cells was determined.

**Antioxidant assay**

*Boesenbergia rotunda* crude extract (CBR) and fractionated *B. rotunda* (FBR) were determined for antioxidant activity using a 2,2-Diphenyl-1-picrylhydrazyl radical scavenging assay, as previously described [14]. The total phenolic and flavonoid contents were determined using a previously described method [15].

**RESULTS**

**Column chromatography and TLC-bioautography**

Figure 1A shows the TLC pattern of CBR and pooled fractions PF1-PF7 under UV 254 nm. Figure 1B



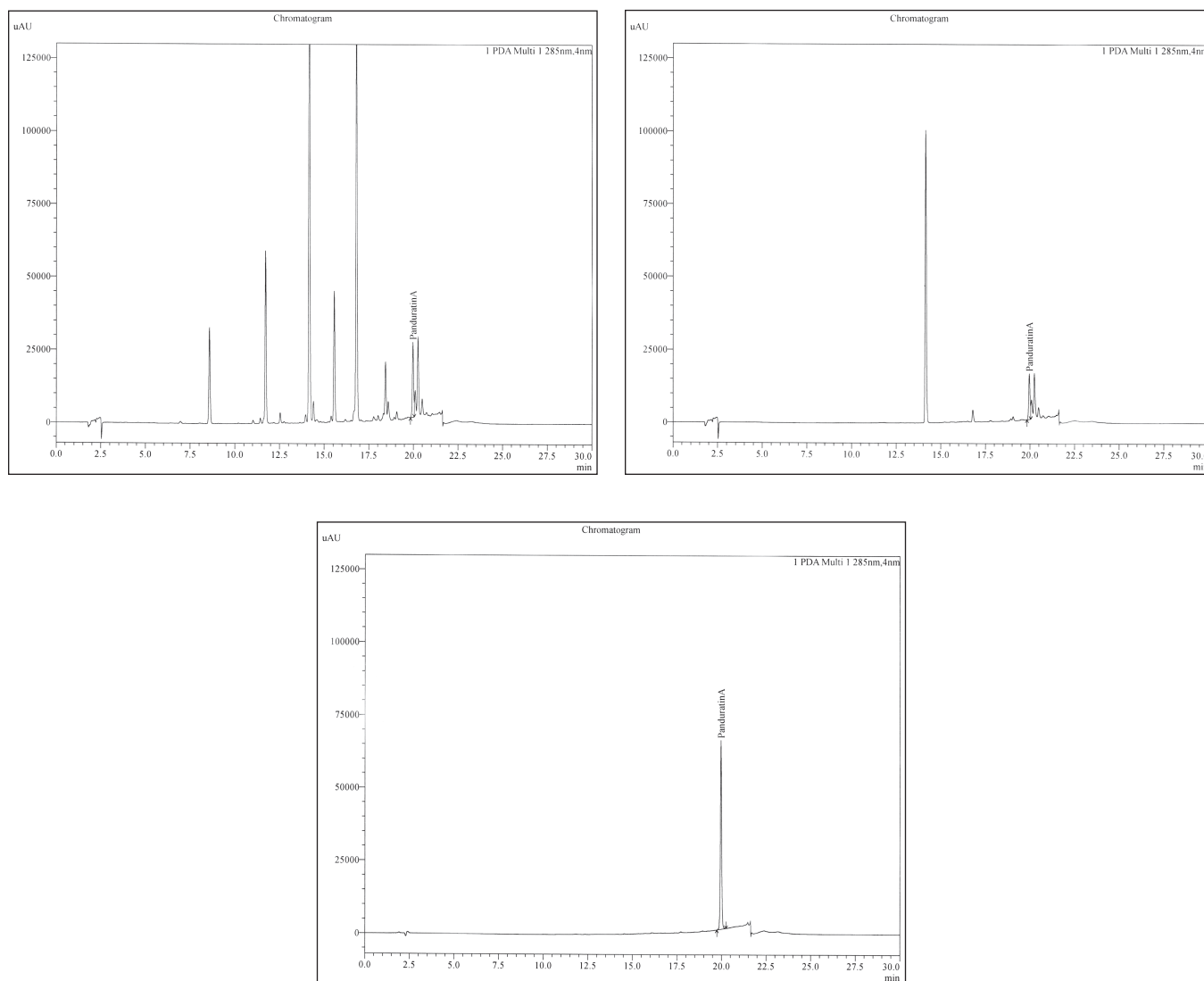**Figure 1.** TLC of CBR and PF1-PF7 (A) and TLC-bioautography against S. mutans (B).



**Figure 2.** HPLC chromatogram of panduratin A in CBR (A), FBR (B), and the standard panduratin A (C).

shows the TLC-bioautography of CBR and PF1-PF7 against *S. mutans*. CBR presented several inhibition zones, whereas PF2-PF4 presented a common inhibition zone at $R_f = 0.66$, as indicated by the arrows. PF3 exhibited the strongest inhibition zone at $R_f = 0.66$, followed by PF2 and PF4. Similar results were obtained for *S. pyogenes* and *S. sobrinus* (data not shown).

### Characterization of panduratin A by HPLC

The chemical composition of PF3 was analyzed by HPLC because it showed the strongest antibacterial activity against the tested bacteria. From this point onward, PF3 is referred to as FBR. Our results showed the presence of panduratin A in CBR and FBR at the same retention time as that of the standard panduratin A (19.9 minutes) (Fig. 2). The identification of panduratin A in both the CBR and FBR samples suggested that it remained stable after column chromatography.

The quantitation of panduratin A in CBR and FBR, as shown in Table 1, revealed concentrations of 3.75 and 13.76 g/100 g, respectively. This indicates that FBR contains 4.3 times more panduratin A than CBR. The high concentration of panduratin A in FBR may be attributed to the fractionation process, which allowed the isolation of the compound from other components in the crude extract.

### Antimicrobial activity

**Table 1.** Quantitation of panduratin A in crude extract and FBR by HPLC.

| Panduratin A | Unit (g/100 g) |
|---|---|
| Crude extract *B. rotunda* | 3.75 |
| Fractionated *B. rotunda* | 13.76 |

In the present study, the antimicrobial activities of CBR and FBR against oral pathogens were evaluated using a microdilution assay. The MIC and MBC values of both extracts against *S. mutans, S. pyogenes, S. sobrinus,* and *C. albicans* are listed in Table 2. The MIC/MBC ratios for CBR against the tested bacteria ranged from 0.01/0.06 to 0.03/0.12 mg/ml, whereas the MIC/MBC ratios for FBR against the tested bacteria ranged from 0.002/0.008 to 0.008/0.03 mg/ml. The MIC/MBC ratios of CBR and FBR against *C. albicans* were 0.25/0.25 and 0.12/0.25 mg/ml, respectively. CBR and FBR demonstrated stronger antimicrobial activity against the tested bacteria than against the tested yeast.

### Antiviral activity and cytotoxicity

Exposure of human coronavirus 229E to CBR and FBR at a concentration of 1,600 µg/ml for 30 minutes resulted in more than 99.99% efficacy, with more than 4 log reduction against the virus. The 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay showed that CBR and FBR exhibited low toxicity toward the Vero cells, with $IC_{50}$ values exceeding 2,000 µg/ml. These findings suggest that the concentration used to eliminate the virus was unlikely to be toxic to the cells.

### Antioxidant activity

The antioxidant potential of CBR and FBR extracts revealed a more pronounced activity in CBR. Both extracts exhibited significant phenolic and flavonoid contents, essential for their antioxidant properties, with a marked concentration of phenolics in FBR as shown in Table 3. Relative to standard antioxidants, the extracts demonstrated considerable antioxidant effectiveness, suggesting their utility in pharmaceutical applications as natural antioxidant sources.

**Table 2.** MIC and MBC of CBR and FBR against oral pathogens by broth dilution assay.

| Sample | MIC/MBC (mg/ml) | | | |
|---|---|---|---|---|
| | *Streptococcus mutans* DMST 18777 | *Streptococcus pyogenes* DMST 30563 | *Streptococcus sobrinus* DMST 35719 | *Candida albicans* DMST 5815 |
| CBR | 0.03/0.12 | 0.03/0.12 | 0.01/0.06 | 0.25/0.25 |
| FBR | 0.008/0.03 | 0.004/0.008 | 0.002/0.008 | 0.12/0.25 |
| Tetracycline | 0.0005/0.002 | 0.0005/0.002 | 0.0005/0.002 | - |
| Nystatin | - | - | - | 0.002/0.005 |

**Table 3.** Antioxidant activity (Inhibitory concentration 50%, $IC_{50}$), total phenolic, and total flavonoid contents of CBR and FBR.

| Sample | IC50 (mg/ml) | Total phenolic (mg GAE/g extract) | Total flavonoid (mg QE/g extract) |
|---|---|---|---|
| CBR | 0.56 | 203.44 ± 3.4 | 23.22 ± 0.63 |
| FBR | 1.01 | 423.33 ± 2.06 | 2.84 ± 0.18 |
| Butylated hydroxytoluene | 0.07 | NA | NA |
| ascorbic acid | 0.01 | NA | NA |
| α-tocopherol | 0.03 | NA | NA |

Butylated hydroxytoluene, ascorbic acid, and α-tocopherol were used as standards for the DPPH assay; NA: not applicable.

## DISCUSSION

Our study corroborates and extends the previous findings [16] by demonstrating that both CBR and FBR exhibit significant antimicrobial activity against oral pathogens, with FBR showing greater potency. These findings align with other researchers [17], who also reported the antimicrobial efficacy of fingerroot extracts. Furthermore, our results are in line with the previous study [18] about the antibacterial activities of *B. pandurata* extracts and suggested a mechanism involving cell wall and membrane disruption.

The direct inhibitory effect of CBR and FBR against human coronavirus 229E adds a new dimension to the antiviral potential of *B. rotunda*. This is consistent with recent studies indicating the pre-and post-infection inhibition of SARS-CoV-2 [16] and the inhibition of the main protease of SARS-CoV-2 by panduratin A [19]. The molecular docking studies previously [20] further underscore the potential of panduratin A in targeting 2′-O-methyltransferase, a crucial enzyme in viral replication.

In addition to these antimicrobial and antiviral properties, our investigation into the antioxidant activities of CBR and FBR aligns with previous findings on the antioxidant potential of *B. rotunda*. Consistent with the previous observation [7], our results highlight the antioxidant properties inherent in *B. rotunda* extracts. In addition, previous studies [16,21] further reinforce the relevance of panduratin A in antioxidant activity and its protective role against oxidative stress in biological systems. These findings collectively emphasize the multifaceted therapeutic potential of *B. rotunda*, especially in combating oxidative stress-related cellular damage.

The safety profile of these extracts, indicated by their low toxicity to Vero cells, suggests their suitability for therapeutic use. However, the translation from *in vitro* efficacy to clinical application necessitates a cautious approach. The mechanisms through which panduratin A exerts its antimicrobial and antiviral effects require further elucidation. *In vivo* studies and clinical trials are essential to confirm these findings and to understand the pharmacokinetics and pharmacodynamics of panduratin A.

## CONCLUSION

This study has demonstrated the significant potential of both CBR and FBR of *B. rotunda* against oral pathogens and human coronavirus 229E. The direct inhibitory effect on human coronavirus 229E notably contributes to its antiviral potential, aligning with recent advancements in antiviral research and the pre- and post-infection inhibition of SARS-CoV-2. This highlights *B. rotunda*'s capacity to address current global health challenges, particularly in the context of emerging viral threats. Furthermore, the substantial antimicrobial activity against *Streptococcus* species and *C. albicans*, as indicated by the MIC and MBC ratios, emphasizes its role in combating oral infections. The antioxidant properties of these extracts, revealed through their phenolic and flavonoid contents, further underscore their therapeutic potential, offering a holistic approach to oral healthcare. In conclusion, *B. rotunda*, particularly through its bioactive components such as Panduratin A, presents itself as a promising natural resource for pharmaceutical development.

This study suggests promising pharmaceutical applications of *B. rotunda* in the treatment of oral infections and viral diseases, positioning it as a valuable herbal medicine for natural and effective treatments in oral and antiviral healthcare.

## AUTHOR CONTRIBUTIONS

NW and DS conceived and designed the project. KB and RW performed the experiments. NW, DS, IF, and PS analyzed the results. NW drafted the manuscript. NW, DS, IF, and PS revised and finalized the manuscript, which was reviewed and approved by all authors.

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## CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

## ETHICAL APPROVAL

This study does not involve experiments on animals or human subjects.

## DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

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