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Original Article

Antioxidant activity and antibacterial effects of *Boesenbergia rotunda* extract against *Staphylococcus pseudintermedius* isolates from canine superficial pyoderma

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Abstract

The rhizome of *Boesenbergia rotunda* (fingerroot) is an edible herb that has antioxidant properties and has been shown to have antimicrobial activity against Gram-positive pathogens including coagulase-positive staphylococci. However, there is no data on its activity against the primary cause of canine superficial pyoderma *Staphylococcus pseudintermedius*. The total phenolic and total flavonoid contents of an ethanolic *B. rotunda* rhizome extract were determined by the Folin-Ciocalteu method and aluminum chloride assay and antioxidant activity was determined in the 2,2-diphenyl-l-1-picrylhydrazil (DPPH) radical scavenging test. The minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations of the extract against 12 clinical isolates of *S. pseudintermedius* causing pyoderma were determined in broth microdilution assays and the time-kill kinetics were examined against *S. pseudintermedius* ATCC 49051. The extract contained high levels of flavonoids (394.01 ± 4.79 mg quercetin equivalent/g extract) and phenolic compounds (85.11 ± 2.68 mg gallic acid equivalent/g extract) and the antioxidant index of the extract was more than 95% at concentrations above 1.25 mg/mL, which was consistent with the activity of the positive control; butylated hydroxytoluene. The $MIC_{50/90}$ (2/8 µg/mL) and $MBC_{50/90}$ (8/32 µg/mL) values for the extract were slightly higher than cephalexin ($MIC_{50/90}$ 1/2 µg/mL and $MBC_{50/90}$ 1/4 µg/mL). In the time-kill assay, the fingerroot extract eliminated 99.9% of viable *S. pseudintermedius* ATCC 49051 within eight hours at 256 × MIC (512 µg/mL). The promising antioxidant effects of the extract combined with its antibacterial activity against pathogenic *S. pseudintermedius* isolates suggest that fingerroot rhizome extract may be an effective novel or supplementary treatment for superficial pyoderma in dogs.

Keywords: antioxidant, canine pyoderma, ethanolic extract, fingerroot, *Staphylococcus pseudintermedius*

1. Introduction

Superficial pyoderma is a common skin disorder in dogs characterized by infection of the epidermis and hair follicles. *Staphylococcus pseudintermedius*, a bacterium that is part of the normal skin flora in dogs, is one of the primary causative agents of this disease (Bannoehr & Guardabassi, 2012). When conditions are favorable, such as when the animal's skin is compromised or when the immune system is weakened, *S. pseudintermedius* can proliferate and cause inflammation and oxidative stress, which are the key facilitators of pyoderma (Kamr *et al*., 2020). If left untreated, the infection can progress to deeper skin layers, causing cellulitis and erythema (Scott, Miller, & Griffin, 2001).

Antibiotics are the primary treatment for canine superficial pyoderma. Commonly used antibiotics include cephalexin, amoxicillin-clavulanic acid, or clindamycin (Hillier *et al*., 2014). Antibacterial shampoos, sprays, and ointments containing mupirocin and fusidic acid can also be effective as well as antiseptic shampoos containing

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chlorhexidine or benzoyl peroxide (Borio *et al*., 2015; Valentine, 2019). However, the use of antibiotics can result in the development of antibiotic resistance in pathogens and there are several reports of increasing rates of antibiotic resistance in *Staphylococcus* strains isolated from dogs with superficial pyoderma (González-Domínguez, Carvajal, Calle-Echeverri, & Chinchilla-Cárdenas, 2020; Silva *et al*., 2021). On the other hand, antiseptic treatments can cause skin irritation. In particular, topical shampoos containing benzoyl peroxide can dry out the skin and cause erythema, pruritus, and pain and chlorhexidine can inhibit wound healing and is not recommended for extended use (Koch, Torres, & Plumb, 2012). Therefore, the development of novel effective treatments for canine superficial pyoderma that are antibacterial, as well as anti-inflammatory and antioxidative is of interest.

Boesenbergia rotunda (L.) Mansf., or fingerroot, is a plant belonging to the Zingiberaceae family native to Southeast Asia, particularly Thailand, Indonesia, and Malaysia. Its rhizomes are widely used in traditional medicine and culinary practices in the region. Fingerroot extract contains several compounds likely to have antioxidant and antibacterial properties such as flavonoids, diarylheptanoids, terpenoids, phenolics, and essential oils (Eng-Chong *et al*., 2012), but there are limited studies investigating the antibacterial properties of fingerroot itself, and none investigating its activity against *S. pseudintermedius* causing canine superficial pyoderma. This study aimed to determine the total phenolic and total flavonoid contents of fingerroot extract and investigate its antioxidant activity and antibacterial activity against *S. pseudintermedius* isolated from dogs with superficial pyoderma.

2. Materials and Methods

2.1 Fingerroot samples and extraction

Fingerroot rhizomes (*B. rotunda* (L.) Mansf.) were purchased in a single batch from a single source at a local market in Khon Kaen Province, Thailand. The botanical identification was confirmed by Pranom Chantaranothai, Faculty of Science, Khon Kaen University (KKU), and a specimen was deposited in the KKU herbarium (voucher specimen J. Aiemsaard *et al*., 05). Fresh rhizomes were first washed with distilled water, cut, dried (at 50°C), and ground to powder. Extraction was then conducted using a Soxhlet apparatus with absolute ethanol as the solvent (RCI Labscan, Thailand). The crude extract was filtered through Whatman filter paper No. 1 (pore size: 11 µm) and the solvent was removed at 60 °C with a rotary evaporator (Heidolph, Germany). The extract was stored at 4 °C until use (Punareewattana, Borlace, Seubsasana, Thongkham, & Aiemsaard, 2023).

2.2 Total phenolic content

The total phenolic content was determined by the Folin-Ciocalteu method as described in a previous study (Punareewattana *et al*., 2023) with some modifications. Briefly, fingerroot extract was dissolved and diluted with 98% v/v ethyl alcohol (Merck, Germany) to give final concentrations of 1, 5, and 10 mg/mL. Then 0.5 mL samples were mixed with 2.5 mL Folin-Ciocalteu reagent (Sigma-Aldrich, Germany). After 5 min, 2 mL of 7.5% sodium carbonate (Loba Chemie, India) was added. The solution was incubated at room temperature in the dark for 1 hour. The absorbance was measured at 760 nm using a visible spectrophotometer (Genesys 10S Vis, Thermo Scientific, USA). The total phenolic content was calculated using a standard calibration curve of gallic acid concentrations of 25, 50, and 100 µg/mL (Sigma-Aldrich, Germany), and the results are expressed as gallic acid equivalent in mg/g (mg GAE/g) with a detection limit of 2 mg GAE/g extract.

2.3 Total flavonoid content

The total flavonoid content was determined by aluminum chloride assay as described in a previous study (Punareewattana *et al*., 2023) with some modifications. Briefly, 0.05 mL of 0.5% w/v sodium nitrite (Loba Chemie, India) was added to 0.5 mL fingerroot extract solution and allowed to stand for 5 min. Then 0.05 mL of 10% w/v aluminum chloride (Loba Chemie, India) was added and the mixture was left for 5 min. Then 0.5 mL of 1 M sodium hydroxide (Loba Chemie, India) was added, and the final volume was adjusted to 1.5 mL with 98% v/v ethyl alcohol. The absorbance was measured at 420 nm by visible spectrophotometer. The total flavonoid content was calculated using a standard calibration curve of quercetin concentrations of 25, 50, and 100 µg/mL (Sigma-Aldrich, Germany), and the results are expressed as quercetin equivalent in mg/g (mg QE/g) with a detection limit of 2 mg QE/g extract.

2.4 Antioxidant activity

The antioxidant activity of fingerroot extract was examined by the 2,2-diphenyl-l-1-picrylhydrazil (DPPH) assay as described in a previous study (Thongkham, Aiemsaard, & Kaenjampa, 2021). Briefly, the extract was serially diluted two-fold (0.5 mL) with absolute ethyl alcohol. Then 0.5 mL of a 0.0004 M DPPH (Fluka, Germany) solution was added and the mixture was incubated in the dark for 25 minutes at room temperature. The absorbance at 517 nm was measured by a visible spectrophotometer. Butylated hydroxytoluene (BHT, Riedel de Haen, Germany) was used as the positive antioxidant control. The percentage of DPPH radical inhibition (% antioxidation index) was calculated according to the following equation:

$$\text{Antioxidant index (\%)} = \frac{(Abs_{control} - (Abs_{sample} - Abs_{blank}) \times 100}{Abs_{control}}$$

Where $Abs_{control}$ represents the absorbance of the solution containing all reagents except the antioxidant. Abs_{sample} is the absorbance of the solution containing antioxidant and DPPH. Abs_{blank} is the absorbance of the solution containing antioxidant without DPPH.

2.5 Bacterial strains and culture conditions

The 12 *S. pseudintermedius* isolates used in this study were collected from dogs with superficial pyoderma and are stored at the Veterinary Pharmacology Laboratory, Faculty of Veterinary Medicine, Khon Kaen University, Thailand.

Isolate identifications were confirmed using PCR-RFLP (Aiemsaard *et al*., 2022). *Staphylococcus pseudintermedius* ATCC 49051 was obtained from the American Type Culture Collection (ATCC), Virginia, USA. Bacteria were cultured in Mueller Hinton broth (MHB, Becton Dickinson, France) and incubated at 37 °C for 24 hours before inocula were prepared.

2.6 Broth microdilution method

Broth microdilution testing was performed according to Clinical and Laboratory Standard Institute Guidelines (2018) with some modifications. The crude fingerroot extract was dissolved in dimethyl sulfoxide (DMSO; RCI Labscan, Thailand) and serially diluted two-fold (50 µL) with MHB in 96-well round-bottomed microtiter plates (Corning Incorporated, USA). A 50 µL inoculum of a standardized microbial suspension (1×10^6 CFU/mL) in MHB was added to each of the wells. Cephalexin (Sigma-Aldrich) and DMSO were used as the standard antimicrobial control and solvent control, respectively (Papich & Lindeman, 2018). The final concentration range of crude fingerroot extract in the microtiter trays was 0.5 to 256 µg/mL with a maximum DMSO concentration of 62.5 µL/mL. The final concentration range for cephalexin was 0.031 to 16 µg/mL and for DMSO it was 0.122 to 62.5 µL/mL. Wells containing MHB with and without microbial inocula were the positive and negative growth controls, respectively. The microtiter plates were incubated at 37 °C for 24 hours. The minimum inhibitory concentration (MIC) was determined from the lowest concentration of the extract inhibiting visible growth after incubation. The minimum bactericidal concentration (MBC) was determined from the lowest concentration of the extract that showed no colonies in 100 µL samples from the MIC wells plated onto Mueller-Hinton agar (MHA, Becton Dickinson, France) plates and incubated at 37 °C for 24 hours. The MIC_{50} or MIC_{90} were MIC for 50% or 90% of tested bacterial samples, and MBC_{50} or MBC_{90} were MBC for 50% or 90% of tested bacterial samples. All tests were performed in triplicate.

2.7 Time-kill test

The time-kill assay was performed according to the method previously described (Punareewattana *et al*., 2023) with some modifications. A 100 µL microbial inoculum containing 5×10^7 CFU/mL of *S. pseudintermedius* ATCC 49051 in MHB was mixed with 900 µL of diluted fingerroot extract dissolved in DMSO and MHB to give final concentrations of 1, 4, 16, 64, and 256 × MIC. After incubation at 37°C for 0, 2, 4, 6, and 8 hours, 100 µL samples were taken and diluted 10-fold with normal saline solution and 100 µL aliquots of the 10^{-1} to 10^{-4} dilutions were inoculated onto MHA plates. After incubation at 37°C for 24 hours the colonies on the plates were counted and the results are expressed as the $\log_{10}$ of the number of viable bacteria (CFU/mL). DMSO (62.5 µL/mL) was used as a negative control. All tests were performed in triplicate.

2.8 Statistical analysis

The normality of the data was assessed by the Shapiro-Wilk test. Differences in the antioxidation index of fingerroot extract and BHT were analyzed by using the independent samples t-test. The MIC_{50}, MIC_{90}, MBC_{50}, and MBC_{90} values are expressed by descriptive statistics. Differences in the reduction of viable bacterial cells by various concentrations of fingerroot extract in the time-kill assay were analyzed by using the one-way analysis of variance (ANOVA). All analyses were performed on IBM SPSS v.28 software using $p < 0.05$ as significant.

3. Results

3.1 Fingerroot extract characteristics

The ethanolic extract of fingerroot was a highly viscous dark red-yellow colored semisolid with a strong unique herbal scent. The yield of the crude extract was 8.42% of the plant material dry weight. Dissolving the crude fingerroot extract for use in the antimicrobial assays required the use of DMSO as a solvent with 62.5 **µ**L/mL the maximum final concentration of DMSO in any fingerroot extract solution used throughout the study.

3.2 Total phenolic and total flavonoid contents

The Folin-Ciocalteu and aluminum chloride assay results demonstrated that the fingerroot extract obtained in this study contained high amounts of flavonoids and phenolics (Table 1). The concentration of flavonoid and phenolic compounds were 394.01 ± 4.79 mg QE/g extract (33.18 ± 0.40 mg QE/g dry plant) and 85.11 ± 2.68 mg GAE/g extract (7.17 ± 0.23 mg GAE/g dry plant), respectively.

3.3 Antioxidant activity

Table 2 shows the results of the DPPH assay. There were no significant differences between fingerroot extract and BHT at the higher tested concentrations (1.25 and 2.5 mg/L) with antioxidant indices greater than 95%. However, at lower concentrations (0.156-0.625 mg/mL), fingerroot extract showed reduced radical scavenging activity (antioxidant index range from 61.06 ± 3.73 to 87.12 ± 2.03) compared to BHT. Antioxidant indices for BHT were above 95% for all tested concentrations.

3.4 Antibacterial activity against *S. pseudintermedius*

The fingerroot extract was first screened for antibacterial activity in broth microdilution assays. For *S. pseudintermedius* ATCC 49051, the fingerroot extract MIC/MBC values were 2 and 4 µg/mL, while the cephalexin MIC/MBC values were both 1 µg/mL. The summary results of the microdilution assays for the 12 clinical *S. pseudintermedius* isolates are presented in Table 3. The $MIC_{50/90}$ values for fingerroot extract were 2 and 8 µg/mL (range 1-8 µg/mL) and the $MBC_{50/90}$ values were 8 and 32 µg/mL (range 2-32 µg/mL). The MBC values were higher than their respective MIC values with one isolate having an MBC 32 times its MIC. For cephalexin, the $MIC_{50/90}$ values for the 12 clinical *S. pseudintermedius* isolates were 1 and 2 µg/mL and the $MBC_{50/90}$ values were 1 and 4 µg/mL. Thus, all of the tested *S. pseudintermedius* isolates had MIC values less

Table 1. Total phenolic and total flavonoid contents in fingerroot extract

Constituent	Amount (per g extract)	Amount (per g dry plant)
Total phenolic	85.11 ± 2.68 mg GAE	7.17 ± 0.23 mg GAE
Total flavonoid	394.01 ± 4.79 mg QE	33.18 ± 0.40 mg QE

Values represent the mean ± SD of triplicate experiments.

Table 2. Antioxidant activity of fingerroot extract and butylated hydroxytoluene (BHT)

Concentration (mg/mL)	Antioxidant index (%)				
	2.5	1.25	0.625	0.313	0.156
Fingerroot extract	96.56 ± 0.22 [a]	95.33 ± 0.60 [a]	87.12 ± 2.03 [b]	75.63 ± 2.89 [b]	61.06 ± 3.73 [b]
BHT	96.63 ± 0.58 [a]	96.91 ± 0.40 [a]	97.08 ± 0.42 [a]	96.54 ± 0.30 [a]	96.68 ± 0.51 [a]

Values represent the mean ± SD of triplicate experiments. Different letters within a column indicate statistically significant differences between the means ($p < 0.05$).

Table 3. Antibacterial activity of cephalexin and fingerroot extract against 12 clinical *S. pseudintermedius* isolates

	Antibacterial activity					
	MIC range	MIC_{50}	MIC_{90}	MBC range	MBC_{50}	MBC_{90}
Cephalexin (µg/ml)	0.5-2	1	2	1-4	1	4
Fingerroot extract (µg/ml)	1-8	2	8	2-32	8	32

Values represent minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) collected from triplicate experiments. MIC_{50} or MIC_{90} = MIC for 50% or 90% of tested samples. MBC_{50} or MBC_{90} = MBC for 50% or 90% of tested samples.

than or equal to 2 µg/mL and were sensitive to cephalexin according to the CLSI breakpoints for 1st generation cephalosporins (Clinical and Laboratory Standard Institute, 2013).

3.5 Time-kill assay against *S. pseudintermedius* ATCC 49051

The bactericidal effects of fingerroot extract were then determined in a time-kill assay. Concentrations of fingerroot extract ranging from 1 × MIC to 256 × MIC were tested against *S. pseudintermedius* ATCC 49051 over 8 hours (Figure 1). While most of the tested concentrations were unable to cause a 3-log_{10} reduction (99.9%) in the number of bacteria (CFU/mL) over the eight-hour time course, it is clear that the antibacterial activity of the fingerroot extract was both time- and concentration-dependent. Increasing the exposure time and increasing the concentration of fingerroot extract increased the antibacterial activity. The 16 × MIC and 64 × MIC concentrations achieved 2-log_{10} reductions (99%) in *S. pseudintermedius* ATCC 49051 CFU/mL after 6 hours and 4 hours, respectively, while the 4 × MIC concentration only caused a 1.6-log_{10} reduction in the CFU/mL after 8 hours, and the 1 × MIC concentration was no different to the control after 8 hours. Only the 256 × MIC concentration of fingerroot extract caused a 3-log_{10} reduction (99.9%) in the number of viable *S. pseudintermedius* ATCC 49051, and this did not happen until the eight-hour time point.

4. Discussion

Previous reports have indicated that different sources, plant parts, and extraction solvents can all influence the quantity of phytochemicals in a plant extract. Thomya *et al*. (2023) examined ethanolic extracts of fingerroot rhizomes sourced from various cultivation areas in northern Thailand and showed higher total phenolic contents (ranging from 16.52–23.79 mg GAE/g dry plant) and total flavonoid contents (ranging from 50.00–95.12 mg catechin equivalent (CE)/g dry plant) than the extract used in our study. Saah, Siriwan, & Trisonthi (2021) examined acetone, ethanol, methanol, and water extracts of fingerroot rhizomes and roots from plants grown in central Thailand. Rhizomes had higher phenolic contents than roots with ethanolic extract of rhizomes providing the highest total phenolic content (90 mg GAE/g dry plant), which is much higher than in our extract. Total flavonoid contents were similar to our extract (37–47 mg QE/g dry plant) across rhizomes and roots and solvent type, but flavonoids were not detected in the aqueous extract. Jing, Mohamed, Rahmat, & Bakar (2010) demonstrated that a methanolic extract of fingerroot rhizomes from plants in the northern part of the island of Borneo, Malaysia had a similar total phenolic content to our extract (6.19 mg GAE/g dry plant) but a much lower total flavonoid content (2.19 mg CE/g dry plant). Yusuf, Annuar, & Khalid (2013) found the content of the fingerroot flavonoid pinostrobin to be 8.22 mg/g dry plant in plants grown on the west coast of Peninsular

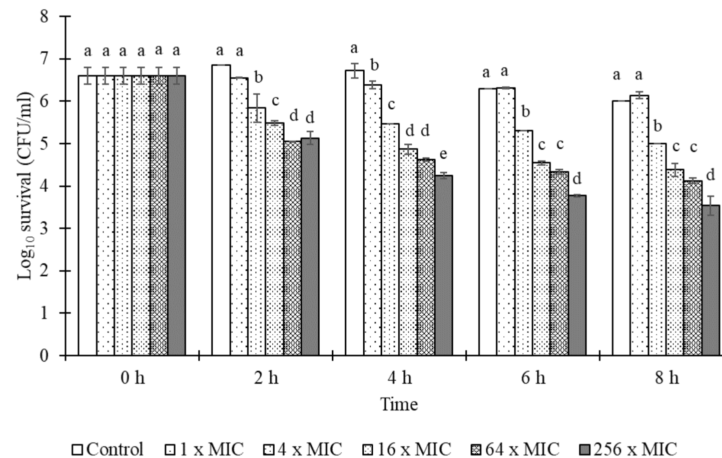

Figure 1. Time-kill assay of fingerroot extract against *S. pseudintermedius* ATCC 49051. MIC = minimum inhibitory concentration. 1 × MIC = 2 µg/mL, 4 × MIC = 8 µg/mL, 16 × MIC = 32 µg/mL, 64 × MIC = 128 µg/mL, and 256 × MIC = 512 µg/mL. Control = 62.5 µ/mL DMSO. Different letters within a time point indicate statistically significant differences between the means ($p < 0.05$).

Malaysia. In addition to pinostrobin, there are several important phenolic and flavonoid derivatives previously identified in fingerroot including pinocembrin, panduratin A, alpinetin, cardamonin, and boesenbergin (Ching *et al*., 2007; Saah *et al*., 2021; Yusuf *et al*., 2013). These compounds have comprehensive pharmacological effects, especially antioxidant, antimicrobial, anti-inflammatory, and anti-cancer effects (Eng-Chong *et al*., 2012).

Canine pyoderma caused by staphylococci induces damage through direct bacterial invasion and oxidative stress (Kubesy, Salem, & Jaheen, 2017). The antioxidant activity tests in the current study showed that the fingerroot extract DPPH free radical scavenging ability was equivalent to BHT at 1.25 mg/mL. Several previous studies have shown that fingerroot extract has good antioxidant activity with 50% inhibitory concentration (IC_{50}) values in the range of 0.21-0.92 mg/mL (Atun, Handayani, & Rakhmawati, 2018; Saah *et al*., 2021; Sundram, Zakaria, & Nasir; 2019). This effect is usually due to the phenols and flavonoids present (Zeb, 2020). The antioxidant effects seen in the current study could be due to flavonoids panduratin A and 4-hydroxypanduratin A and pinostrobin (Chahyadi, Hartati, Wirasutisna, & Elfahmi, 2014; Rithichai, Jirakiattikul, Poljan, Youngvises, & Itharat, 2022). It will be of interest to investigate the anti-inflammatory activity of fingerroot extract in canine pyoderma, as an ethanolic extract of fingerroot was recently shown to exhibit strong anti-inflammatory and antioxidant effects in cardiac tissues in a doxycycline-induced rat model (Zhang, Jiang, Wang, Jaisi, & Olatunji, 2023).

Previous studies have found a range of activities for fingerroot extracts against bacteria and yeast as well as filamentous fungi and the protozoan parasite giardia (Eng-Chong *et al*., 2012). While results from different studies can be somewhat contradictory, in general, ethanolic extracts of fingerroot have shown antibacterial activity in disc diffusion and broth microdilution assays against Gram-positive bacteria, with less effect on Gram-negative bacteria. In particular, fingerroot has shown activity against coagulase-positive staphylococci, which include *Staphylococcus aureus*, *Staphylococcus epidermidis,* and *S. pseudintermedius*. Studies have reported MIC values of 0.16 mg/mL against *S. epidermidis* and 0.31 mg/mL against *S. aureus* for an ethanolic extract of fingerroot rhizome (Jitvaropas *et al*., 2012), and inhibition zones of 8.5-10.5 mm for a 1 mg/mL ethyl acetate extract of fingerroot rhizome against *S. epidermidis*, *S. aureus* and methicillin-resistant *S. aureus* (Sopitthummakhun *et al*., 2021). In the current study, the range of the inhibitory concentrations of our ethanolic extract of fingerroot rhizomes against 12 clinical *S. pseudintermedius* isolates was much lower, in the microgram per millilitre range (MIC_{90} 8 µg/mL) rather than the milligram per millilitre range reported in these other studies. Interestingly, the range of the cephalexin MIC and MBC values (0.5-2 and 1-4 µg/mL) were 2 to 8-fold smaller than they were for the fingerroot extract (1-8 and 2-32 µg/mL) indicating some variation in the antimicrobial effects of fingerroot extract among the tested *S. pseudintermedius* isolates. Our MIC values are closer to those reported for the antimicrobial activity of purified panduratin A, a natural chalcone compound isolated from the rhizomes of fingerroot. Panduratin A exhibited MIC values of 2-4 µg/mL against oral bacteria, and MIC_{90} values of 1-2 µg/mL against clinical isolates of staphylococci and enterococci (Rukayadi, Han, Yong, & Hwang, 2010; Rukayadi, Lee, Han, Yong, & Hwang, 2009). Similarly, three flavanone compounds from an ethanolic extract of fingerroot rhizomes were found to generate zones of inhibition in disc diffusion testing against *Streptococcus mutans*, *S. epidermidis*, *S. aureus* and *Escherichia coli* with concentrations in the microgram per millilitre range (Atun *et al*., 2018). Fingerroot extract has also shown anti-candida activity (Taweechaisupapong, Singhara, Lertsatitthanakorn, & Khunkitti, 2010). As one of the main microbial causes of canine pyoderma after *S. pseudintermedius* is the yeast *Malassezia pachydermatis*, it would be interesting to investigate the activity of our fingerroot ethanolic extract against *M. pachydermatis* as any anti-fungal activity would be beneficial, particularly in a topical preparation such as an ointment, cream or shampoo.

The time-kill testing indicated that the fingerroot extract showed bactericidal activity at a concentration of 256 × MIC after 8 hours, which corresponds with 512 µg/mL. For

use against canine pyoderma, a topical preparation that could be used at mg/mL concentrations would be more bactericidal and effective against *S. pseudintermedius*, which is a concentration more appropriate for use as a topical treatment. Despite the prolonged contact time required for the extract to attain the desired effect, it is possible to enhance the potency of the active constituents by designing formulations that employ drug carriers and controlled drug delivery systems, and by investigating synergistic interactions with other substances (de Araujo & Padula, 2023; Trucillo, 2021; Vaou *et al*., 2022). Resistance to β-lactam antibiotics appears to be increasing in *S. pseudintermedius*, but a recent study found that ethanolic extract of fingerroot rhizome showed a synergistic effect when used with β-lactam antibiotics against β-lactam resistant staphylococci (Teethaisong, Pimchan, Srisawat, Hobbs, & Eumkeb, 2018). The effects of topical preparations of fingerroot and an examination of any synergistic effects with beta-lactam antibiotics should be investigated in *S. pseudintermedius* canine superficial pyoderma.

5. Conclusions

Ethanolic extract of rhizomes of fingerroot (*B. rotunda*) showed promising *in vitro* antioxidant activity and antimicrobial effects against *S. pseudintermedius*, the primary causative agent for canine superficial pyoderma. Inhibitory and bactericidal concentrations were in the microgram per millilitre range and a 512 µg/mL concentration of extract effectively eliminated 99.9% of viable *S. pseudintermedius* within eight hours in a time-kill assay. Thus, this extract could be an effective novel treatment for pyoderma in dogs. The promising antioxidant activity and potential anti-inflammatory activity of fingerroot extract provide the basis for further studies to elucidate these activities and mechanisms. Further studies to determine the identity of the compounds in our crude extract that are responsible for these actions would be interesting as would a trial investigating the effects of a topical preparation of crude fingerroot extract in dogs with superficial pyoderma.

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