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Research Article
Antibacterial and Anti-Inflammatory Properties of Flavonoids from *Streptomyces chartreusis* RH3.5

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Abstract

Background and Objective: The RH3.5 was isolated from the rhizosphere of *Boesenbergia rotunda* (L.) Mansf. and identified to be *Streptomyces chartreusis* via analysis of its 16S rDNA sequence, chemotaxonomy and morphology. The aim of this study was to identify the major compounds of RH3.5 and assess their biological activities. **Materials and Methods:** Silica gel column chromatography and thin-layer chromatography were used to purify major compounds, elucidate 5,7,2'-trihydroxy-8-methoxyflavanone (compound **1**) and 5',2',5'-trihydroxy-7,8-dimethoxyflavanone (compound **2**). Subsequently, mass spectrometry and NMR techniques were used to identify the structure of these compounds. Antimicrobial, anti-inflammatory and cytotoxic properties were carried out using *in vitro* assays. **Results:** The bioassays revealed the antimicrobial effect of compounds **1** and **2** on MRSA and *Staphylococcus aureus*. The minimum inhibitory concentration and minimum bactericidal concentration was calculated in the range of 32-64 and 128-256 µg/mL, respectively. The compounds **1** and **2** also exhibited anti-inflammatory potential by inhibiting NO, IL-1β and TNF-α production in LPS-stimulated RAW264.7 cells in a dose-dependent manner. Additionally, they had mild cytotoxic action against Vero and L929 cell lines with IC_{50} values greater than 512 µg/mL. **Conclusion:** These findings showed that flavonoids of *Streptomyces chartreusis* RH3.5 exhibited antibacterial and anti-inflammatory activities with low cytotoxicity against healthy cells. Thorough research on these compounds could result in the creation of useful methods for treating microbial infections and acute inflammatory responses.

Key words: Antimicrobial, anti-inflammatory, *Boesenbergia rotunda* (L.) Mansf., flavonoids, *Streptomyces chartreusis* RH3.5

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Boesenbergia rotunda (L.) Mansf. (Zingiberaceae), is a medicinal plant commonly found in Southeast Asia. It is frequently used as a folk medicine to cure a variety of ailments and is used all over the world as a culinary flavouring[1]. The different classes of chemical compounds of *Boesenbergia rotunda* have been previously reported[2]. According to the literature, *Boesenbergia rotunda* has the following biological properties: Antibacterial[3,4], anticancer[5,6], anti-allergic[7], anti-inflammatory[8], antioxidant[9], antiviral[10-12], anti-ulcer[13] activities and wound healing[14]. It also acts as pupicidal and larvicidal[15]. Moreover, it can use to treat hepatic disease[16]. Furthermore, endophytic actinomycetes have been isolated using this plant[17-19]. Endophytic actinomycetes are promising because they produce unique antibiotics to fight resistant bacteria, promote plant growth and offer eco-friendly pest control. Several endophytic actinomycetes produce a remarkable array of bioactive compounds with a proven track record as antimicrobial and anti-inflammatory agents in pharmaceutical applications. They are a promising source of novel bioactive compounds with the potential to combat antibiotic-resistant microorganisms[20].

The endophytic actinomycetes resulted in the identification of the RH3.5 strain, which has demonstrated inhibitory properties towards Gram-positive bacterial growth. The present study, investigated the bioactive components of the RH3.5 strain and then purified and identified the major molecules present. It also investigated the antimicrobial properties against the clinical strain of MRSA, assessed for their effects on LPS-stimulated RAW264.7 cells and evaluated their cytotoxicity using the MTT assay on African green monkey kidney cells (Vero) and mouse fibroblast cells (L929) using MTT assay.

MATERIALS AND METHODS

Study area: The study was carried out at the Departments of Microbiology and Chemistry, Silpakorn University, Nakhon Pathom, Thailand, from May-June, 2023.

Isolation and screening for antimicrobial activities: Total twelve samples were isolated from the *Boesenbergia rotunda* rhizosphere collected from the three locations of Nakhon Pathom (13.8189417'N and 100.0413870'E), Thailand. Samples were treated with 0.01% SDS for 30 min at 30°C and dried in oven at 45°C for 24 hrs. Distilled water was used to make a tenfold dilution, which was then applied to humic-acid vitamin agar containing cycloheximide (50 µg/mL) and nalidixic acid (20 µg/mL). Following the standard procedure, actinomycetes strains were isolated and identified[19]. A total of 36 actinomycete strains were tested for their antimicrobial properties against *Bacillus subtilis* (ATCC 6633), *B. cereus* (ATCC 7064), *S. aureus* (ATCC 25923) and the clinical isolates of MRSA (Sp2, Sp3, RI and T2)[21]. The antimicrobial activity of actinomycetes strains was carried out using soft-agar overlay method with slight modifications[22]. Of the 36 actinomycete strains, the RH3.5 exhibited the most potent antibacterial property. It was identified based on morphological, physiological and chemotaxonomic characterizations in accordance with the methods developed by Cassarini *et al.*[23]. The strain RH3.5 was cultured on ISP-2 agar for 15 days at 32°C, then extraction with ethyl acetate[24]. Antibacterial compounds in the crude extract were detected using thin-layer chromatography (TLC)-based bioautography[25].

Compound purification and characterisation: Fractionation of the crude extract (9.21 g) was carried out using silica gel-column chromatography and collected with 15-20% methanol in chloroform with increasing polarity. The fractions eluted, exhibited antibacterial activity by disk diffusion method. The compounds were then purified by thin-layer chromatography using ethyl acetate:chloroform:methanol (5:3:1) and afforded compound **1** (32.75 mg) and compound **2** (20.25 mg). The chemical structures of purified compounds were elucidated using the following methods. Melting points of the compounds were measured using a Stuart SMP20 apparatus (Stuart, United Kingdom). The UV spectra were acquired in methanol using a PerkinElmer Lambda 35 Spectrophotometer (PerkinElmer Life and Analytical Sciences, United States of America) for further structural characterization. To elucidate the detailed structures of the compounds, ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectra were acquired using a Bruker AVANCE 300 spectrometer (Bruker, Germany). The mass spectrometry analysis was also performed on a POLARIS Q mass spectrometer (Thermo Fisher Scientific, United States of America) to determine the molecular weights of the compounds.

Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC): The MIC and MBC of the crude extract and the purified compounds against relevant

microbial strains were determined following the established protocols by Pfaller *et al.*[26].

MTT assay: To evaluate the potential cytotoxicity of the crude extract and purified compounds, their effects on the viability of Vero (African green monkey kidney), L929 (murine epithelial) and RAW264.7 (murine macrophage) cells lines were assessed using the MTT assay[24]. The cytotoxicity of the purified compounds was evaluated by treating RAW264.7 macrophages with various concentrations for 24 hrs, followed by an MTT assay to determine cell viability. The cells were treated with various concentrations of the purified compounds for 24 hrs, followed by an MTT assay to determine cell viability.

Anti-inflammatory assay: To evaluate their anti-inflammatory effects, RAW 264.7 macrophages (obtained from Korea Cell Line Bank) were pre-treated with purified compounds (15-60 µg/mL) for 1 hr followed by LPS stimulation (1 µg/mL) for 24 hrs. Nitrite production (indicating NO levels) in the culture supernatant was measured using Griess reagent[27]. The ELISA assays quantified the secretion of pro-inflammatory cytokines (IL-1β and TNF-α (PeproTech, New Jersey, USA))[28,29] after LPS stimulation.

Statistical analyses: Each experiment was performed in triplicate. Statistical analysis was performed using SPSS statistics (Version 20.0, IBM, Armonk, New Jersey, USA) with a significance level of $p<0.05$.

RESULTS

A total of 36 strains were collected from the rhizosphere soil of *Boesenbergia rotunda* and further purified using the ISP-2 growth media. Using the soft-agar overlay method, the isolates were evaluated for their antimicrobial effect. Following a 24 hrs incubation at 37°C, the antimicrobial activity of the actinomycete colonies was evaluated by measuring the diameter of clear zones surrounding the colonies, indicating the extent of microbial growth inhibition. Among all, RH3.5 strain depicted the highest inhibition of actinomycetes over *B. subtilis* ATCC 6633, *B. cereus* ATCC 7064 and MRSA (Fig. 1a-b).

The RH3.5 strain was cultured on ISP-2 growth media and characterised by cream-coloured colonies with white aerial mycelium. The RH3.5 strain's cell wall was composed of the meso-isomer of 2,6-diaminopimelic acid. Microscopic examination showed the strain possessed typical *Streptomyces* morphology, characterized by a flexuous, spiral-shaped chain of oval spores, consistent with observations of other *Streptomyces* species (Fig. 2a-b). The 16S rDNA (1533 nt) sequence analysis using BLAST showed that the RH3.5 strain is closely related to *S. chartreusis* (NRRL 3882) with 99.67% similarity.

Phylogenetic tree of the 16S rDNA sequence shared a cluster with *Streptomyces chartreusis* (NRRL 3882) (Fig. 3). Under the accession number LC726344, the 16S rDNA sequence mentioned in this study is accessible in GenBank. The TLC-based bioautography of the extract eluted

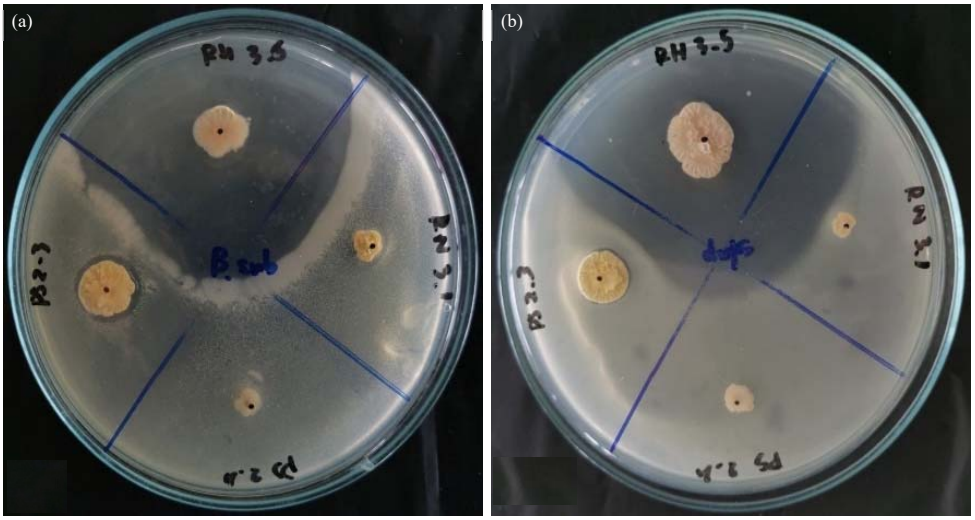

Fig. 1(a-b): After 24 hrs of incubation at 37°C, the actinomycetes colony's clear zone were examined to check for antibacterial activity using the soft-agar overlay technique, (a) *Bacillus subtilis* and (b) *Staphylococcus aureus* were added to the 7-day-old preculture of *Streptomyces chartreusis* RH3.5 on ISP-2 medium.

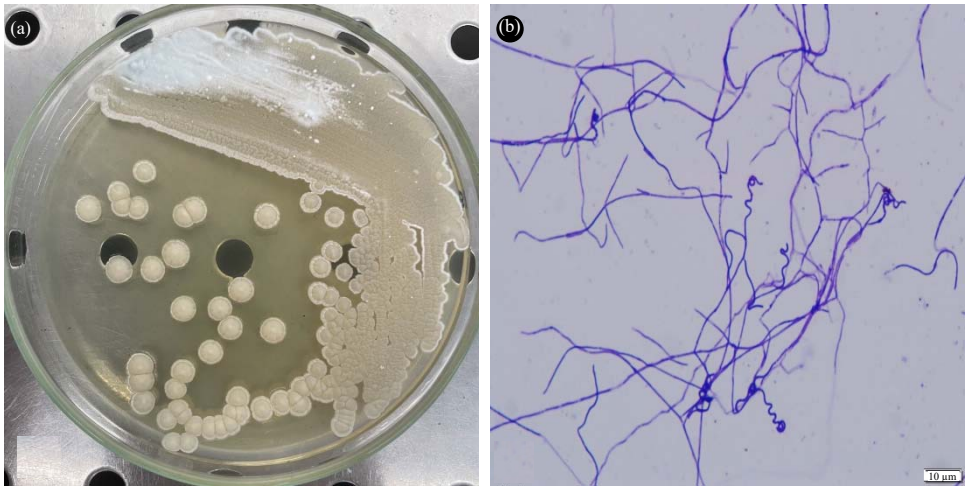

Fig. 2(a-b): Morphological characteristics of the *Streptomyces chartreusis* RH3.5 on the ISP-2 agar after 21 days of growth at 30°C incubation, (a) Colony appearance with cream-coloured and (b) Light micrograph showed spore production in long chains with retinaculiaperti type morphology.
Bar = 10 µm.

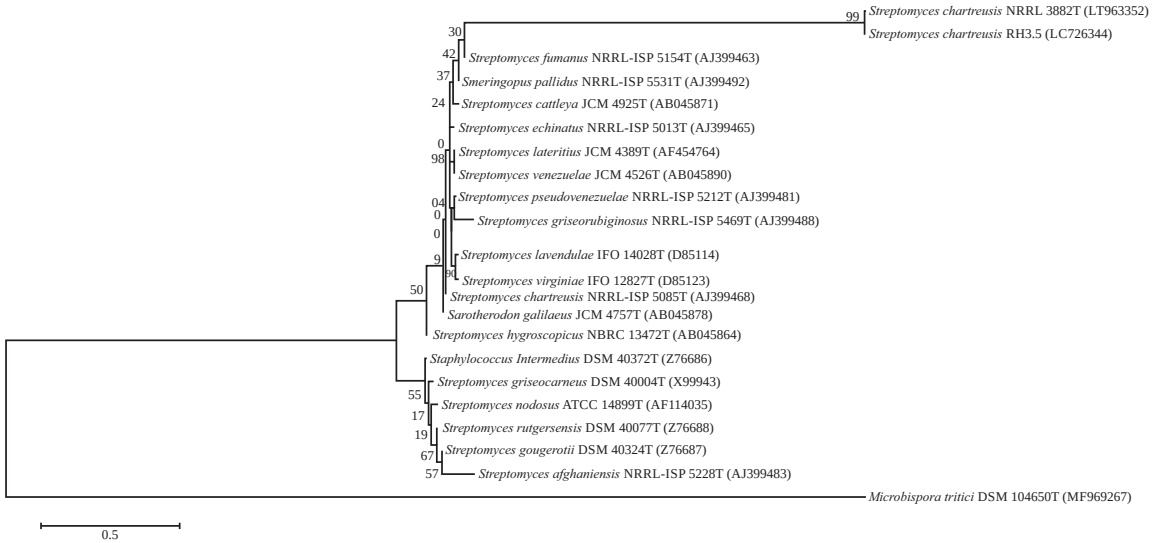

Fig. 3: Phylogenetic tree of the *Streptomyces chartreusis* RH3.5 and closely related strains. GenBank was used to extract the 16SrDNA gene sequences of *S. chartreusis* RH3.5 and related strains. Using MEGA6 software and the neighbour-joining method, a phylogenetic tree was created using the accession numbers in parentheses.
Percentage values are provided for the bootstrap (1000 replicates) Bar 0.5 substitutions per site.

with ethyl acetate:chloroform:methanol (5:3:1) tested against the bacterial strains revealed that there were at least two compounds with antibacterial activity in this crude extract (Fig. 4). After isolation of the compounds, the compounds **1** and **2** were available in enough amounts for the further studies. Their ^{1}H- and ^{13}C-NMR spectral data were identical with those of 2(5)-5,7,2-trihydroxy-8-methoxyflavanone and 2 (S)-5,2,5-trihydroxy-7,8-dimethoxyflavanone. The chemical structures were elucidated as 5,7,2'-trihydroxy-8-methoxyflavanone (1) and 5,2',5'-trihydroxy-7,8-dimethoxyflavanone (2) (Fig. 5).

The compound **1**: 5,7,2'-trihydroxy-8-methoxy flavanone, white needles, MP 197-199°C, IRν_{max} cm^{-1}: 3468, 1637, 1612, UV (MeOH) λ_{max} nm (log ε): 290 (4.26), 342 (3.67); (MeOH+$AlCl_3$) 315 (4.41), 402 (3.68); (MeOH+$AlCl_3$+HCl) 312 (4.35), 398 (3.65); (MeOH+NaOMe) 330 (4.45); (MeOH+NaOAc) 329 (4.44). The ^{1}H- and ^{13}C-NMR data were shown in Table 1.

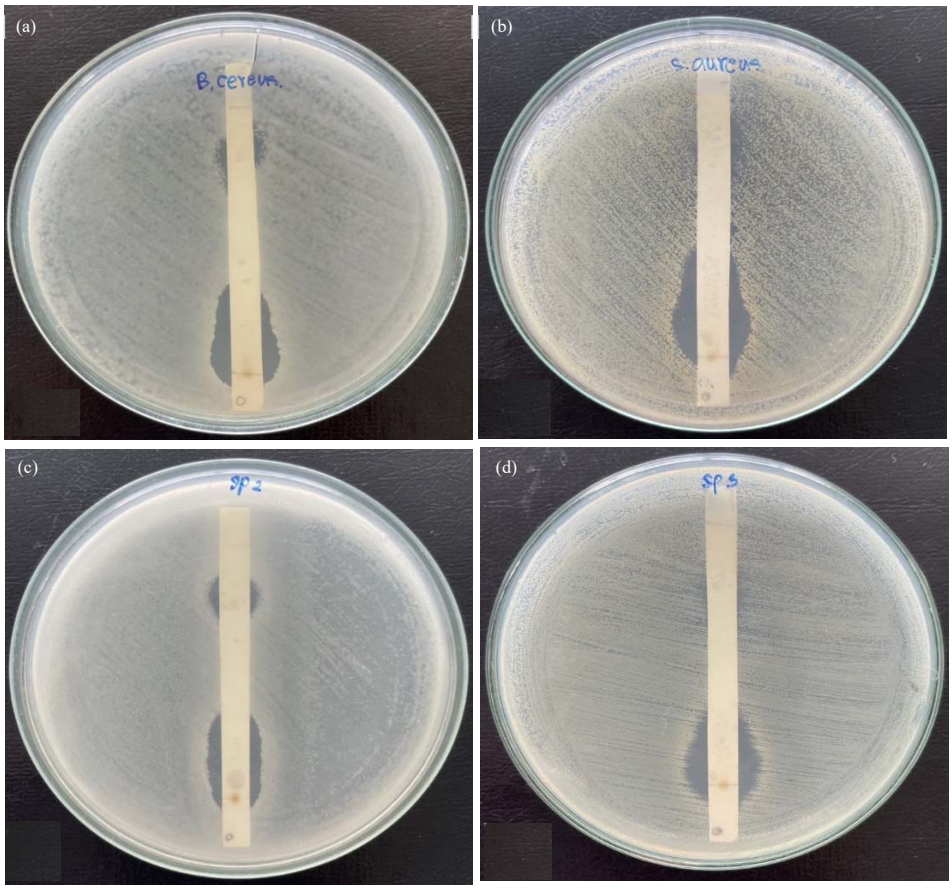

Fig. 4(a-d): TLC-based bioautography of the crude extract tested against, (a) *Bacillus cereus* ATCC 7064, (b) *Staphylococcus aureus* ATCC 25923, (c) Methicillin-resistant *Staphylococcus aureus* Sp2 and (d) Methicillin-resistant *Staphylococcus aureus* Sp3.

TLC of the crude extract was eluted with ethyl/acetate/chloroform/methanol (5/3/1) and carried out to localise the antibacterial activity on chromatogram after 24 hrs at 30°C incubation against the bacterial strains.

The compound **2**: 5,2',5'-trihydroxy-7,8-dimethoxy-flavanone, white needles, MP 191-194°C, IRν_{max} cm^{-1}: 3382, 1631, 1604, 1591 and 1215 cm^{-1}. The UV (MeOH) λ_{max} nm (log ε): 290 (3.94), 346 (3.41); (MeOH+$AlCl_3$) 312 (4.38), 406 (3.41); (MeOH+$AlCl_3$+HCl) 312 (4.03), 402 (3.35); (MeOH+NaOMe) 286 (3.82), 411 (3.55). The ^{1}H- and ^{13}C-NMR data were shown in Table 1.

Table 2 summarized the antimicrobial activity of the crude extract and purified compounds, as determined by MIC and MBC values. The studied bacteria were inhibited by the crude extract, with MICs and MBCs of 32-128 and 256–512 µg/mL, respectively. Compounds **1** and **2** displayed promising antibacterial activity by reducing the growth of *S. aureus*, including MRSA. While they exhibited some growth inhibition against *B. subtilis* and *B. cereus*, these compounds lacked bactericidal activity against these *Bacillus* species. The crude extract and purified compounds were assessed for cytotoxicity using Vero and L929 cell lines. All samples exhibited low cytotoxicity, with IC_{50} values greater than 512.00 µg/mL, indicating minimal cell growth inhibition at these concentrations. The RAW264.7 macrophages treated with purified compounds at concentrations of 15-60 µg/mL for 24 hrs showed no significant changes in cell viability compared to the control group upon subsequent LPS stimulation (Fig. 6). Similarly, no significant cytotoxicity was observed at concentrations up to 120 µg/mL (data not shown). Based on these results, subsequent experiments utilized purified compound concentrations ranging from 15-60 µg/mL.

The compounds **1** and **2** exhibited a dose-dependent suppression of pro-inflammatory mediators (NO, IL-1β and TNF-α) in LPS-activated RAW 264.7 macrophages. The LPS increased NO production, measured as nitrite concentration using the Griess reagent assay. Compound **1** at the concentrations of 15, 30 and 60 µg/mL dramatically decreased NO formation by 41.4±6.3, 60.7±5.2 and 74.7±6.5%, respectively, compared to LPS-stimulated control cells.

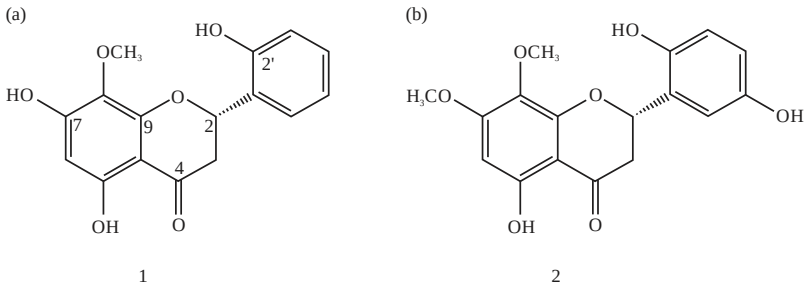

Fig. 5(a-b): Structures of the compounds, (a) 5,7,2'-trihydroxy-8-methoxyflavanone and (b) 5,2',5'-trihydroxy-7,8-dimethoxyflavanone.

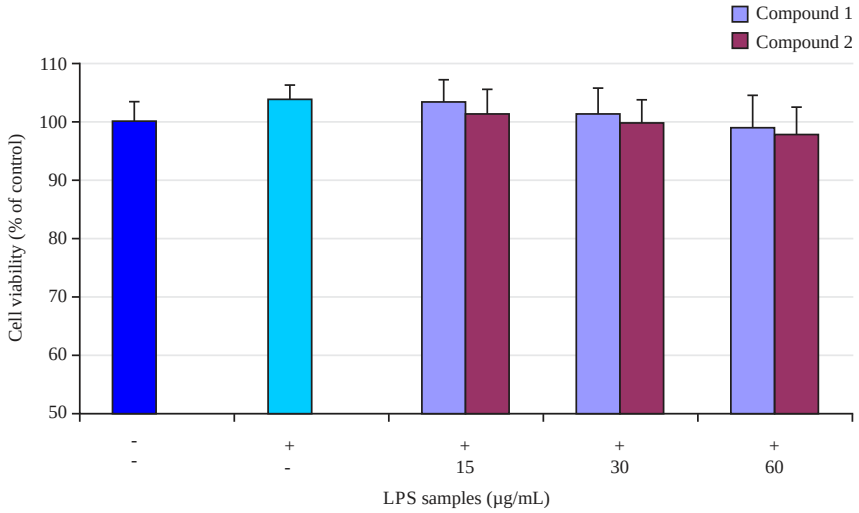

Fig. 6: Relative cell viability of RAW264.7 cells treated with different concentrations of purified compounds for 1 hrs and then stimulated with 1 µg/mL LPS for 24 hrs using the MTT assay.

Results are presented as the Means ± SD of triplicate experiments. Statistical analysis was performed using One-way Analysis of Variance (ANOVA) with Tukey's test for multiple comparison using SPSS statistics for windows, version 20.0. There is no statistically significant difference between groups.

Table 1: Spectroscopic data of compounds using ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz).

	Compounds			Compounds	
H-atom position	1	2	C-atom position	1	2
2	5.68 dd (3.0, 13.0)	5.65 dd (2.8, 12.8)	2	75.0	75.2
$3_{equ.}$	2.73 dd (3.0, 17.2)	2.74 dd (2.8, 17.2)	3	41.8	42.0
$3_{axi.}$	3.16 dd (13.2, 17.2)	3.14 dd (13.0, 17.2)	4	197.4	197.6
6	5.95 s	6.22 s	5	159.4	159.6
3'	6.80 m	6.68 d (8.4)	6	95.6	93.8
4'	7.16 td (1.2, 7.8)	6.58 dd (1.2,8.6)	7	160.9	161.5
5'	6.86 m	-	8	129.2	129.4
6'	7.45 dd (1.2, 7.6)	6.88 d (1.2)	9	155.0	154.6
OCH_3	3.64 s (C-8)	3.62 s (C-8)	10	102.6	102.8
		3.84 s (C-7)	1'	125.8	126.0
			2'	153.0	147.8
			3'	116.3	116.7
			4'	130.1	116.4
			5'	119.6	150.4
			6'	127.3	113.8
			OCH_3	61.2 (C-8)	57.8 (C-7)
					61.4 (C-8)

DMSO-d6, δ-values depicted in ppm and coupling constant in Hz.

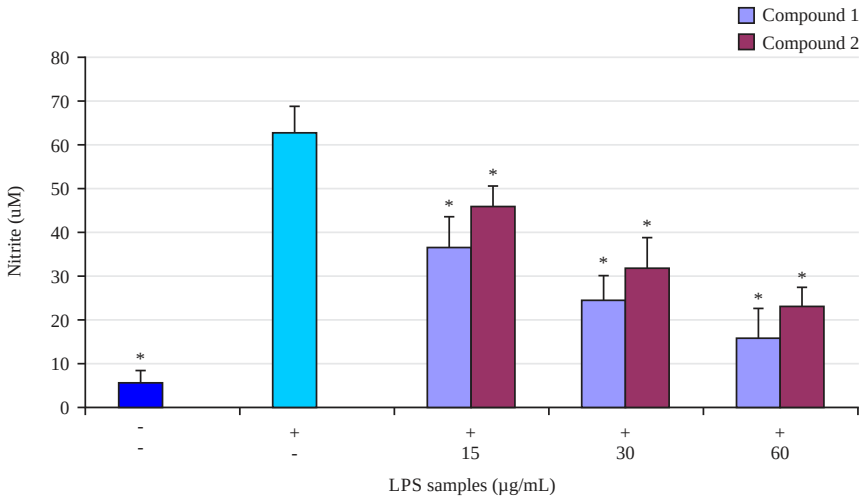

Fig. 7: Effects of purified compounds on LPS-induced nitric oxide (NO) production in RAW 264.7 cells. The cells were pretreated with purified compounds at different concentrations for 1 hr and then stimulated with 1 µg/mL LPS for 24 hrs. Nitrite accumulated in the culture supernatant was measured as an indicator of NO production using Griess reagent.
Values are presented as Means±SD of three independent experiments and *p<0.05, compared to the LPS-treated group.

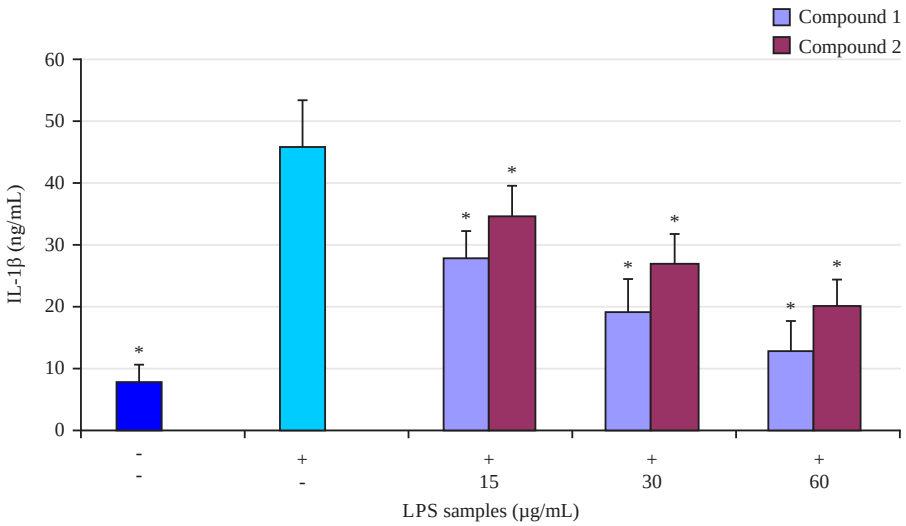

Fig. 8: Effects of purified compounds on LPS-induced IL-1β production. The RAW 264.7 cells were pretreated with purified compounds at different concentrations for 1 hr and then stimulated with 1 µg/mL LPS for 24 hrs. The IL-1β production was detected by ELISA.
Values are presented as Mean±SD of three independent experiments and *p<0.05, compared to the LPS-treated group.

In contrast, compound **2** at the same concentrations decreased NO production by 27.2±5.6 and 49.2±6.5 and 60.1±7.3%, respectively (Fig. 7). The LPS also increased the secretion of pro-inflammatory cytokines IL-1β and TNF-α. Both compounds dose-dependently suppressed the production of these cytokines, with compound **1** again showing a stronger effect at the highest concentration (60 µg/mL) as measured by ELISA. At the highest concentration of compounds **1**, the release of IL-1β and TNF-α was reduced by up to 71.9±4.3 and 74.6±6.2%, respectively, compared to LPS-stimulated control cells. In contrast, compound **2**, the release of IL-1β and TNF-α was reduced by up to 56.3±4.3 and 63.9±6.2%, respectively, which was reduced lower than compound **1** (Fig. 8 and 9).

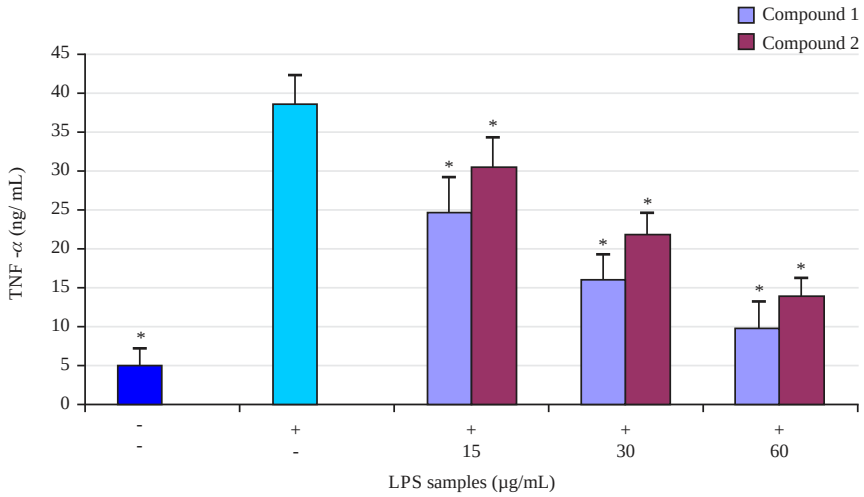

Fig. 9: Effects of purified compounds on LPS-induced TNF-α production. The RAW 264.7 cells were pretreated with purified compounds at different concentrations for 1 hr and then stimulated with 1 µg/mL LPS for 24 hrs. The TNF-α production was detected by ELISA.
Values are presented as Mean±SD of three independent experiments and *p<0.05, compared to the LPS-treated group.

Table 2: MBC and MIC of the crude extract and pure compounds against tested microbial strains.

	MIC (µg/mL)							MBC (µg/mL)						
Test substances	B.s.	B.c.	S.a.	MRSA Sp2	MRSA Sp3	MRSA RI	MRSA T2	B.s.	B.c.	S.a.	MRSA Sp2	MRSA Sp3	MRSA RI	MRSA T2
Crude extract	256	256	32	64	64	128	128	>512	>512	256	512	512	>512	>512
Compound **1**	256	256	32	32	32	64	64	>512	>512	128	128	128	256	256
Compound **2**	256	256	32	32	32	64	64	>512	>512	128	128	128	256	256
Chloramphenicol	2	2	1	2	8	8	8	4	4	2	4	16	16	16

B.s: *Bacillus subtilis* (ATCC 6633), B.c: *Bacillus cereus* (ATCC 7064), (ATCC 6633), S.a: *Staphylococcus aureus* (ATCC 25923) and MRSA: Methicillin-resistant *Staphylococcus aureus* Sp2, Sp3, RI and T2 (the clinical isolates).

Therefore, it is Interesting to note that compounds **1** and **2** showed toxicity against tested bacterial cells and have significant anti-inflammatory properties with no toxicity in normal cells which imply their potential as antimicrobial and anti-inflammatory agents. In the future, a more thorough investigation will be needed to comprehend their mechanism of actions.

DISCUSSION

A plant's rhizosphere is rich with nutrients that promote the development of microbes, some of which are called endophytes. Actinomycetes are well-known to generate enormous amounts of biologically active compounds. A herbal plant called *Boesenbergia rotunda* (L.) Mansf. contains approximately a hundred different compounds, including flavonoids, chalcones, esters, kawains, terpenes and terpenoids[2]. This plant has been used to isolate the endophytic actinomycetes which produced bioactive compounds[18].

In this study, *S. chartreusis* RH3.5 isolated from the *Boesenbergia rotunda* rhizosphere soil could produce the flavonoids which were determined as 5,2',5'-trihydroxy -7,8-dimethoxyflavanone **(1)** and 5,7,2'-trihydroxy-8-methoxyflavanone **(2)** based on a comparison of the spectral data in this study to those of previous studies[30,31]. These flavonoids possessed antibacterial activity against MRSA and *in vitro* anti-inflammatory activity in LPS-stimulated RAW264.7 cells. These compounds related of flavonoids which have been reported to possess anti-bacterial activity[32]. It is believed that current work is the first to report the isolation of 5,2',5'-trihydroxy-7,8-dimethoxyflavanone and 5,7,2'-trihydroxy-8-methoxyflavanone from the crude extract of microorganism. However, several related compounds have been isolated from the root of *Scutellaria indica*, a plant historically used in traditional Chinese medicine. These compounds exhibited cytotoxic activity against various tumor cell lines[33].

Flavonoids are often abundant in the plant kingdom, especially in fruit, vegetables, nuts, seeds, stems and flowers[34].

However, flavonoids in microorganisms have been reported for example in *Escherichia coli*[35,36], *Saccharomyces cerevisiae*[37] and *Streptomyces*[38-40] and the presence of metabolic pathways of flavonoid biosynthesis in microorganisms has been confirmed[41]. Flavonoids have been recognized for their wide range of pharmacological effects, such as antioxidant, antitumor, antimicrobial, antiallergic, antiviral and anti-inflammatory effects. Flavonoids' antimicrobial effect was primarily linked to 3 mechanisms: (1) Deceleration of nucleic acid biosynthesis, (2) Cytoplasmic membrane function inhibition and (3) Limiting the energy metabolism[42,43]. The effect of a number of flavone-based analogues on the DNA gyrase of *Escherichia coli* has been examined and it has been shown that the inhibitors act by intercalating into DNA to reduce gyrase activity[44]. It has been shown that certain flavonoids interact with the porin protein of *E. coli* outer membrane, blocking the porin's primary function the transport of tiny hydrophilic molecules like glucose and ultimately preventing the development of *E. coli*[45]. Some flavonoids, including glabrol, have been shown to rapidly enhance bacterial membrane permeability and reduce the force of proton movement. Glabrol also reduced the activity of phosphatidylglycerol, peptidoglycan and cardiolipin. Glabrol forms hydrogen bonds with phosphatidylglycerol and cardiolipin during binding, according to a molecular docking study[46]. A similar mechanism has also been suggested for tea compounds' ability to inhibit the *Streptococcus mutans* from adhering to tooth surfaces and forming a biofilm.

It has been proposed that flavonoids coat cell surfaces, thereby affecting cell surface characteristics and impairing interactions between bacterial cells and the surface of the substrate[47]. The antibacterial activity of flavonoids is influenced by the presence of hydroxyl groups on their aromatic rings. Studies have shown that hydroxylation, particularly at positions C5 and C7, enhances the ability of flavonoids to inhibit bacterial growth. Conversely, methoxylation at these same positions weakens their antibacterial action[48]. Flavonoids exhibit anti-inflammatory properties by targeting various pathways[49]. They achieve this through several mechanisms: (1) Modulating enzyme activity, they can regulate enzymes like cyclooxygenase (COX), lipoxygenase (LOX) and Inducible Nitric Oxide Synthase (iNOS) involved in inflammatory processes and (2) Reducing inflammatory mediators, flavonoids can decrease the production of molecules that promote inflammation, such as prostaglandins (PGs), leukotrienes (LTs), nitric oxide (NO) and pro-inflammatory cytokines like IL-1β, TNF-α, IL-6 and IL-8. The structure of the flavonoid molecule itself also plays a crucial role in its anti-inflammatory activity.

In this study, we observed that 5,2',5'-trihydroxy-7,8-dimethoxyflavanone **(1)** Prevented NO, IL-1β and TNF-α induction from LPS-treated RAW 264.7 cells better than 5,7,2'-trihydroxy-8-methoxyflavanone and **(2)** According to the findings of hydroxy group at C7 favoured the inhibition of NO production[50] and hydroxy group at C2' exerted NO inhibition as well[51]. Studies have shown that hydroxyl groups at positions C7 and C2' of flavonoid structures are critical for inhibiting nitric oxide (NO) production and pro-inflammatory cytokine production.

Taken together, current study findings reported here suggest that 5,2',5'-trihydroxy-7,8-dimethoxyflavanone and 5,7,2'-trihydroxy-8-methoxyflavanone showed significant antimicrobial activity against MRSA and *S. aureus* and also have anti-inflammatory activity. They could be used to treat some bacterial infection and decrease inflammation and also showed mild cytotoxic action on healthy cells.

CONCLUSION

The purified compounds of *Streptomyces chartreusis* RH3.5 displayed notable antimicrobial and anti-inflammatory activities. Importantly, it also exhibited low cytotoxicity against healthy L929 and Vero cells, indicating good biocompatibility. These two primary flavonoids; 5,7,2'-trihydroxy-8-methoxyflavanone (compound **1**) and 5,2',5'-trihydroxy-7,8-dimethoxyflavanone (compound **2**) were elucidated. They exhibited potent antibacterial activity, especially against Staphylococcus aureus and the clinical strain of MRSA and also decreased inflammation. These results suggest that these compounds might be clinically important for the treatment of MRSA and *Staphylococcus aureus* infections and acute inflammation.

SIGNIFICANCE STATEMENT

This study investigated *Streptomyces chartreusis* strain RH3.5, isolated from the rhizosphere of the *Boesenbergia rotunda* (L.) Mansf. Notably, this strain was found to produce two unique flavonoid compounds: 5,7,2'-trihydroxy-8-methoxyflavanone (compound **1**) and 5,2',5'-trihydroxy-7,8-dimethoxyflavanone (compound **2**). The isolated compounds displayed both antimicrobial and anti-inflammatory activities with minimal cytotoxicity toward healthy cells. This finding suggests their potential as alternative therapeutic agents for treating bacterial infections and managing acute inflammation.

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