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LIPOXYGENASE INHIBITORY ACTIVITY OF PHYTOCONSTITUENTS ISOLATED FROM THE RHIZOMES OF *Boesenbergia albosanguinea* (Ridl.) Loes.

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ABSTRACT. This study aimed to investigate the compounds isolated from the rhizome extract of *Boesenbergia albosanguinea*. The extraction was performed using Soxhlet extraction of the dried powdered rhizome, following a polarity gradient of *n*-hexane, dichloromethane, and methanol. The phytochemicals were purified using chromatography techniques, and their structures were confirmed through spectroscopic analyses (IR, NMR, and MS) and comparison with existing literature. Additionally, the isolated compounds were evaluated for their lipoxygenase inhibitory activity. The isolation process successfully yielded eight phytochemicals belonging to various classes, including phenylpropanoid, chalcones, kavalactone, flavanones, flavonol, and flavone. These compounds were identified as elemicin (**1**), panduratin A (**2**), isopanduratin A (**3**), 5,6-dehydrokawain (**4**), pinostrobin (**5**), pinocembrin (**6**), kaempferol (**7**), and luteolin (**8**). Among these, luteolin (**8**) and kaempferol (**7**) demonstrated the most potent lipoxygenase inhibitory activity, with percentage inhibitions of 88.5% and 86.2%, respectively. These findings highlight the potential of *B. albosanguinea* as a natural source of lipoxygenase inhibitors, particularly in comparison to previously studied plant-based inhibitors. The strong inhibitory effects of luteolin (**8**) and kaempferol (**7**) underscore their relevance in the development of anti-inflammatory and nutraceutical formulations. This study contributes to the growing body of research on *Boesenbergia* species, offering a foundation for further pharmacological and industrial applications.

KEY WORDS: Zingiberaceae, *Boesenbergia albosanguinea*, Constituent, Flavonoid, Lipoxygenase

INTRODUCTION

Lipoxygenases (LOXs) are a group of monomeric enzymes responsible for catalyzing the oxidation of polyunsaturated fatty acids (PUFAs), such as linoleic, linolenic, and arachidonic acids, leading to the formation of hydroperoxides. These enzymes are broadly distributed across animals, plants, fungi, and cyanobacteria [1]. Moreover, 5-LOX is ubiquitous in mammalians and oxygenates carbon-5 of arachidonic acid, while 9-LOX and 13-LOX are plant LOXs that catalyze the oxygenation of linoleic and linolenic acids [2]. Lipoxygenases, inherent in the human body, play a vital role in stimulating inflammatory reactions. Overexposure to reactive oxygen species can result in inflammation, which then stimulates the synthesis of cytokines and the activation of

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LOXs. Numerous illnesses, including cancer, stroke, cardiovascular, and neurological conditions, are associated with inflammation [3, 4]. Leukotriene and prostaglandin production is aided by LOXs. They are linked to the onset of the disease, and blocking them is considered an essential first step in the prevention of the disease [5]. Enzyme activity inhibitors may be better candidates for chemopreventive intervention because these enzymes' inhibition directly reduces fatty acid metabolite production, with simultaneous reduction of the related inflammatory, proliferative, and metastatic processes that lead to the development of cancer [6, 7]. Thus, the discovery of new lipoxygenase inhibitors with potent inhibitory activity is needed. In fact, in a few studies, LOX inhibitors have prevented carcinogen-induced lung adenomas and rat mammary gland cancer.

Zingiberaceae, commonly known as a ginger family, belongs to the order Zingiberales. It is a family of flowering plants made up of about 50 genera with a total of about 1600 known species, distributed throughout tropical Africa, Asia, and the Americas. Some parts of the plants from the Zingiberaceae family are usually aromatic, well-known for their ornamental and unique flavours. It is also a common ingredient in food, spices, medicines, dyes, and perfumes [8, 9]. For instance, rhizome extracts of *Zingiber zerumbet* have been used in Malay traditional medicine to treat inflammation and pain-mediated diseases, worm infestation, and diarrhea [10]. Besides, *Alpinia galanga* is useful against lumbago, rheumatic pain, sore throat, diabetes, tubercular glands, bronchitis, and catarrhal affection [11]. Furthermore, the leaves of *Curcuma aromatica* and *C. longa* are utilized for treating bruises, sprains, snake bites, skin ailments, and a range of inflammatory conditions [12].

Boesenbergia is a genus of the Zingiberaceae family, characterised by its small-sized species. The genus is classified within the subtribe *Zingiberae* of the family Zingiberoideae. The *Boesenbergia* genus currently includes about 99 species, with Thailand and Borneo being the main centres of diversity. Many researchers have proven that the rhizome part of *Boesenbergia* species displayed health-benefit properties. Besides, *Boesenbergia* serves a range of purposes, including food, medicinal applications, and ornamental use [13]. The extracts of this genus are rich in flavonoids, diterpenoids, and polyphenols, all of which possess a wide range of pharmacological activities such as anticancer, anti-inflammatory, antimicrobial, antiviral, antidiabetic, antiallergic, antiobesity, hepatoprotective, vasorelaxant, and aphrodisiac activities [14].

Boesenbergia albosanguinea is mainly distributed in Malaysia (Langkawi, Pulau Langgun) and Thailand (Satun Province). It can be found on limestone outcrops in shaded habitats in close proximity to the sea. In the Langkawi populations, the plants tend to be generally less robust, shorter in height with narrower, shorter leaves. The leaf sheaths are red but the color does not extend onto the rachis. The inflorescences are more cylindrical, less flattened with slightly longer, narrower bracts which sometimes deflex slightly away from the rachis on some plants. Flower shape and color are nearly identical to the Thai populations, albeit slightly smaller [15]. Recently, investigation of the essential oil revealed the presence of 34 components, accounting for 96.7% of the total oil. The most abundant components were elemicin (44.0%), α-gurjunene (9.3%), β-caryophyllene (4.5%), and safrole (4.1%). Besides, the rhizome oil showed a moderate inhibitory effect was observed for acetylcholinesterase and butyrylcholinesterase enzymes with IC_{50} values of 90.5 and 110.8 μg/mL, respectively [16].

In our attempt to isolate natural products for drug discovery and development using a rational approach as a part of a laboratory project to re-investigating medicinal plants, we initiated a study to isolate constituents from the rhizome of *Boesenbergia albosanguinea*. To the best of our knowledge, no report exists on their chemical constituents and its lipoxygenase inhibitory activity.

RESULTS AND DISCUSSION

Eight compounds were successfully identified from the rhizome of *B. albosanguinea*, which were characterized as elemicin (**1**), panduratin A (**2**), isopanduratin A (**3**), 5,6-dehydrokawain (**4**),

pinostrobin (**5**), pinocembrin (**6**), kaempferol (**7**), and luteolin (**8**). They were all identified by analyzing their spectroscopic data and comparing them with the reported literature. Their chemical structures are shown in Figure 1.

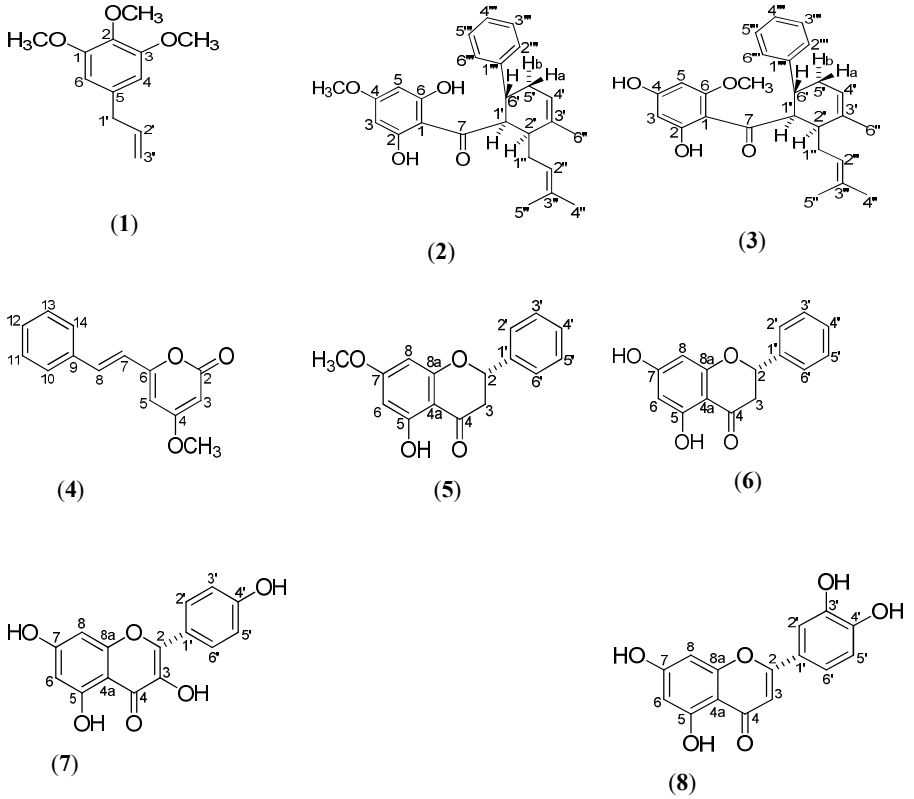

Figure 1. Chemical structures of isolated constituents from *B. albosanguinea.*

Compound (**1**) was identified as elemicin, it exhibited distinctive signals in its NMR spectrum, confirming its identity. The ^{1}H NMR spectrum reveals a three singlet signal, δ 3.87 and 3.85 corresponding to the methoxyl group at C-1, C-2, and C-3. Another singlet signal resonance at δ 6.43 indicates the presence of aromatic protons of H-4 and H-6. A doublet signal was appeared at δ 3.36 with a coupling constant of 6.8 Hz, characteristic of the methylene proton of H-1′. Besides, a doublet of doublet signal was displayed at δ 5.11 which attributed to protons H-3′a and H-3′b. A multiplet signal at δ 5.98 integrated for was assigned to the methine proton, H-2′. The ^{13}C NMR spectrum showed the presence of twelve carbons which attributed to four quaternary, three methine, two methylene, and three methoxyl carbons. The DEPT spectra revealed the existence of quaternary carbons resonated at δ 153.2 (C-1/C-3) and 136.4 (C-2). The methine carbons were observed at δ 105.5 (C-4/C-6) and 137.2 (C-2′). The methylene carbons resonate at δ 40.5 (C-1′) and 115.9 (C-3′). The methoxyl carbons were presented at δ 56.0 (1/3-OCH_3) and 60.8 (2-OCH_3).

Compound (**2**) was identified as panduratin A. The ^{1}H NMR spectrum revealed singlet signals assignable to three methyls at δ 1.51 (H-4″ and H-5″) and 1.77 (H-6″). Two set doublets of doublet

of doublet were appeared at δ 2.05/2.38 (H-5′a/H-5′b) and δ 2.16/2.27 (H-1″a/H-1‴b) which attributed to methylene protons. Besides, three methine protons were displayed at at δ 2.62 (ddd), 3.42 (ddd), and 4.67 (dd), which were corresponded to H-2′, H-6′, and H-1′, respectively. Two tri-substituted olefins were resonated at δ 4.85 (H-2″) as triplet (J = 6.9 Hz) and 5.42 (H-4′) as doublet of doublet (J = 3.0 and 5.0 Hz). In addition, seven aromatic protons were discovered at δ 5.87 (H-3/H-5), 7.10 (H-4‴), 7.21 (H-2‴/H-6‴/H-3‴/H-5‴), together with a methoxyl group at δ 3.74 (4-OCH_3). The ^{13}C NMR spectrum exhibited 26 signals of carbon present as one methoxyl carbon at δ 55.0 (4-OCH_3), one carbonyl at δ 207.0 (C-7), two methylene carbons 29.6 (C-1″), 35.8 (C-5′), three methyl carbons at δ 17.3 (C-5″), 22.9 (C-6″), 25.5 (C-4″), seven quaternary carbons δ 41.8 (C-2′), 105.6 (C-1), 137.3 (C-3′), 145.7 (C-1‴), 163.0 (C-2/C-6), 167.6 (C-4), and twelve methine carbons at δ 37.1 (C-6′), 41.8 (C-2′), 54.0 (C-1′), 96.6 (C-3/C-5), 122.6 (C-4′), 124.1 (C-2″), 125.6 (C-4‴), 127.2 (C-2‴/C-6‴), and 127.9 (C-3‴/C-5‴). The relative stereochemistry of C-l′, C-2′, and C-6′ was established by comparing the coupling constants with those of saggenon C and D [18]. These data lead to the conclusion that H-1′ and H-2′ are *cis*-oriented (J = 4.7 Hz) and H-1′/H-6′ have a *trans* relationship (J = 11.4 Hz).

Compound (**3**) was identified as isopanduratin A. The ^{1}H NMR spectrum was closely resembled to the ^{1}H NMR spectrum of panduratin A (**1**) except the position of the methoxyl group. Compound (**2**) features a methoxy group at C-6 position, while compound (**1**) features a methoxy group at C-4 position. The HMBC spectrum confirmed this position by showing the correlation of 6-OCH_3 (δ 55.7) with C-6 (δ 162.4). The ^{13}C NMR spectrum and DEPT spectra showed the presence of 26 signals attributed to 26 carbons, which were found similar to those of compound (**1**).

Compound (**4**) was identified as 5,6-dehydrokawain, displaying characteristic signals in its ^{1}H NMR spectrum, revealing the presence of a methoxyl group at 4-OCH_3 were detected at δ 3.85. Two doublets observed at δ 6.61 (J = 16.0 Hz) and 7.54 (J = 16.0 Hz) were assigned to olefinic protons H-7 and H-8, respectively. The large coupling constant, J = 16.0 Hz suggests that these protons are in a *trans*-orientation. Another two sets of doublets appeared at δ 5.52 and 5.97 (J = 2.2 Hz) were attributed to olefinic protons H-3 and H-5, of pyran-2-one respectively. The aromatic protons (H-10 to H-14) were observed as multiplet signals at δ 7.36-7.42. The ^{13}C NMR and DEPT spectra revealed the presence of fourteen carbons; one methoxy carbon at δ 55.9 (4-OCH_3), nine methine carbons at δ 88.8 (C-3), 101.3 (C-5), 118.6 (C-7), 127.4 (C-10/C-14), 128.9 (C-11/C-13), 129.4 (C-12), and 135.2 (C-8), three quaternary carbons at δ 135.8 (C-9), 163.9 (C-6), and 158.6 (C-4), and a carbonyl carbon at δ 171.0 (C-2).

Compound (**5**) was identified as pinostrobin, which exhibited characteristic signals in its ^{1}H NMR spectrum, confirming the presence of a hydroxyl group which was represented by a singlet signal at δ 12.05. Another singlet peak was observed at δ 3.84, assigned to a methoxy group (7-OCH_3). The *meta*-coupled signals appeared at δ 6.19 and 6.11 (J = 2.2 Hz), which were attributed to aromatic protons H-6 and H-8, respectively. In addition, three set doublets of doublet signal, each integrated for one proton were observed at δ 2.85 (J = 3.0 Hz and 17.1 Hz), 3.12 (J = 13.0 Hz and 17.1 Hz) and 5.45 (J = 3.0 Hz and 13.1 Hz,) were assigned for H-3a, H-3b and H-2, respectively. In addition, a multiplet signal resonated at δ 7.42-7.47 integrating for five protons of aromatic protons, H-2′–H-6′ of ring B. Besides, based on the chemical shift pattern comparison of H-2, H-3a, and H-3b, the configuration of H-3 was deduced as R configuration [19]. The ^{13}C NMR and DEPT spectra indicated the presence of sixteen carbons including one methyl, one methylene, one carbonyl carbon, eight methine, and five quaternary carbon atoms. The carbon signal of methoxy group (7-OCH_3) was clearly assigned at δ 55.7, while the carbonyl carbon (C-4) was also observed at δ 195.8. The single signal position of methylene carbon (C-3a/C-3b) was observed at δ 43.4. Eight methine carbons were detected at δ 79.3 (C-2), 94.3 (C-8), 95.2 (C-6), 126.2 (C-2′/C-6′) and 128.9 (C-3′/C-4′/C-5′) as well as five quaternary carbons at δ 103.2 (C-4a), 138.4 (C-1′), 162.8 (C-8a), 164.2 (C-5) and 168.0 (C-7).

Compound (**6**) was identified as pinocembrin. The ^{1}H NMR spectrum was almost identical to compound (**5**). The similarities of those spectra were observed as the presence of meta-coupled signals, the multiplet peak for aromatic protons, and the singlet peak at the downfield region (5-OH). The only difference between both spectra was the absence of a methoxy signal at C-7 with additional proton OH in the ^{1}H NMR spectrum of compound (**6**). Besides, based on the chemical shift pattern comparison of H-2, H-3a, and H-3b, the stereochemistry of H-3 was deduced as R configuration [20]. The ^{13}C NMR and DEPT spectra exhibited the presence of fifteen carbons, embracing of one methylene carbon, eight methine carbons, five quaternary carbons, and a carbonyl carbon.

Compound (**7**) was identified as kaempferol. The ^{1}H NMR spectrum showed one proton singlet at δ 6.15 which was assigned for the aromatic proton at H-6 and one proton singlet δ 6.40 for the aromatic proton at H-8. The peak with two protons at δ 8.00 appeared as the doublet of doublet was assigned to H-2' and H-6'. Another doublet of doublet peak with two protons at δ 6.89 was assigned for H-5' and H-3'. The ^{13}C NMR spectrum established the resonances of fifteen carbon atoms. Characterization of these signals as six methines appeared at δ 98.7 (C-6), 94.0 (C-8), 115.9 (C-3'/C-5'), 130.0 (C-2'/C-6') and nine quaternary carbons resonated at δ 103.5 (C-4a), 122.1 (C-1'), 136.2 (C-3), 147.3 (C-2), 159.7 (C-4'), 156.7 (C-8a), 161.2 (C-5), 161.2 (C-7), and 176.4 (C-4).

Compound (**8**) was identified as luteolin, displaying characteristic signals in its NMR spectrum, similar to kaempferol (**7**) except for an additional singlet signal at δ 6.47 which was attributed to H-3. In addition, the ABX aromatic spin system signals which were observed at δ 7.31 (1H, d, J = 2.3), 7.28 (1H, d, J = 1.7 Hz), and 6.74 (1H, d, J = 8.1 Hz), and were assigned to methine protons of H-2′, H-6′, and H-5′ in B ring, respectively. The fifteen carbons established from the ^{13}C NMR spectrum were categorized into six methines, nine quaternary carbons, and one carbonyl.

Compounds (**1–8**) have been previously reported from several *Boesenbergia* species. Compound (**1**) has been reported as a major component in the roots of *B. pulcherrima* [21] and *n*-hexane extract of *B. xiphostachya* [22]. Compounds (**2-7**) were reported previously from the rhizomes of *B. rotunda* [23], whereas compound (**8**) was isolated from *B. armeniaca* [24].

As a summary, the chemical constituents of *B. albosanguinea* from Malaysia were found to be rich in flavonoids. Flavonoids represent one of the largest classes of plant secondary metabolites and are involved in a multitude of physiological functions, including UV protection, insect attraction, pathogen defense, symbiosis, and variation of flower color [25]. Flavonoids have been associated with many favorable agronomic traits and health benefits to humans, and their metabolic engineering is therefore an important target for plant biotechnology [26]. To the best of our knowledge, all compounds were isolated from *B. albosanguinea* for the first time.

Recently, attention has been paid to the preventative and medicinal value of dietary components, especially secondary metabolites with known inhibitory properties against LOXs. There is evidence that at least several hydrogen-donating organic molecules are co-oxidized by plant and mammalian LOXs. These observations lead to the conclusion that LOX is a versatile biocatalyst for biotransformation of endobiotics and xenobiotics [27]. Based on the relevant potential of plants as a source of anti-inflammatory compounds, in the current study, the isolated constituents from *B. albosanguinea* were subjected to lipoxygenase inhibitory activity, and the results are shown in Table 1.

All isolated constituents showed strong lipoxygenase inhibitory activity, which gave inhibition values in the range of 73.0–88.5 µg/mL at a concentration of 1 mg/mL. Among them, luteolin (**8**) and kaempferol (**7**) have shown the highest an inhibition, which gave inhibition of 88.5% and 86.2%, respectively. The result was in line with previous data which reported that luteolin as the most potent inhibitor of the mammalian enzyme with an IC_{50} value of 0.6 µM [28]. Comparatively, synthetic inhibitors such as zileuton, a well-established 5-lipoxygenase inhibitor used in asthma management, exhibit IC_{50} values in the micromolar range (~0.5–2 µM). While

direct IC_{50} comparisons are necessary for a definitive conclusion, flavonoids like luteolin have previously shown IC_{50} values around 2–5 μM in LOX inhibition studies, suggesting competitive potency with synthetic alternatives. Remarkably, even flavone was found to be inhibitory, showing that the general presence of phenolic hydroxyl groups is not a pre-requisite for the inhibition. In particular, the 3-OH proved to be not essential but rather interfering, as indicated by the higher potency of the flavone luteolin than that of the flavonol quercetin. Presence of catechol in ring A or B enhanced the inhibitory potency but was not essential for it [29]. Meanwhile, recent studies demonstrated that several prenylated chalcones and hydroxychalcones were potent free radical scavengers and also inhibited the expression of adhesion molecules and iNOS by blocking the activation of NF-kB [30]. In a previous study, panduratin A (**2**) showed significant anti-inflammatory activity in a skin-inflammatory animal model [31]. Inflammation is still a condition that is harmful to public health, which brings great pain to patients. LOX not only refers to the oxidation of lipids, but also the involvement in producing leukotrien, which mediates the occurrence of inflammation. Inhibiting LOX activity is a prospective method to treat inflammation, for the reason that many specific compounds were designed and synthesized as LOX inhibitors.

Table 1. Lipoxygenase inhibitory activity of the isolated constituents.

Compounds	% inhibition at 1 mg/mL
Elemicin (**1**)	73.2
Panduratin A (**2**)	76.2
Isopanduratin A (**3**)	84.9
5,6-Dehydrokawain (4)	73.0
Pinostrobin (**5**)	73.2
Pinocembrin (**6**)	76.0
Kaempferol (**7**)	86.2
Luteolin (**8**)	88.5
Standard - Quercetin	81.9

EXPERIMENTAL

Plant material

The rhizomes of *B. albosanguinea* were collected from Langgun Island, Langkawi (August 2023) and identified by Dr Shamsul Khamis from Universiti Kebangsaan Malaysia (UKM). The voucher specimen (PL-13/2) was deposited at UKM Herbarium, UKM.

General experimental procedures

A Soxhlet extraction technique was applied to extract the phytochemicals from the dried rhizome using different polarity solvents (*n*-hexane, dichloromethane (DCM) and methanol). Column chromatography (CC) was performed using Merck silica gel 60 (70-230 mesh) as the stationary phase. Thin layer chromatography (TLC) analysis was performed on Merck precoated silica (SiO_2) gel F_{254} plates (0.22 mm thickness) to detect and monitor the presence of compound samples. The spots were visualized under UV light (254 and 365 nm) and included with spraying reagent vanillin sulphuric acid in MeOH followed by heating. Melting points were measured by comparing them with other literature. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a Bruker Avance 500 Spectrometer. Chemical shifts were reported in ppm and $CDCl_3$ as solvent. The residual solvent was used as an internal standard. The IR spectra were recorded on Perkin Elmer ETR and 1600 spectrophotometer series as KBr discs or thin film of NaCl discs. Mass spectral data were obtained from Orbitrap Exploris 240 Mass Spectrometer.

Extraction and isolation

The dried rhizome of *B. albosanguinea* (500 g) was grinded into powder and extracted with *n*-hexane, DCM, and methanol sequentially by soxhlet extraction. The extract was concentrated using rotary evaporation to afford the crude extracts. The *n*-hexane extract (BARH, 8 g) was fractionated by VLC and eluted with *n*-hexane:DCM: EtOAc to afford 10 major fractions (F1-F10). Fraction F2, F4, and F6 was purified by CC and eluted with *n*-hexane:DCM to afford compounds (**1**) (20 mg), (**2**) (40 mg) and (**3**) (30 mg), respectively. Purification of the DCM extract (BARE, 12 g) by CC eluted with *n*-hexane:EtOAc:MeOH afforded 8 fractions (F1-F8). Fraction F3 was crystallized and successfully yielded compound (**4**) (18 mg), whereas fraction F6 yielded compound (**5**) (15 mg). Fraction F7 was subjected to CC using *n*-hexane:EtOAc as the eluent, yielding compound (**6**) (14 mg). Purification of the methanol extract (BARM, 15 g) by CC eluted with *n*-hexane:EtOAc:MeOH afforded 9 fractions (F1-F9). The fractions F4 and F5 were further purified by PTLC and successfully afforded compounds (**7**) (8 mg) and (**8**) (8 mg), respectively.

Elemicin (**1**): colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.36 (2H, d, J = 6.8 Hz, H-1'), 3.85 (3H, s, 2-OCH_3), 3.87 (6H, s, 1/3-OCH_3), 5.11 (1H, dd, J = 10.1 and 1.8 Hz, H-3'a), 5.13 (1H, dd, J = 17.0 and 1.8 Hz, H-3'b), 5.98 (1H, m, H-2'), 6.43 (2H, s, H-4/H-6); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 40.5 (C-1'), 56.0 (1-OCH3), 56.0 (3-OCH_3), 60.8 (2-OCH_3), 105.5 (C-4/C-6), 115.9 (C-3'a/C-3'b), 136.4 (C-2), 137.2 (C-2'), 153.2 (C-1/C-3), 135.7 (C-5); EIMS: *m/z* 208 [M^+, $C_{12}H_{16}O_3$].

Panduratin A (**2**): white crystalline needle. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.51 (6H, s, H-4"/H-5"), 1.77 (3H, s, H-6"), 2.05 (1H, ddd, J = 3.0, 11.8, 18.0 Hz, H-5'b), 2.16 (1H, ddd, J = 7.0, 7.5, 15.0 Hz, H-1″a), 2.27 (1H, ddd, J = 7.3, 7.3, 14.9 Hz, H-1″b), 2.38 (1H, ddd, J = 4.6, 7.0, 17.7 Hz, H-5'a), 2.62 (1H, ddd, J = 4.7, 7.4, 7.8 Hz, H-2'), 3.42 (1H, ddd, J = 6.2, 10.8, 10.9 Hz, H-6'), 3.74 (3H, s, 4-OCH_3), 4.67 (1H, dd, J = 4.7 Hz, H-1'), 4.85 (1H, t, J = 6.9 Hz, H-2"), 5.42 (1H, dd, J = 3.0, 5.0 Hz, H-4'), 5.87 (2H, s, H-3/H-5), 7.10 (1H, m, H-4'"), 7.21 (4H, m, H-2'"/H-3'"/H-5'"/H-6'"); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 17.3 (C-5"), 22.9 (C-6"), 25.5 (C-4"), 29.6 (C-1"a/C-1"b), 35.8 (C-5'a/C-5'b), 37.1 (C-6'), 41.8 (C-2'), 54.0 (C-1'), 55.0 (4-OCH_3), 96.6 (C-3/C-5), 105.6 (C-1), 122.6 (C-4'), 124.1 (C-2"), 125.6 (C-4'"), 127.2 (C-2'"/C-6'"), 127.9 (C-3'"/C-5'"), 132.4 (C-3"), 137.3 (C-3'), 145.7 (C-1'"), 167.6 (C-4), 167.6 (C-2), 207.0 (C-7); EIMS: *m/z* 407 [M^+, $C_{26}H_{30}O_4$].

Isopanduratin A (**3**): yellow oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.52 (6H, s, H-4"/H-5"), 1.81 (3H, s, H-6"), 2.05 (1H, m, H-5'b), 2.10 (1H, m, H-1'a), 2.26 (1H, m, H-1'b), 2.43 (1H, m, H-5'a), 2.52 (1H, m, H-2'), 3.44 (1H, m, H-6'), 3.92 (3H, s, 6-OCH_3), 4.52 (1H, dd, J = 4.7 and 11.3 Hz, H-1'), 4.88 (1H, t, J = 7.0 Hz, H-2"), 5.45 (1H, br.s, H-4'), 5.94 (1H, s, H-3), 5.96 (1H, s, H-5), 7.11 (1H, m, H-4'"), 7.21 (2H, m, H-2'"/H-6'"), 7.23 (2H, m, H-3'"/H-5'"); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 17.8 (C-5"), 22.9 (C-6"), 25.6 (C-4"), 28.9 (C-1"a/b), 35.8 (C-5'a/b), 37.1 (C-6'), 42.6 (C-2'), 54.1 (C-1'), 55.7 (6-OCH_3), 90.9 (C-5), 96.8 (C-3), 106.6 (C-1), 120.9 (C-4'), 124.1 (C-2"), 125.5 (C-4'"), 127.0 (C-2'"/6'"), 128.3 (C-3'"/5'"), 131.8 (C-3"), 137.2 (C-3'), 147.1 (C-1'"), 162.7 (C-4/6), 167.5 (C-2), 206.3 (C-7); EIMS: *m/z* 405 [M+H^+, $C_{26}H_{30}O_4$].

5,6-Dehydrokawain (**4**): white solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.85 (3H, s, 4-OCH_3), 5.52 (1H, d, J = 2.2 Hz, H-3), 5.97 (1H, d, J = 2.1 Hz, H-5), 6.61 (1H, d, J = 16.0 Hz, H-7), 7.36-7.42 (1H, m, H-10/H-11/H-12/H-13/H-14), 7.54 (1H, m, H-8); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 55.9 (4-OCH_3), 88.8 (C-3), 101.3 (C-5), 118.6 (C-7), 127.4 (C-10/C-14), 128.9 (C-11/C-13), 129.4 (C-12), 135.2 (C-8), 135.8 (C-9), 158.6 (C-4), 163.9 (C-6), 171.0 (C-2); EIMS: *m/z* 229 [M^+, $C_{14}H_{12}O_3$].

Pinostrobin (**5**): white solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.85 (1H, dd, J = 3.0, 17.1 Hz, H-3b), 3.12 (1H, dd, J = 13.0, 17.1 Hz, H-3a), 3.84 (3H, s, 7-OCH_3), 5.45 (1H, dd, J = 3.0, 13.1 Hz, H-2), 6.09 (1H, d, J = 2.3 Hz, H-8), 6.11 (1H, d, J = 2.2 Hz, H-6), 7.42 (1H, m, H-3'/H-4'/H-5'), 7.47 (1H, m, H-2'/H-6'), 12.05 (1H, s, 7-OH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 43.4 (C-3a/b), 55.7

(7-OCH_3), 79.3 (C-2), 94.3 (C-8), 95.2 (C-6), 103.2 (C-4a), 126.2 (C-2'/C-6'), 128.9 (C-3'/C-4'/C-5'), 138.4 (C-1'), 162.8 (C-8a), 164.2 (C-5), 168.0 (C-7), 195.8 (C-4); EIMS: *m/z* 271 [M^+, $C_{16}H_{14}O_4$].

Pinocembrin (**6**): yellow solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.86 (1H, dd, J = 3.0, 17.1 Hz, H-3b), 3.12 (1H, dd, J = 3.0, 17.1 Hz, H-3a), 5.45 (1H, dd, J = 3.0, 13.0 Hz, H-2), 6.00 (2H, s, H-8), 6.03 (2H, s, H-6), 7.41-7.49 (1H, m, H-2'/H-3'/H-4'/H-5'/H-6'), 12.07 (1H, s, 5-OH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 43.4 (C-3a/b), 79.3 (C-2), 95.5 (C-8), δ 96.8 (C-6), 103.3 (C-4a), 126.2 (C-2'/C-6'), 128.9 (C-3'/C-4'/C-5'), 138.3 (C-1'), 163.1 (C-8a), 164.4 (C-5), 164.5 (C-7), 195.8 (C-4); EIMS: *m/z* 257 [M^+, $C_{15}H_{12}O_4$].

Kaempferol (**7**): yellow crystalline solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.15 (1H, d, J = 2.0 Hz, H-6), 6.40 (1H, d, J = 2.0 Hz, H-8), 6.89 (1H, d, J = 8.8 Hz, H-3'/H-5'), 8.00 (1H, d, J = 8.8 Hz, H-2'/H-6'), 12.44 (1H, s, 5-OH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 94.0 (C-8), 98.7 (C-6), 103.5 (C-4a), 115.9 (C-3'/C-5'), 122.2 (C-1'), 130.0 (C-2'/C-6'), 136.2 (C-3), 147.3 (C-2), 156.7 (C-8a), 159.7 (C-4'), 161.2 (C-5), 164.4 (C-7), 176.4 (C-4); EIMS: *m/z* 287 [M^+, $C_{15}H_{10}O_6$].

Luteolin (**8**): yellow crystalline solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.00 (1H, d, J = 2.0 Hz, H-6), 6.25 (1H, d, J = 2.0 Hz, H-8), 6.47 (1H, s, H-3), 6.74 (1H, d, J = 8.1 Hz, H-5'), 7.28 (1H, d, J = 1.7 Hz, H-6'), 7.31 (1H, d, J = 2.3 Hz, H-2'), 12.89 (1H, s, 5-OH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 94.8 (C-8), 100.1 (C-6), 102.3 (C-3), 102.8 (C-4a), 112.8 (C-2'), 116.3 (C-5'), 119.4 (C-6'), 120.4 (C-1'), 146.9 (C-3'), 152.4 (C-4'), 158 (C-8a), 161.8 (C-5/C-7), 164.1 (C-2); EIMS: *m/z* 285 [M^+, $C_{15}H_{14}O_6$].

Lipoxygenase inhibitory activity

Evaluation of lipoxygenase (LOX) assay of isolated constituents was prepared according to the standard protocol (Lipoxygenase inhibitor screening assay kit, Item No. 760700, Cayman Chemicals Co) [17]. Stock solution of the samples was prepared to obtain a concentration of 1 mg/mL. The prepared solutions were then introduced onto 96-well plates where the cells were distributed as blanks 1A-2A-1D (triplicate), positive control 1B-2B (duplicate), and 100% initial activity wells 1C-2C-2D (triplicate). The remaining wells were designated for inhibitor (tested samples) solutions in duplicate. The addition of the reagents was done according to the standard protocol, according to which 100 µL of assay buffer was added to the blank wells and 90 µL of lipoxygenase (15-LOX) enzyme and 10 µL of assay buffer were added to positive control wells. For the 100% initial activity wells, 90 µL of lipoxygenase enzyme and 10 µL of solvent (DMSO) were added. The inhibitor (tested samples) wells were charged with 90 µL of lipoxygenase enzyme and 10 µL of respective stock (tested samples) solution. The reaction was initiated by adding 10 µL of the substrate (AA) to all wells. The plate was then shaken for 5 min on an orbital shaker. Ultimately, 100 µL of chromogen solution (prepared according to standard protocol) was added to each well to stop the enzyme catalysis. The plate was incubated for 30 min and was read at 500 nm. The percentage inhibitions (I%) of the tested samples were calculated using the following equation: $I\% = [A_{\text{initial activity}} - A_{\text{inhibitor}} / A_{\text{initial activity}}] \times 100$; where $A_{\text{initial activity}}$ is the absorbance of 100% initial activity wells without sample and $A_{\text{inhibitor}}$ is the absorbance of sample/reference.

CONCLUSION

In the current study, the phytochemical investigation of the rhizome of *B. albosanguinea* has afforded eight compounds, which comprised of diarylheptanoids (panduratin A, isopanduratin A), kavalactone (5,6-dehydrokawain), chalcones (pinostrobin, pinocembrin), flavonol (kaempferol), flavone (luteolin), and phenylpropanoid (elemicin). Likewise, the process of isolating and identifying phytochemicals can enhance our understanding of the compositional variations of phytochemicals in this species, which could potentially serve as an anti-inflammatory agent. There is a growing body of evidence to suggest that 5-LOX derived ROS are involved in a variety

of pathological and inflammatory responses. However, the detailed signaling mechanisms through which LOX metabolites mediate the specific signaling pathways that are involved in ROS generation, have yet to be clearly demonstrated. Taken together, our results shed light on the anti-inflammatory activity of isolated constituents and provide further evidence for the potential use of *Boesenbergia* species as a functional food. However, the stability and bioavailability of the active principles must be thoroughly investigated before any application can be realized.

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