Structural characterization and antioxidant and anti-inflammatory activities of new chemical constituent from the seeds of bambara groundnut (Vigna subterranea (L.) Verdc.)

Dutsadee Chinnapun and Natee Sakorn

School of Medicine, Walailak University, Nakhon Si Thammarat, Thailand; The Center for Scientific and Technological Equipment, Walailak University, Nakhon Si Thammarat, Thailand

ABSTRACT

A new compound (C20H18O6) was isolated from bambara groundnut seeds (Vigna subterranea (L.) Verdc.) and identified as a luteolin glycoside based on 1H, 13C and 2D nuclear magnetic resonance spectroscopy data and further analytical data. This compound exhibited similar scavenging activity for DPPH radical to Trolox and had a higher inhibitory effect on superoxide anion radicals than gallic acid and Trolox. C20H18O6 exhibited an inhibitory effect on DPPH and superoxide anion radicals, with IC50 values of 2.25 ± 0.14 µg/mL and 9.8 ± 0.3 µg/mL, respectively. However, C20H18O6 displayed low activities for FRAP and metal chelation. C20H18O6 had a higher inhibitory effect on lipoxigenase (IC50 = 2.16 ± 0.14 µg/mL) than nordihydroguaiaretic acid but had a lower inhibitory effect on xanthine oxidase than allopurinol. These results indicate that bambara groundnut seeds can be natural sources of antioxidants for humans and can serve as lipoxigenase inhibitor to reduce inflammation.

1. Introduction

For many years, legumes have been essential sources of dietary protein. Phytotoxins isolated from legumes are considered to have potential antioxidant, anti-inflammatory, and antihyperglycemic activities (Bhadkaria et al., 2021; Lam et al., 2019; Xu & Chang, 2010). Several phytochemicals purified from legumes have already been studied for biological activities. For example, daidzein, aescin, and quercetin isolated from Vigna vexillata were found to inhibit superoxide anion and elastase release (Leu et al., 2012). Three new compounds isolated from the methanol extract of Vigna luteola exhibited superoxide anion inhibition and anti-inflammatory activity (Lam et al., 2019).

Lipoxigenases and xanthine oxidase present in the human body and are associated with inflammatory reactions (Dash et al., 2020; Mashima & Okuyama, 2015). Lipoxigenases play an important role in prostaglandin and leukotriene synthesis (Ding et al., 2003; Rådmark et al., 2015). Xanthine oxidase is an important enzyme for purine catabolism. Xanthine oxidase catalyzes purine into uric acid, which causes gout disease (Dash et al., 2020). Several extracts from plants of the Fabaceae family inhibit the activity of lipoxigenases and xanthine oxidase. Extracts from Erythrina senegalensis (stem bark and root) (Souleymane et al., 2016), Sophora tonkinensis (Yoo et al., 2017), Cassia alata (leaves) (Oso & Karigidi, 2019), and Pterocarpus erinaceus (root bark) (Noufou et al., 2017) have been found to inhibit lipoxigenases. Pure polyphenolic compounds isolated from Vicia faba and Lotus edulis have been found to inhibit xanthine oxidase (Spanou et al., 2012).

The legume Vigna subterranea (L.) Verdc. (bambara groundnut) is widely cultivated in Africa and Asia. Bambara groundnut is an economically important legume in sub-Saharan Africa (Ajllogba et al., 2022) because of production...
by communities for household use (Aviara et al., 2013). However, commercial production and industrial utilization of bambara groundnut are low (Aviara et al., 2013). In Thailand, bambara groundnut is cultivated for domestic consumption and export to Malaysia. Bambara groundnut has high nutrient levels, a good balance of essential amino acids, a high carbohydrate content, and sufficient quantities of fats such as linoleic acid and linolenic acid (Minka & Bruneters, 2000). Moreover, the extracts of bambara groundnut seeds have been reported to display antioxidant activities. These seeds contain a variety of components associated with antioxidant activities, such as flavonoids, tannins, and phenolics (Chinnapun, 2018). However, few studies have been performed on the chemical constituents of bambara groundnut seeds. Therefore, the chemical constituents associated with the antioxidant and anti-inflammatory activities of bambara groundnut seed extracts were studied in this study.

2. Materials and methods

2.1. Chemicals

The following chemicals were purchased from Sigma–Aldrich (Thailand): acetic acid, anisaldehyde-sulfuric acid, boric acid, dichloromethane (CH₂Cl₂), dimethyl sulfoxide (DMSO), 2,2'-diphenyl-1-picryl-hydrazyl (DPPH), ethanol, ethyl acetate (EtOAc), ethylenediaminetetraacetic acid (EDTA), ferrous chloride (FeCl₂), ferrozine, hydrochloric acid, linoleic acid, lipoygenase, methanol (MeOH), nicotinamide adenine dinucleotide (NADH), nitroblue tetrazolium (NBT), nordihydroguaiaretic acid (NDGA), phenazine methosulfate, rutin, 2,4,6-tris(2-pyridyl)-s-triazine, Trolox, xanthine and xanthine oxidase. Gallic acid and iron (III) chloride hexahydrate (FeCl₃·6H₂O) were purchased from Merck Millipore (Thailand).

2.2. Plant material

Bambara groundnut seeds (Songkhla 1 variety) were investigated in this study. The seeds were obtained from a market in Trang Province, Thailand. The plant was identified by Dr. Rumrada Meeboonya, a plant taxonomist working on the genus Vigna Savi for the Flora of Thailand project and Dr. Witsanu Saisorn (School of Science, Walailak University, Thailand). The plant specimen was deposited at the Prince of Songkla University Herbarium (PSU Herbarium) with herbarium number PSU 019182. The bambara groundnut seeds were cleaned with water. The peel of the seeds was removed before performing experiments.

2.3. Crude extraction

The bambara groundnut seeds were ground using a grinder. A sample of the powder (8 kg) was continuously extracted by reflux extraction with 100% ethanol (1:1 w/v). The sample was re-extracted 3 times for 1 hour each time. The solution was filtered and concentrated under reduced pressure by a rotary evaporator at a boiling point of 40°C and 175 mbar. The remaining filtrated solution was freeze-dried to obtain the powdered crude extract.

2.4. Partition extraction

The crude extract was partitioned by liquid-liquid extraction. The crude extract (300 g) was suspended in 1 L of 80% MeOH, partitioned with hexane, and successively partitioned with CH₂Cl₂, EtOAc and water. Each partition was evaporated to dryness by a rotary evaporator to produce residues of the hexane, CH₂Cl₂, EtOAc, and aqueous fractions. Each fraction was held at 4°C to further isolate the constituents by column chromatography.

2.5. Bioassay-guided fractionation

Thin-layer chromatography (TLC) profiles (TLC plates viewed at 254 nm and 365 nm with a UV lamp) were used in this study, and an anisaldehyde-sulfuric acid reagent was used as the primary indicator of the pooled fraction.

2.6. Isolation of compounds from the ethyl acetate fraction

The EtOAc fraction (5.0 g) with the highest DPPH free radical scavenging activity was column chromatographed on silica gel. The column was eluted in ascending polarity with CH₂Cl₂: EtOAc; 1: 1, CH₂Cl₂: EtOAc; 2: 8, EtOAc 100%, EtOAc: MeOH; 9:8: 0.2, CH₂Cl₂: MeOH; and 8: 2, MeOH 100% to obtain 106 fractions. A volume of 75 mL was collected of each fraction. All 106 fractions were guided with TLC to identify six fractions (C₁F₁–C₁F₆) (Figure 1).

Based on the results obtained from DPPH free radical scavenging activity experiments, the C₁F₄ fraction, which had the highest DPPH free radical scavenging activity, was further column chromatographed on silica gel. The column was eluted in ascending polarity with CH₂Cl₂ 100%, CH₂Cl₂: EtOAc; 9: 1, CH₂Cl₂: EtOAc; 8: 2, CH₂Cl₂: EtOAc; 1: 1, EtOAc 100%, EtOAc: MeOH; and 8: 2 to obtain 6 fractions (C₂F₄S₁–C₂F₄S₆) (Figure 1).

Compound 1 was obtained from the precipitation of C₂F₄S₄ and recrystallization (Figure 1). Compound 1 was a yellow crystalline solid.

2.7. Identification of extracted compounds

The structure of isolated compound was determined and identified by comparing the ¹H, ¹³C and 2D nuclear magnetic resonance spectroscopy (NMR) spectral data with previously reported data.

2.8. LC–MS/MS analysis

The samples were analyzed using an Agilent Technologies 6490 Triple Quad LC/MS instrument with a Zorbax SB-C18 column (2.1 x 50 mm, 1.8 µm: Agilent Technologies, USA). The injection volume was 10 μL. The mobile phase was composed of water with 0.1% formic acid and 10 mM ammonium acetate (A) and acetoni trile (B). The mobile phase A was prepared by dissolving 10 mM ammonium acetate in water and adding formic acid (0.1%, v/v). The flow rate was 0.7 ml/min at 27°C. The following gradient elution program was used: 0–8 min, 10% B; 8.1–11.1 min, 95% B; 11.2–14 min, 10% B. The analysis was carried out in negative ion mode, with a fragment of 380 V.
2.9. Antioxidant activity

2.9.1. Ferric reducing antioxidant power (FRAP) assay
FRAP assay was performed as previously described (Sasipriya & Sidduraju, 2012). Ten microliters of each sample dissolved in DMSO were mixed with 190 µL of fresh FRAP reagent in 96-well microplates. Each mixture was incubated at 37°C for 30 min in the dark. The absorbance of the reaction was measured at a wavelength of 593 nm with a microplate reader. The positive controls for this assay were gallic acid and rutin. The ferrous sulfate standard curve was used to calculate the quantity of Fe²⁺ in the samples.

2.9.2. Determination of DPPH free radical scavenging activity
The DPPH free radical scavenging activity of the samples was measured using a method described by Blois (1958). One hundred microliters of each sample dissolved in DMSO at various concentrations were mixed with 100 µL of 0.1 mM DPPH in methanol. The reaction was incubated in the dark for 20 min at 27°C. The absorbance of the reaction was measured at a wavelength of 517 nm with a microplate reader. The positive controls for this assay were gallic acid, rutin, and Trolox. One hundred microliters of DPPH solution mixed with 100 µL of methanol was used as a control. The scavenging activity of a sample against DPPH free radicals was calculated using the formula given below.

\[
\text{DPPH free radical scavenging activity(%) = \left[ \frac{A_{S17sample} - A_{S17control}}{A_{S17control}} \right] \times 100}
\]

2.9.3. Determination of metal chelating activity
The metal chelating activity of the samples was determined following a previously reported procedure (Dinis et al., 1994). Twenty microliters of 0.2 mM FeCl₂ in water were mixed with 140 µL of samples dissolved in DMSO at various concentrations in 96-well microplates. The mixture was incubated at room temperature for 30 s. Forty microliters of 1 mM ferrozine in water were added to the mixture. The mixture was incubated at room temperature for 10 min. The absorbance of the reaction was measured at 562 nm with a microplate reader. The positive control for this assay was EDTA. Twenty microliters of 0.2 mM FeCl₂ mixed with 40 µL of 1 mM ferrozine and 140 µL of distilled water were used as a control. The metal chelating activity was calculated using the formula given below.

\[
\text{Metal chelating activity(%) = \left[ \frac{A_{562sample} - A_{562control}}{A_{562control}} \right] \times 100}
\]

2.9.4. Determination of superoxide anion radical scavenging activity (SOSA)
The superoxide anion radical scavenging activity of the samples was determined following the method of Robak and Gryglewski (1988). All chemical solutions for the determination of SOSA (150 µM NBT, 60 µM phenazine methosulfate, and 468 µM NADH) were prepared by dissolving chemical reagents in a 0.1 M phosphate buffer pH 7.4. Fifty microliters of 150 µM NBT were mixed with 50 µL of 60 µM phenazine methosulfate and 50 µL of 468 µM NADH. Then, 50 µL of each sample dissolved in DMSO at various concentrations were added to the mixture. Each mixture was incubated at room temperature for 5 min in the dark. The absorbance of the reaction was measured at 560 nm with a microplate reader. The positive controls for this assay were gallic acid, rutin, and Trolox. Fifty microliters of 150 µM NBT mixed with 50 µL of 60 µM phenazine methosulfate, 50 µL of 468 µM NADH, and 50 µL of a 0.1 M phosphate buffer pH 7.4 were used as a control. The SOSA was calculated using the formula given below.

\[
\text{Superoxide anion scavenging ability(%) = \left[ \frac{A_{560control} - A_{560sample}}{A_{560control}} \right] \times 100}
\]
2.10. Anti-inflammatory activity

2.10.1. Lipoxygenase inhibition assay

Linoleic acid (250 μM) was used as a substrate for this assay. The substrate was prepared by mixing 10 μL of linoleic acid with 30 μL of ethanol and 120 mL of a 0.2 M borate buffer pH 9.0. Thirteen microliters of each sample dissolved in DMSO at various concentrations were mixed with 487 μL of 400 U/mL lipoxygenase. Each mixture was incubated at room temperature for 5 min. Five hundred microliters of 250 μM linoleic acid were added to each mixture. The absorbance of the reaction was measured at 234 nm every 30 seconds for 5 minutes with a spectrophotometer. The positive control for this assay was NDGA. The lipoxygenase activity was calculated using the formulas given below.

\[
\text{Lipoxygenase activity (Units)} = \frac{(OD_{5\text{ min}} - OD_{2\text{ min}})}{3}/0.001
\]

\[
\text{Lipoxygenase activity (}) = \frac{(A_{234\text{ control}} - A_{234\text{ sample}})/A_{234\text{ control}}}{100}
\]

2.10.2. Xanthine oxidation inhibition assay

Twenty microliters of each sample dissolved in DMSO at various concentrations were mixed with 100 μL of 0.2 U/mL xanthine oxidase. Each mixture was incubated at room temperature for 5 min. Seven hundred and eighty microliters of a 0.2 M phosphate buffer pH 7.5 and 100 μL of a 0.4 mM xanthine solution were added to each mixture. The reaction was incubated at 37°C for 30 min. The absorbance of the reaction was measured at 290 nm with a spectrophotometer. The positive control for this assay was allopurinol. The xanthine oxidase activity was calculated using the formula given below.

\[
\text{Xanthine oxidase activity (}) = \frac{(A_{290\text{ control}} - A_{290\text{ sample}})/A_{290\text{ control}}}{100}
\]

2.11. Statistics

All assays were performed three times with three different sample preparations. SPSS version 17.0 was used for statistical analysis. The results are expressed as the means ± standard deviations of three determinations. The statistical significance of the differences between the groups (P < 0.05) was determined by one-way ANOVA followed by Tukey’s post-hoc test.

Table 1. The percentage yields of crude extract, partition extracts, and isolated compound from bambara groundnut seeds.

| Samples                  | Percentage yield (%) |
|--------------------------|-----------------------|
| Crude and partition extracts |                        |
| Crude                    | 4.06                  |
| Hexane                   | 19.71                 |
| CH₂Cl₂                   | 0.70                  |
| EtOAc                    | 1.85                  |
| Aqueous                  | 65.80                 |
| Isolated compound        |                       |
| Compound 1               | 0.08                  |

CH₂Cl₂, dichloromethane; EtOAc, ethyl acetate.
CH₂Cl₂, chloroform; EtOAc, acetato de etilo.

3. Results

3.1. Extraction yield of bambara groundnut seeds

The crude ethanol extract yield percentage was 4.06%, which was calculated based on the weight of bambara groundnut seeds without their peels (Table 1). An amount of 300 g of the crude ethanol extract was further partitioned into its hexane, CH₂Cl₂, EtOAc, and aqueous fractions. The percentage yields of the partitioned extracts were based on the weight of the crude ethanol extract, as shown in Table 1. The yield of the aqueous fraction (65.80%) was the highest among the four fractions, whereas the yield of the CH₂Cl₂ fraction (0.70%) was the lowest. The yields of the four fractions of the partitioned extract were different and decreased in the following order: aqueous fraction > hexane fraction > EtOAc fraction > CH₂Cl₂ fraction. Based on the results obtained from the DPPH free radical scavenging activity experiment, the EtOAc fraction with the highest DPPH free radical scavenging activity was further column chromatographed on silica gel (Table 2). The EtOAc fraction (5.0 g) was further fractionated by column chromatography to obtain compound 1. The percentage yield of compound 1 was based on the weight of the EtOAc fraction, as shown in Table 1.

3.2. Identification and structural elucidation

Identification and structural elucidation of the isolated compound were performed based on spectroscopic data analyses. The chemical structure of compound 1 is shown in Figure 2. Compound 1 was isolated as a yellow crystalline solid. The molecular formula C₂₀H₁₈O₂₆ was assigned to compound 1 on the basis of NMR spectral analytical data. On the ¹H-NMR spectrum, H-1 to H-5 were aromatic protons present on aromatic rings A and C at δ 7.76 (1 H, d, 2 Hz), 7.67 (1 H, dd, 8, 2 Hz), 6.89 (1 H, d, 8 Hz), 6.76 (1 H, d, 2 Hz), and 6.44 (1 H, d, 2 Hz), respectively. H-6 was methine present on aromatic ring B at δ 5.56 (1 H, d, 1 Hz). H-7 to H-10 were methine present on pentose at δ 4.02 (1 H, m), 3.84 (1 H, q, 5 Hz), 3.61 (1 H, m), and 3.48 (1 H, t, 10 Hz), respectively. H-11 was a methyl doublet present on pentose at δ 1.26 (3 H, d, 8 Hz). The ¹³C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra showed ten quaternary carbons at δ 176.10 (C-4), 161.91 (C-9), 160.90 (C-5), 156.34 (C-7), 147.68 (C-3”), 144.90 (C-4”), 122.49(C-5”), 114.62 (C-1”), 107.18 (C-2”), and 104.76 (C-10); nine methane carbons at δ 120.47 (C-2”), 114.88 (C-6”), 98.51 (C-8), 98.48 (C-6), 93.85 (C-3”), 72.22 (C-1”), 70.69 (C-2”), 70.34 (C-3”), and 69.83 (C-4”); and one methyl carbon at δ 16.68 (C-5”). All proton and carbon signal assignments were achieved by the combination of 2D NMR experiment (the spectral data are provided in the supplemental material). The chromatogram of compound 1 obtained by LC-MS/MS analysis in negative ion mode exhibited [M–H]⁻ ion at m/z 413.0000 and a fragment ion at m/z 113.0000 (Figure 3). Based on the experimental results, compound 1 was identified as a luteolin glycoside of the flavone class. In previous studies, different flavonoid compounds of bambara groundnut seeds were found: quercetin (C₃₅H₂₇O₁₁), isorhapontin (C₃₅H₂₇O₁₁), kaempferol (C₂₇H₂₂O₇), luteolin (C₁₇H₁₀O₇), rutin (C₂₉H₂₂O₁₁), myricetin (C₁₇H₁₄O₆), catechin (C₁₇H₁₄O₆), epicatechin (C₁₇H₁₄O₆), catechin hexoside (C₂₇H₁₄O₇), catechin hexoside (C₁₇H₁₂O₇), procyanidin dimer B₁ (C₃₅H₂₂O₁₂), quercetin 3-galactoside 7-rhamnoside (C₂₉H₂₉O₁₆), and quercetin...
Table 2. FRAP, DPPH, metal chelating, and superoxide anion radical scavenging activity of crude extract, partition extracts, column chromatography fractions, and isolated compound from bambara groundnut seeds.

| Samples                     | FRAP (µmol Fe(III)/g extract) | DPPH (IC50 µg/mL) | Metal chelating (IC50 µg/mL) | Superoxide anion radical scavenging (IC50 µg/mL) |
|-----------------------------|--------------------------------|-------------------|-----------------------------|-----------------------------------------------|
| Positive controls           |                                |                   |                             |                                               |
| Gallic acid                 | 15,655 ± 107                   | 0.54 ± 0.07       | ND                          | 12 ± 1                                        |
| Rutin                       | 2570 ± 129                     | 1.52 ± 0.23       | ND                          | 7.29 ± 0.43                                  |
| Trolox                      | ND                             | 2.28 ± 0.42       | ND                          | 203 ± 19                                     |
| EDTA                        | ND                             | ND                | 0.89 ± 0.16                 | ND                                            |
| Crude and partition extracts|                                |                   |                             |                                               |
| Crude                      | 201 ± 12                      | 50 ± 9h           | 474 ± 24h                   | 35 ± 6f                                       |
| Hexane                     | 31 ± 7                      | 423 ± 65c         | 354 ± 19h                   | NF                                            |
| CH2Cl2                     | 290 ± 26d                    | 38 ± 7ab          | 214 ± 18ab                  | 20 ± 2b                                       |
| EtOAc                      | 2913 ± 19b                   | 1.93 ± 0.23a      | 665 ± 75a                   | 11 ± 2b                                       |
| Aqueous                    | 120 ± 17b                    | 77 ± 2b           | 212 ± 21a                   | 74 ± 6b                                       |
| Isolated compound           |                                |                   |                             |                                               |
| Compound 1                  | 253 ± 33                      | 2.25 ± 0.14       | 367 ± 13                    | 9.8 ± 0.3                                     |

Values are means of three independent samples analyzed in triplicate (n = 3) ± standard deviation. Values in the same column sharing different letters (a-e: crude and partition extracts, f-j: column chromatography fractions) are significantly different (p < .05). EDTA, ethylenediaminetetraacetic acid; CH2Cl2, dichloromethane; EtOAc, ethyl acetate; ND, not determined; NF, not found.

3.3. Antioxidant activity

3.3.1. FRAP activity
The FRAP activity of the samples is presented in Table 2. The EtOAc fraction was found to have significantly higher (p < .05) FRAP value than the partition extract samples, followed by the CH2Cl2, aqueous, and hexane fractions. The EtOAc and CH2Cl2 fractions had significantly higher (p < .05) FRAP values than the crude extract. Among the column chromatography fractions, the highest FRAP activity was observed for the C1F3 fraction. The FRAP activity value for compound 1 was 253 ± 33 µmol Fe(III)/g extract. The FRAP activity of the isolated compound was lower than those of the positive controls.

3.3.2. DPPH free radical scavenging activity
Among the partition extract fractions, the EtOAc fraction exhibited the highest inhibitory effect, with an IC50 value of 1.93 ± 0.23 µg/mL, followed by the CH2Cl2, aqueous, and hexane fractions (Table 2). Therefore, the EtOAc fraction was subjected to column chromatography on silica gel to obtain the C1F1-C1F6 fractions. Among the column chromatography fractions, the C1F4 fraction had the highest DPPH free radical scavenging activity, with an IC50 value of 2.55 ± 0.58 µg/mL. The tested column chromatography fractions could be arranged according to their IC50 value in the

Figure 2. Structure of compound 1 isolated from bambara groundnut seeds and identified by the 1H, 13C and 2D nuclear magnetic resonance spectroscopy data.

Figura 2. Estructura del compuesto 1 aislado de las semillas de cacahuete bambara e identificado por los datos de espectroscopia de resonancia magnética nuclear de 1H, 13C y 2D.

hexoside (C21H19O12) (Adedayo et al., 2021; Harris et al., 2018; Mubaiva et al., 2019; Salawu, 2016).
following order: C1F4 < C1F3 < C1F6 < C1F2 < C1F5 < C1F1 (Table 2). Compound 1, isolated from the C1F4 fraction, had an IC50 value for DPPH free radical scavenging activity of 2.25 ± 0.14 µg/mL. The scavenging activity of compound 1 was similar to that of Trolox but lower than those of gallic acid and rutin (Table 2). The results of the present study show that the isolated compound strongly decreased the levels of DPPH radical.

3.3.3. Metal chelating activity

The CH2Cl2 and aqueous fractions had significantly highest (p < 0.05) metal chelating activity compared to partition extract samples, followed by the hexane and EtOAc fractions, respectively (Table 2). Compared to the metal chelating activity of the crude extract, those of the CH3Cl2 and aqueous, and hexane fractions were significantly higher (p < 0.05), whereas that of the EtOAc fraction was significantly lower (p < 0.05) (Table 2). These results indicate low metal chelating activity for the EtOAc fraction. Therefore, the column chromatography fractions obtained from the EtOAc fraction were not tested. The isolated compound, compound 1 had metal chelating activity, with an IC50 value of 367 ± 13 µg/mL. The metal chelating activity of the isolated compound was lower than that of EDTA.

3.3.4. Superoxide anion radical scavenging activity

The SOSA results are presented in Table 2. Among the partition extract fractions, the EtOAc fraction exhibited significantly higher (p < 0.05) SOSA than the other partition extracts, with an IC50 value of 11 ± 2 µg/mL. The IC50 values for scavenging of superoxide anion radical were 20 ± 2 µg/mL and 74 ± 6 µg/mL for the CH2Cl2 and aqueous fractions, respectively. The EtOAc and CH3Cl2 fractions had significantly higher (p < 0.05) SOSA than the crude extract. Among the column chromatography fractions, the highest SOSA was noted for the C1F3, C1CF4 and C1F5 fractions (Table 2). The results displayed that compound 1 exhibited SOSA, with an IC50 value of 9.8 ± 0.3 µg/mL. The SOSA of compound 1 was similar to that of rutin and higher than those of gallic acid and Trolox, which means that compound 1 is an excellent scavenger of superoxide anion radical.

3.4. Anti-inflammatory activity

3.4.1. Inhibition of lipoxygenase activity

Table 3 shows the results for the inhibition of the lipoxygenase activity of the crude extract, partition extracts, and isolated compound from bambara groundnut seeds. Among the partition extract fractions, the EtOAc fraction exhibited significantly higher (p < 0.05) inhibition of lipoxygenase activity than the hexane, CH2Cl2 and aqueous fractions, with an IC50 value of 23 ± 3 µg/mL. The EtOAc fraction exhibited significantly higher (p < 0.05) inhibition of
lipoxigenase activity than the crude extract. Compound 1 exhibited inhibition of lipoxigenase activity, with an IC\textsubscript{50} value of 2.16 ± 0.14 µg/mL. The inhibition of lipoxigenase activity of compound 1 was higher than NDGA, showing that compound 1 is an excellent inhibitor of lipoxigenase activity.

3.4.2. Inhibition of xanthine oxidase activity

Table 3 shows the results for the inhibition of xanthine oxidase activity by the crude and partition extracts of bambara groundnut seeds. All the partition extracts, except for the hexane fraction, exhibited significantly higher (p < .05) inhibition of xanthine oxidase than the crude extract. Among the partition extract fractions, the CH\textsubscript{3}Cl and ETOAc fractions exhibited significantly higher (p < .05) inhibition of xanthine oxidase than the aqueous fraction, with an IC\textsubscript{50} value of 149 ± 10 µg/mL and 191 ± 4 µg/mL, respectively. Compound 1 inhibited xanthine oxidase activity, with an IC\textsubscript{50} value of 30.46 ± 0.77 µg/mL. The isolated compound exhibited lower inhibition of xanthine oxidase activity than allopurinol.

4. Discussion

In previous studies, plants of the Vigna genus were found to include many flavonoids, such as the three flavonoids, aglycons kaempferol, quercetin, and isorhamnetin, that have been found in Vigna unguiculata L. Walp (Lattanzio et al., 2000); vitesin and isovitesin that have been found in Vigna radiate (Tao et al., 2021); and myricetin-3, 4”-O-diglucoside, quercetin-3-O-sambubioside-7-O-glucose, quercetin-3-O-galactoside-7-O-glucose, quercetin-3,7-O-diglucoside, kaempferol-3-O-sambubioside-7-O-glucose, quercetin-3-O-(feruloyl)hexoside, kaempferol-3,4”-O-diglucoside, kaempferol-3,7-O-diglucoside, quercetin 3-O-glucoside that have been found in cowpea pod (Vigna unguiculata L.) (Li et al., 2020). Flavonoids are present mainly as glycosides and accumulate in plants in response to many factors. Bambara groundnut seeds have been found to be composed of various flavonoid glycoside compounds, such as quercetin, isoquercitrin, catechin hexoside, quercetin 3-galactoside 7-rhamnoside, and quercetin hexoside (Adedayo et al., 2021; Harris et al., 2018; Mubaiwa et al., 2019; Salawu, 2016).

However, the chemical structure of the compound isolated from bambara groundnut seeds in this study had not been reported previously. Compound 1 was isolated from bambara groundnut seeds for the first time in this study. The molecular formula of compound 1 is C\textsubscript{20}H\textsubscript{18}O\textsubscript{9}. Compound 1 was identified as luteolin glycoside. Several compounds have the same molecular formula as compound 1, such as cetraric acid, quinizarin monoglucoside and apigenin 7-xyloside. However, the chemical structure of these compounds is different from that of compound 1. The structure of cetraric acid is non-glycoside. Quinizarin monoglucoside and apigenin 7-xyloside have glycoside structures that are different from that of compound 1. Quinizarin monoglucoside has an anthraquinone glycoside structure. Apigenin 7-xyloside has a similar structure to compound 1 (luteolin glycoside), except for a hydroxyl group in the 3”-position of the B ring and a methyl group in the sugar structure (Figure 2). The LC–MS/MS chromatogram of compound 1 exhibited [M–H]” ion at m/z 413.0000 and a fragment ion at m/z 113.0000. The molecular weight of compound 1 determined by LC–MS/MS differs from the theoretical molecular weight. The theoretical molecular weight of compound 1 is 402.36 g/mol. There are several factors that influence the absolute mass spectrometry response, such as ion source flow rates, the cleanliness of the ion source and ion suppression (Pitt, 2009). Kachlicki et al. (2016) reported that the sensitivity achieved of each compound for LC–MS analysis depends on energy transferred to the molecules during the ionization process. Excess energy causes fragmentation and decrease quantity in the mass spectrum.

The high FRAP activity of the EtOAc fraction may originate from the polar fraction of the bambara groundnut seed extract; previous studies found that the more polarity of plant extracts is the more antioxidant activity (Brahmi et al., 2012). Gallic acid and rutin, the positive controls, exhibited higher FRAP activity than all the active fractions and isolated compound because of containing more chemical groups that play a role in antioxidant activity, such as aromatic rings with hydroxyl groups, hydroxyl groups, carbonyl groups, and double bonds (Ozgen et al., 2016).

Gallic acid and rutin contained more active groups (OH) and therefore exhibited higher activity for DPPH free radical scavenging than compound 1. Previous studies found that the more active group of compound structure is the more antioxidant activity (Bendary et al., 2013). Moreover, the role of the structure–antioxidant relationship activity depends on the position of the active groups. The ortho position, para position, and meta position were found to be the more active, respectively due to its ability to form intramolecular hydrogen bonding (Bendary et al., 2013).

Previous studies found that carbonyl and hydroxyl functional groups exhibit Fe\textsuperscript{2+} chelating activity; for example, ferrous ions could be chelated via the carbonyl and hydroxyl functional groups of curcumin (Ak & Gulcin, 2008). Another study found that L-adrenaline binds iron ions through its amine and hydroxyl groups (Gulcin, 2009). Therefore, the presence of a large number of carbonyl and hydroxyl functional groups of compounds effect to metal chelating activity.

The high SOSA of rutin and compound 1 may have resulted from the presence of glycosides. A previous study found that the presence of acteoside in Plantago lanceolata L. extract produced a high SOSA (Al-Mamun et al., 2007). The SOSA is also related to the number of phenolic hydroxyl groups in phenylpropanoid glycosides (Wang et al., 1996). Moreover, the hydroxyl groups at C-5 and C-7 are important for radical scavenging activity (Fazlatun et al., 2005).

A previous study found that a hydroxyl group at C-5 and C-7, C2 and C3 double bond, and C-4”+ or C-3”, C-4”+ of compounds belonging to the flavonoids was essential for anti-inflammatory and lipoxigenase inhibitory activity (Kim et al., 2004). Therefore, the presence of a hydroxyl group at C-5, C-3” and C-4” of compound 1 resulted in high lipoxigenase inhibitory activity.

Some flavonoids, such as kaempferol, quercetin glycosides, and flavonol glycosides, are known xanthine oxidase inhibitors. However, luteolin glycoside compound isolated from bambara groundnut seed had low xanthine oxidase inhibitory activity compared with allopurinol. The presence of a hydroxyl group at C-5 and C-7 of chrysir and luteolin compounds has been found to be important for the inhibition of xanthine oxidase (Nagao et al., 1999). Moreover, the activity of xanthine oxidase inhibition was decreased by
substituting the hydroxyl group at C-7 of the flavonoid structure with a glycoside (Spanou et al., 2012). Therefore, the presence of the glycoside at C-7 of compound 1 resulted in low xanthine oxidase inhibitory activity.

The results of this study suggest that luteolin glycoside isolated from bambara groundnut seeds can be a natural source of antioxidants and a lipoxigenase inhibitor. Therefore, the potential of luteolin glycoside for the treatment of inflammatory-related diseases should be explored further.

5. Conclusion

The results of this study showed that bambara groundnut seeds contain luteolin glycoside (C_{15}H_{16}O_{6}). The structure of luteolin glycoside extracted from bambara groundnut seeds has not been previously found in plants and is reported for the first time in this study. Luteolin glycoside exhibited strong DPPH free radical scavenging activity and SOSA but low FRAP activity and metal chelating activity. The anti-inflammatory activity of luteolin glycoside was high for lipoxigenase but low for xanthine oxidase. These results indicate that luteolin glycoside isolated from bambara groundnut seeds can serve as a natural source of antioxidants to protect humans from free radicals and as a lipoxigenase inhibitor to reduce inflammation.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

ORCID

Dutsadee Chinnapun http://orcid.org/0000-0002-4553-5557

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