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Characterization of Phenolic Compounds from *Pavetta graciliflora* Wall. ex Ridl. (Rubiaceae) and their Lipoxygenase Inhibitory Activity

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Pavetta genus belongs to the family Rubiaceae. The genus is used in traditional medicine to treat various diseases, including hemorrhoids, headaches, urinary conditions, ulcerated nose, and drops. The present study describes the investigation of the phytochemicals from *Pavetta graciliflora* and their lipoxygenase inhibitory activity. The isolation and purification of the compounds were carried out on the extracts using various chromatographic methods and identified by directly comparing their spectroscopic data with the respective published data. The isolated compounds were tested for anti-inflammatory activity by using lipoxygenase assay. Fractionation and purification of the leaves extract of *P. graciliflora* led to the isolation and identification of seven phenolics; (+)-catechin **(1)**, (+)-epicatechin **(2)**, quercetin **(3)**, kaempferol **(4)**, myricetin **(5)**, apigenin **(6)**, and luteolin **(7)**, together with β-sitosterol **(8)**, β-sitostenone **(9)**, and lupeol **(10)**. Isolated compounds demonstrated remarkable lipoxygenase inhibitory activity with IC_{50} values ranging from 3.4 to 59.2 µM. Phenolic compounds with high content in *P. graciliflora* can probably be used as a chemical marker for this *Pavetta* species.

Keywords: Rubiaceae; *Pavetta graciliflora*; flavonoid; lipoxygenase

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Diabetes is increasingly prevalent, and type 2 diabetes (T2D) accounts for the vast majority of cases of diabetes. Although insulitis, the presence of immune cells around the pancreatic islet cells, has traditionally been associated with type 1 diabetes (T1D), it is a phenomenon that is also observed in T2D. Inflammation is present in the pathogenesis of not only T1D and T2D but also immune checkpoint inhibitor-induced diabetes. In T2D, inflammation contributes to insulin resistance and is seen in organ systems, including adipose tissue (AT) and the liver. Both for T1D and T2D, therapies targeting inflammation have been and are currently being investigated. Globally, 382 million individuals have been diagnosed with diabetes, and 600 million are predicted to have the disease by 2023 [1]. About 4.9 million people die every year due to diabetes, and 50% of this death toll is a consequence of diabetic complications. An association between inflammation, vascular complications, and hyperglycemia has been observed in diabetic persons. Gradually, the pancreatic β-cell dysfunction increases, and persistent hyperglycemia activates the immune system, thus leading to an increase in the inflammatory response. Thus, inflammation contributes to the pathogenesis of T2D [2]. Moreover, in diabetes, high blood plasma glucose and increased levels of free fatty acids could arouse inflammation processes that further increase glucose consumption. It is also believed that oligomers of polypeptides (amyloid) of the pancreas might trigger inflammation [3].

Various species from the Rubiaceae family have proven to be a promising source of new bioactive substances, which may lead to the development of new products as active molecules or drug prototypes due to their structural diversity and pharmacological activities. One peculiar characteristic of this family is that they contain a wide range of secondary metabolites, such as alkaloids, flavonoids, terpenes, anthraquinones, and coumarins, having good pharmacological properties. These classes of secondary metabolites have been associated with antimicrobial, anti-malarial, anti-inflammatory, hepatoprotective,

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antioxidant, and many other interesting biological activities [4].

Pavetta genus belongs to the family Rubiaceae. The genus is used in traditional medicine to treat various diseases, including hemorrhoids, headaches, urinary conditions, ulcerated nose, and drops [5]. Previous phytochemical investigations on *Pavetta* species led to the isolating of several dimeric, trimeric, tetrameric, and oligomeric proanthocyanidins [6] and phenolic compounds [7]. Meanwhile, the extracts have shown cytotoxicity [6], antioxidant [8], anti-inflammatory [9], anti-mycobacterial [10], and anti-diabetic properties [11]. *P. lanceolata* shows good inhibition of nitric oxide (NO) production [12], and *P. crassicaulis* showed excellent anti-inflammatory activity compared to the standard [9].

Pavetta graciliflora can be found in evergreen, dry evergreen, and mixed deciduous forests at 0–750 m altitude. It is a shrub or small tree up to 5 m high. It is distributed in lowland and hill forests up to 900 m above sea level (asl) and is found mainly in Malaysia, Myanmar, and Thailand. It is also known as *khem-kliang* in Thailand and 'kaca piring' in Malaysia [13]. In addition, we have recently reported the chemical compositions and anticholinesterase inhibitory activity of the essential oil from this species. The essential oil revealed the existence of 20 components, which made up 92.85% of the total oil. The essential oil is composed mainly of β-caryophyllene (42.52%), caryophyllene oxide (25.33%), β-pinene (8.67%), and α-pinene (6.52%). The essential oil showed weak inhibitory activity on acetylcholinesterase and butyrylcholinesterase assays, with a percentage inhibition of 62.5% and 65.4%, respectively [14]. In continuation of our search for chemical compounds from *Pavetta* species, we have investigated the phytochemical study of the leaves of *P. graciliflora* collected from Peninsular Malaysia, and the isolated compounds were subjected to anti-inflammatory experiments using the lipoxygenase (LOX) assay method.

EXPERIMENTAL

Plant Material

The dried leaves of *Pavetta graciliflora* were collected from Behrang, Perak, in September 2019 and identified by Shamsul Khamis from Universiti Kebangsaan Malaysia (UKM). The voucher specimen (SK28/19) was deposited at UKM Herbarium.

Chemical Reagents and Solvents

Analytical grade *n*-hexane, chloroform, dichloromethane, ethyl acetate, acetone, diethyl ether, and methanol were purchased from R&M Chemicals, UK. Silica gel 60 (70-230 and 230-400 mesh) and deuterated chloroform were purchased from Merck, Germany. LOX inhibition was purchased using an enzyme immunoassay (EIA) kit (Catalog No. 760700, Cayman Chemical, USA).

General Experimental Procedures

The soxhlet extraction technique was applied to extract the phytochemicals from the dried sample using different polarity solvents (*n*-hexane, dichloromethane, and methanol). Vacuum liquid chromatography (VLC) was performed using Merck silica gel 60 (230-400 mesh), while column chromatography (CC) using Merck silica gel 60 (70-230 mesh) which were used as the stationary phase, respectively. Thin-layer chromatography (TLC) analysis was performed on Merck precoated silica (SiO_2) gel F_{254} plates (0.2 mm thickness) to detect and monitor the presence of compounds in the samples. The TLC and PTLC spots were visualized under UV light (254 and 366 nm). Melting points were measured using a melting point apparatus equipped with a microscope, Leica Gallen III, and were uncorrected. The ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a Bruker Avance 400 Spectrometer. Chemical shifts were reported in ppm and $CDCl_3$ as the solvent. The residual solvent was used as an internal standard. The IR spectra were recorded on Perkin Elmer ATR and 1600 spectrophotometer series as KBr discs. The mass spectra were obtained from LCMS-IT-TOF, Shimadzu and GC-MS (Agilent GC-MS 7890A/5975C Series MSD).

Extraction and Isolation

The dried powder leaves (300 g) of *P. graciliflora* were ground and extracted exhaustively for 12 hours by Soxhlet extraction with *n*-hexane, followed by dichloromethane (DCM) and MeOH. The *n*-hexane extract (4.0 g) was subjected to column chromatography over silica gel using DCM gradually enriched with methanol (MeOH) to yield 10 fractions. Fractions were combined based on TLC behavior. Fractions 2-4 and 5-7 were further purified using PTLC afforded compound **1** (5 mg) (PTLC; DCM:MeOH 95:5; R_f 0.26) and compound **2** (4 mg) (PTLC; DCM:MeOH 95:5; Rf 0.3), respectively. The DCM extract (5.0 g) was submitted to column chromatography and obtained 20 fractions. Fraction 3-5 produced compound **3** (6 mg) (PTLC; DCM:MeOH 95:5; R_f 0.45) and compound **4** (6 mg) (PTLC; DCM:MeOH 95:5; R_f 0.50). Fraction 7-9 produced compound **5** (5 mg) (PTLC; EtOAc:MeOH 95:5; R_f 0.4). The MeOH extract (8 g) was separated on a silica gel column using a gradient Hex:DCM to give 10 fractions. Fraction 5-6 produced compound **6** (6 mg) (PTLC; DCM:MeOH 97:3; R_f 0.45) and compound **7** (PTLC; DCM:MeOH 96:4; R_f 0.50). Other fractions were also isolated β-sitosterol **8** (20 mg), β-sitostenone **9** (15 mg), and lupeol **10** (10 mg).

(+)-Catechin (1). Colourless solid. MS *m/z* 291.1165 (calculated 291.1186 for $C_{15}H_{15}O_6$); IR (KBr) ν_{max}: 3310, 2926, 1605, 1512, 1450, 1090,

1060 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): δ 2.45 (1H, ddd, J = 16.4, 8.0, 2.0 Hz, H-4b), 2.80 (1H, dd, J = 16.4, 5.6 Hz, H-4a), 3.95 (1H, ddd, J = 8.0, 7.2, 5.6 Hz, H-3), 4.55 (1H, d, J = 7.2 Hz, H-2), 5.82 (1H, d, J = 2.4 Hz, H-6), 5.88 (1H, d, J = 2.4 Hz, H-8), 6.65 (1H, dd, J = 8.4, 2.0 Hz, H-6'), 6.72 (1H, d, J = 8.4 Hz, H-5'), 6.78 (1H, d, J = 2.0 Hz, H-2'); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): δ 28.8 (C-4 axi/eq), 68.4 (C-3), 82.8 (C-2), 95.5 (C-8), 96.2 (C-6), 100.7 (C-4a), 115.3 (C-2'), 115.7 (C-5'), 120.1 (C-6'), 132.3 (C-1'), 145.6 (C-3'), 145.7 (C-4'), 156.7 (C-8a), 157.1 (C-5), 157.6 (C-7) [6].

(+)-Epicatechin (2). Colourless solid. MS *m/z* 291.1654 (calculated 291.1611 for $C_{15}H_{15}O_6$); IR (KBr) ν_{max}: 3306, 2925, 1606, 1518, 1449, 1095, 1062 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): δ 2.72 (1H, dd, J = 16.5, 3.6 Hz, H-4β), 2.88 (1H, dd, J = 16.5, 4.5 Hz, H-4α), 4.20 (1H, m, H-3), 4.88 (1H, s, H-2), 5.95 (1H, d, J = 1.2 Hz, H-6), 6.05 (1H, d, J = 1.5 Hz, H-8), 6.85 (2H, m, H-5', 6'), 7.08 (1H, s, H-2'); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): δ 29.2 (C-4 axi/eq), 67.1 (C-3), 79.6 (C-2), 95.5 (C-8), 96.3 (C-6), 100.0 (C-4a), 115.5 (C-2'), 115.7 (C-5'), 119.6 (C-6'), 132.5 (C-1'), 145.5 (C-4'), 145.6 (C-3'), 157.4 (C-8a), 157.8 (C-5), 157.9 (C-7) [6].

Quercetin (3). Yellow amorphous powder. MS *m/z* 303.1470 (calculated 303.1485 for $C_{15}H_{11}O_7$); IR (KBr) ν_{max}: 3318, 2920, 1610, 1510, 1455, 1094, 1065 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): δ 6.15 (1H, d, J = 2.0 Hz, H-6), 6.40 (1H, d, J = 2.0 Hz, H-8), 6.85 (1H, d, J = 8.5 Hz, H-5'), 7.55 (1H, dd, J = 2.0, 8.0 Hz, H-6'), 7.65 (1H, d, J = 2.0 Hz, H-2'), 9.33 (1H, s, 3'-OH), 9.34 (1H, s, 4'-OH), 9.55 (1H, s, 3-OH), 10.77 (1H, s, 7-OH), 12.46 (1H, s, 5-OH); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): δ 93.5 (C-8), 98.2 (C-6), 103.0 (C-10), 115.4 (C-2'), 115.8 (C-5'), 120.2 (C-6'), 122.0 (C-1'), 135.8 (C-3), 145.2 (C-3'), 147.0 (C-4'), 147.8 (C-2), 156.5 (C-9), 160.7 (C-5), 164.2 (C-7), 176.0 (C-4) [15].

Kaempferol (4). Yellow powder. MS *m/z* 287.1286 (calculated 287.1245 for $C_{15}H_{11}O_6$); IR (KBr) ν_{max}: 3312, 2929, 1612, 1510, 1454, 1094, 1062 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): δ 6.25 (1H, d, J = 2.0, H-6), 6.56 (1H, d, J = 2.0, H-8), 7.02 (2H, d, J = 8.8, H-3', 5'), 8.15 (2H, d, J = 8.8, H-2', 6'); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): δ 146.8 (C-2), 136.5 (C-3), 176.5 (C-4), 157.6 (C-5), 98.7 (C-6), 165.0 (C-7), 94.2 (C-8), 160.2 (C-9), 103.8 (C-10), 123.1 (C-1¢), 130.2 (C-2', 6'), 116.0 (C-3', 5'), 161.8 (C-4') [16].

Myricetin (5). Yellow amorphous powder. MS *m/z* 319.1195 (calculated 319.1154 for $C_{15}H_{11}O_8$); IR (KBr) ν_{max}: 3318, 2928, 1610, 1512, 1450, 1095, 1065 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): δ 6.18 (1H, d, J = 2.4 Hz, H-6), 6.37 (1H, d, J = 1.8 Hz, H-8), 7.24 (2H, s, H-2′, 6′); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): δ 93.2 (C-8), 98.2 (C-6), 102.8 (C-10), 107.1 (C-2′, 6′), 120.7 (C-1′), 135.8 (C-4′), 135.9 (C-3), 145.7 (C-3′, 5′), 146.7 (C-2), 156.0 (C-9), 160.6 (C-5), 164.0 (C-7), 175.7 (C-4) [17].

Apigenin (6). Pale yellow amorphous powder. MS *m/z* 271.1852 (calculated 271.1822 for $C_{15}H_{11}O_5$); IR (KBr) ν_{max}: 3325, 2930, 1620, 1515, 1450, 1095, 1061 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): δ 6.75 (1H, s, H-3), 6.15 (1H, d, J = 1.9 Hz, H-5), 6.44 (1H, d, J = 1.9 Hz, H-8), 7.91 (2H, d, J = 9.0 Hz, H-2′/H-6′), 6.90 (2H, d, J = 9.0 Hz, H-3′/H-5′), 12.94 (OH); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): δ 163.7 (C-2), 102.8 (C-3), 181.7 (C-4), 161.2 (C-5), 98.9 (C-6), 164.5 (C7), 94.0 (C-8), 157.3 (C-9), 103.5 (C-10), 121.1 (C-1′), 128.5 (C-2′/C-6′), 116.0 (C-3′/C-5′), 161.4 (C-4′) [18].

Luteolin (7). Yellow amorphous powder. MS *m/z* 287.1125 (calculated 287.1196 for $C_{15}H_{11}O_6$); IR (KBr) ν_{max}: 3322, 2932, 1618, 1512, 1455, 1082, 10485 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): δ 12.97 (1H, s, 5-OH), 7.42 (1H, d, J = 2.0 Hz, H-2′), 7.40 (1H, dd, J = 2.0, 8.5 Hz, H-6′), 6.89 (1H, d, J = 8.0 Hz, H-5′), 6.66 (1H, s, H-3), 6.44 (1H, d, J = 2.0 Hz, H-8), 6.19 (1H, d, J = 1.5 Hz, H6); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): δ 167.6 (C-2), 101.8 (C3), 181.0 (C-4), 161.3 (C-5), 99.6 (C-6), 163.6 (C-7), 94.3 (C-8), 157.5 (C-9), 102.3 (C-10), 119.8 (C1'), 112.2 (C-2'), 146.4 (C-3'), 152.0 (C-4'), 115.8 (C-5'), 118.9 (C-6') [19].

β-Sitosterol (8). White needles; m.p 133-134°C; MS *m/z* 414 [M^+, $C_{29}H_{50}O$]. IR (KBr) ν_{max}: 3435, 2966, 1461, 1376 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.70 (3H, s, H-18), 0.84 (3H, d, J = 6.6 Hz, H-27), 0.86 (3H, d, J = 6.6 Hz, H-26), 0.88 (3H, d, J = 3.9 Hz, H-29), 0.95 (3H, d, J = 6.3 Hz, H-21), 1.03 (3H, s, H-19), 1.27−2.31 (29H, m, overlapping CH and CH_2), 3.54 (1H, m, H-3), 5.37 (1H, d, J = 4.8 Hz, H-6); ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.8 (C-29), 11.9 (C-18), 18.7 (C-21), 19.0 (C-27), 19.3 (C-19), 19.8 (C-26), 21.0 (C-11), 23.0 (C-28), 24.3 (C-15), 26.0 (C-23), 28.2 (C-7), 29.1 (C-25), 31.6 (C-2), 31.9 (C-8, C-16), 33.9 (C-22), 36.1 (C-20), 36.5 (C-10), 37.2 (C-1), 39.7 (C-12), 42.3 (C-4), 42.3 (C-13), 45.8 (C-24), 50.1 (C-9), 56.0 (C-17), 56.7 (C-14), 71.8 (C-3), 121.7 (C-6), 140.7 (C-5) [20].

β-Sitostenone (9). White solids; m.p 77-79°C; MS *m/z* 412 [M^+, $C_{29}H_{48}O$]. IR (KBr) ν_{max}: 3444, 2960, 1687, 1460, 1414, 1222 cm^{-1} ; ^1H NMR (400 MHz, $CDCl_3$): δ 0.73 (3H, s, H-18), 0.82 (3H, d, J = 6.4 Hz, H-27), 0.84 (3H, d, J = 6.4 Hz, H-26), 0.86 (3H, t, J = 7.6 Hz, H-29), 0.93 (3H, d, J = 6.8 Hz, H-21), 1.18 (3H, s, H-19), 1.25−2.44 (29H, m, overlapping CH and CH_2), 5.74 (1H, s,

H-4); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.2 (C-29), 14.6 (C-18), 15.9 (C-19), 17.0 (C-21), 18.4 (C-27), 20.8 (C-26), 23.0 (C-11), 24.3 (C-28), 26.2 (C-15), 28.1 (C-23), 29.5 (C-16), 31.9 (C-25), 33.8 (C-7), 35.2 (C-6), 36.2 (C-22), 38.7 (C-2), 39.8 (C-8), 42.5 (C-1), 43.6 (C-20), 44.9 (C-10), 46.0 (C-12), 48.2 (C-13), 49.3 (C-24), 52.0 (C-9), 53.9 (C-17), 56.0 (C-14), 123.7 (C-4), 171.4 (C-5), 198.8 (C-3) [21].

Lupeol (10). Colorless needles; m.p 215-216°C; MS *m/z* 426 [M^+, $C_{30}H_{50}O$]. IR (KBr) ν_{max}: 3434, 2927, 1634, 1070 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.71 (1H, d, J = 9.2 Hz, H-5), 0.77 (3H, s, H-28); 0.80 (3H, s, H-25), 0.94 (3H, s, H-27), 0.96 (3H, s, H-23), 0.98 (3H, s, H-24), 1.00 (3H, s, H-26), 1.67 (3H, s, H-30), 1.95 (2H, m, H-21), 2.36 (1H, dt, J = 11.2, 5.6 Hz, H-19), 3.19 (1H, dd, J = 11.2, 5.4 Hz, H-3), 4.58 (1H, s, H-29), 4.70 (1H, s, H-29); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.5 (C-27), 15.3 (C-24), 15.9 (C-26), 16.1 (C-25), 18.0 (C-28), 18.3 (C-6), 19.3 (C-30), 20.9 (C-11), 25.1 (C-12), 27.3 (C-23), 27.4 (C-2), 27.9 (C-15), 29.8 (C-21), 34.3 (C-7), 35.5 (C-16), 37.1 (C-10), 38.0 (C-13), 38.7 (C-1), 38.8 (C-4), 40.0 (C-22), 40.8 (C-8), 42.8 (C-14), 43.0 (C-17), 47.9 (C-19), 48.3 (C-18), 50.4 (C-9), 55.3 (C-5), 79.0 (C-3), 109.3 (C-29), 150.9 (C-20) [22].

Lipoxygenase (LOX) Inhibitory Activity

LOX inhibition was determined using an enzyme immunoassay (EIA) kit according to the manufacturer's instructions and literature [23]. The Cayman Chemical lipoxygenase inhibitor screening assay detects and measures the hydroperoxides produced in the lipoxygenation reaction using purified lipoxygenase. Stock solutions of the samples were dissolved in a minimum volume of DMSO and were diluted using the supplied buffer solution (0.1 M Tris-HCl, pH 7.4). To a 90 µL solution of 1-LOX enzyme in 0.1 M Tris-HCl and pH 7.4 buffer, 10 µL of various concentrations of test samples (final volume of 210 µL) were added, and the lipoxygenase reaction was initiated by the addition of 10 µL (100 µM) of arachidonic acid. After maintaining the 96-well plate on a shaker for 5 min, 100 µL of chromogen was added, and the plate was retained on a shaker for 5 min. The lipoxygenase activity was determined after measuring the absorbance at a wavelength of 500 nm. The percentage inhibition (I%) of the samples was calculated as follows:

$$I\% = [\ A_{\text{initial activity}} - A_{\text{inhibitor}} / A_{\text{initial activity}}] \times 100;$$

where $A_{\text{initial activity}}$ is the absorbance of 100% initial activity wells without sample, and $A_{\text{inhibitor}}$ is the absorbance of samples/reference. All tests were carried out in triplicate and expressed as means ± SD.

RESULTS AND DISCUSSION

Literature reviews indicated that only a few species in the genus *Pavetta* have been investigated for their chemical compounds and biological activities. To date, only a few species have been studied, including *P. tomentos*a [24], *P. indica* [5], *P. crassipes* [25], and *P. crassicaulis* [24]. In addition, phytochemical investigations on *Pavetta* species led to the isolation of several dimeric, trimeric, tetrameric, and oligomeric proanthocyanidins and phenolic compounds [6,7]. Given the attributed medicinal properties, studies were undertaken on the leaf parts of *P. graciliflora*, which resulted in the isolation and structure elucidation of flavonoids and pentacyclic triterpenes. Seven flavonoids have been isolated including (+)-catechin **(1)**, (+)-epicatechin **(2)**, quercetin **(3)**, kaempferol **(4)**, myricetin **(5)**, epigenin **(6),** and luteolin **(7)** together with three pentacyclic triterpenes identified as β-sitosterol **(8)**, β-sitostenone **(9)** and lupeol **(10)**. The chemical structure of the isolated compounds is shown in Figure 1.

All secondary metabolites were identified by analyzing their spectroscopic data and comparing them with the literature data. To our knowledge, all compounds were isolated for the first time from this species. These isolated flavonoids **1** and **2** were previously isolated from the stembark of *P. owariensis* [5]. Meanwhile, compounds **3** and **4** were reported to be present in the roots of *P. indica* [26] and leaves of *P. carymbosa*, respectively [27, 28]. In addition, compound **(8)** has been previously isolated from the leaves of *P. crassicaulis* [9]. Furthermore, flavonoid compounds were also reported from other Rubiaceae genera, such as *Uncaria* [29], *Augusta* [30], *Morinda* [31], and *Galianthe* [32] genera. The presence of those valuable flavonoids in various species enriches their chemical diversity and provides evidence for chemotaxonomic studies of *Pavetta* species and the family Rubiaceae.

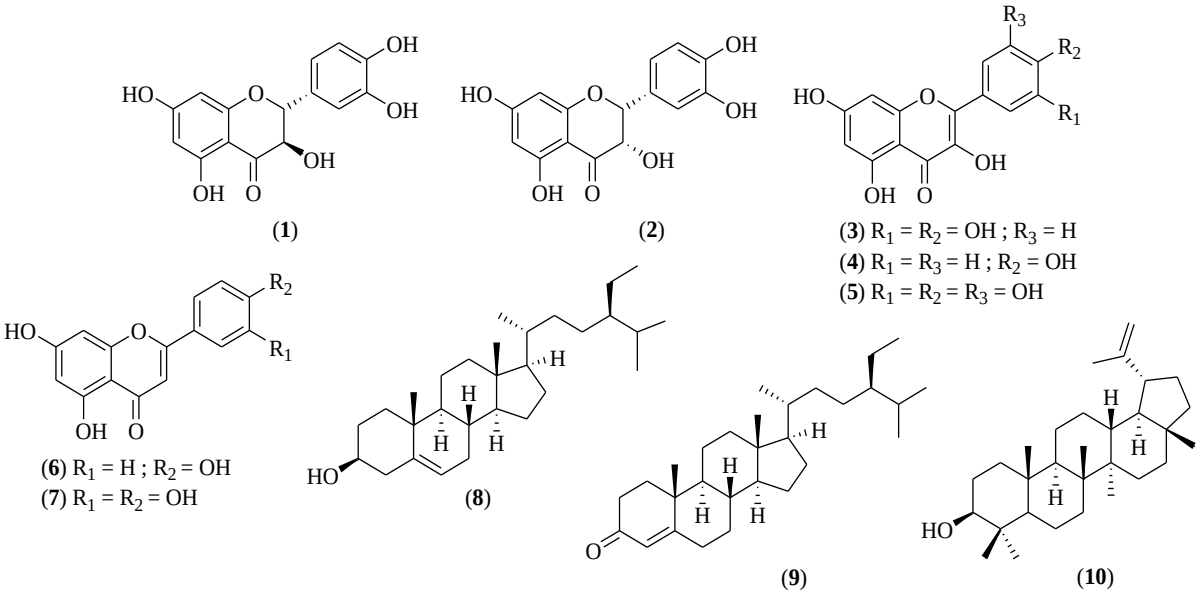

Figure 1. Chemical structures of isolated compounds from *P. graciliflora*.

Inflammation can result from the over-production of prostaglandins or leukotrienes through the enzymatic pathways known as cyclooxygenase (COX) or lipoxygenase (LOX) pathways, respectively. Lipoxygenase (LOX) are non-heme iron-containing enzymes that are responsible for stereo and region-specific oxygenation of polyunsaturated fatty acids containing 1,4-pentadiene systems such as arachidonic acid or linoleic acid to form 1-hydroperoxy-1,4-pentadiene products. The mammalian LOX are classified as 5, 12, and 15-LOX depending on the position of deoxygenation of that fatty acid. The primary product of the deoxygenation of arachidonic acid by 15-LOX is 15-hydroproxyeicosatetraenoic acid (HPETEs), which is then reduced to 15-hydroxyeicosatetraenoic acid (HETEs) or converted into various types of eicosanoids such as leukotrienes. The products of 15-LOX have been implicated among the causes of various human diseases, such as diabetes mellitus, ulcers, asthma, skin cancer, rheumatoid arthritis, and Alzheimer's disease [33].

Isolated compounds obtained from *P. graciliflora* were screened to determine their inhibitory effect on the soybean lipoxygenase-1 enzyme (LOX-1). Isolated compounds demonstrated remarkable inhibitory activity with IC_{50} values ranging from 3.4 to 59.2 µM with statistically significant SD values less than 0.05, represented in Table 1. It was observed that among the isolated compounds, compounds **(1)**, **(3)**, **(5)**, **(6),** and **(7)** show good anti-inflammatory activity through lipoxygenase (LOX) enzyme inhibition. In this study, all hydroxyflavonoids are found to be active. It was proposed that hydroxyflavonoids of the planar skeleton have a significant role in determining their inhibitory capacity [34]. In addition, flavonoids containing a hydroxyl group at C-3′ and C-4′ in ring B and a double bond at C2-C3 in ring C are attributable to the inhibitory effect of LOX. On the other hand, the presence of a hydroxyl group at C3 of the C-ring reduced the inhibitory activity. However, the presence of the hydroxyl group in ring A does not appear to be a factor for the inhibition [35].

Table 1. IC_{50} of lipoxygenase (LOX) of isolated compounds of *P. graciliflora.*

Compounds	**IC_{50} (µM)**
(+)-Catechin **(1)**	14.1 ± 1.4
(+)-Epicatechin **(2)**	59.2 ± 0.3
Quercetin **(3)**	4.1 ± 0.1
Kaempferol **(4)**	21.5 ±0.2
Myricetin **(5)**	13.8 ± 0.7
Apigenin **(6)**	4.2 ± 0.1
Luteolin **(7)**	3.4 ± 1.1
Baicalein (Positive control)	23.4 ± 0.5

Results are represented as percent of control ± SD, n = 3

CONCLUSION

This study revealed that *P. graciliflora* could be a potential source of lipoxygenase inhibitors, which could be used to prevent lipoxygenase diseases. Among the numerous molecules involved in the inflammatory response, leukotrienes are important chemical mediators in the onset of some allergic disorders. Hence, the isolated compounds could be further evaluated to develop safe agents to be introduced in modern therapy. In addition, a more comprehensive understanding should be made to reveal the mode of action of *P. graciliflora*, which might help understand the possible roles in human physiology.

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