purity and could be the good replacement in Vietnam as an analytical standard in identifying the components of various plant extracts.

6. Conclusion

The procedure for the quantification of stachyose in *Stachys affinis* rhizomes by LC-MS was validated and met the requirements of the ICH and AOAC guidelines so that it can be applied to quality control of the input materials. Besides, this method was applied for determination of the content of the standard according to the standard setting procedure from which the assigned value is given. Stachyose has been established as a reference material with a purity of 97.3% calculated on the inoculant as-is, which meets the requirements of a standard used in the quality control of stachyose in raw materials and products.

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MICROSCOPIC CHARACTERISTICS, ANTIOXIDANT ACTIVITIES, AND CHEMICAL COMPOSITIONS OF *OCHNA INTEGERRIMA* LEAVES AND STEMS

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Summary

Microscopic Characteristics, Antioxidant Activities, and Chemical Compositions of *Ochna integerrima* Leaves and Stems

Ochna integerrima is a typical and precious plant for spring in Vietnam. The plant has been used traditionally for treatments of various ailments. The microscopic transverse section of its leaf and stem reveals the presence of phloem arcs from the leaf midrib. The upper and lower arcs are continuous and separated. The sclerenchyma contains two rings with a continuous outer and an interrupted inner in the stem. The MeOH extracts of the leaf and stem possess significant total phenolic

contents of 114.11 ± 2.1 and 127.6 ± 3.45 mg GAE/g, respectively. These results corresponded to IC_{50} values of 17.2 ± 0.15 and 11.9 ± 0.37 µg/ml for DPPH inhibitory activity, respectively. These results corresponded to the remarkable DPPH-scavenging activity with IC_{50} values of 17.2 ± 0.15 and 11.9 ± 0.37 µg/ml, respectively. Methanol stem extract displayed higher antioxidant activity than methanol leaf extract. Chromatographic separation of MeOH extract of stems led to the isolation of four compounds, including 6-γ,γ-dimethyl-allylkaempferol 7-*O*-β-D-glucopyranoside (**1**), 6-γ,γ-dimethylallyldihydrokaempferol 7-*O*-β-D-glucoside (**2**), iriskumaonin-3′-methyl ether (**3**), calodenone (**4**). The results from this study suggest that *O. integerrima* and its isolates could be good candidates for further investigation of antioxidant bioactivities.

Keywords: *O. integerrima stems and leaves, Botanical microscopic studies, Total phenol content, Antioxidant activity.*

1. Background

Ochna integerrima (Lour.) Merr symbolizes spring in Southern Vietnam and belongs to the genus *Ochna*. It was used as an ornamental plant in the house, especially during New Year named "Mai vàng". This species is widely distributed in the Central (from Quang Nam, Da Nang to Khanh Hoa) and the South of Vietnam (Mekong Delta. Its yellow flowers bloom from December to February, which brings prosperity and happiness for a whole year.

The plant has been used traditionally to treat various ailments. The leaves are used for asthma, dysentery, epilepsy, gastric disorders, ulcers, and edible vegetables; the barks and roots are for digestive stimulation; and the flowers are for chest tightness, tuberculosis, cough, burns, sore throats, dizziness, and loss of appetite [1]. Edible oils can be extracted from this genus [2]. According to the survey, this plant has about 26-35 genera and 495-600 species [3]. A microscopic investigation is necessary for the medicinal material. Flavonoids, biflavonoids, anthranoids, triterpenes, and steroids have been found in the genus *Ochna* [4],[5],[6],[7]. It is noticeable that this species provides a massive amount of flavonoid, a promising antioxidant agent for slowing the aging process. This plant has been reported to possess various biological effects, including anti-HIV [5], antimalarial [1], and antioxidant activities [8]. Moreover, the microscopic examination of the stem and leaf, chemical investigation, and bioactivities have been limited. Therefore, the objectives of this study are:

1. to provide an accurate analysis of the microscopic characteristics of its powder and transverse sections for stem and leaf.

2. to carry out the total phenolic content and antioxidant activity of the MeOH extracts from leaves and stems and the fractions of stem extract.

3. to investigate the chemical constituents of the active fractions.

2. Object and research method

2.1. Plant material

O. integerrima (leaves and stems) was collected during the blooming time from Binh Thuan province, Vietnam in December 2019, identified by the botanist Dr. Van Son Dang at the Institute of Tropical Biology, Vietnam Academy of Science and Technology, Ho Chi Minh City, Vietnam. A voucher specimen (BT-OI-01) was deposited at the Faculty of Pharmacy, Ton Duc Thang University, Vietnam.

2.2. General procedures

NMR spectra were acquired on a Bruker Avance NEO spectrometer (Bruker, USA) (400 MHz for ^{1}H, and 100 MHz for ^{13}C) with TMS as an internal standard. LC-MS was analyzed on Agilent 1260 Infinity II HPLC system (USA) equipped with an Agilent G6125B single-quad MSD. HR-ESI-MS was recorded on Shimazu LCMS-8040 (Japan). The powder microscopy were estimated by Olympus CX23 (Japan). The percentage DPPH scavenging effect was recorded using a Thermo Fisher Scientific UV-Vis spectrophotometer, U.S. *Silica gel* (60 N, spherical, neutral, 40-50 µm, Kanto Chemical Co., Inc., Tokyo, Japan), and Cosmosil 75C18-OPN (Nacalai Tesque Inc., Kyoto, Japan) was used for open column chromatography (CC). Spots were visualized by spraying with a solution of 10% H_2SO_4 in ethanol, followed by heating at 100°C. Ascorbic acid was purchased from the Institute of Drug Quality Control, Ho Chi Minh, Vietnam. Chemical reagents were purchased from Sigma Aldrich (USA).

2.3. Transverse section examination

The leaves and stems of *O. integerrima* were cut into thin slices by using a blade. The sliced sections were then bleached with Javel's solution for 20 min to remove the color. The slices were then washed with water and soaked with 10% acetic acid to remove the trace of bleach before being dyed with Alum Carmine-Methyl Green reagent for 20 min. The dyed slices were washed with water to remove the excessed reagents before being examined under the light microscope [9].

2.4. Microscopic study

Powder microscopy was performed according to the procedure by Khandelwal [10]. The leaf and stem of *O. integerrima* were dried at the temperature of 60°C. The dried materials were pulverized and sieved by stainless mesh (No. 507) to obtain the fine powder, then examined under a light microscope.

2.5. Preliminary phytochemical screening

Before bioassay activities and isolation,

preliminary qualitative chemical screening of MeOH extract was examined using the procedure described by Kokate [11].

2.6. Antioxidant assays

Total phenolic content (TPC): Folin-Ciocalteu method with slight modification was used to determine total phenolic contents in the extracts [12]. The final concentrations (50 µg/mL) of MeOH extract from the leaves and stems were prepared by dissolving them in water. 2.0 mL of this solution was mixed with 0.25 mL of 25% Folin-Ciocalteu reagent and added 0.25 µL of 10% sodium carbonate. The mixtures were vortexed for 20 s and allowed to stand for 30 min at room temperature to avoid light. Then absorbance was recorded at a 760 nm UV-spectrophotometer. The TPC was expressed as mg of gallic acid (GAE) equivalent per 1 g dry weight. The final concentrations of GAE (1.56 to 25.0 µg mL^{-1}) were used for a calibration curve.

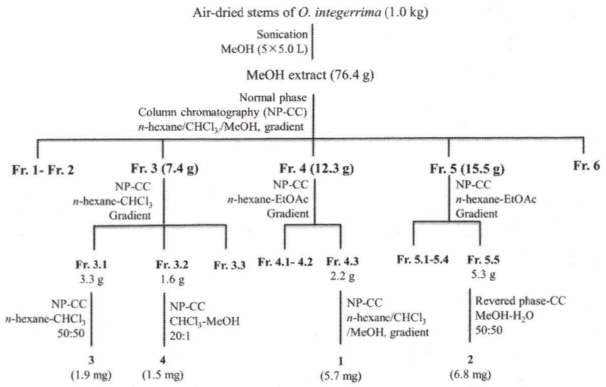
DPPH radical scavenging assay: Free radical scavenging ability of the MeOH extracts from the leaves and stems was measured by DPPH assay according to Mishra et al. with slight modifications [13]. The presence of antioxidants changed the color from purple to yellow by donating the hydrogen atom. 0.1 mM DPPH in methanol was prepared and added with five concentrations of extract and fractions (3.125 to 100.0 µg/ml). The reaction mixture was vortexed slightly and left in the darkness for 30 min. The absorbance (Abs) of the mixture was measured at 517 nm. Ascorbic acid was used as a positive control. All experiments were performed in triplicate. The IC_{50} was calculated by the equation curves. The following equation determined the percentage inhibition of the DPPH radical:

DPPH inhibition (%) = [(Abs $_{control}$ - Abs $_{extract}$)/Abs $_{control}$] × 100

2.7. Extraction and isolation

The air-dried stems of *O. integerrima* (1.0 kg) were extracted with MeOH (5×5.0 L) for three days each with sonication to obtain MeOH extract (76.4 g). This crude extract was applied to a normal phase *silica gel* column and eluted with increasing polarities of solvents to obtain six sub-fractions (Fr. 1-Fr. 6). Fraction Fr. 3 (7.4 g) was subjected to a normal phase open CC and eluted with increasing polarities of *n*-hexane-$CHCl_3$ to afford three sub-fractions (Fr. 3.1-Fr. 3.3). Fraction Fr. 3.1 (3.3 g) was re-chromatographed on a normal phase open CC, eluted with *n*-hexane-$CHCl_3$ (50:50) to obtain **3** (1.9 mg). Fraction Fr. 3.2 (1.6 g) was re-chromatographed on a normal phase open CC, eluted with $CHCl_3$-MeOH (20:1) to obtain **4** (1.5 mg).

Fraction Fr. 4 (12.3 g) was fractionated by normal phase open CC, eluted with *n*-hexane-EtOAc (70:30 to 0:100) to obtain three sub-fractions (Fr. 4.1-Fr. 4.3). Fraction Fr. 4.3 (2.2 g) was further purified on a normal phase open CC with increasing polarities *n*-hexane-$CHCl_3$ and $CHCl_3$-MeOH to give **1** (5.7 mg). Fraction Fr. 5 (15.5 g) was further separated by normal phase open CC, eluted with *n*-hexane-EtOAc (60:40 and 0:100) to obtain five sub-fractions (Fr. 5.1-Fr. 5.5). Fraction Fr. 5.5 (5.3 g) was re-chromatographed on a C-18 open column using MeOH-H_2O (50:50) as eluent to yield **2** (6.8 mg) (Scheme 1).



Scheme 1: Extraction and isolation procedure of **1-4** from *O. integerrima*

Compound **1**: Yellow powder; LRESIMS *m/z* 551.2 $[M+Cl]^-$ [5, 14].

^{1}H NMR (400 MHz, CD_3OD): δ_H 8.14 (1H, d, *J* = 8.8, H-6′), 6.91 (1H, d, *J* = 8.8, H-5′), 6.90 (1H, d, *J* = 8.8, H-3′), 8.12 (1H, d, *J* = 8.8, H-2′), (3.5, 3.48, m, H-1″), 5.27 (1H, t, *J* = 7.2, H-2″), 1.65 (3H, s, H-4″), 1.81 (3H, s, H-5″), 5.1 (1H, d, *J* = 7.6, H-1‴), 3.55 (1H, m, H-2‴); 3.40 (1H, t, *J* = 8.8, H-3‴), 3.40 (1H, t, *J* = 9.6, H-4‴), 3.50 (m, H-5‴), [3.95 (1H, dd, *J* = 12.0, 2.0), 3.71 (1H, dd, *J* = 12.4, 6.0), H-6‴)].

^{13}C NMR (100 MHz, CD_3OD): δ_C 160.8 (C-2), 137.5 (C-3), 177.6 (C4), 106.0 (C4a), 158.6 (C-5), 114 (C-6), 160.8 (C-7), 94.2 (C-8), 156.0 (C-8a), 123.7 (C-1′), 132.2 (C-6′), 116.4 (C-5′), 148.7 (C-4′), 116.4 (C-3′), 130.9 (C-2′), 22.5 (C-1″), 123.6 (C-2″), 130.9 (C-3″), 26.0 (C-4″), 18.1 (C-5″), 101.7 (C-1‴), 75.0 (C-2‴), 78.5 (C-3‴), 71.4 (C-4‴), 78.5 (C-5‴), 62.6 (C-6‴).

Compound **2**: Pale yellow powder; HRESIMS *m/z* 517.1741 $[M-H]^-$ (calcd. for $C_{26}H_{29}O_{11}$ 517.1709) [5, 14].

^{1}H NMR [400 MHz, CD_3OD]: δ_H 5.01 (1H, d, H-2), 4.6 (1H, d, *J* = 11.6 , H-3), 6.28 (1H, s, H-8), 7.36 (1H, d, *J* = 8.4, H-2′), 6.84 (1H, d, *J* = 8.8, H-3′), 6.84 (1H, d, *J* = 8.8, H-5′), 7.36 (1H, d, *J* = 8.4, H-6′), [3.39 (1H, dd, *J* = 12.8, 8.8),

3.36 (1H, dd, J = 12.8, 8.8), H-1″], 5.22 (1H, t, J = 7.2, H-2″), 1.65 (s, H-4″), 1.78 (s, H-5″), 5.01 (1H, d, J = 7.2, H-1‴), 3.46 (m, H-2‴), 3.44 (m, H-3‴), 3.67 (m, H-4‴), 3.50 (m, H-4‴), 3.50 (m, H-5‴), [3.88 (m), 3.83 (m), H-6‴].

^{13}C NMR [100 MHz, CD_3OD]: δ_C 83.8 (C-2), 72.5 (C-3), 198.2 (C-4), 101.8 (C4a), 160.0 (C-5), 110.7 (C-6), 163.5 (C-7), 93.9 (C-8), 161.0 (C-8a), 127.8 (C-1′), 129.0 (C-2′), 114.7 (C-3′), 157.9 (C-4′), 114.0 (C-5′), 129.0 (C-6′), 20.7 (C-1″), 122.2 (C-2″), 130.6 (C-3″), 24.5 (C-4″), 16.6 (C-5″), 99.9 (C-1‴), 73.4 (C-2‴), 76.9 (C-3‴), 69.8 (C-4‴), 76.9 (C-5‴), 61.0 (C-6‴).

Compound **3**: white amorphous solid; HRESIMS *m/z* 357.0975 $[M+H]^+$ (calcd. for $C_{19}H_{17}O_7$, 357.0978).

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.81 (1H, s, H-2), 7.21 (1H, d, J = 2.5, H-2′), 7.0 (1H, dd, J = 8.4, 2.5, H-6′), 6.90 (1H, d, J = 8.4, H-5′), 6.64 (s, H-8), 6.07 (2H, s, OCH_2O), 4.09 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 3.90 (3H, s, OCH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 175.5 (C-4), 154.7 (C-8a), 152.8 (C-7), 150.4 (C-2), 149.1 (C-4′), 148.7 (C-3′), 141.7 (C-5), 135.5 (C-6), 125.4 (C-3), 124.6 (C-1′), 121.3 (C-6′), 113.8 (C-4a), 112.8 (C-2′), 111.0 (C-5′), 102.2 (OCH_2O), 93.2 (C-8), 61.3 (OCH_3), 56.0 (OCH_3), 55.9 (OCH_3) [15].

Compound **4**: white amorphous solid; HRESIMS *m/z* 525.1541 $[M+H]^+$ (calcd. for $C_{31}H_{25}O_8$ 525.1550).

^{1}H NMR [400 MHz, MeOD]: δ_H 8.20 (1H, s, H-2), 7.87 (1H, d, J = 9.2, H-5), 6.83 (1H, dd, J = 2.0, 8.8, H-6), 6.68 (1H, d, J = 2.0 , H-8), 6.01 (1H, d, J = 12.4, H-11), 6.29 (1H, d, J = 2.8, H-15), 6.47 (1H, dd, J = 2.4; 9.0, H-17), 8.22 (1H, d, J = 9.0, H-18), 4.68 (1H, d, J = 12.0, H-19), 7.15 (2H, d, J = 8.4, H-21, H-25), 6.58 (2H, d, J = 8.4, H-22, H-24), 7.15 (2H, d, J = 2.4, H-27, H-31), 6.55 (1H, d, J = 2.4, H-28, H-30), 3.79 (s, OCH_3).

^{13}C NMR [100 MHz, MeOD]: δ_C 156.8 (C-2), 122.4 (C-3), 177.1 (C-4), 117.1 (C-10), 128.1 (C-5), 116.7 (C-6), 165.7 (C-7), 103.4 (C-8), 159.6 (C-9), 45.0 (C-11), 205.4 (C-12), 115.0 (C-13), 166.9 (C-13), 101.8 (C-15), 168.1 (C-16), 108.5 (C-17), 133.9 (C-18), 54.3 (C-19), 134.8 (C-20), 130.5 (C-21), 116.1 (C-22), 156.7 (C-23), 116.1 (C-24), 130.5 (C-25), 135.9 (C-26), 129.8 (C-27), 116.7 (C-28), 157.3 (C-29), 116.1 (C-30), 129.8 (C-31), 56.2 (C-OCH_3) [16-18].

2.8. Statistical analysis

The data are expressed as mean ± standard deviation of at least three replicates. The statistical analyses were carried out using OriginPro 2019.

3. Results

3.1. Powder microscopic investigation and transverse section analysis of leaf and stem

Powder microscopy of leaf and stem of *O. integerrima*: Leaf powder is dark green with a slightly bitter taste. Many pieces of epidermises, palisade, brown resin masses, vasculature with stomata, and the bundle of fibers were observed in the leaves (Fig. 2). The stem powder is brown. It possesses palisade, brown resin masses, epidermis with thick wall trichomes, scalariform vessels, thick wall trichomes, bundle of fibers, and calcium oxalate crystals (Fig. 1).

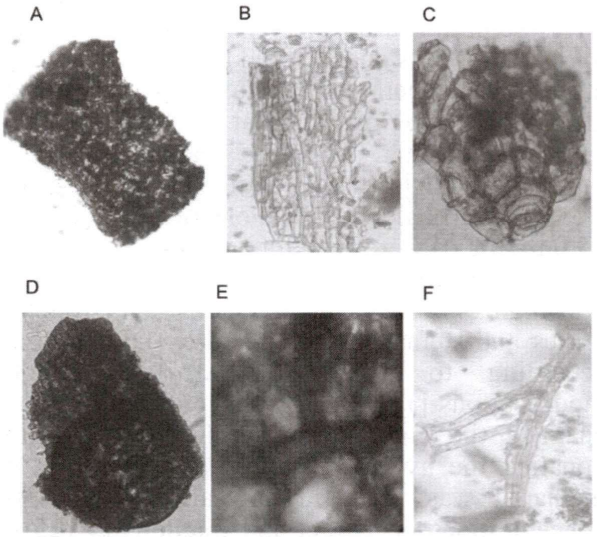


Fig. 1. Microscopic characters of the leaf powder of *O. integerrima*
A. Palisade, B. Epidermis, C. B. Epidermis with stomata, D. Brown resin masses, E. Vasculature with stomata, F. Bundle of fibers

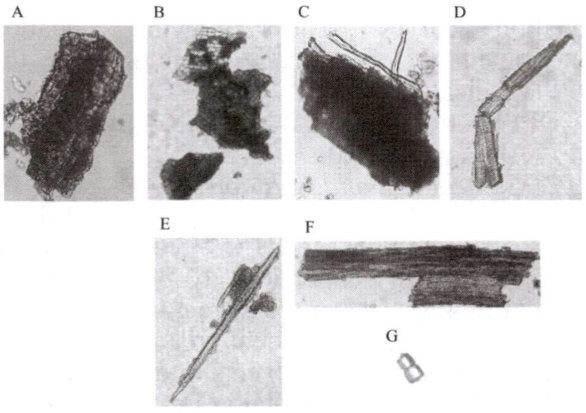


Fig. 2. Microscopic characters of the stem powder of *O. integerrima*
A. Palisade, B. Brown resin masses, C. Epidermis with thick wall trichomes, D. Scalariform vessels, E. Thick wall trichome, F. Bundle of fibers, G. Calcium oxalate crystals

Transverse section analysis: the leaf and stem of *O. integerrima* revealed the presence of various

diagnostic features. Transverse section of leaf midrib: upper epidermal consists of a layer of rectangular cells covered with cuticles. Lower epidermal cells are smaller. The upper parenchyma is narrow with 3 - 4 layers of the circular cell, while the lower parenchyma is larger with bigger cells. The pericyclic fibers arcs surround the main central vascular bundle. The upper arc of the phloem is narrow and continuous, while the lower is separated by sclerenchyma cells. Xylem consists of radially arranged big, lignified vessels. The pith is narrow with circular parenchymal cells with scattering lignified cells.

Transverse section of leave lamina: Upper and lower epidermal consists of a layer of rectangular cells, covered with cuticles. Stomata are rarely observed in the upper epidermis, palisade with a layer of columnar-shaped cells. Spongy cells with 3 - 5 layers of circular-shaped cells arranged loosely with airspaces (Fig. 3).

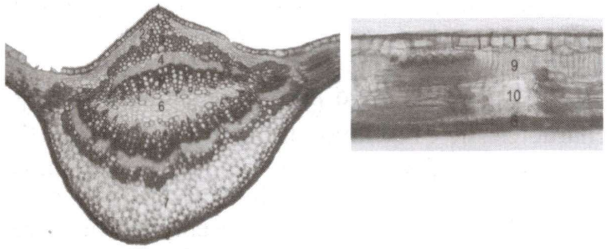


Fig. 3. Transverse section of the leaf (× 10): 1. Upper epidermis; 2. Upper parenchyma; 3. Fiber; 4. Phloem; 5. Xylem; 6. Parenchyma, 7. Lower parenchyma; 8. Lower epidermis; 9. Palisade mesophyll; 10. Spongy mesophyll.

Transverse section of stem: cork cells consist of 8 - 10 layers of sub-square cells, arranged in order. Two rings of sclerenchyma, the outer is continuous while the inner is interrupted. The outer cortex is relatively narrow, consisting of 8 - 10 parenchymatous cell layers with two medullary bundles. Pericyclic fiber cells with 3 - 4 layers arranged in an interrupted ring above the phloem. Phloem is narrow, with the cambium indistinct. Xylem consists of big, lignified cells arranged irregularly. Pith is relatively large with lignified parenchyma cells (Fig. 4).

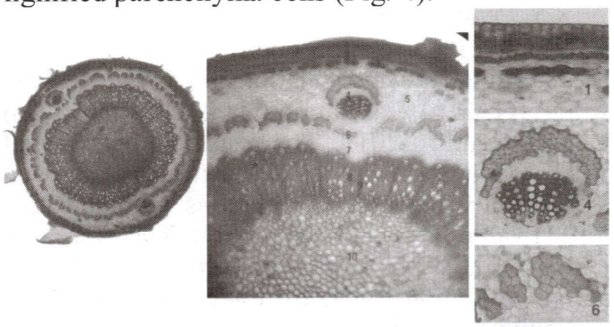


Fig. 4. Transverse section of stem (× 10): 1. Cork; 2. Periderm; 3 Sclerenchyma; 4. Medullary bundle; 5. Cortex; 6. Fiber; 7. Secondary Phloem; 8. Secondary xylem; 9. Medullary ray; 10. Pith

3.2. Preliminary phytochemical screening

Preliminary phytochemical screening showed the presence of flavonoids (γ-pyron and proanthocyanidin), steroids, polysaccharides, saponin glycosides, tannins, and the absence of alkaloids and anthocyanidins in MeOH extracts of the leaves and stems (Table 1).

Table 1. Preliminary phytochemical screening of MeOH extracts from the leaves and stems

Chemical constituents	Reagents	MeOH extract	
		Leaves	Stems
Alkaloids	Dragendorff	-	-
Flavonoids (γ-pyron)	Shinoda	+	+
Flavonoids (anthocyanidin)	HCl 10% + NaOH 10%	-	-
Flavonoids (proanthocyanidin)	HCl 10%	+	+
Steroids	Liebermann-Burchard	+	+
Polysaccharides	Fehling	+	+
Saponin glycosides	Foam test	+	+
Tannins	Ferric chloride	+	+

"-": negative "+": positive.

3.3. The total polyphenol contents

The value of TPC was expressed by the GAE equivalent, GAE (regression equation of the gallic acid calibration curve: $y = 0.0625x + 0.0374$, $R^2 = 0.999$), mg of GA/g of dried extract. The result showed that the MeOH extract of the leaves (OD = 0.394), and the stems (OD = 0.436) displayed high values of TPC (114.11 ± 2.10 and 127.6 ± 3.45 mg GAE/g, respectively). It suggested that these MeOH extracts may display potent antioxidant secondary metabolites.

3.4. DPPH assay of the extracts

The antioxidant capacity of MeOH extracts of the leaves and stems was determined by the DPPH radical scavenging assay. The result revealed that MeOH extract showed potent antioxidant activity with an IC_{50} 11.9 ± 0.37 from the stems, followed by 17.52 ± 0.15 μg/ml from

the leaves compared to ascorbic acid with an IC_{50} 1.83 μg/ml (1 μg/ml ≈ 5.68 μM) (Table 2).

Table 2. IC_{50} values of MeOH extract of the leaves and stems

Samples	IC_{50} ± SD (μg/ml)
MeOH extracts	
Leaves	17.2 ± 0.15
Stems	11.9 ± 0.37
Ascorbic acid (positive control)	1.83 ± 0.072

Data are expressed as the means ±SD of three experiments.

3.5. Pearson analysis

Pearson's correlation coefficient (r) was used to determine the interrelationship between TPC value (GAE/g) and antioxidant activity (IC_{50}) using OriginPro 2019. The results indicated that TPC value of MeOH extracts showed significantly negative correlations with the IC_{50} DPPH value ($r = -0.944$, $p < 0.01$). Therefore, the increase of TPC led to the high antioxidant activities demonstrated by lower IC_{50} of DPPH scavenging activity. This finding indicated that phenolic compounds may contribute to antioxidant activity.

3.6. DPPH assay of the fractions

The DPPH assay indicated that the MeOH extract from the stems was more active than that from the leaves. Therefore, MeOH extract from the stems was selected for further isolation. In fact, among fractions (Fr. 1- Fr. 6), Fr. 4 displayed the highest antioxidant activity with an IC_{50} 8.67 ± 0.16 μg/ml, followed by Fr. 5 (IC_{50} 12.21 ± 0.46 μg/ml), Fr. 3 (IC_{50} 12.54 ± 0.32 μg/ml), Fr. 2 (15.56 ± 0.31 μg/ml), Fr. 6 (IC_{50} 29.21 ± 2.11 μg/ml), and Fr .1 (IC_{50} 64.6 ± 5.0 μg/ml) (Table 3).

Table 3. IC_{50} values of MeOH fractions

Fractions	IC_{50} ± SD (μg/ml)
Fr. 1	64.6 ± 5.0
Fr. 2	15.56 ± 0.31
Fr. 3	12.54 ± 0.32
Fr. 4	8.67 ± 0.16
Fr. 5	12.21 ± 0.46
Fr. 6	29.21 ± 2.11
Ascorbic acid (positive control)	1.83 ± 0.072

Data are expressed as the means ±SD of three experiments.

3.7. The elucidation of isolated compounds 1-4

The MeOH extract of *O. integerrima* stem was selected for further isolation because of the significantly high antioxidant activity compared to the leaves. Fractionation of MeOH extract of *O. integerrima* stems by open CC obtained four known compounds **1-4**, including 6-γ,γ-dimethylallylkaempferol 7-*O*-β-D-glucopyranoside (**1**), 6-γ,γ-Dimethylallyldihydro-kaempferol-*O*-β-D-glucoside (**2**), iriskumaonin- 3′-methyl ether (**3**), and calodenone (**4**). The structures of isolated compounds (**1-4**) were determined by comparing their spectroscopic data with the literature (Fig. 5). Compounds **1** and **2** were also isolated from the flowers of this species in our previous publication[14]. In detail, compounds **1-4** were isolated from the active fractions (Fr.3 and Fr. 4) of MeOH extract from the stems, which suggested being responsible for their antioxidant capacities. The isolates prenylated flavonoids and bioflavonoids **1-4** which reportedly exerted significant antioxidant properties can be assumed as bioactive constituents [14],[19]. The previous studies reported that *O. integerrima* was a great natural antioxidant source, which agrees with our results from the present study [8].

Compound **1** was obtained as a yellow powder. The ESI-MS showed a quasi-molecular ion peak with *m/z* 551.2 $[M+Cl]^-$, indicating the molecular formula of **1** is $C_{26}H_{28}O_{11}$. The signals at δ_H 8.14 (1H, d, J = 8.8, H-6′), 6.91 (1H, d, J = 8.8, H-5′), 6.90 (1H, d, J = 8.8, H-3′), 8.12 (1H, d, J = 8.8, H-2′), 6.29 (1H, s, H-8) displayed the characteristic of a flavonol moiety. Moreover, the presence of a prenyl group at δ_H (3.5, 3.48, m, H-1″), 5.27 (1H, t, J = 7.2, H-2″), 1.65 (3H, s, H-4″), 1.81 (3H, s, H-5″) was observed. The sugar unit in **1** was identified as β-form by the presence of anomeric proton at δ_H 5.1 (1H, d, J=7.6, H-1‴). The signals at δ_H 3.40-3.71 (4H, m, H-2‴-H-5‴) and [3.95 (1H, dd, J = 12.0, 2.0), 3.71 (1H, dd, J = 12.4, 6.0), H-6‴)] were assigned to the methine and methylene protons of the glucose unit. The 13C-NMR revealed 15 typical signals of flavonol including δ_C 160.8 (C-2), 137.5 (C-3), 177.6 (C4), 106.0 (C4a), 158.6 (C-5), 114 (C-6), 160.8 (C-7), 94.2 (C-8), 156.0 (C-8a), 123.7 (C-1′), 132.2 (C-6′), 116.4 (C-5′), 148.7 (C-4′), 116.4 (C-3′), 130.9 (C-2′), 5 signals of a prenyl group at δ_C 22.5 (C-1″), 123.6 (C-2″), 130.9 (C-3″), 26.0 (C-4″), 18.1 (C-5″) and sugar moiety with anomeric carbon at δ_C 101.7 (C-1‴) and δ_C 75.0 (C-2‴), 78.5 (C-3‴), 71.4 (C-4‴), 78.5 (C-5‴), 62.6 (C-6‴). Compound **1** was assigned as 6-γ,γ-Dimethylallylkaempferol 7-*O*-β-D-glucopyranoside by comparison to the previous publication.

Compound **2** was isolated as yellow powder with the molecular formula $C_{26}H_{30}O_{11}$, deduced from the HR-ESI-MS *m/z* 553.1485 $[M+Cl]^-$ (calcd. for $C_{26}H_{30}O_{11}Cl$ 553.1477). The ^1H and ^{13}C NMR spectroscopic data of **2** were closely similar to those of **1**, except for the presence of proton at δ_H 5.01 (1H, d (overlapped), H-2), 1 proton at δ_H 4.60 (1H, d, J=11.6, H-3) as well as

two signals of carbons at δ_C 83.8 (C-2); 72.5 (C-3). By comparison with published data[5], compound **2** was determined as 6-γ,γ-Dimethylallyldihydrokaempferol 7-*O*-β-D-glucopyranoside.

Compound **3** was obtained as a white amorphous solid with the molecular formula $C_{21}H_{20}O_{10}$, ESI-MS *m/z*: 357.0978 [M-H]. The ^{1}H NMR data of **3** revealed the presence of three methoxy groups at δ_H 3.90, 3.92 and 4.09, a methylenedioxy group at δ_H 6.07, the 2′,5′,6′-trisubstituted aromatic protons at δ_H 6.90 (1H, d, *J*=8.4, H-5′), 7.21 (1H, d, *J*=2.5, H-2′) and 7.0 (1H, dd, *J*=8.4, 2.5, H-6′), a substituted aromatic ring at δ_H 6.64 (s, H-8) and an isoflavone at δ_H 7.81 (1H, s, H-2). The ^{13}C NMR data of **1** showed resonances for 16 C atoms of isoflavone skeleton with one methylene, five methine and ten quaternary carbons. The presence of three methoxy groups at δ_C 56.0, 55.9 and 61.3 was observed in ^{13}C-NMR data. Comparing their NMR and MS data with reported literature [15], compound **3** was named iriskumaonin-3′-methyl ether.

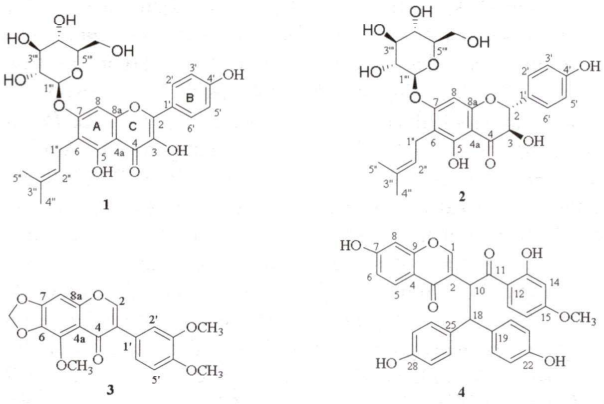


Fig. 5. Structures of isolated compounds (**1-4**)

Compound **4** was obtained as a white amorphous solid; Its molecular formula of $C_{31}H_{24}O_8$ was determined by HR-ESI-MS ion peak at *m/z* 525.1541 $[M+H]^+$ (calcd. for $C_{31}H_{25}O_8$ 525.1550). The ^{1}H NMR data of **3** displayed the signals of 14 aromatics, one vinylic, two methinic protons, and one methoxy proton. The resonance of 31 carbons including two carbonyl groups at δ_C 177.1 (C-3), 205.4 (C-11), 11 quaternary carbons at δ_C 122.4 (C-2), 117.1 (C-4), 165.7 (C-7), 159.6 (C-9), 115.0 (C-12), 166.9 (C-13), 168.1 (C-15), 134.8 (C-19), 156.7 (C-22), 135.9 (C-25), 157.3 (C-28), 15 tertiary carbons at δ_C 156.8 (C-1), 128.1 (C-5), 116.7 (C-6), 103.4 (C-8), 101.8 (C-14), 108.5 (C-16), 133.9 (C-17), 130.5 (C-20), 116.1 (C-21), 116.1 (C-23), 130.5 (C-24), 129.8 (C-26), 116.7 (C-27), 116.1 (C-29), 29.8 (C-30), one methoxy group at δ_C 56.2 (C-OCH_3), and two aliphatic methines at δ_C 45.0 (C-10), 54.3 (C-18). Compound **4** was a derivative of lophirone A. The structure was confirmed by comparing the NMR data with that reported data [17],[18]. It was identified as calodenone.

4. Discussion

The microscopic evaluation of the species was undertaken to provide its detailed features. Standardizing herbal medicinal plants is vital in drug preservation [20]. The characteristics of a plant are beneficial in pharmacognostic evaluation, which helps set the standards for proper identification, authentication, and standardization of crude drug material. It is also one of the essential criteria for raw material selection. Microscopic observation is one of the simplest and cheapest methods to identify the quality of medicinal plants [21]. Next, the plant extract was then analyzed for the presence of secondary metabolites. The phytochemical screening of the MeOH extract indicated the presence of tannins, saponins, triterpenoids, number of phenols and flavonoids while it had no positive indication for alkaloids. Based on the results, each secondary metabolite harbors a different biological function and pharmacological significance [22]. Among them, phenolic compounds and flavonoids are considered the primary elements for antioxidant properties and are widely distributed among various plant species. Furthermore, the TPC accounts for the chief factor encountering oxidative stress because of its redox properties. *O. integerrima* has contained abundant flavonoids leading to free radical scavengers and reducing oxidative stress [5]. The effective total phenolic content contributed significantly to the antioxidant activity of MeOH extracts evaluated by the Pearson correlation coefficient. In this study, *O. integerrima* stem was chosen for isolation because of the significant antioxidant activity compared to the leaves. In detail, compounds **1-4** were isolated from the active fractions of MeOH extract of the stems, which suggested being responsible for their antioxidant capacities. The amount of antioxidant capacity of fractions in the order F4 > F5 ≈ F3> F2 > F6> F1 may result in the high content of antioxidant compounds (**1-4**). Reportedly, the isolates **1-4** having prenylated flavonoids and bioflavonoids exerted significant antioxidant properties [19],[23]. It could therefore be assumed that the pharmacologically active constituents. The previous studies reported that *O. integerrima* was a remarkable natural antioxidant source, which agrees with our results from the present study [8]. However, the present study had some limitations. The microscopic identification of the other species

Ochna is needed to compare their significant features in the following study. Further phytochemical investigation from the leaves and their antioxidant activities of isolated compounds are required to elucidate the pharmacological properties.

5. Conclusions

The present investigation revealed noteworthy features of the leaves and stems of *O. integerrima*. This work reports for the first time the microscopic characteristics of leaves and stems and the antioxidant properties of the leaves. In the leaf, the upper phloem arc is a continuous form wherein its lower is separate strands accompanied by sclerenchyma cells. In the stem, sclerenchyma has two rings with continuous outer and interrupted inner. Moreover, the MeOH extracts of the leaves and stems displayed potent antioxidant activity using DPPH radical scavenging with IC_{50} values of 11.9 and 17.52 µg/mL with high TPC values. Due to bioassay-guided extracts and fractions, the four compounds 6-γ,γ-dimethylallylkaempferol 7-*O*-β-D-glucopyranoside (**1**), 6-γ,γ-dimethylallyldihydrokaempferol 7-*O*-β-D-glucoside (**2**), iriskumaonin-3′-methyl ether (**3**), and calodenone (**4**) were isolated from the active antioxidant extracts, particularly in sub-fractions (Fr. 3 and Fr. 4) from the stems. Taken together, *O. integerrima* species is a promising candidate for further isolation and profound antioxidant studies.

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