HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm

Original research http://dx.doi.org/10.1016/j.apjtm.2017.06.002

# Xanthine oxidase inhibitors from *Archidendron clypearia* (Jack.) I.C. Nielsen: Results from systematic screening of Vietnamese medicinal plants

Nguyen Thuy Duong[1], Pham Duc Vinh[1], Phuong Thien Thuong[2], Nguyen Thi Hoai[3], Le Nguyen Thanh[4], Tran The Bach[5], Nguyen Hai Nam[6✉], Nguyen Hoang Anh[1✉]

[1]*Department of Pharmacology, Hanoi University of Pharmacy, 13 Le Thanh Tong, Hoan Kiem, Hanoi, Viet Nam*

[2]*Department of Pharmaceutical Analysis and Standardization, National Institute of Medicinal Materials, 3B Quang Trung, Hoan Kiem, Hanoi, Viet Nam*

[3]*Department of Pharmacognosy, Hue University of Medicine and Pharmacy, 6 Ngo Quyen, Vinh Ninh, Hue, Viet Nam*

[4]*Institute of Marine Biochemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Caugiay, Hanoi, Viet Nam*

[5]*Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Caugiay, Hanoi, Viet Nam*

[6]*Department of Medicinal Chemistry, Hanoi University of Pharmacy, 13 Le Thanh Tong, Hoan Kiem, Hanoi, Viet Nam*

ARTICLE INFO

*Article history:*
Received 25 Jan 2017
Received in revised form 8 Apr 2017
Accepted 26 May 2017
Available online 15 Jun 2017

*Keywords:*
Xanthine oxidase
Inhibitor
Medicinal plants
Vietnam

ABSTRACT

**Objective:** To screen Vietnamese medicinal plants for xanthine oxidase (XO) inhibitory activity and to isolate XO inhibitor(s) from the most active plant.

**Methods:** The plants materials were extracted by methanol. The active plant materials were fractionated using different organic solvents, including *n*-hexane, ethyl acetate, and *n*-butanol. Bioassay-guided fractionation and column chromatography were used to isolate compounds. The compounds structures were elucidated by analysis of spectroscopic data, including IR, MS, and NMR.

**Results:** Three hundreds and eleven methanol extracts (CME) belonging to 301 Vietnamese herbs were screened for XO inhibitory activity. Among these plants, 57 extracts displayed XO inhibitory activity at 100 μg/mL with inhibition rates of over 50%. The extracts of *Archidendron clypearia* (*A. clypearia*), *Smilax poilanei*, *Linociera ramiflora* and *Passiflora foetida* exhibited the greatest potency with $IC_{50}$ values below 30 μg/mL. Chemical study performed on the extract of *A. clypearia* resulted in the isolation of six compounds, including 1-octacosanol, docosenoic acid, daucosterol, methyl gallate, quercitrin and (−)-7-O-galloyltricetiflavan. The compound (−)-7-O-galloyltricetiflavan showed the most potent XO inhibitory activity with an $IC_{50}$ value of 25.5 μmol/L.

**Conclusions:** From this investigation, four Vietnamese medicinal plants were identified to have XO inhibitory effects with $IC_{50}$ values of the methanol extracts below 30 μg/mL. Compound (−)-7-O- galloyltricetiflavan was identified as an XO inhibitor from *A. clypearia* with $IC_{50}$ value of 25.5 μmol/L.

## 1. Introduction

Xanthine oxidase (XO) is an enzyme catalyzing the formation of uric acid from xanthine and hypoxanthine which in turn is produced from purine catabolism [1]. Therefore, any defect in the purine degradation pathway may result in an increase in the uric acid level recognized as the single most important risk factor for the development of gout [2]. Moreover, reactive oxygen species (ROS) generated from XO-catalyzed reactions may also contribute to a range of pathological conditions including inflammation, metabolic disorders, cellular aging, and carcinogenesis [3]. As a result, inhibition of XO activity is suggested to be one of the therapeutic strategies for not only

First author: Nguyen Thuy Duong, Department of Pharmacology, Hanoi University of Pharmacy, 13 Le Thanh Tong, Hoan Kiem, Hanoi, Viet Nam.
E-mail: duongnt@hup.edu.vn
✉Corresponding authors: Nguyen Hai Nam, Department of Medicinal Chemistry, Hanoi University of Pharmacy, 13 Le Thanh Tong, Hoan Kiem, Hanoi, Viet Nam
Nguyen Hoang Anh, Department of Pharmacology, Hanoi University of Pharmacy, 13 Le Thanh Tong, Hoan Kiem, Hanoi, Viet Nam.
E-mails: namnh@hup.edu.vn, anh90tk@yahoo.com

Peer review under responsibility of Hainan Medical University.
Foundation project: This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 106.99-2012.90.

hyperuricemia but also various other diseases. Although XO inhibitors are currently considered the first-line therapy for long-term management of gout and related disorders, treatment choices are limited to allopurinol and febuxostat [4]. Allopurinol is associated with severe adverse events (e.g., hypersensitivity, hepatitis, interstitial nephritis, and eosinophilia) which reduce the tolerance and long-term adherence with the treatment. Febuxostat is better tolerated, however it also possesses the risk of undesirable effects and access to the drug may be restricted in developing countries due to its cost [5]. These unmet medical needs require more efforts in finding novel XO inhibitors with greater effectiveness and a better safety profile.

In the past two decades, a number of research groups have carried out screening for XO inhibitors from native medicinal plants and this approach was shown to be effective [6–10]. In 2004, Nguyen *et al.* found some plant extracts which exhibited potential XO inhibition through a screening of 96 Vietnamese herbs collected in a mountainous area in the south central provinces of Vietnam [6]. Since Vietnam is a country possessing abundant biological resources and rich knowledge of folk medicines, it is likely that many other indigenous medicinal plants remain potential as a source of potent bioactive compounds, including XO inhibitors. Therefore, focus on the research for novel XO inhibitors from natural products, we have screened 301 plant species used in Vietnamese traditional medicines for treatment of arthropathies, inflammatory disorders, and pain but almost uninvestigated in the earlier study. This paper describes the results obtained from this screening and chemical investigation carried out on *Achidendron clypearia* (Jack) I.C. Neilsen (syn. *Pithecellobium clypearia* Benth), the most active plant in inhibiting XO activity found from the samples screened.

## 2. Materials and methods

### 2.1. Chemicals and reagents

XO from bovine milk (X1875-25UN, 7.2 IU/mL), xanthine powder, potassium oxonate and allopurinol biological grade were purchased from Sigma–Aldrich Co (St Louis, MO, USA). Solvents for extraction and fractionation and other chemicals used for preparing buffers were purchased from Merck (Darmstadt, Germany).

### 2.2. Plant materials

All plant samples used in this study were collected from different provinces in Vietnam and botanically identified by Dr. Nguyen The Cuong (Institute of Ecology and Biological Resources VAST). Voucher specimens were deposited at three herbaria located respectively at the Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology; the Department of Pharmacognosy, Hue University of Medicine and Pharmacy; and at the Vietnam National Institute of Medicinal Materials.

### 2.3. Preparation of the methanol extracts

Each dried plant sample (100 g) was ground coarsely and extracted twice with 200 mL of methanol under reflux (2 h each time). The extracts were filtered and combined. The solvent was evaporated completely under reduced pressure to give a crude methanol extract.

### 2.4. Extraction and isolation of the active compounds from A. clypearia

The dried, powdered plant materials of *A. clypearia* (5 kg) was extracted three times with MeOH by maceration at room temperature (each time 15 L for 1 week). The extracts were combined and solvent was evaporated under reduced pressure to give a MeOH residue (750 g), which was suspended in water and then successively partitioned with *n*-hexane, chloroform, ethyl acetate, and *n*-butanol. Evaporation of solvents under reduced pressure gave *n*-hexane (H, 100 g), chloroform (C, 145 g), ethyl acetate (E, 250 g), *n*-butanol (B 105 g) and water (W, 145 g) residues, respectively. The E extract, which showed the most potent XO inhibition ($IC_{50}$ value of 4.4 μg/mL) was selected for further chemical study.

The E extract (240 g) was chromatographed on a silica gel column and eluted with a gradient solvent system of *n*-hexane–acetone (100:1–0:1) to afford eight fractions E1–E8, respectively. The fraction E3 was further chromatographed on a silica gel column eluting with chloroform/MeOH (5:1, v/v) to give five fractions E3A–E3E. Fraction E3D was separated by Sephadex LH-20 column chromatography eluted with MeOH/water (4:1, v/v) to give smaller fractions E3D1–E3D4. The fraction E3D2 was subjected to chromatography on a reverse phase silica gel (C-18) column, and eluted with acetone/water/formic acid (6:15:0.5) to afford sub-fractions E3D2A–E3D2E. The fraction E3D2A was purified by Sephadex LH-20 column chromatography, eluted with MeOH followed by column chromatography on silica gel eluting with chloroform/MeOH/water (3:1:0.1, v/v) to give compound 1 (15 mg). The fraction E3D2D was separated with Sephadex LH-20 column chromatography and eluted with MeOH to yield compound 2 (20 mg).

The fraction E4 was separated by column chromatography on silica gel eluting with a chloroform/acetone (10:1) to give five fractions E4A–E4E. Fraction E4B was chromatographed on a reverse phase silica gel column and eluted with acetone/water (3:2) to afford sub-fractions E4B1–E4B4. Repeated column chromatography of fraction E4B3 with a gradient solvent system of chloroform/ethyl acetate/methanol (15/1/0.1, v/v) followed by Sephadex LH-20 column chromatography using MeOH as an eluant yielded compound 3 (25 mg). The fraction E4D was purified by Sephadex LH-20 column chromatography and eluted with MeOH to give four smaller fractions E4D1–E4D4. The fraction E4D2 was chromatographed on reverse phase silica gel column eluting with MeOH/water (8:1) to afford sub-fractions E4D2A–E4D2D. Fraction E4D2B was separated by silica gel preparative TLC eluting with chloroform/MeOH/formic acid (5:1:0.1) to give compound 4 (12 mg).

The fraction E6 was chromatographed on silica gel column eluting with chloroform-acetone (1:1, v/v) to give five fractions E6A–E6E. The fraction E6B was subjected to a reverse phase silica gel column chromatography and eluted with MeOH-water (2:1) to afford sub-fractions E6B1–E6B4. The fraction E6B2 was separated by Sephadex LH-20 column chromatography and eluted with MeOH to give sub-fractions E6B2A–E6B2E. The fraction E6B2A was purified by silica gel column chromatography eluting with chloroform/MeOH/formic acid (6:1:0.1) followed by reverse phase silica gel column chromatography eluting with MeOH/water/formic acid (1:1:0.05) to yield

compound 5 (105 mg). The fraction E6B2C was chromatographed on silica gel column eluting with chloroform/MeOH (3:2, v/v) to give three fractions E6B2C1–E6B2C3. Compound 6 (20 mg) was obtained from fraction E6B2C2 by recrystallization in acetone.

### 2.5. In vitro XO assay

The XO inhibitory activity was assayed spectrophotometrically in 96-well plates, based on a modified protocol described previously by Noro *et al.* with minor modifications [11]. In brief, reagents were 70 nmol/L phosphate buffer pH 7.5, 150 nmol/L xanthine solution dissolved in the phosphate buffer (pH 7.5) and 0.01 IU/mL XO solution (diluted from a stock enzyme solution into the buffer solution). The methanol extracts were prepared initially in dimethyl sulfoxide (DMSO) followed by dilution with the buffer. A mixture consisting of 35 μL of the phosphate buffer, 50 μL test solution and 30 μL of XO solution was pre-incubated for 15 min at 25 °C. Subsequently, the reaction was initiated by the automatic addition of 60 μL of xanthine solution into the above mixture. The final reaction mixture was incubated for 30 min at 25 °C. The reaction was stopped by adding 25 μL of HCl 1 mol/L solution prior to measuring the absorbance at 290 nm on a Bio Tek Epoch microplate spectrophotometer. A blank was prepared in the same way, however the order of adding the substrate and HCl solution was reversed.

XO inhibitory activity was expressed as the inhibitory percentage (I) calculated as using the formula:

$$\text{Inhibitory percentage } (I) = (\Delta OD_{\text{control}} - \Delta OD_{\text{sample}})/\Delta OD_{\text{control}} \times 100.$$

where, $\Delta OD_{\text{control}}$ and $\Delta OD_{\text{sample}}$ are blank-corrected optical density values of control wells (in absence of test materials) and test wells (in presence of test samples). Based on the inhibitory percentage values at various concentrations, $IC_{50}$ values of potential plant samples were determined by Graphpad Prism version 5.0 Software with the sigmoidal dose–response model.

All experiments were performed in triplicate. Allopurinol, a known XO inhibitory agent, served as positive control. DMSO presented in the final reaction mixture with concentration of below 0.25% at which this solvent was demonstrated to have negligible effect on XO activity.

### 2.6. Lineweaver–Burk plots

Enzyme kinetic was determined in the absence and presence of compounds isolated from *A. clypearia*, using the XO assay methodology as described above with a range of substrate concentrations between 5 and 25 μmol/L.

### 2.7. Evaluation of in vivo anti-hyperuricemic effects

#### 2.7.1. Mice

The adult male Swiss mice (8 weeks old, weighing 28–30 g) were obtained from Centre of Experimental Animals, National Institute of Hygiene and Epidemiology (Hanoi, Vietnam). Animals were used and processed according to the suggested ethical guidelines for the care of laboratory animals, and the experimental protocols in this study were approved by the Scientific and Ethical Committee of Hanoi University of Pharmacy (701/QD-QLKH). The mice were acclimatized one week to adapt to their environment before any experimental manipulation. They were housed in 612 mm × 345 mm × 216 mm cages (Tecniplast 2000P) in an animal room (Department of Pharmacology, Hanoi University of Pharmacy) with a temperature of (25 ± 1) °C, humidity of 55%–60%, regular 12/12 h light/dark cycle, and access to standard laboratory diet and tap water freely until used. All the samples from animals subjected to the treatments were included in the data analysis.

#### 2.7.2. Drug and sample dosage and administration route

The crude methanol extract of *A. clypearia* (CME) was suspended in water. This preparation was given to mice with oral doses of 1 120, 3 730 and 11 180 mg/kg body weight ($n$ = 9). Treatment was applied once daily at 8:00 am for 5 consecutive days. In the same period, mice in a negative control group ($n$ = 10) were administrated with 0.5% sodium carboxymethylcellulose (CMC-Na), while those in a positive control group were administered allopurinol at a dose of 10 mg/kg ($n$ = 9).

#### 2.7.3. Protocols

Hyperuricemia in mice was induced by an uricase inhibitor, potassium oxonate, as described by Tung *et al.* with minor modifications [12]. Briefly, a suspension of potassium oxonate prepared in 0.5% CMC-Na was intraperitoneally injected to mice with dose of 500 mg/kg at 1 h before administration of test samples. Whole blood samples were collected from the tail vein of the mice 1 h after the final administration. The blood was allowed to clot for approximately 1 h at room temperature and then centrifuged at 3 000 r/min for 10 min and the serum was separated. The serum was stored at −20 C until used. Serum uric acid levels were measured by the phosphotungstic acid method, using standard diagnostic kits purchased from Biosystems S.A (Barcelona, Spain).

### 2.8. Statistical analysis

Data was expressed as mean ± standard error (SE). An ANOVA analysis followed by *LSD* or Dunnett's T3 post-hoc test was performed to determine the significance of differences among groups. The values of $P < 0.05$ were considered to be statistically significant. All computations were made with the statistical software SPSS 16.0.

## 3. Results

### 3.1. Screening Vietnamese medicinal plants for XO inhibitory activity

Three hundreds and eleven (311) extracts from 301 Vietnamese herbs were assayed for XO inhibition. Among these, 234 extracts showed XO inhibitory activity at 100 μg/mL (Table 1). At 50 μg/mL, 123 extracts possessed the activity while 61 were found to be active at 10 μg/mL (data not shown). Fifty seven extracts which displayed the activity at 100 μg/mL with inhibition rates of 50% or higher were subsequently evaluated to determine the $IC_{50}$ values and the results are shown in Table 1. The crude extracts possessing XO inhibitory activity with $IC_{50}$ values of less than 30 μg/mL included *Passiflora foetida* (*P. foetida*) ($IC_{50}$ 25.5 μg/mL), *Linociera ramiflora* (*L. ramiflora*)

**Table 1**

The xanthine oxidase (XO) inhibition of the potential herbal extracts.

| Scientific name | Local name | Family | Part(s) used | XO inhibition (μg/mL) | |
|---|---|---|---|---|---|
| | | | | $IC_{50}$ | 95% *CI* |
| *Archidendron clypearia* (Jack) I.C. Nielsen | Man dia | Mimosaceae | Leaf, twig | 15.6 | 14.4–17.4 |
| *Smilax poilanei* Gagnep. | Kim cang poilane | Smilacaceae | Rhizome | 20.0 | 11.7–28.1 |
| *Linociera ramiflora* (Roxb.) Wall. | Ho bi | Oleaceae | Leaf, twig | 25.4 | 17.3–33.9 |
| *Passiflora foetida* L. | Lac tien | Passifloraceae | Aerial part | 25.5 | 17.2–34.3 |
| *Clinacanthus nutans* (Burm.f.) Lindau | Manh cong | Acanthaceae | Aerial part | 30.4 | 21.7–36.6 |
| *Equisetum diffusum* D.Don | Co thap but | Equisetaceae | Aerial part | 30.8 | 24.9–37.4 |
| *Hibiscus sagittifolius* (Kurz) Merr. | Bao sam | Malvaceae | Rhizome | 31.1 | 23.2–40.0 |
| *Bridelia retusa* (L.) A.Juss. | Dom lom | Euphorbiaceae | Leaf, twig | 32.5 | 23.8–42.8 |
| *Dillenia indica* L. | So ba | Dilleniaceae | Leaf, twig | 33.9 | 26.0–43.2 |
| *Fallopia multiflora* (Thunb.) Haraldson | Ha thu o do | Polygonaceae | Rhizome | 34.3 | 21.3–51.1 |
| *Anisoptera costata* Korth. | Ven ven | Dipterocarpaceae | Leaf, twig | 36.4 | 28.8–45.4 |
| *Sabia parviflora* Wall. | Hoa khe cay nho goc | Sabiaceae | Root | 40.0 | 30.5–42.8 |
| *Leucaena leucocephala* (Lam.) De Wit | Keo dau | Mimosaceae | Seed | 43.7 | 32.8–59.7 |
| *Buchanania arborescens* (Blume) Blume | Chay lon | Anacardiaceae | Leaf, twig | 43.8 | 32.1–56.8 |
| *Wikstroemia indica* (L.) C.A Mey | Niet do | Thymelaeaceae | Root | 44.6 | 36.3–55.6 |
| *Uncaria acida* (Hunter) Roxb. | Cau dang | Rubiaceae | Twig | 46.3 | 36.8–59.5 |
| *Fernandoa brilletii* (Dop) Steenis. | Dinh thoi | Bignoliaceae | Leaf, twig | 46.3 | 29.2–44.7 |
| *Elaeocarpus grandiflorus* Sm. | Com hoa lon | Elaeocarpaceae | Leaf, twig | 48.1 | 36.2–61.8 |
| *Pandanus tectorius* Parkinson ex Du Roi | Dua dai | Pandaceae | Stem | 49.3 | 41.0–60.3 |
| *Smilax perfoliata* Lour. | Chong chong | Similacaceae | Rhizome | 49.3 | 42.1–57.0 |
| *Dracaena gracilis* Salisb. | Phat du manh | Asparagaceae | Root | 50.2 | 41.0–62.9 |
| *Leea aequata* L. | Goi hac bang | Leeaceae | Root | 50.7 | 41.4–63.6 |
| *Mussaenda cambodiana* Pierre ex Pit. | Buom bac Campuchia | Rubiaceae | Twig, leaf, flower | 51.6 | 41.4–63.7 |
| *Verbena officinalis* L. | Co roi ngua | Verbenaceae | Whole plant | 52.4 | 25.9–114.4 |
| *Rauvolfia verticillata* (Lour.) Baill. | Ba gac | Apocynaceae | Root | 53.1 | 43.1–67.6 |
| *Bauhinia lakhonensis* Gagnep. | Mong bo long xam | Caesalpiniaceae | Leaf, stem | 53.3 | 32.6–80.2 |
| *Homalomena occulta* (Lour.) Schott | Thiên niên kiện | Araceae | Rhizome | 58.1 | 49.8–67.5 |
| *Eurya ciliata* Merr. | Sum long | Theaceae | Twig, leaf | 61.9 | 53.0–73.1 |
| *Alangium chinense* (Lour.) Harms | Quang | Alangiaceae | Whole plant | 65.0 | 50.8–90.1 |
| *Baeckea frutescens* L. | Choi xue | Myrtaceae | Aerial part | 66.2 | 33.1–202.9 |
| *Macrosolen cochinchinensis* (Lour.)Tiegh. | Tam gui | Loranthaceae | Leaf, stem | 68.9 | 54.3–88.7 |
| *Anoectochilus roxburghii* (Wall.) Lindl. | Kim tuyen Sapa | Orchidaceae | Whole plant | 69.1 | 57.4–85.2 |
| *Allamanda cathartica* L. | Day huynh | Apocynaceae | Leaf, stem | 71.2 | 59.6–85.6 |
| *Osmanthus matsumuranus* Hayata | Vo san la dai | Oleaceae | Twig, leaf | 73.6 | 63.1–87.4 |
| *Lagerstroemia loudonii* Teysm. & Binn. | N/A | Lythraceae | Leaf, twig | 74.2 | 62.5–88.7 |
| *Selaginella uncinata* (Desv. ex Poir.) Spring | Quyen ba | Selaginellaceae | Whole plant | 75.4 | 47.1–147.7 |
| *Diospyros crumenata* Thwaites | Thi den | Ebenaceae | Leaf, twig | 77.2 | 63.6–95.3 |
| *Solanum torvum* Sw. | Ca dai hoa trang | Solanaceae | Stem, leaf | 77.9 | 46.8–179.9 |
| *Callicarpa arborea* Roxb. | Tu hu go | Verbenaceae | Leaf, twig | 78.3 | 61.8–102.8 |
| *Flueggea spirei* Beille | N/A | Euphorbiaceae | Leaf, twig | 80.0 | 37.4–219.8 |
| *Cissus triloba* (Lour.) Merr. | Chia voi | Vitaceae | Whole plant | 80.9 | 54.6–144.9 |
| *Saraca dives* Pierre | Vang anh | Fabaceae | Leaf, twig | 83.4 | 47.1–200.0 |
| *Elsholtzia penduliflora* W.W. Sm. | Kinh gioi ru | Lamiaceae | Aerial part | 83.5 | 64.8–115.9 |
| *Persicaria lanigera* (R.Br.) Soják | Nghe trang | Polygonaceae | Leaf | 84.8 | 74.3–98.4 |
| *Piper thomsonii* (C. DC.) Hook. f. | Tieu ba vi | Piperaceae | Aerial part | 85.7 | 69.4–111.4 |
| *Elaeocarpus stipularis* Blume | Com la kem | Elaeocarpaceae | Leaf, twig | 85.9 | 44.3–269.5 |
| *Eriobotrya bengalensis* (Roxb.) Hook.f. | Ty Ba Nam A | Rosaceae | Leaf, twig | 91.3 | 73.2–119.7 |
| *Triumfetta bartramia* L. | N/A | Tiliaceae | Whole plant | 91.5 | 74.2–118.0 |
| *Isodon lophanthoides* (Buch.-Ham. ex D.Don) H.Hara | Mat gau | Lamiaceae | Whole plant | 94.5 | 69.4–148.2 |
| *Helicteres hirsuta* Lour. | An dien long | Rubiaceae | Whole plant | 94.5 | 78.9–118.1 |
| *Crotalaria assamica* Benth. | Luc lac la oi | Fabaceae | Twig, leaf | 94.9 | 64.0–183.3 |
| *Homalium dasyanthum* Warb. | Cha ran hoa nham | Flacourtiaceae | Leaf, twig | 97.3 | 76.4–132.8 |
| *Ardisia gracillima* K. Larsen & C.M.Hu | Sang trang | Myrsinaceae | Aerial part | 98.2 | 81.8–123.6 |
| *Decaspermum parviflorum* (Lam.) A.J.Scott. | Thap tu hoa thua | Myrtaceae | Leaf, twig | 100.6 | 66.5–207.9 |
| *Uncaria cordata* (Lour.) Merr. | Cau dang | Rubiaceae | Twig with hook | 110.3 | 89.1–156.2 |
| *Hedyotis capitellata* Wall. ex G.Don | Da cam | Rubiaceae | Aerial part | 121.4 | 108.1–139.8 |
| *Prunella vulgaris* L. | Ha kho thao | Lamiaceae | Spike | 123.0 | 93.7–199.2 |

A total of 57 crude methanol extracts which displayed the XO inhibitory activity at 100 μg/mL with inhibition rates of 50% or higher in a screening experiment were subjected to *in vitro* XO assay based on spectrophotometric measurement of uric acid formation from xanthine to determine the $IC_{50}$ values. These values and their 95% confidence intervals (95% *CI*) were calculated using Graphpad Prism version 5.0 Software with the sigmoidal dose–response model. The results are sorted in order of decreasing potency.

($IC_{50}$ 25.4 μg/mL), *Smilax poilanei* (*S. poilanei*) ($IC_{50}$ 20.0 μg/mL) and *Archidendron clypearia* ($IC_{50}$ 15.6 μg/mL). In addition, 17 others also possessed XO inhibitory activity with $IC_{50}$ values below 50 μg/mL.

### 3.2. XO inhibitory activity of the fractions from the methanol extract of A. clypearia

All fractions obtained from the methanol extract of *A. clypearia* were assayed for XO inhibitory activity. The results (Table 2) showed that fraction E possessed the most potent XO inhibition ($IC_{50}$ 4.4 μg/mL), which followed by the fraction H ($IC_{50}$ 22.4 μg/mL), C ($IC_{50}$ 23.6 μg/mL) and B ($IC_{50}$ 42.3 μg/mL). The activity remained in aqueous residue was negligible. From these results, the fraction E was chosen for further phytochemical investigation.

### 3.3. Isolation of compounds from A. clypearia

Six compounds were isolated from the E extract of *A. clypearia*. Below are physicochemical parameters and spectroscopic data of the isolated compounds. Based on the analysis of the spectroscopic data and in comparison with those in the literature, the structures of compounds were identified to be 1-octacosanol (1) [13], docosenoic acid (2) [14], daucosterol (3) [13], methyl gallate (4) [15], quercitrin (5) [16] and (−)-7-O-galloyltricetiflavan (6) [17].

1-Octacosanol (1). White needles. mp 72–73 °C. ESI-MS *m/z* 411.1 $[M+H]^+$ ($C_{28}H_{58}O$). $^1$H-NMR (500 MHz, $CDCl_3$) δ (ppm): 3.64 (2H, t, *J* = 7.0 Hz, H-1), 1.57 (2H, m, H-2), 1.25–1.34 (50H, H-3 to H-27), 0.88 (3H, t, *J* = 7.0, H-28). $^{13}$C-NMR (125 MHz, $CDCl_3$) δ (ppm): 63.1 (C-1), 32.8 (C-2); 31.9 (C-3); 29.7 (C-4 to C-24); 29.4 (C-25); 25.8 (C-26); 22.7 (C-27); 14.1 (C-28) [13].

Docosenoic acid (2). White solid. mp 33–34 °C. ESI-MS *m/z* 337.1 $[M-H]^+$ ($C_{22}H_{42}O_2$) $^1$H-NMR (500 MHz, $CDCl_3$), δ (ppm), *J* (Hz): 5.34 (2H, m, CH=CH), 2.34 (2H, t, *J* = 7.5 Hz, H-2), 2.00 (4H, m), 1.63 (2H, m), 1.14–1.42 (28H, m), 0.88 (3H, t, *J* = 7.5 Hz, H-22). $^{13}$C-NMR (125 MHz, $CDCl_3$, δ, ppm): 180.2 (C-1), 129.9 (C-11), 129.6 (C-12), 34.0 (C-2), 31.9 (C-3), 29.7–29.0 (12C), 27.2, 27.1, 24.6, 22.6, 14.0 (C-22) [14].

Daucosterol (3) White crystals. ESI-MS *m/z* 577.1 $[M+H]^+$ ($C_{35}H_{60}O_6$). $^1$H-NMR (500 MHz, $CDCl_3$+ $CD_3OD$), δ (ppm): 5.37 (1H, d, *J* = 8.0 Hz, H-6), 4.41 (1H, d, *J* = 7.5 Hz, Glc-1), 3.84 (1H, dd, *J* = 12.0; 5.0 Hz, Glc-6), 3.76 (1H, dd, *J* = 12.0; 3.0 Hz, Glc-6), 3.58 (1H, m, H-3), 3.26–3.47 (4H, m, Glc-2; Glc-3, Glc-4, Glc-5), 1.01 (3H, s, H-19), 0.93 (3H, d, *J* = 6.5 Hz, H-21), 0.85 (3H, t, *J* = 8.0 Hz, H-29), 0.83 (3H, d, *J* = 6.5 Hz, H-27), 0.82 (3H, d, *J* = 6.5 Hz, H-26), 0.69 (3H, s, H-18). $^{13}$C-NMR (125 MHz, $CDCl_3$+ $CD_3OD$) δ (ppm): 139.9 (C-5), 121.5 (C-6), 100.7 (Glc-1), 78.6 (C-3), 76.1 (Glc-3), 75.6 (Glc-5), 73.2 (Glc-2), 69.8 (Glc-4), 61.3 (Glc-6), 56.3 (C-14), 55.6 (C-17), 49.8 (C-9), 45.5 (C-24), 41.9 (C-13), 39.3 (C-12), 38.2 (C-4), 36.8 (C-1), 36.2 (C-10), 35.7 (C-20), 33.5(C-22), 31.5 (C-7), 31.5 (C-8), 29.2 (C-2), 28.7 (C-25), 27.7 (C-16), 25.6 (C-23), 23.8 (C-15), 22.6 (C-28), 20.6 (C-11), 19.1 (C-27), 18.7 (C-18), 18.3 (C-26), 18.2 (C-21), 11.2 (C-19), 11.2 (C-29) [13].

Methyl gallate (4) Yellow oil. ESI-MS *m/z* 185.1 $[M+H]^+$. $^1$H-NMR (500 MHz, $CD_3OD$), δ (ppm): 7.06 (2H, s, H-2, H-6), 3.83 (3H, s, OMe). $^{13}$C-NMR (125 MHz, $CD_3OD$) δ (ppm): 169.0 (C-7), 146.4 (C-3 and C-5), 139.7 (C-4), 121.5 (C-1), 110.1 (C-2 and C-6), 52.2 (OMe) [15].

Quercitrin (5) Yellow powder; ESI-MS *m/z* 447.1 $[M-H]^-$. $^1$H-NMR (500 MHz, $CD_3OD$) δ (ppm): 7.35 (1H, d, *J* = 2.0 Hz, H-2′), 7.32 (1H, dd, *J* = 2.0, 8.0 Hz, H-6′), 6.93 (1H, d, *J* = 8.0 Hz, H-5′), 6.38 (1H, d, *J* = 2.0 Hz, H-8), 6.21 (1H, d, *J* = 2.0 Hz, H-6), 5.31 (1H, d, *J* = 1.5 Hz, H-1″), 4.24 (1H, m, H-2″), 3.77 (1H, m, H-3″), 3.09 (2H, m, H-4″, 5″), 0.96 (d, *J* = 6.5 Hz, H-6″). $^{13}$C-NMR ($CD_3OD$) δ (ppm): 176.8 (C-4), 166.2 (C-7), 161.0 (C-5), 156.7 (C-2), 156.2 (C-9), 149.2 (C-4′), 145.7 (C-3′), 133.6 (C-3), 121.9 (C-6′), 120.7 (C-1′), 115.5 (C-2′), 115.4 (C-5′), 102.0 (C-10), 101.7 (C-1″), 99.8 (C-6), 94.2 (C-8), 71.3 (C-4″), 70.9 (C-5″), 70.4 (C-3″), 70.2 (C-2″), 17.5 (C-6″) [16].

(−)-7-O-Galloyltricetiflavan (6). brown amorphous powder; $[\alpha]_{20}^{D}$ −5.3 (c 0.8, MeOH); ESI-MS *m/z* 443.1443.1 $[M+H]^+$ ($C_{22}H_{18}O_{10}$). $^1$H-NMR (500 MHz, $CD_3OD$) δ (ppm): 7.19 (2H, s, H-2″, H-6″), 6.45 (2H, s, H-2′, 6′), 6.21 (1H, d, *J* = 2.0 Hz, H-6), 6.21 (1H, d, *J* = 2.0 Hz, H-8), 4.82 (1H, dd, *J* = 2.0 Hz, 10.0 Hz, H-2), 2.74–2.77 (1H, m, H-4a), 2.64–2.71 (1H, m, H-4b), 2.14–2.18 (1H, m, H-3a), 1.96–2.00 (1H, m, H-3a). $^{13}$C-NMR ($CD_3OD$) δ (ppm): 167.1 (C-7″), 157.7 (C-9), 157.2 (C-5), 151.4 (C-7), 146.9 (C- 3″, C-5″), 146.6 (C-3′, C-5′), 140.4 (C-4″), 134.0 (C-1′), 133.7 (C-4′), 120.8 (C-1″), 110.5 (C-2″, C-6″), 108.6 (C-10), 106.2 (C-2′, C-6′), 102.4 (C-8), 101.5 (C-6), 79.0 (C-2), 30.4 (C-4), 20.3 (C-3) [17].

### 3.4. In vitro XO inhibition of purified compounds from A. clypearia

Six compounds isolated from the fraction E of *A. clypearia* were subjected to the XO inhibitory assay. Among them, only two demonstrated remarkable XO inhibitory activity with $IC_{50}$ values of 25.5 μmol/L ((−)-7-O-galloyltricetiflavan, 95% *CI* 21.5–29.8 μmol/L) and 244.8 μmol/L (methyl gallate, 95% *CI* 206.1–274.6 μmol/L). Others did not possess the inhibitory activity which is potent enough to determine significant $IC_{50}$ values.

To elucidate the inhibition mode of the compound 6, which exhibited the most potential activity, Lineweaver–Burk plots were constructed in the presence (3 μg/mL) or absence of this compound (Figure 1). The results indicated this compound to be a noncompetitive inhibitor, which is different from allopurinol, a known competitive inhibitor with respect to XO. Therefore, this compound inhibited XO by binding only to the enzyme–substrate complex.

**Table 2**
Xanthine oxidase (XO) inhibition of fractions from *A. clypearia*.

| No. | Fractions | Amount (g) | XO inhibition (μg/mL) | |
|---|---|---|---|---|
| | | | $IC_{50}$ | 95% *CI* |
| 1 | *n*-Hexane (H) | 100 | 22.4 | 20.7–24.8 |
| 2 | Chloroform (C) | 145 | 23.6 | 20.3–30.8 |
| 3 | Ethyl acetate (E) | 250 | 4.4 | 3.6–5.2 |
| 4 | *n*-Butanol (B) | 105 | 42.3 | 26.3–54.1 |
| 5 | Residue (W) | 145 | >500.0 | |

The methanol extract of *A. clypearia* (obtained from 5 kg of dried plant materials) was extracted successively with *n*-hexane, chloroform, ethyl acetate, *n*-butanol under reflux to yield respective fractions. $IC_{50}$ and 95% confidence interval (95% *CI*) for XO inhibition of these fractions were calculated on the basis of inhibitory percentage values at various concentrations, using Graphpad Prism version 5.0 Software with the sigmoidal dose–response model.

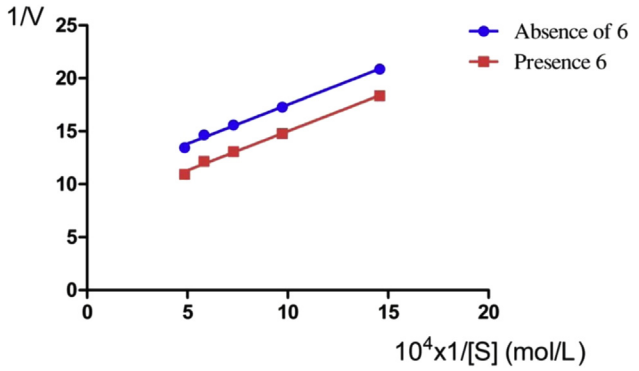


**Figure 1.** Lineweaver–Burk plots in the absence and in the presence of (−)-7-O-galloyltricetiflavan.
In the absence of (−)-7-O-galloyltricetiflavan, $K_m$ 8.97 μmol/L and $V_{max}$ 0.089; in the presence of (−)-7-O-galloyltricetiflavan, $K_m$ 7.27 μmol/L and $V_{max}$ 0.074. V, μmol substrate metabolized/(mg enzyme·min); S, substrate.

### 3.5. Anti-hyperuricemic effects of A. clypearia

Based on the present screening, the methanol extract of *A. clypearia* exhibited the strongest XO inhibitory activity with the $IC_{50}$ value of 15.6 μg/mL. To verify whether this *in vitro* XO inhibitory activity transferred into antiuricemic activity *in vivo*, the methanol extract of *A. clypearia* was evaluated by measurement of serum uric acid levels in potassium oxonate-induced hyperuricemic mice. As shown in Figure 2, compared to the hyperuricemic mice group (140.69 μmol/L), serum uric acid concentrations of groups treated with 1 120, 3 730 and 11 180 mg/kg of the methanol extract of *A. clypearia* were reduced by 22.8% ($P = 0.979$), 63.6% ($P < 0.001$) and 68.3% ($P < 0.001$), respectively. The results revealed that the anti-hyperuricemic activity of the methanol extract of *A. clypearia* was dose-dependent and only exhibited significantly with higher doses (3 730 and 11 180 mg/kg). Also, the anti-hyperuricemic effects of the methanol extract of *A. clypearia* at any dose levels were still inferior to that of allopurinol at 10 mg/kg which displayed an inhibition of 82.9% of the serum uric acid concentration compared to the negative control.

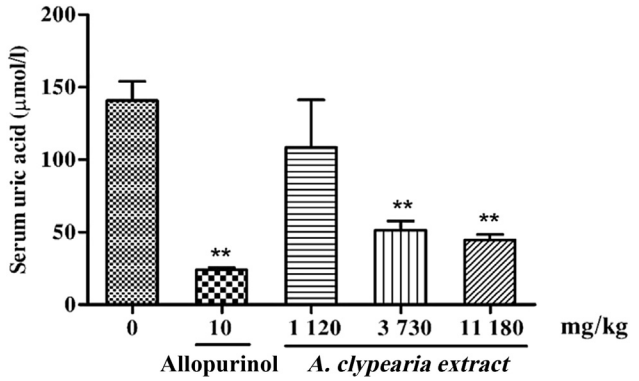


**Figure 2.** The effects of the methanol extract of *Archidendron clypearia* on serum urate levels in hyperuricemic mice.
Hyperuricemia was induced by a potassium oxonate intraperitoneal injection 1 h before the last drug administration. The methanol extract of *A. clypearia* at 1 120, 3 730 and 11 180 mg/kg and allopurinol at 10 mg/kg were orally administrated once a day for 5 consecutive days ($n = 9$ for each treatment group). The control group were orally administered with 0.5% sodium carboxymethylcellulose (CMC-Na) ($n = 10$). The serum uric acid levels of mice were measured by the phosphotungstic acid method. Values are expressed as mean ± SEM. $^{**}P < 0.01$ vs. control using ANOVA analysis.

## 4. Discussion

XO is well known as an important target in the pharmacological approach for the treatment of gout disease and hyperuricemia. Recently, there has been an emerging interest in links between increased XO activity and oxidative stress which may significantly contribute to the development of cardiovascular diseases, cancer, and many other medical conditions [18,19]. From this fact, screening of XO inhibitory activity from medicinal plants might be an effective tool to find new potential candidates for therapy of these major diseases. In the current work, we screened 311 extracts from 301 Vietnamese herbs which had been used empirically for various conditions including inflammation, pain and gout. Four medicinal plants displaying the greatest XO inhibitory activities include *P. foetida*, *L. ramiflora*, *S. poilanei* and *A. clypearia*. As far as our knowledge, this is the first report on the XO inhibitory activity of these four medicinal plants.

The aerial part of *P. foetida* is widely used to treat insomnia, diarrhea and certain inflammatory conditions in Vietnam [20]. Phytochemical studies on this plant species reported several flavonoids (e.g., apigenin, luteolin, quercetin-4′-methyl ether, vitexin, kaempferol) as major constituents, together with alkaloids, glycosides, fatty acid, and some other compounds [21,22]. Flavonoids are well known antioxidants that have been studied extensively as possible medicinal agents for diseases mediated by free radicals. Furthermore, some flavonoids including apigenin, quercetin and their derivatives also exhibited XO inhibitory activity either *in vitro* or in experimental animals [23–26]. Thus, the flavonoid constituents could be responsible for XO inhibitory activity of *P. foetida*.

Limited available data revealed that *L. ramiflora*, also known as *Chionanthus ramiflorus*, has been ethnopharmacological used in a few countries, such as Papua New Guinea and Thailand, for treatment of malnutrition (bark and leaf), uterus infection (root), and cancer (stem) [27,28]. While a decoction from the stem of *L. ramiflora* was reported to have immunosuppressant activity [29], no publication mentioned the XO inhibition-related effects of this plant. Likewise, its phytochemistry remains to be investigated. Another potent herb, identified as *S. poilanei*, appears to have not been previously recorded any bioactivity. The rhizome of this plant, however, was empirically used in management of arthritis in Vietnamese folk medicine. Moreover, anti-hyperuricemic effects of two other *Smilax* species, including *Smilax riparia* and *Smilax china*, were observed in mice [30,31]. These results suggested that *Smilax* genus with more than 300 plant species will be a promising source for screening XO inhibitory activity and therapeutic effects of gout disease.

The most active extract found in the present screening was the methanol extract from leaves and twigs of *A. clypearia*, which is traditionally used as a medicinal plant for detoxicating, cooling, reducing edema, and treating diarrhea in Vietnam, Malaysia, and Indonesia [32,33]. Recently, there have been some studies on the anti-inflammatory potency of *A. clypearia* [34,35]. However to the best of our knowledge, gout-relating activity of this plant has not been reported previously. Thus, further investigations were conducted to examine the anti-hyperuricemic effects of *A. clypearia*. The results demonstrated that

*A. clypearia* reduced the serum uric levels in a dose-dependent manner in the mouse model of hyperuricemia induced by potassium oxonate, an uricase inhibitor. Based on the *in vitro* assay for XO inhibition and the fact that *A. clypearia* also showed a reduction in hepatic XO activities in another independent experiment (data not shown), it could be suggested that there existed a close correlation between XO inhibition and the hypouricemic effects. Taking into consideration of this relationship, isolation and identification of the active constituents were guided by the XO inhibitory activity.

Since the ethyl acetate-soluble portion (fraction E) was the only fraction of the four fractions displaying superior *in vitro* activity compared to the original extract (methanol extract), the fraction E was selected for chemical investigation. Various chromatographic separation and purification carried out on the fraction E led to the isolation of six compounds, including 1-octacosanol docosenoic acid, daucosterol, methyl gallate, quercitrin, and (−)-7-O-galloyltricetiflavan. Among these, two compounds, methyl gallate and 7-O-galloyltricetiflavan, were demonstrated to be responsible, at least in part, for the XO inhibitory activity of fraction E. Methyl gallate was also isolated from some other plants and was previously reported to have a relatively weak inhibitory activity against XO [36]. On the contrary, although the conflicting data on XO inhibitory activity of quercitrin were recorded, this flavonoid derivative showed to be almost inactive in the assays [37,38]. Meanwhile, (−)-7-O-galloyltricetiflavan was reported to have antiviral activity against respiratory syncytial virus [17] and *in silico* soluble epoxide hydrolase inhibitory activity [39] but its XO inhibition has not been evaluated. Thus, this is a new finding of the biological activity of this compound.

Our present results also demonstrated (−)-7-O-galloyltricetiflavan as an uncompetitive inhibitor, which is different from allopurinol, a known competitive inhibitor with respect to XO. Accordingly, this compound inhibited XO by binding only to the enzyme–substrate complex. It should be noted, however, that the potency of (−)-7-O-galloyltricetiflavan was not stronger than that of the ethyl acetate portion ($IC_{50}$ value 11.3 vs. 4.4 μg/mL, respectively). Therefore, a synergistic addition of the XO inhibitory activity from some different constituents present in this portion, including minor constituents that have not yet been identified in our study, might be relevant. Recently, Thao *et al.* reported the isolation of several flavonoids with similar structural features compared to (−)-7-O-galloyltricetiflavan [39]. It is likely that these flavonoids are also promising as XO inhibitors.

In conclusion, our systematic *in vitro* screening study of Vietnamese medicinal plants allowed to identify a range of species possessing the XO inhibitory activity. Chemical investigation carried out on *A. clypearia*, the most active plant obtained from the screening, led to the identification of 7-O-galloyltricetiflavan as a potential XO inhibitor. Further studies on other active plants should warrant more promising XO inhibitors.

## Conflict of interest statement

We declare that we have no conflict of interest.

## Acknowledgement

This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 106.99–2012.90.

## References

[1] Maiuolo J, Oppedisano F, Gratteri S, Muscoli C, Mollace V. Regulation of uric acid metabolism and excretion. *Int J Cardiol* 2016; **213**: 8-14.
[2] Fravel MA, Ernst ME, Clark EC. Gout and hyperuricemia. In: DiPiro JT, Talbert RL, Yee GC, Matzke GR, Wells BG, Posey LM, editors. *Pharmacotherapy: a pathophysiologic approach*. 9th ed. New York: McGraw Hills; 2014, p. 3565-3601.
[3] Bove M, Cicero AF, Veronesi M, Borghi C. An evidence-based review on urate-lowering treatments: implications for optimal treatment of chronic hyperuricemia. *Vasc Health Risk Manag* 2017; **13**: 23-28.
[4] Wilson L, Saseen JJ. Gouty arthritis: a review of acute management and prevention. *Pharmacotherapy* 2016; **36**(8): 906-922.
[5] Schlesinger N. The safety of treatment options available for gout. *Expert Opin Drug Saf* 2017; **16**(4): 429-436.
[6] Nguyen MT, Awale S, Tezuka Y, Tran QL, Watanabe H, Kadota S. Xanthine oxidase inhibitory activity of Vietnamese medicinal plants. *Biol Pharm Bull* 2004; **27**(9): 1414-1421.
[7] Orban-Gyapai O, Lajter I, Hohmann J, Jakab G, Vasas A. Xanthine oxidase inhibitory activity of extracts prepared from Polygonaceae species. *Phytother Res* 2015; **29**(3): 459-465.
[8] Vanyolos A, Orban-Gyapai O, Hohmann J. Xanthine oxidase inhibitory activity of Hungarian wild-growing mushrooms. *Phytother Res* 2014; **28**(8): 1204-1210.
[9] Umamaheswari M, AsokKumar K, Somasundaram A, Sivashanmugam T, Subhadradevi V, Ravi TK. Xanthine oxidase inhibitory activity of some Indian medical plants. *J Ethnopharmacol* 2007; **109**(3): 547-551.
[10] Kong LD, Cai Y, Huang WW, Cheng CH, Tan RX. Inhibition of xanthine oxidase by some Chinese medicinal plants used to treat gout. *J Ethnopharmacol* 2000; **73**(1–2): 199-207.
[11] Noro T, Oda Y, Miyase T, Ueno A, Fukushima S. Inhibitors of xanthine oxidase from the flowers and buds of *Daphne genkwa*. *Chem Pharm Bull (Tokyo)* 1983; **31**(11): 3984-3987.
[12] Tung YT, Lin LC, Liu YL, Ho ST, Lin CY, Chuang HL, et al. Antioxidative phytochemicals from *Rhododendron oldhamii* Maxim. leaf extracts reduce serum uric acid levels in potassium oxonate-induced hyperuricemic mice. *BMC Complement Altern Med* 2015; **15**: 423-433.
[13] Aydin T, Cakir A, Kazaz C, Bayrak N, Bayir Y, Taskesenligil Y. Insecticidal metabolites from the rhizomes of *Veratrum album* against adults of Colorado potato beetle, *Leptinotarsa decemlineata*. *Chem Biodivers* 2014; **11**(8): 1192-1204.
[14] Singh S, Sharma SK, Ali M. Fatty acid esters from roots of amaranthus hybridus Linn. *Asian J Chem* 2011; **23**(1): 232-234.
[15] Madikizela B, Aderogba MA, Finnie JF, Staden JV. Isolation and characterization of antimicrobial compounds from *Terminalia phanerophlebia* Engl. & Diels leaf extracts. *J Ethnopharmacol* 2014; **156**: 228-234.
[16] Yang NY, Tao WW, Duan JA. Antithrombotic flavonoids from the faeces of *Trogopterus xanthipes*. *Nat Prod Res* 2010; **24**(19): 1843-1849.
[17] Li Y, Leung KT, Yao F, Ooi LS, Ooi VE. Antiviral flavans from the leaves of *Pithecellobium clypearia*. *J Nat Prod* 2006; **69**(5): 833-835.
[18] Okafor ON, Farrington K, Gorog DA. Allopurinol as a therapeutic option in cardiovascular disease. *Pharmacol Ther* 2017; **172**: 139-150.
[19] Pelin M, De Iudicibus S, Londero M, Spizzo R, Dei Rossi S, Martelossi S, et al. Thiopurine biotransformation and pharmacological effects: contribution of oxidative stress. *Curr Drug Metab* 2016; **17**(6): 542-549.
[20] Quattrocchi U. *CRC world dictionary of medicinal and poisonous plants: common names, scientific names, eponyms, synonyms, and etymology*. Boca Raton: CRC Press, Taylor and Francis Group; 2012, p. 2803-2804.
[21] Dhawan K, Dhawan S, Sharma A. Passiflora: a review update. *J Ethnopharmacol* 2004; **94**(1): 1-23.
[22] Nguyen YT, To DC, Tran MH, Lee JS, Lee JH, Kim JA, et al. Anti-inflammatory flavonoids isolated from *Passiflora foetida*. *Nat Prod Commun* 2015; **10**(6): 929-931.

[23] Cao H, Pauff JM, Hille R. X-ray crystal structure of a xanthine oxidase complex with the flavonoid inhibitor quercetin. *J Nat Prod* 2014; **77**(7): 1693-1699.
[24] Huang J, Wang S, Zhu M, Chen J, Zhu X. Effects of genistein, apigenin, quercetin, rutin and astilbin on serum uric acid levels and xanthine oxidase activities in normal and hyperuricemic mice. *Food Chem Toxicol* 2011; **49**(9): 1943-1947.
[25] Huo LN, Wang W, Zhang CY, Shi HB, Liu Y, Liu XH, et al. Bioassay-guided isolation and identification of xanthine oxidase inhibitory constituents from the leaves of *Perilla frutescens*. *Molecules* 2015; **20**(10): 17848-17859.
[26] Su ZR, Fan SY, Shi WG, Zhong BH. Discovery of xanthine oxidase inhibitors and/or alpha-glucosidase inhibitors by carboxyalkyl derivatization based on the flavonoid of apigenin. *Bioorg Med Chem Lett* 2015; **25**(14): 2778-2781.
[27] Jorim RY, Korape S, Legu W, Koch M, Barrows LR, Matainaho TK, et al. An ethnobotanical survey of medicinal plants used in the eastern highlands of Papua New Guinea. *J Ethnobiol Ethnomed* 2012; **8**: 47.
[28] Chuakul W, Saralamp P, Boonpleng A. Medicinal plants used in the Kutchum District, Yasothon province, Thailand. *Thai J Phytopharm* 2002; **9**(1): 22-49.
[29] Prasitpuriprecha C, Sripanidkulchai B, Lulitanond V, Saguansermsri J. Evaluation of immunomodulating activity of selected Thai medicinal plants by lymphocytes proliferation assay. *ISAN J Pharm Sci* 2006; **2**(1): 53-62.
[30] Chen L, Yin H, Lan Z, Ma S, Zhang C, Yang Z, et al. Anti-hyperuricemic and nephroprotective effects of *Smilax china* L. *J Ethnopharmacol* 2011; **135**(2): 399-405.
[31] Wu XH, Wang CZ, Wang SQ, Mi C, He Y, Zhang J, et al. Anti-hyperuricemia effects of allopurinol are improved by *Smilax riparia*, a traditional Chinese herbal medicine. *J Ethnopharmacol* 2015; **162**: 362-368.
[32] Anderson EF. Ethnobotany of hill tribes of northern Thailand. II. Lahu medicinal plants. *Econ Bot* 1986; **40**(4): 442-450.
[33] Cheng SC. *Chinese medicinal herbs of Hong Kong*. HongKong: Commercial Press; 1994, p. 6.
[34] Yang WS, Jeong D, Nam G, Yi YS, Yoon DH, Kim TW, et al. AP-1 pathway-targeted inhibition of inflammatory responses in LPS-treated macrophages and EtOH/HCl-treated stomach by *Archidendron clypearia* methanol extract. *J Ethnopharmacol* 2013; **146**(2): 637-644.
[35] Yang WS, Lee J, Kim TW, Kim JH, Lee S, Rhee MH, et al. Src/NF-kappaB-targeted inhibition of LPS-induced macrophage activation and dextran sodium sulphate-induced colitis by *Archidendron clypearia* methanol extract. *J Ethnopharmacol* 2012; **142**(1): 287-293.
[36] Pham AT, Malterud KE, Paulsen BS, Diallo D, Wangensteen H. DPPH radical scavenging and xanthine oxidase inhibitory activity of *Terminalia macroptera* leaves. *Nat Prod Commun* 2011; **6**(8): 1125-1128.
[37] Cioffi G, D'Auria M, Braca A, Mendez J, Castillo A, Morelli I, et al. Antioxidant and free-radical scavenging activity of constituents of the leaves of *Tachigalia paniculata*. *J Nat Prod* 2002; **65**(11): 1526-1529.
[38] Jung SJ, Kim DH, Hong YH, Lee JH, Song HN, Rho YD, et al. Flavonoids from the flower of *Rhododendron yedoense* var. *poukhanense* and their antioxidant activities. *Arch Pharm Res* 2007; **30**(2): 146-150.
[39] Thao NP, Luyen BT, Kim JH, Jo AR, Dat NT, Kiem PV, et al. Identification, characterization, kinetics, and molecular docking of flavonoid constituents from *Archidendron clypearia* (Jack.) Nielsen leaves and twigs. *Bioorg Med Chem* 2016; **24**(14): 3125-3132.