Xanthine Oxidase Inhibitory Activity of Vietnamese Medicinal Plants

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Among 288 extracts, prepared from 96 medicinal plants used in Vietnamese traditional medicine to treat gout and related symptoms, 188 demonstrated xanthine oxidase (XO) inhibitory activity at 100 $\mu\text{g/ml}$, with 46 having greater than 50% inhibition. At 50 $\mu\text{g/ml}$, 168 of the extracts were active, with 21 possessing more than 50% inhibition. At 25 $\mu\text{g/ml}$, 146 extracts exhibited inhibitory activity, with 8 showing over 50% inhibition, while 126 extracts presented activity at 10 $\mu\text{g/ml}$, with 2 having greater than 50% inhibition. The MeOH extracts of *Artemisia vulgaris*, *Caesalpinia sappan* (collected at the Seven-Mountain area), *Blumea balsamifera* (collected in Lam Dong province), *Chrysanthemum sinense* and MeOH–H₂O extract of *Tetracera scandens* (Khanh Hoa province) exhibited strong XO inhibitory activity with IC₅₀ values less than 20 $\mu\text{g/ml}$. The most active extract was the MeOH extract of the flower of *C. sinense* with an IC₅₀ value of 5.1 $\mu\text{g/ml}$. Activity-guided fractionation of the MeOH extract led to the isolation of caffeic acid (1), luteolin (2), eriodictyol (3), and 1,5-di-*O*-caffeoylquinic acid (4). All these compounds showed significant XO inhibitory activity in a concentration-dependent manner, and the activity of 2 was more potent (IC₅₀ 1.3 μM) than the clinically used drug, allopurinol (IC₅₀ 2.5 μM).

Key words Vietnamese medicinal plant; xanthine oxidase inhibitory activity; gout; *Chrysanthemum sinense*

Natural products are excellent sources of lead compounds in the search for new medicaments for the treatment of diseases. The largest present underexplored source of such materials lies in tropical and subtropical regions of the world. In these areas, a long tradition of ethnobotanical medicine often exists and offers a rich and relatively untapped source for the discovery of new drugs from natural products. Vietnam, a tropical Southeast Asian country, also has a long history of traditional medicine systems.¹⁾ However, systematic exploitation of these natural resources for their human health benefits has not been carried out to a significant degree.

Gout is a common disease with a worldwide distribution. Hyperuricemia, which is associated with gout, results from the overproduction or underexcretion of uric acid and is greatly influenced by a high dietary intake of foods rich in nucleic acids, such as meats (especially organ meats), leguminous seeds, some types of seafood, and food yeasts.^{2,3)} During the last step of purine metabolism, xanthine oxidase (XO) catalyses the oxidation of xanthine and hypoxanthine into uric acid.⁴⁾ Uricosuric drugs which increase the urinary excretion of uric acid, or XO inhibitors which block the terminal step in uric acid biosynthesis, can lower the plasma uric acid concentration, and are generally employed for the treatment of gout.⁵⁾ Allopurinol is a clinically used XO inhibitor in the treatment of gout, but this drug suffers from many side effects such as hepatitis, nephropathy, and allergic reactions.⁶⁾ Thus, new alternatives with increased therapeutic activity and less side effects are desired. Moreover, superoxide anion radicals generated by XO are involved in various pathological states such as hepatitis, inflammation, ischemia-reperfusion, carcinogenesis, and aging.^{2,7)} Thus, the search for novel XO inhibitors would be beneficial not only to treat gout but also to combat various other diseases.

To identify potential XO inhibitory agents from natural sources, we have tested 288 extracts prepared from 96 selected medicinal plants, which are used by the indigenous people in Vietnam for the treatment of gout or diseases asso-

ciated with symptoms such as rheumatism, arthritis and inflammation. In addition, the active constituents of *Chrysanthemum sinense*, which showed the most potent XO inhibitory activity, have been determined.

MATERIALS AND METHODS

Plant Materials Vietnamese medicinal plants used in this study were collected at the Seven-Mountain area in An Giang province on March 1998, Lam Dong province in May 1998 and November 2001, Khanh Hoa province in November 2002, and Phu Yen province in November 2002 (Table 1). The plants collected at the Seven-Mountain area were identified by Prof. Le Cong Kiet, Department of Botany, University of Ho Chi Minh City, Ho Chi Minh City, Vietnam, while other plants were identified by Mr. Nguyen Duy Chinh, Department of Resource and Environment, Dalat University, Dalat City, Lam Dong province, Vietnam. The voucher specimens are preserved at the Museum of Materia Medica, Research Center for Ethnomedicines, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan.

Chemicals Xanthine oxidase (EC 1.2.3.2) from bovine milk (10 units/ml) and xanthine were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Allopurinol was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Other reagents were of the highest grade available.

Preparation of Samples Each medicinal plant (10–213 g) was cut into small pieces and extracted successively with MeOH (200–300 ml, reflux, 2 h, $\times 3$), MeOH–H₂O (1 : 1, 200–300 ml, reflux, 2 h, $\times 2$), and water (200–300 ml, reflux, 2 h). The MeOH solution was evaporated under reduced pressure to give a MeOH extract, while MeOH–H₂O (1 : 1) and water solutions were concentrated under reduced pressure and lyophilized to give MeOH–H₂O (1 : 1) and H₂O extracts, respectively.

Assay of XO Activity The XO inhibitory activity was

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assayed spectrophotometrically under aerobic conditions, based on the procedure reported by Noro *et al.*,⁸⁾ with modification by using 96-well plates. The assay mixture consisting of 50 μ l of test solution, 35 μ l of 70 mM phosphate buffer (pH=7.5), and 30 μ l of enzyme solution (0.01 units/ml in 70 mM phosphate buffer, pH=7.5) was prepared immediately before use. After preincubation at 25 °C for 15 min, the reaction was initiated by the addition of 60 μ l of substrate solution (150 μ M xanthine in the same buffer). The assay mixture was incubated at 25 °C for 30 min. The reaction was stopped by adding 25 μ l of 1 N HCl, and the absorbance at 290 nm was measured with a Perkin-Elmer HTS-7000 Bio Assay Reader (Norwalk, CT, U.S.A.). A blank was prepared in the same way, but the enzyme solution was added to the assay mixture after adding 1 N HCl. One unit of XO is defined as the amount of enzyme required to produce 1 μ mol of uric acid/min at 25 °C.

XO inhibitory activity was expressed as the percentage inhibition of XO in the above assay system, calculated as $(1-B/A) \times 100$, where *A* and *B* are the activities of the enzyme without and with test material. IC₅₀ values were calculated from the mean values of data from four determinations.

The crude extracts were dissolved initially in DMSO followed by dilution with the buffer; the final concentration of DMSO was less than 0.25%. Allopurinol, a known inhibitor of XO, was used as a positive control.

Extraction and Isolation of the Active Compounds from the Flower of *Chrysanthemum sinense* Dried flower (10.7 g) of *C. sinense* was extracted with MeOH (300 ml, $\times 3$) under reflux for 2 h, to yield a MeOH extract (1.4 g; IC₅₀ value, 5.06 μ g/ml). The MeOH extract (650 mg) was chromatographed on silica gel with a MeOH-CHCl₃ solvent system to give six fractions: fr. 1 (107 mg; IC₅₀, >100 μ g/ml), fr. 2 (152 mg; IC₅₀, >100 μ g/ml), fr. 3 (49.6 mg; IC₅₀, 1.3 μ g/ml), fr. 4 (49.6 mg; IC₅₀, 6.3 μ g/ml), fr. 5 (49.6 mg; IC₅₀, 11.9 μ g/ml), fr. 6 (49.6 mg; IC₅₀, 78.8 μ g/ml). Fraction 3 was separated by reversed-phase preparative TLC with CH₃CN-MeOH-H₂O (1:1:2) to give caffeic acid²⁾ (1, 3.6 mg) and luteolin⁹⁾ (2, 4.9 mg). Fraction 4 was separated by reversed-phase preparative TLC with CH₃CN-MeOH-H₂O (1:1:2) to give eriodictyol¹⁰⁾ (3, 1.7 mg), while fraction 5 was subjected to reversed-phase preparative TLC with acetone-CH₃CN-H₂O (2:2:7) to give 1,5-di-*O*-caffeoylquinic acid¹¹⁾ (4, 6.4 mg). Their structures were identified by spectral analysis and comparison of their data with those in the literature.

RESULTS AND DISCUSSION

Xanthine oxidase (XO) is an enzyme that has been investigated for decades. Natural XO inhibitors were reported from a variety of plants used in traditional herbal medicines for the treatment of gout and rheumatism in China,¹²⁾ Australia,¹³⁾ North America,³⁾ Chile,¹⁴⁾ Paraguay,¹⁵⁾ and Panama.¹⁶⁾ Vietnam, a country possessing a long history of traditional medicine system, also has a number of medicinal plants used for gout and rheumatism, but no systematic investigations have been reported until now. In the present study, 96 plants from Vietnam were selected based on their ethnomedical use for the treatment of rheumatism, arthritis, and gout by the natives of this region (Table 1) and were successively extracted

with MeOH, MeOH-H₂O (1:1) and H₂O to give 288 crude extracts. All of these extracts were tested for their XO inhibitory activity to identify potential anti-gout agents. The assay was carried out at four different concentrations of extract ranging from 10–100 μ g/ml (Table 2).

Of the extracts assayed, 188 extracts (65.3%) demonstrated XO inhibitory activity at 100 μ g/ml, among which 46 (24.5%) showed an inhibition rate greater than 50%. Altogether, 168 extracts (58.3%) were found to be active at a concentration of 50 μ g/ml, among which 21 (12.5%) showed inhibition of more than 50%. At 25 μ g/ml, 146 extracts (50.7%) were active, and eight (5.5%) showed an inhibition of over 50%. Of the extracts assayed, 126 (43.8%) displayed activity at 10 μ g/ml, including two (1.6%) of over 50% inhibition. In total, 49 [31 MeOH, 15 MeOH-H₂O (1:1), and three H₂O] extracts showed IC₅₀ values below 100 μ g/ml. In general, the MeOH extracts were found to be more active than the MeOH-H₂O and H₂O extracts.

The crude extracts possessing XO inhibitory activity with IC₅₀ values less than 20 μ g/ml were MeOH extracts of *Artemisia vulgaris* (IC₅₀, 14.7 μ g/ml) and *Caesalpinia sappan* (IC₅₀, 14.2 μ g/ml) from the Seven-Mountain area, *Blumea balsamifera* (IC₅₀, 6.0 μ g/ml) from Lam Dong province, and *Chrysanthemum sinense* (IC₅₀, 5.1 μ g/ml) from Khanh Hoa province and the MeOH-H₂O extract of *Tetracera scandens* from Khanh Hoa province (IC₅₀, 15.6 μ g/ml). Although these plants are used in Vietnamese folk medicine to treat of rheumatism and inflammatory diseases,¹⁷⁾ this is the first report on their XO inhibitory activity.

The aerial part of *A. vulgaris* is widely used for the treatment of rheumatism and fever in Vietnam. The phytochemical studies on this plant species reported that it contains a number of flavonoids (e.g., apigenin, eriodictyol, kaempferol, luteolin) as the major constituents, together with monoterpenes, sesquiterpene lactones, and other compounds.^{18–20)} Flavonoids are well known antioxidants and attract a tremendous amount of interest among researchers as possible therapeutic agents for diseases mediated by free radicals. Flavonoids are also effective inhibitors of several enzymes including XO, cyclooxygenase, and lipooxygenase.^{21–23)} Thus, the putative therapeutic effects of *A. vulgaris* and its XO inhibitory activity are ascribed to its flavonoid constituents. Another Asteraceae plant, *B. balsamifera*, is also reported to contain a number of flavonoids inhibiting XO.^{21–25)}

It is interesting to note that the wood of *C. sappan* possesses various biological activities such as antioxidative, anti-inflammatory, hepatoprotective, cytotoxic, and hypoglycemic activity. Among the activities, the antioxidative activity is the most widely studied, and was attributed to the presence of phenolic compounds such as brazilin and flavanoids.^{26,27)} Two phenolic compounds isolated from *C. sappan*, 1',4'-dihydro-spiro[benzofuran-3(2H),3'-[3H-2]benzopyran]-1',6',6',7'-tetrol and 3-[[4,5-dihydroxy-2-(hydroxymethyl)-phenyl]methyl]-2,3-dihydro-3,6-benzofurandiol, were reported to inhibit XO.²⁸⁾ Thus, the phenolic constituents may play an essential role in the inhibition of XO by *C. sappan*. Although all three extracts of *T. scandens* showed XO inhibitory activity, there are no scientific reports on their chemical constituents and biological activity. However, the presence of flavonoids such as derivatives of quercetin, kaempferol, apigenin, luteolin, and myricetin, have been

Table 1. Vietnamese Medicinal Plants Used in This Study, Their Families, Part Used, Local Name, Therapeutic Applications, and Voucher Specimen Number (TMPW No.)

Plant name	Family	Part used	Local name	Therapeutic application	TMPW No.
Collected at the Seven-Mountain Area, Tinh Bien District, An Giang Province (1998)					
<i>Angelica dahurica</i> (Fisch. ex Hoffm.) Benth. et Hook. f.	Umbelliferae	Aerial part	Bach chi	Fever, rheumatism, leucorrhea	20415
<i>Artemisia vulgaris</i> L.	Asteraceae	Leaf	Ngai diep	Antibacteria, fever, rheumatism, leucorrhea	20442
<i>Asparagus cochinchinensis</i> (Lour.) Merr.	Liliaceae	Root	Thien mon	Tuberculosis, inflammation, diabetes, breast cancer	20421
<i>Borassus flabellifer</i> L.	Araceae	Flower	Thot not bong	Diuretic, anthelmintic, inflammation	20419
<i>Caesalpinia sappan</i> L.	Caesalpiniaceae	Wood	To moc	Diarrhoea, inflammation, rheumatism	20454
<i>Ceiba pentandra</i> (L.) Gaertn.	Bombacaceae	Fruit	Gon	Diarrhoea, inflammation, malaria	20428
<i>Cinnamomum iners</i> Reinw. ex Blume	Lauraceae	Bark	Hau phac nam	Rheumatism, tonic for stomach	20431
<i>Combretum quadrangulare</i> Kurz.	Combretaceae	Aerial part	Tram bau	Anthelmintic, hepatitis, inflammation	20456
<i>Cyperus rotundus</i> L.	Cyperaceae	Rhizome	Co gau	Menstrual disorders, uterus inflammation, diarrhea	20423
<i>Desmodium heterophyllum</i> (Willd.) DC.	Fabaceae	Aerial part	Han the	Fever, inflammation	20430
<i>Elsholtzia ciliata</i> (Thunb.) Hyland.	Lamiaceae	Aerial part	Kinh gioi	Tuberculosis, antibacterial	20436
<i>Euphorbia tirucalli</i> L.	Euphorbiaceae	Stem	Xuong kho	Inflammation, antibacterial	20459
<i>Glycyrrhiza uralensis</i> Fisch.	Fabaceae	Root	Cam thao	Tonic, inflammation, diarrhea, Addison's disease	20420
<i>Hedyotis diffusa</i> Willd.	Rubiaceae	Aerial part	Luoi ran trang	Inflammation, hepatitis, antitumor	20416
<i>Hydnophytum formicarum</i> Jack.	Rubiaceae	Rhizome	Bi ky nam	Hepatitis, rheumatism, diarrhea	20418
<i>Lasia spinosa</i> (L.) Thw.	Araceae	Whole plant	Mop gai	Inflammation, rheumatism	20440
<i>Leonurus heterophyllum</i> Sweet.	Lamiaceae	Aerial part	Ich mau	Menstrual disorder, inflammation, tonic for women	20433
<i>Lavanga scandens</i> (Roxb.) Buch.-Ham.	Rutaceae	Branchlet	Than xa huong	Rheumatism, fever	20453
<i>Melaleuca leucadendra</i> (L.) L.	Myrtaceae	Stem, fruit	Tram	Fever, rheumatism, diarrhea	20455
<i>Mitusa velutina</i> (Dun.) Hook. f. et Thoms.	Amonaceae	Wood	Co sen	Inflammation, antibacterial	20424
<i>Nauclaea officinalis</i> (Pitt.) Merr.	Rubiaceae	Fruit	Huynh ba	Inflammation	20432
<i>Orthosiphon stamineus</i> Benth.	Lamiaceae	Aerial part	Rau meo	Diuretic, inflammation	20448
<i>Panicum repens</i> L.	Poaceae	Aerial part	Cu co ong	Rheumatism, inflammation, leucorrhea	20425
<i>Parameria laevigata</i> (Juss.) Moldenk.	Apocynaceae	Stem	Do trong day	Rheumatism, hypertension	20460
<i>Polanisia chelidonii</i> (L. f.) A. DC	Capparaceae	Aerial part	Man ri tia	Fever, inflammation	20438
<i>Rhinacanthus nasutus</i> (L.) Kurz	Acanthaceae	Aerial part	Kien co	Tuberculosis, inflammation, rheumatism, hypertension	20434
<i>Sansevieria cylindrica</i> Bojer.	Agavaceae	Leaf	Ngai nga	Inflammation	20443
<i>Tinospora cordifolia</i> (Willd.) Miess.	Menispermaceae	Stems	Than thong	Malaria, fever, inflammation	20452
Collected at Da Lat City, Lam Dong Province (1998)					
<i>Ageratum conyzoides</i> L.	Compositae	Aerial part	Cay cut lon	Inflammation	20462
<i>Andrographis paniculata</i> (Burm. f.) Nees	Acanthaceae	Aerial part	Xuyen tam lien	Inflammation, hypertension	20626
<i>Artemisia apiacea</i> Hance ex Walp.	Compositae	Aerial part	Thach hao	Malaria, inflammation	20621
<i>Codonopsis javanica</i> (Blume.) Hook. f.	Campanulaceae	Root	Dang sam	Tonic, leukemia, inflammation, hepatitis	20466
<i>Coscinium fmesistratum</i> (Gaertn.) Colber.	Menispermaceae	Stem	Hoang dang	Malaria, diarrhea, inflammation	20606
<i>Datura metal</i> L.	Solanaceae	Flower	Ca doc duoc	Asthma, inflammation	20463
<i>Eleutherine bulbosa</i> (Müll.) Urb.	Iridaceae	Bulb	Sam dai hanh	Cough, inflammation	20618
<i>Eucommia ulmoides</i> Oliv.	Eucommiaceae	Bark	Do trong	Hypertension, rheumatism	20468
<i>Gynopetatum cochinchinensis</i> Kurz	Cucurbitaceae	Stem, leaf	Cut qua	Cough, inflammation	20465
<i>Heliotropium indicum</i> L.	Boraginaceae	Aerial part	Voi voi	Inflammation	20625
<i>Kalanchoe pinnata</i> (Lam.) Pers.	Crassulaceae	Aerial part	Truong sinh	Antibacteria, inflammation	20624
<i>Lonicera japonica</i> Thunb.	Caprifoliaceae	Flowers	Kim ngan	Inflammation, rheumatism	20609
<i>Luffa cylindrica</i> (L.) Roem.	Cucurbitaceae	Fruit	Muop gai	Cough, inflammation	20614
<i>Mimosa pudica</i> L.	Mimosaceae	Aerial part	Mac co	Inflammation, hepatitis, hypertension	20612
<i>Phyllanthus amarus</i> Schum. et Thonn.	Euphorbiaceae	Aerial part	Cho de rang cua	Inflammation, hepatitis	20464
<i>Piper lolot</i> C. DC.	Piperaceae	Aerial part	La lot	Rheumatism, diarrhea	20610
<i>Plantago major</i> L.	Plantaginaceae	Aerial part	Ma de	Inflammation	20613
<i>Polyscias frutescens</i> (L.) Harms	Araliaceae	Stem, leaf	Dinh lang	Tonic, inflammation	20467
<i>Schefflera octophylla</i> (Lour.) Harms	Araliaceae	Bark	Ngu gia bi	Tonic, inflammation	20615
<i>Smilax glabra</i> Roxb.	Smilacaceae	Rhizome	Tho phuc linh	Inflammation	20623
<i>Sophora flavescens</i> Ait.	Fabaceae	Root	Kho sam	Inflammation, diarrhea	20608

Table 1. (continued)

Plant name	Family	Part used	Local name	Therapeutic application	TMPW No.
<i>Xanthium strumarium</i> L. Collected at Da Lat City, Lam Dong Province (2001)	Compositae	Fruit	Ke dau ngua	Inflammation, malaria	20607
<i>Acorus calamus</i> L.	Araceae	Rhizome	Thuy xuong bo	Diuretic, rheumatism, fever, cold	22131
<i>Angelica dahurica</i>	Umbelliferae	Root	Bach chi	Fever, rheumatism, leucorrhea	22085
<i>Angelica tuhao</i>	Umbelliferae	Root	Doc hoat	Rheumatism	22144
<i>Aretium lappa</i> L.	Asteraceae	Fruit	Nguu bang	Fever, rheumatism, digestive disorder	22118
<i>Arctiodes macrocephala</i>	Asteraceae	Rhizome	Bach truat	Tonic, diarrhea, diabetes, diuretic, inflammation	22166
<i>Bambusa</i> sp.	Gramineae	Leaf	La tre	Antibiotic, fever, inflammation	22110
<i>Begonia jimbrispalata</i> HANCE.	Begoniaceae	Whole plant	Hong thien qui	Rheumatism, inflammation	22145
<i>Blumea balsamifera</i> L.	Asteraceae	Aerial part	Dai bi	Rheumatism, inflammation, menstrual pain	22177
<i>Cajanus cajan</i> L.	Fabaceae	Seed	Dau sang	Fever, rheumatism, cough	22142
<i>Centella asiatica</i>	Umbelliferae	Whole plant	Rau ma	Fever, inflammation, leucorrhea	22121
<i>Cibotium barometz</i>	Dicksoniaceae	Root	Cau tich	Rheumatism	22138
<i>Cinnamomum cassia</i>	Lauraceae	Bark	Que	Rheumatism, digestive diseases	22153
<i>Curcuma longa</i> L.	Zingiberaceae	Rhizome	Nghe	Menstrual disorders, inflammation, rheumatism	22150
<i>Cyrtomium fortunei</i>	Polypodiaceae	Rhizome	Quan chung	Fever, hypertension, inflammation	22120
<i>Dracaena cambodiana</i>	Dracaenaceae	Stem	Huyet giac	Congestion, rheumatism	22147
<i>Eclipta alba</i> Hassk.	Asteraceae	Aerial part	Co muc	Hepatitis, inflammation	22159
<i>Elephantopus scaber</i> LINN.	Asteraceae	Whole plant	Dia dom	Hepatitis, inflammation, diarrhea	22163
<i>Fibraurea tinctoria</i> L.	Menispermaceae	Root	Hoang dang	Malaria, diarrhea, inflammation	22171
<i>Gadennia jasminoides</i>	Rubiaceae	Seed	Danh danh	Hepatitis, inflammation, diuretic	22160
<i>Imperata cylindrica</i>	Poaceae	Rhizome	Co tranh	Fever, urinary tract infection, hypertension, inflammation	22090
<i>Juncus effusus</i> L.	Juncaceae	Stem	Bac den	Fever, urinary tract infection, inflammation	22081
<i>Kaempferia galanga</i> L.	Zingiberaceae	Rhizome	Dia lien	Rheumatism, cough	22143
<i>Lophatherum gracile</i>	Gramineae	Whole plant	Dam truc diep	Fever, urinary tract infection, inflammation	22099
<i>Lycopodium japonicum</i>	Lycopodiaceae	Whole plant	Than canh thao	Urinary tract infection, hypertension, inflammation	22169
<i>Morus alba</i> L.	Moraceae	Bark	Dau	Rheumatism, asthma	22095
<i>Morus alba</i> L.	Moraceae	Leaf	Dau	Fever, cough, hypertension, inflammation	22096
<i>Phyllanthus urinaria</i> L.	Euphorbiaceae	Aerial part	Cho de rang cua	Inflammation, hepatitis	22158
<i>Plantago asiatica</i>	Plantaginaceae	Aerial part	Ma de	Inflammation	22165
<i>Polygonum cuspidatum</i> SIEB.	Polygonaceae	Rhizome	Tam that can	Rheumatism, inflammation, hepatitis	22167
<i>Pueraria thomsoni</i> BENTH.	Fabaceae	Root	San day	Fever, inflammation	22123
<i>Rehmania glutinosa</i>	Scrophulariaceae	Root	Dia hoang	Inflammation, menstrual disorders	22100
<i>Sargentodoxa cuneata</i>	Sargentodoxaceae	Stem	Huyet dang	Menstrual disorders, rheumatism	22146
<i>Taraxacum officinale</i>	Asteraceae	Whole plant	Bo cong anh	Hepatitis, inflammation, nephropathy	22157
<i>Xanthium strumarium</i> L. Collected at Nha Trang City, Khanh Hoa Province (2002)	Asteraceae	Whole plant	Ke dau ngua	Fever, rheumatism	22106
<i>Achyranthes bidentata</i> BLUME.	Amaranthaceae	Root	Nguu tat	Rheumatism	22152
<i>Chrysanthemum sinense</i> SABINE.	Asteraceae	Flower	Cuc hoa	Inflammation, fever, rheumatism	22092
<i>Dendrobium</i> sp.	Orchidaceae	Stem	Thach hoc	Fever, rheumatism	22126
<i>Homalomena aromatica</i>	Araceae	Root	Thien nien kien	Rheumatism	22155
<i>Lonicera japonica</i>	Caprifoliaceae	Flower	Kim ngan	Inflammation, rheumatism	22107
<i>Scrophularia buergeriana</i> Mro.	Scrophulariaceae	Root	Huyen sam	Fever, inflammation	22105
<i>Tetracera scandens</i> L.	Dilleniaceae	Root, stem	Day chieu	Inflammation, hepatitis	22162
Collected at Tuy Hoa Township, Phu Yen Province (2002)					
<i>Artemisia vulgaris</i> L.	Asteraceae	Aerial part	Ngai diep	Antibacteria, fever, rheumatism, leucorrhea	22117
<i>Crinum asiaticum</i> L.	Amaryllidaceae	Whole plant	Trinh nu h. cung	Inflammation, fever	22134
<i>Cuscuta chinensis</i> LAMM.	Cuscutaceae	Seed	Day to hong	Rheumatism, impotence	22140
<i>Perilla ocymoides</i> L.	Lamiaceae	Leaf	Tia to	Cough, fever, rheumatism	22133
<i>Polycarpaea arenaria</i>	Apiaceae	Root	Nam sai ho	Malaria, inflammation	22174

Table 2. Xanthine Oxidase Inhibitory Activity of Vietnamese Medicinal Plants

Plant name	XO Inhibition (%)											
	100 µg/ml				50 µg/ml				25 µg/ml			
	M	M-W	W		M	M-W	W		M	M-W	W	
Collected at the Seven-Mountain Area, Tinh Bien District, An Giang Province (1998)												
<i>Angelica dahurica</i>	13.9	—	—	5.1	—	—	—	—	3.0	—	—	—
<i>Artemisia vulgaris</i>	89.3	44.2	—	79.7	30.5	6.0	—	—	53.5	45.3	1.5	*
<i>Asparagus cochinchinensis</i>	13.2	7.4	9.3	7.7	4.4	—	—	—	6.5	—	—	*
<i>Borassus flabellifer</i>	50.9	62.1	46.8	44.8	60.7	46.8	25.3	—	33.3	2.1	18.8	*
<i>Cesalpinia sappan</i>	78.5	70.0	72.2	63.8	51.6	47.2	47.7	—	58.0	46.1	92.6	29.3
<i>Celba pentandra</i>	45.2	32.4	26.3	27.9	26.2	18.4	11.5	—	24.2	—	14.2	36.0
<i>Cinnamomum iners</i>	63.2	54.7	42.3	57.8	48.5	25.2	15.4	—	48.2	12.3	33.5	69.5
<i>Combretum quadrangulare</i>	37.4	57.4	21.0	29.2	40.6	30.4	15.6	—	20.3	—	12.8	72.4
<i>Cyperus rotundus</i>	79.4	52.8	28.5	43.0	35.7	5.0	6.5	—	20.3	52.9	91.8	*
<i>Desmodium heterophyllum</i>	2.1	1.8	—	1.3	0.7	0.3	—	—	0.9	0.5	—	*
<i>Elsholtzia ciliata</i>	35.9	43.3	18.4	18.7	42.8	29.2	—	—	10.3	—	—	*
<i>Euphorbia tirucalli</i>	14.8	—	—	12.4	—	—	—	—	10.9	—	—	*
<i>Glycyrrhiza uralensis</i>	64.4	33.1	—	47.3	30.5	10.2	—	—	31.9	8.8	54.9	*
<i>Hedyotis diffusa</i>	55.9	38.9	12.6	38.6	31.6	15.1	—	—	26.8	6.6	78.9	*
<i>Hydrophyllum formicarum</i>	11.3	34.6	—	10.3	32.5	—	—	—	9.8	8.7	—	*
<i>Lasia spinosa</i>	28.1	33.7	26.2	18.3	32.8	20.9	14.1	—	15.2	7.4	10.4	*
<i>Leonurus heterophyllus</i>	12.6	—	—	4.6	—	—	—	—	1.7	0.5	—	*
<i>Levurea scandens</i>	19.4	15.9	—	14.1	12.4	9.8	8.7	—	13.3	11.8	12.1	*
<i>Melaleuca leucadendra</i>	64.6	64.2	41.7	36.4	26.4	38.5	21.8	—	25.6	11.5	20.4	78.9
<i>Mitella velutina</i>	10.9	8.6	15.3	8.3	8.0	—	8.7	—	7.5	6.3	6.1	*
<i>Nauclea officinalis</i>	51.1	31.4	11.6	26.2	26.9	13.9	4.4	—	18.0	10.9	9.8	*
<i>Orthosiphon stamineus</i>	37.6	37.7	21.9	32.0	26.5	13.4	5.6	—	17.1	4.7	3.2	*
<i>Panicum repens</i>	2.4	—	2.1	1.2	—	—	—	—	0.6	0.4	—	*
<i>Parameria laevigata</i>	41.0	57.9	26.1	29.5	53.3	31.2	16.3	—	24.8	18.7	23.4	45.1
<i>Polanisia chelidoni</i>	16.9	9.7	15.5	16.1	8.4	6.2	11.3	—	14.1	13.6	4.8	*
<i>Rhinacanthus nasutus</i>	14.7	25.1	20.1	12.5	16.9	15.0	15.1	—	11.2	10.4	11.4	*
<i>Sansevieria cylindrica</i>	59.0	22.6	18.0	50.5	17.2	16.0	14.6	—	37.2	22.3	49.0	*
<i>Tinospora cordifolia</i>	63.5	7.8	—	44.3	4.9	3.8	—	—	23.0	9.2	62.2	*
Collected at Da Lat City, Lam Dong Province (1998)												
<i>Ageratum conyzoides</i>	1.2	—	0.8	0.7	—	—	0.6	—	0.4	0.2	—	*
<i>Andrographis paniculata</i>	—	1.3	—	—	0.7	0.5	—	—	—	—	—	*
<i>Artemisia apiacea</i>	57.4	34.3	—	34.5	15.9	—	—	—	14.9	2.5	—	79.9
<i>Codonopsis javanica</i>	3.9	—	8.0	—	—	—	7.1	—	—	—	2.2	*
<i>Coscinium fneistratum</i>	—	2.4	—	—	1.9	1.2	—	—	—	—	—	*
<i>Datura metel</i>	20.9	17.2	—	8.3	12.1	—	—	—	5.3	3.6	—	*
<i>Eleutherine</i>	8.5	—	2.7	7.2	6.7	—	1.5	—	4.2	1.7	—	*
<i>Eucommia ulmoides</i>	22.3	11.8	—	—	—	5.5	—	—	—	—	4.3	*
<i>Gymnopetalum cochinchinensis</i>	7.1	—	15.9	—	—	—	10.0	—	—	—	2.1	*
<i>Heliotropium indicum</i>	13.7	9.8	—	5.2	8.4	—	—	—	4.2	3.1	—	*
<i>Kalanchoe pinnata</i>	68.1	60.7	36.8	51.7	50.3	37.2	11.6	—	38.3	28.2	9.6	49.2
<i>Lonicera japonica</i>	—	2.3	—	—	1.9	1.1	—	—	1.9	0.4	—	*
<i>Luffa cylindrica</i>	3.1	—	—	2.7	—	—	—	—	1.9	0.4	—	*
<i>Mimosa pudica</i>	65.5	9.5	51.0	47.5	7.4	4.7	19.2	—	33.4	12.5	13.2	96.9
<i>Phyllanthus amarus</i>	52.4	55.4	17.8	21.1	43.8	17.7	—	—	11.2	96.2	75.0	*
<i>Piper lolot</i>	—	2.6	—	—	1.8	1.2	—	—	—	—	—	*
<i>Plantago major</i>	—	7.5	6.9	—	6.6	5.5	3.7	—	—	—	—	*
<i>Polyscias fruticosa</i>	13.8	17.1	12.2	12.7	12.9	8.3	7.3	—	11.0	9.7	3.6	*
<i>Schefflera octophylla</i>	2.5	—	—	1.9	—	—	—	—	1.3	0.2	—	*
<i>Smilax glabra</i>	52.7	31.1	12.9	44.9	27.3	7.4	—	—	31.3	12.5	76.2	*
<i>Sophora flavescens</i>	8.4	11.5	2.9	7.7	10.8	—	1.3	—	5.5	3.3	—	*
<i>Xanthium strumarium</i>	24.1	17.4	—	19.8	15.5	9.3	—	—	11.6	8.1	—	*

Table 2. (continued)

Plant name	XO Inhibition (%)												IC ₅₀ (μg/ml)		
	100 μg/ml				50 μg/ml				25 μg/ml				10 μg/ml		
	M	M-W	W		M	M-W	W		M	M-W	W		M	M-W	W
Collected at Da Lat City, Lam Dong Province (2001)															
<i>Acorus calamus</i>	55.1	22.1	—		24.2	19.0	—		16.8	7.9	—		12.0	4.8	—
<i>Angelica dahurica</i>	20.3	19.1	9.7		19.3	17.1	8.6		16.4	12.8	6.2		14.2	10.8	5.9
<i>Angelica tuhuo</i>	7.7	2.0	9.3		4.6	1.6	8.8		2.8	1.3	3.6		1.3	—	1.5
<i>Arctium lappa</i>	25.9	6.7	—		5.0	4.3	—		1.4	0.6	—		0.8	—	—
<i>Arctostaphylos macrocephala</i>	—	—	2.3		—	—	1.6		—	—	0.7		—	—	—
<i>Bambusa</i> sp.	33.7	7.3	—		12.7	1.7	—		2.6	—	—		0.9	—	—
<i>Begonia jimbirsitipalata</i>	10.2	14.1	20.4		5.0	10.8	19.2		3.8	9.2	15.6		—	4.3	12.8
<i>Blumea balsamifera</i>	80.9	52.2	6.0		76.6	33.7	—		70.8	9.0	—		58.5	—	—
<i>Cajanus cajan</i>	23.7	29.2	20.9		21.6	27.2	15.6		13.9	14.9	13.4		10.9	13.8	12.4
<i>Centella asiatica</i>	27.2	22.8	—		2.4	8.1	—		—	—	—		—	—	—
<i>Cibotium barometz</i>	8.9	10.3	—		5.4	7.0	—		0.9	5.5	—		—	4.8	—
<i>Cinnamomum cassia</i>	55.8	47.5	27.4		35.1	41.3	22.5		31.4	26.2	16.0		16.3	16.1	7.7
<i>Curcuma longa</i>	5.9	4.1	—		4.1	2.0	—		2.4	—	—		0.3	—	—
<i>Cyrtanthium fortunei</i>	51.4	51.3	36.4		41.7	44.8	23.2		25.8	23.3	5.0		10.4	5.9	—
<i>Dracaena cambodiana</i>	62.9	—	45.0		28.4	—	26.2		7.5	—	4.9		3.5	—	1.1
<i>Eclipta alba</i>	34.0	33.9	—		14.1	18.0	—		2.5	—	—		—	—	—
<i>Elephantopus scaber</i>	31.5	11.9	—		22.6	6.0	—		20.5	—	—		0.8	—	—
<i>Fibraurea tinctoria</i>	38.2	31.8	17.5		27.4	26.7	15.2		25.2	18.9	13.4		20.2	14.4	11.6
<i>Gadennia jasminoides</i>	27.4	17.5	22.2		19.2	15.3	18.9		18.6	11.6	12.7		—	10.7	8.7
<i>Imperata cylindrica</i>	5.8	3.9	—		—	2.3	—		41.9	0.8	9.2		—	—	—
<i>Juncus effusus</i>	61.4	26.8	20.7		58.0	17.1	18.8		—	6.7	10.0		25.6	—	7.5
<i>Kaempferia galanga</i>	90.6	8.7	13.4		45.4	4.9	12.7		—	3.9	2.7		—	1.8	7.4
<i>Lophatherum gracile</i>	—	10.1	6.3		—	2.7	5.3		19.4	—	—		—	—	—
<i>Lycopodium japonicum</i>	46.4	15.1	—		32.0	6.0	—		49.7	0.8	—		13.5	0.3	—
<i>Morus alba</i> (barks)	76.5	62.4	11.3		66.2	51.7	10.6		26.9	26.9	9.0		29.3	22.8	7.8
<i>Morus alba</i> (leaves)	12.0	17.9	16.4		6.6	12.2	13.3		5.0	11.6	11.9		3.4	9.4	10.5
<i>Phyllanthus urinaria</i>	34.1	46.1	6.1		14.1	19.9	—		3.3	6.3	—		—	2.4	—
<i>Plantago asiatica</i>	4.9	—	—		2.8	—	—		1.1	—	—		0.7	—	—
<i>Polygonum cuspidatum</i>	60.2	55.6	—		41.5	48.5	—		17.4	7.2	—		4.3	—	—
<i>Pueraria thomsoni</i>	2.4	4.1	3.3		1.9	3.1	2.7		1.1	0.9	1.9		0.7	—	1.5
<i>Rehmania glutinosa</i>	—	1.9	—		—	1.5	—		33.6	42.2	5.5		14.0	0.3	—
<i>Sargentodoxa cuneata</i>	61.2	59.0	31.2		50.5	50.4	18.3		1.5	—	—		0.2	22.5	3.6
<i>Taraxacum officinale</i>	14.8	—	—		3.5	—	—		—	—	—		—	—	—
<i>Xanthium strumarium</i>	11.8	13.4	—		5.8	3.2	—		—	1.9	—		—	0.7	—
Collected at Nha Trang City, Khanh Hoa Province (2002)															
<i>Achyranthes bidentata</i>	1.9	4.1	—		—	2.5	—		—	1.7	—		—	0.8	—
<i>Chrysanthemum sinense</i>	82.9	23.4	13.6		79.6	21.8	7.1		79.4	10.5	5.7		62.3	6.3	2.9
<i>Dendrobium</i> sp.	11.3	—	4.7		2.8	—	2.5		—	—	1.4		—	—	0.6
<i>Homolomena aromatica</i>	21.3	17.0	14.8		10.6	11.3	12.3		9.7	6.7	10.3		5.7	4.1	4.0
<i>Lonicera japonica</i>	59.3	10.5	—		42.0	8.7	—		24.4	3.5	—		9.0	—	—
<i>Scrophularia buergeriana</i>	—	2.1	—		—	1.5	—		—	0.7	—		—	0.2	—
<i>Tetracera scandens</i>	73.6	83.0	75.6		70.3	82.8	65.0		48.1	72.9	49.6		8.4	32.3	16.0
Collected at Tuy Hoa Township, Phu Yen Province (2002)															
<i>Artemisia vulgaris</i>	70.2	29.5	—		62.6	22.3	—		48.0	4.0	—		19.6	—	—
<i>Crinum asiaticum</i>	9.7	4.3	—		—	2.5	—		2.4	1.7	—		0.9	0.6	—
<i>Cuscuta chinensis</i>	16.5	—	—		5.4	—	—		44.2	—	—		—	—	—
<i>Perilla ocymoides</i>	79.9	52.3	17.1		64.3	41.2	11.9		10.0	14.4	8.5		22.0	6.4	—
<i>Polycarpaea areolaria</i>	26.7	11.9	—		13.1	9.0	—		—	4.5	—		5.1	0.4	—
<i>Alloporrhizol</i>	—	—	—		—	—	—		—	—	—		—	—	—

M: MeOH extract; M-W: MeOH-H₂O (1:1) extract; W: H₂O extract; —: <0.1% XO inhibition; *: IC₅₀ value >100 μg/ml.

Fig. 1. Structures of the Compounds Isolated from the Flower of *Chrysanthemum sinense*

Fig. 2. Dose-Dependent XO Inhibition by Compounds 1–4 and Allopurinol

The data represent the mean $\pm$ S.D. of four determinations.

reported together with the antioxidative and hepatoprotective activities in other *Tetracera* species.^{29–32} Thus, the XO inhibitory activity of *T. scandens* also may be due to the presence of phenolic constituents.

The most active extract found in the present screening was the MeOH extract of the flower of *C. sinense* (IC₅₀, 5.1 μ g/ml), which is used for the treatment of rheumatism and inflammatory diseases. To date, no chemical constituents have been reported from *C. sinense*, and thus we carried out further investigation to isolate and identify the active constituents. The MeOH extract of the flower of *C. sinense* was subjected to silica gel column chromatography to give six fractions. Further separation and purification of the active fractions with reversed-phase preparative TLC led to the isolation of caffeic acid²⁾ (1), luteolin⁹⁾ (2), eriodictyol¹⁰⁾ (3), and 1,5-di-*O*-caffeoylquinic acid¹¹⁾ (4) (Fig. 1). These compounds were examined for their XO inhibitory activity (Fig. 2). Among them, luteolin (2) displayed more potent inhibitory activity (IC₅₀, 1.3 μ M) than the positive control allopurinol (IC₅₀, 2.5 μ M), while the IC₅₀ values of 1 and 4 were 85.3 μ M and 64.4 μ M, respectively. The increase in XO inhibitory activity of 2 compared with 3 (IC₅₀, 43.8 μ M) suggests the importance of the C₂–C₃ double bond in flavonoids for the activity.

In order to determine the inhibition mode of 2, further ki-

netic studies were carried out. Lineweaver–Burk plots indicated 2 to be a competitive inhibitor, the same as allopurinol, with respect to the substrate, xanthine. The inhibition constant (K_i) of 2 was 0.92 μ M, which was less than that of allopurinol (1.88 μ M). Further HPLC analysis of the crude extract of *C. sinense* suggested the most active constituent 2 to be the major constituent (106 μ g/mg of extract). Thus, the traditional use of *C. sinense* for the treatment of rheumatism and inflammatory diseases in Vietnam might be attributable to the XO inhibitory activity of flavonoid constituents such as 2.

In conclusion, we have carried out a systematic investigation of Vietnamese medicinal plants for XO inhibitory activity. The results indicate a number of medicinal plants that may be useful for the treatment of hyperuricemia and gout, and provide the basis for further investigation on these medicinal plant species to isolate active constituents and drug development.

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REFERENCES

- 1) World Health Organization Regional Office for Western Pacific, Institute of Materia Medica, "Medicinal Plants in Viet Nam," Science and Technology Publishing House, Hanoi, 1998.
- 2) Chiang H. C., Lo Y. J., Lu F. J., *J. Enz. Inhibit.*, **8**, 61–71 (1994).
- 3) Owen P. L., Johns T., *J. Ethnopharmacol.*, **64**, 149–160 (1999).
- 4) Oettl K., Reibnegger G., *Biochim. Biophys. Acta*, **1430**, 387–395 (1999).
- 5) Ishibuchi S., Morimoto H., Oe T., Ikebe T., Inoue H., Fukunari A., Kamezawa M., Yamada T., Naka Y., *Bioorg. Med. Chem. Lett.*, **11**, 879–882 (2001).
- 6) Osada Y., Tsuchimoto M., Fukushima H., Takahashi K., Kondo S., Hasegawa M., Komoriya K., *Eur. J. Pharmacol.*, **241**, 183–188 (1993).
- 7) Cos P., Ying L., Calomme M., Hu J. P., Cimanga K., Van Poel B., Pieters L., Vlietinck A. J., Berghe D. V., *J. Nat. Prod.*, **61**, 71–76 (1998).
- 8) Noro T., Oda Y., Miyase T., Ueno A., Fukushima S., *Chem. Pharm. Bull.*, **31**, 3984–3987 (1983).
- 9) Ternai B., Markham K. R., *Tetrahedron*, **32**, 565–569 (1976).
- 10) Wagner H., Chari V. M., *Tetrahedron Lett.*, **21**, 1799–1802 (1976).
- 11) Merfort I., *Phytochemistry*, **31**, 2111–2113 (1992).
- 12) Kong L. D., Cai Y., Huang W. W., Cheng C. H. K., Tan R. X., *J. Ethnopharmacol.*, **73**, 199–207 (2000).
- 13) Sweeney A. P., Wyllie S. G., Shalliker R. A., Markham J. L., *J. Ethnopharmacol.*, **75**, 273–277 (2001).
- 14) Theoduloz C., Pacheco P., Hirschman G. S., *J. Ethnopharmacol.*, **33**, 253–255 (1991).
- 15) Theoduloz C., Franco L., Ferro E., Hirschman G. S., *J. Ethnopharmacol.*, **24**, 179–183 (1988).
- 16) Gonzalez A. G., Bazzocchi I. L., Moujir L., Ravelo A. G., Correa M. D., Gupta M. P., *J. Ethnopharmacol.*, **46**, 25–29 (1995).
- 17) Do T. L., "Vietnamese Medicinal Plants," Medicine Publisher, Hanoi, 2001.
- 18) Macro J. A., Sanz J. F., Hierro P. D., *Phytochemistry*, **30**, 2403–2404 (1991).
- 19) Geissman T. A., *Phytochemistry*, **9**, 2377–2381 (1970).
- 20) Lee S.-J., Chung H.-Y., Maier C. G., Wood A. R., Dixon R. A., Mabry T. J., *J. Agric. Food Chem.*, **46**, 3325–3329 (1998).
- 21) Hoorn D. E. C. V., Nijveldt R. J., Leeuwen P. A. M. V., Hofman Z., M'Rabet L., Bont D. B. A. D., Norren K. V., *Eur. J. Pharmacol.*, **451**,

- 111—118 (2002).
- 22) Hayashi T., Sawa K., Kawasaki M., Arisawa M., Shimizu M., Morita N., *J. Nat. Prod.*, **51**, 345—348 (1988).
- 23) Ruangrunsi N., Tappayuthpijarn P., Tantivatana P., *J. Nat. Prod.*, **44**, 541—545 (1981).
- 24) Barua N. C., Sharma R. O., *Phytochemistry*, **31**, 4040 (1992).
- 25) Barua N. C., Sharma R. O., Thyagarajan G., Herz W., Govindan S. V., *Phytochemistry*, **18**, 2003—2006 (1979).
- 26) Badami S., Moorkoth S., Rai S. R., Kannan E., Bhojraj S., *Biol. Pharm. Bull.*, **26**, 1534—1537 (2003).
- 27) Moon C.-K., Park K.-S., Kim S.-G., Won H.-S., Chung J.-H., *Drug Chem. Toxicol.*, **15**, 81—91 (1992).
- 28) Safitri R., Tarigan P., Freisleben H. J., Rumampuk R. J., Murakami A., *Biofactors*, **19**, 71—77 (2003).
- 29) Harrison L. J., Sia G.-L., Sim K.-Y., *Planta Med.*, **60**, 493—494 (1994).
- 30) Harborne J. B., *Phytochemistry*, **8**, 419—423 (1969).
- 31) Kukongviriyapan V., Janyacharoen T., Kukongviriyapan U., Laupattarakasaem P., Kanokmedhakul S., Chantaranonthai P., *Phytother. Res.*, **17**, 717—721 (2003).
- 32) Gurni A. A., Konig W. A., Kubitzki K., *Phytochemistry*, **20**, 1057—1059 (1981).