SHORT COMMUNICATION

Chemical constituents of *Breynia glauca* leaves

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Abstract

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Chemical constituents of *Breynia glauca* leaves
Songklanakarin J. Sci. Technol., 2005, 27(Suppl 2) : 563-567

From the leaves of *Breynia glauca*, eight compounds including friedelin (1), 3-oxo-^{4,5}-sitosterone (2), friedelan-3β-ol (3), β-sitosterol (4), kaempferol (5), arbutin (6), kaempferol-3-*O*-rutinoside (7) and quercetion-3-*O*-glucoside (8) were isolated. The presence of these compounds supports the traditional use of this plant as a detoxifying agent. In addition, all of these chemical constituents were tested for antiviral potential against herpes simplex virus types 1 and 2, but were found to be inactive at 50 μg/ml.

Key words : *Breynia glauca*, chemical constituents, herpes simplex virus, detoxification

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Received, 19 October 2004 Accepted, 23 January 2005

บทคัดย่อ

บุษราคัม ทรัพย์อุดมผล สัมพันธ์ วงศ์เสรีพิพัฒนา และ กิตติศักดิ์ ลิขิตวิทยาวุฒิ

องค์ประกอบทางเคมีของระงับพิษ

ว. สงขลานครินทร์ วทท. 2548 27(ฉบับพิเศษ 2) : 563-567

การศึกษาใบระงับพิษสามารถแยกได้สารบริสุทธิ์คือ friedelin (1), 3-oxo- ^{4,5}-sitosterone (2), friedelan-3β-ol (3), β-sitosterol (4), kaempferol (5), arbutin (6), kaempferol-3-*O*-rutinoside (7) and quercetion-3-*O*-glucoside (8) การมีอยู่ของสารเหล่านี้นับได้ว่าเป็นข้อมูลที่สนับสนุนประโยชน์ของพืชนี้ ซึ่งในยาตำรับยาไทยใช้ระงับพิษ นอกจากนี้ ได้ทำการทดสอบฤทธิ์ของสารทั้งหมดในการต้านไวรัสเริม แบบที่ 1 และ 2 แต่พบว่าสารทุกชนิดไม่แสดงฤทธิ์ที่ 50 μg/ml

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Breynia glauca Craib is a shrub widely distributed in Thailand, having the local name "Rangap-phit" which means "detoxification" (Royal Forest Department, 2001). In Thai traditional medicine, the leaves of *B. glauca* have been reputed to have anti-inflammatory, antipyretic and detoxifying properties (Mahidol University Foundation, 2000). Previous reports on the chemical constituents of the leaves of this plant described the presence of the triterpenoid aborinone and the steroid β-sitosterol, together with long-chain hydrocarbons as well as several unidentifiable components (Kongkatip, B., 1974, Boonyaratavej *et al*., 1992). In this communication, we report the results of our re-examination of the constituents of the leaves of *B. glauca*.

Experimental

General procedures: Melting points were measured on a Fisher-Johns melting point apparatus. UV spectra were measured on a Melton Roy spectrophotometer. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a Bruker DPX 300 spectrometer. EI and FAB mass spectra were taken on a Fison Micromass VG Platform II and a Micromass LCT (LC/MS) mass spectrometer, respectively.

Extraction and isolation: *Plant material*. Dried leaves of *B. glauca* were collected from Chanthaburi province in April 1999. The plant material was identified by the authors. A voucher specimen has been deposited at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

Isolation and purification: The dried leaves of *B. glauca* (5 kg) were extracted with hexane (3×15 L), EtOAc (3×15 L) and MeOH (3×15 L) to give a hexane (47 g), an EtOAc (63 g) and a MeOH (89 g) extract, respectively. The EtOAc extract was fractionated by vacuum liquid chromatography (SiO_2, hexane-EtOAc gradient) to give six fractions (I-VI). Fraction I (2 g) was further separated on a silica gel column (hexane-EtOAc 95:5) and then on a Sephadex LH-20 column ($CHCl_3$-MeOH 1:1) to give friedelin (**1**, 26 mg). Separation of fraction III on a silica gel column (hexane-EtOAc gradient) gave fractions IIIa and IIIb. Fraction IIIa was purified by gel filtration (Sephadex LH-20, MeOH) to yield 3-oxo- ^{4,5}-sitosterone (**2**, 2 mg). Fraction IIIb was further separated on a Sephadex LH-20 column to give friedelan-3β-ol (**3**, 36 mg) and β-sitosterol (**4**, 21 mg). The MeOH extract was partitioned between BuOH and water. The BuOH extract (21 g) was then chromatographed over a silica gel column ($CHCl_3$-MeOH gradient) to give nine fractions (A-H). Fraction B was further separated on a Sephadex LH-20 column (MeOH) to give kaempferol (**5**, 4 mg). Fraction D was re-separated on Sephadex LH-20 (MeOH) to give arbutin (**6**,

16 mg). Separation of fraction F on Sephadex LH-20 (MeOH) furnished kaempferol-3-*O*-rutinoside (**7**, 6 mg) and quercetin-3-*O*-glucoside (**8**, 4 mg).

Structure identifications of the isolated compounds were done by comparison of ^{1}H and ^{13}C NMR, and MS data with reported values for **1** (Umehara *et al*., 1988; Akihisa *et al*., 1992), **2** (Onocha *et al*., 1995), **3** (Pradhan *et al*., 1991; Duwiejua, M., 1999), **4** (Castola *et al*., 2002), **5** (Wagner *et al*., 1976), **6** (Dommisse *et al*., 1986; Perry *et al*.,1996), **7** (Chaurasia and Wichtl, 1987), and **8** (Chaurasia and Wichtl, 1987; Merfort and Wendisch, 1987). Antiviral activity assays were conducted according to previously described protocols (Lipipun *et al*., 2003; Sotanaphun *et al*., 2004).

Results and Discussion

The leaves of *B. glauca* were extracted with hexane, EtOAc and MeOH. Separation of the EtOAc extract using several chromatographic techniques resulted in the isolation of four isoprene-derived compounds, including friedelin (**1**), 3-oxo-^{4,5}-sitosterone (**2**), friedelan-3β-ol (**3**) and β-sitosterol (**4**) (Figure 1). The MeOH extract was partitioned between BuOH and water. Repetitive chromatography of the BuOH fraction led to the isolation of four phenolic compounds, namely kaempferol (**5**), arbutin (**6**), kaempferol-3-*O*-rutinoside (**7**) and quercetin-3-*O*-glucoside (**8**). The structure determinations of these chemical constituents were done by analysis of the ^{1}H and

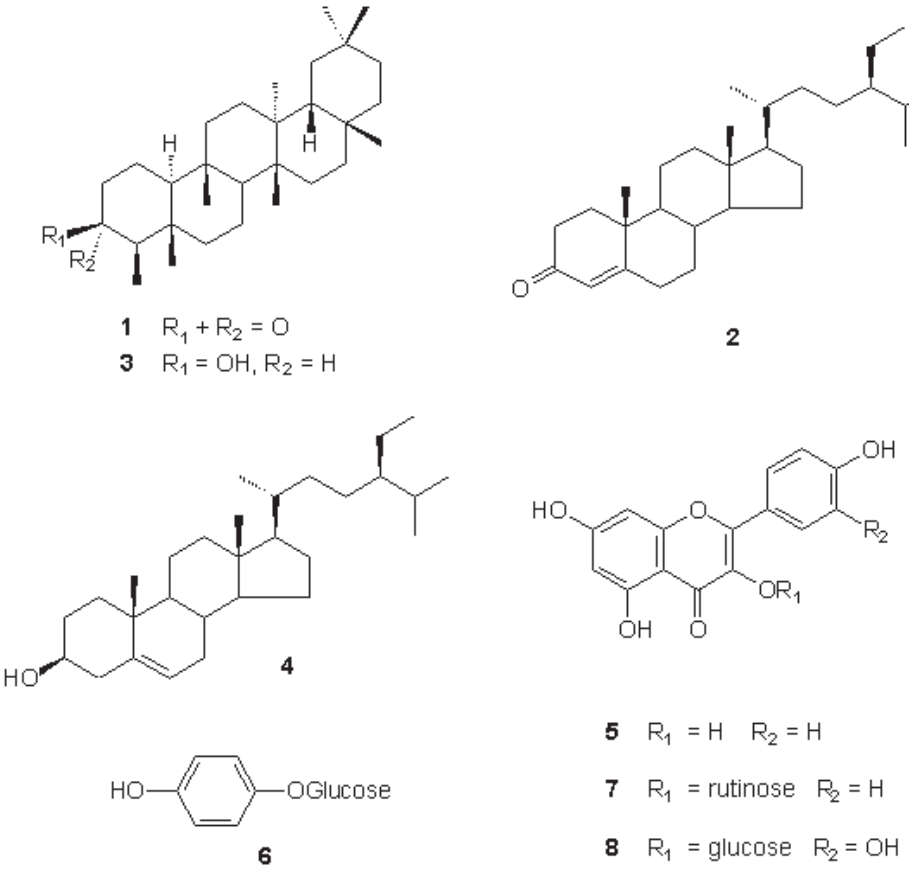

Figure 1. Compounds isolated from *B. Glauca.*

^{13}C NMR, and MS spectra. Compounds **2, 3,** and **5-8** were not found in the earlier investigations (Kongkatip, 1974; Boonyaratavej *et al*., 1992). It should be noted that friedelin (**1**) and β-sitosterol (**4**) have been reported to demonstrate 40 and 70% protection, respectively, against the lethal action of the venom of jararaca snakes (*Bothrops jararaca*) on mice (Mors *et al*., 2000). Anti-inflammatory activity has been observed for friedelan-3β-ol (**3**), β-sitosterol (**4**) and kaempferol (**5**) (Duwiejua *et al*., 1999; Goel *et al*., 1988; Villasenor *et al*., 2002). Furthermore, it is known that flavonoids, including kaempferol-3-*O*-rutinoside (**7**) and quercetin-3-*O*-glucoside (**8**), possess antioxidative activity (Samhan-Arias *et al*., 2004; Parejo *et al*., 2004; Rice-Evans *et al*., 1996). The above-mentioned pharmacological and biological activities of these compounds are consistent with the medicinal claims of this plant, and therefore provide supporting evidence for its traditional use. In this investigation, all of the isolated compounds (**1-8**) were further studied for their antiviral activity against herpes simplex virus types 1 and 2; however, none of these compounds showed inhibition at the concentration of 50 μg/ml.

Acknowledgements

We are grateful to the Faculty of Pharmaceutical Sciences, Chulalongkorn Univerisity, for a research grant. Thanks are due to Dr.Vimolmas Lipipun for antiviral activity assays.

References

Akihisa, T., Yamamoto, K., Tamura, T., Kimura, Y., Iida, T., Nambara, T., and Chang, F.C. 1992. Triterpenoid ketones from *Lingnania chungii* McClure: arborinone, friedelin and glutinone. Chem. Pharm. Bull., 40: 789-791.

Boonyaratavej, B., Siriwarodom, S., and Sucharit, V. 1992. Chemical constituents of the leaves of *Breynia glauca* Craib. Abstracts of senior projects, Department of Chemistry, Faculty of Science, Chulalongkorn University, (www.sc.chula.ac.th/department/chemistry/npru/6Senior/Abs-senior.html; access date: March 16, 2005).

Castola, V., Bighelii, A., Rezzi, S., Melloni, G., Gladiali, S., Desjobert, J.-M., and Casanova, J. 2002. Composition and chemical variability of the triterpene fraction of dichloromethane extracts of cork (*Quercus suber* L.). Ind. Crop. Prod., 15: 15-22.

Chaurasia, N., and Wichtl, M. 1987. Flavonolglykoside aus *Urtica dioca* Planta Med., 53: 432-434.

Dommisse, R.A., van Hoof, L., and Vlietinck, A.J. 1986. Structural analysis of phenolic glucosides from Salicaceae by NMR spectroscopy. Phytochemistry, 5: 1201-1204.

Duwiejua, M., Zeitlin, I.J., Gray, A.I., and Waterman, P.G. 1999. The anti-inflammatory compounds of Polygonum bistorta: isolation and characterisation. Planta Med., 65: 371-374.

Goel R.K., Pandey V.B., Dwivedi S.P., and Rao Y.V. 1988. Antiinflammatory and antiulcer effects of kaempferol, a flavone, isolated from *Rhamnus procumbens*. Indian J Exp Biol., 26: 121-4.

Kongkatip, B. 1974. Study of the chemical constituents of *Breynia glauca* and *Arfeuillea aborescens*. Master's thesis, Mahidol University, Bangkok, Thailand.

Lipipun, V., Kurokawa, M., Suttisri, R., Taweechotipatr, P., Pramyothin, P., Hattori, M., and Shiraki, K., 2003. Efficacy of Thai medicinal plant extracts against herpes simplex virus type 1 infection in vitro and in vivo. Antivir. Res., 60: 175-180.

Mahidol University Foundation, 2000. Encyclopedia of Herbal Medicine Vol. IV: Herbal Medicine of the Northeast. Amarin Printing and Publishing, Bangkok.

Merfort, I., and Wendisch, D. 1987. Flavonoidglycoside aus Arnica Montana und *Arnica chamissonis*. Planta Med., 53: 434-437.

Mors, W.B., do Nascimento, M.C., Pereira, B.M.R., and Pereira, N.A. 2000. Plant natural products active against snake bite- the molecular approach. Phytochemistry, 55: 627-642.

Onocha, P.A., Okorie, D.A., Connolly, J.D., and Roycroft, D.S. 1995. Monoterpene diol, iridoid glucoside and dibenzo-α-pyrone from *Anthocleista djalonensis*. Phytochemistry, 40: 1183-1189.

Parejo, I., Viladomat, F., Bastida, J., Schmeda-Hirschmann, G., Burillo, J., and Codina, C. 2004. Bioguided isolation and identification of the nonvolatile antioxidant compounds from fennel (*Foeniculum vulgare* Mill.) waste. J. Agric. Food Chem., 52: 1890-7.

Perry, N.B., Benn, M.H., Foster, L.M., Routledge, A., and Weavers, R.T. 1996. The glycosidic precursor of (*Z*)-5-ethylidine-2(5*H*)-furanone in *Halocarpus biformis* juvenile foliage. Phytochemistry, 42: 453-459.

Pradhan, B.P., Ghosh, R.K., and Ghosh, P. 1991. Studies on oxidation of triterpenoids: Part XI-Oxygenation of friedelin in presence of potassium *t*-butoxide in *t*-butanol. Indian J. Chem., 30B: 7-12.

Rice-Evans, C.A., Miller, N.J., and Paganga, G. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Rad. Biol. Med., 20: 933-956.

Royal Forest Department 2001. Thai Plant Names-Tem Smitinand, Prachachon Ltd., Bangkok.

Samhan-Arias, A.K., Martin-Romero, F.J., and Gutierrez-Merino, C. 2004. Kaempferol blocks oxidative stress in cerebellar granule cells and reveals a key role for reactive oxygen species production at the plasma membrane in the commitment to apoptosis. Free Rad. Biol. Med., 37: 48-61.

Sotanaphun, U., Lipipun, V. and Bavovada, R., 2004. Constituents of the pericarp of *Glyptopetalum sclerocarpum* Fitoterapia, 75: 606-608.

Villasenor, I.M., Angelada, J., Canlas, A.P., Echegoyen, D. 2002. Bioactivity studies on β-sitosterol and its glucoside. Phytother. Res., 16: 417-21.

Wagner, H., Chari, V.M., and Sonnenbichler, J. 1976. ^{13}C NMR-Spektren natürlich vorkommender flavonoide. Tetrahedron Lett., 21: 1799-1802.

Umehara, K., Hattori, I., Miyase, T., Ueno, A., Hara, S., and Kageyama, C. 1988. Studies on *Citrus unshiu* Marcov. Chem. Pharm. Bull., 36: 5004-5008.