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Studies on the Chemical Constituents of *Torreya grandis* Fort. Ex Lindl

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Abstract: Natural products play a very important role in the medicine of the world's population. Therefore, it is important that natural product chemistry continues to explore natural resources in search of new natural products. The phytochemical analysis of *Torreya grandis* leaves led to the isolation of five compounds using column chromatography, Preparative High-Performance Liquid Chromatography (PHPLC) and Thin Layer Chromatography (TLC). The structures elucidation of the isolated compounds were performed by spectroscopic techniques (1H NMR, LC-MS) and identified as kaempferol (1) acacetin (2), chrysoeriol (3), wogonin (4) and dibutyl phthalate (DBP) (5). Among these, compounds 1 and 5 have not been reported from this plant.

Key words: Phytochemical analysis, isolation, flavonoids, kaempferol, DBP, *Torreya grandis*

INTRODUCTION

Natural products are naturally derived metabolites and/or by-products from microorganisms, plants, or animals (Baker *et al*., 2000). These products have been exploited for human use for thousands of years and plants have been the chief source of compounds used for medicine. Traditional Chinese medicine is used in medicines as well as in daily dietary supplements in Asia. Today the largest users of traditional medicines are the Chinese, with more than 5,000 plants and plant products in their pharmacopoeia (Tina *et al*., 2006). More recently a study by the World Health Organization (WHO) has shown that about 80% of the world's population still relies on traditional medicine. These natural products also play a very important role in the medicine of the remaining 20% of the world's population. (Strobel and Daisy, 2003).

In China, over 10,000 compounds were identified from 11,146 kinds of plants belonging to traditional Chinese medicines (Guo *et al*., 2002). These compounds, not only play an important physiological and ecological role, but are also of commercial interest because of their multitude of applications in the food and pharmaceutical industries (Ortuno *et al*., 2006). For example, kaempferol (3,4',5,7-tetrahydroxyflavone) is a member of the flavone family subclass of flavonoids that are present at high levels in many plants including *Torreya grandis*. It has several biological activities including antiinflamation (Jing Xu *et al*., 2005) antioxidant (Subhashinee *et al*., 2006), anti-bactarial (Sevda *et al*., 2005), monoamine oxidase inhibitor and potential neuroprotectant (Sloley *et al*., 2000). Dibutyl phthalate (DBP) had a pharmacological activity in eliminating tumor cells and could be used as a purging agent in autologous bone marrow transplantation and also induce apoptosis in MO7e and U937 leukemia cell lines (Li-Sheng *et al*., 2002) and used as plasticizer in infusion tubing, infusion bag, blood storage bag, intestinal tubings about 20% (Hans *et al*., 1999). Therefore, it is important that natural product chemistry continues to explore natural resources in search of new natural products.

Torreya grandis Fort ex. Lindl (Taxaceae), common name nutmeg yew tree, ornamental plant common in China and Japan is a large sized ever green coniferous tree with dioecious flowers dioecious (occasionally monoecious); branches whorled; branchlets subopposite or subwhorled, base with bud scales not persistent; winter buds with several pairs of decussate bud scales. Leaves decussate or subopposite, and drup-like fruits with nut seeds (Saeed *et al*., 2006). It is an indigenous medicinal plant due to its anthelmintic, antitussive, carminative, laxative, antifungal, antibacterial and antitumer activity (Huang *et al*., 2001).

The present research describes the extraction and isolation of metabolites from *Torreya grandis,* followed by the identification of these components using high-performance liquid chromatography (HPLC), electro spray ionization mass spectrometry (ESI-MS) and nuclear magnetic resonance (NMR).

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MATERIALS AND METHODS

The experiment was conducted at the isolation and extraction laboratory, department of chemistry, School of Life Sciences and Technology, Beijing Institute of Technology China. The duration of study was nine month from September 2005 to May 2006.

General experimental procedure: HPLC analysis was performed using HPLC DAD-230 Elite, separation was performed on a Scienhome kromasil C18, 5 μ column (250 mm × 4.6 mm. The mobile phase was ACN-H_2O (5-100%, gradient, 40 min) and the flow rate was 1.0 mL min^{-1}. ESI spectra were obtained on a LC-MSD-Trap-SL Agilent 1100, the NMR spectra were taken on a NMR spectrometer ARX-400 and TLC was carried out on silica gel 60 F254 (Merk). Column chromatography was performed over polyamide resin (Merk, particle size 100-200 mesh) and Sephadex LH-20 (pharmacia).

Plant material and collection: The leaves of *Torreya grandis* plant was collected from southern area of China. The plant was taxonomically identified with the help of botanist using taxonomic rules and specimen has been deposited in the herbarium of school for future references.

Extraction and isolation: Air dried and powdered leaves of *T. grandis* (30 g) were extracted with 80% ethanol at 80°C for 3 h. The above procedures were repeated three time. The combined ethanolic extract was evaporated in *vacuo* to yield the total extract (9 g). This extract was then suspended in distilled water and partitioned sequentially with petroleum ether, chloroform, ethyl acetate and n-butanol.

The (2 g) extract of chloroform, ethyl acetate and butanol fractions respectively, were subjected to polyamide (mesh size 100-200 μm) column chromatography and washed with 1 L of 95% (v/v) aqueous ethanol and water (both at a rate of 40 mL min^{-1}). Then eluted with 20-95 % (v/v) aq. ethanol. When the $CHCl_3$ extract was eluted with 60% aq. ethanol, the 6 fractions were collected and these eluted fractions were concentrated to dryness at 70°C under a reduced pressure. The relevant fractions (No. 3-4) (0.05 g) containing mixture of same compounds were combined and rechromatographed with Sephadex LH-20 (MeOH-H_2O = 7:3) to provide 4 fractions (sub fraction No. 3) (25 mg) to give compound-1.

The ethyl acetate extract eluted with 20-95 % (v/v) aq. ethanol 20 fractions were collected and these eluted fractions were concentrated to dryness at 70°C under a reduced pressure. The relevant fractions (No. 4-9) (0.08 g) containing compound (2,3) were combined and further purified semi preparative RP-HPLC (Shimdazu 4 μm, 250×10 mm, acetonitrile-water = 1:1), providing compound-2 (3 mg) and compound-3 (4.5 mg). Fractions (No. 12-17) (0.028 g) were rechromatographed with sephadex LH-20 (MeOH-H_2O = 8:2) to provide 9 fractions (sub fractions No. 4-6) (7 mg) to give compound-4.

When the butanol extract was eluted with 95% aq. ethanol, the 15 fractions were collected Compond-5 (1.4 mg) was obtained from the combined fraction (No. 5-11) (0.02 g) by preparative TLC using Pet.ether-EtOAc (9:1) as a mobile phase which was further purified from Sephadex LH-20 with methanol. All the chemical, reagents and solvents using in this study were purchased from Sigma Chemical (St. Louis, MO, USA) unless specified.

RESULTS AND DISCUSSION

Phytochemical investigation of *Torreya grandis* leaves yielded to the isolation of three known and two unknown compounds from this plant. Using column chromatography, HPLC and TLC. These compounds were tentatively identified by means of a combination of the UV and mass spectra obtained by HPLC-DAD, LCMS and NMR spectrometer.

Compound 1 identified as 3,4',5,7-tetrahydroxyflavone; $C_{15}H_{10}O_6$ (Fig. 1), the yellowish crystal has a melting point of 275-278°C. Soluble in ether or 100% ethanol (hot); slightly soluble in water. EI-MS m/z 285 [M] - (*R*t 46.3 min) (Fig. 2), UV λmax nm: 266, 366 (MeOH); which is typical of a kaempferol skeleton. 1H-NMR (400 MHz) were recorded on a Bruker ARX-400 spectrometer with TMS as internal standard and dimethyl sulfoxide (DMSO) d6 as solvent. δ (ppm): 12.49 (1H, s, OH-5), 10.20 (1H, s, OH-7), 10.18 (1H, s, OH-4'), 10.05 (1H, s, OH-3), δ: 8.06 (2H, d, J = 8.8 Hz, H-2', 6'), 6.94 (2H, d, J = 8.8 Hz, H-3', 5'), 6.43 (1H, d, J = 1.84 Hz, H-8), 6.19 (1H, d, J = 1.84 Hz, H-6).

The 1H-NMR spectra of the compound showed a typical kaempferol type flavonoid. Two doublets at 8.06 ppm and 6.94 ppm, both of which integrated for two protons, were due to protons of the B-ring. These protons signals appeared as two pairs of ortho coupled doubles with J values of 8.8 Hz. Signals at 6.19 ppm (d,1H, J = 1.84 Hz) and 6.43 ppm (d, IH, J = 1.84 Hz) were due to the protons attached to C-6 and C-8, respectively. These protons, which are on the A-ring of kaempferol skeleton, have a low J values because they are meta to each other. On the basis of these spectral data, it was identified as kaempferol. Our finding are same as described by Slavica *et al.* (2004); Rafael *et al.* (2003). Similar results are reported by Jing Xu *et al.* (2005) and Miean *et al.* (2001).

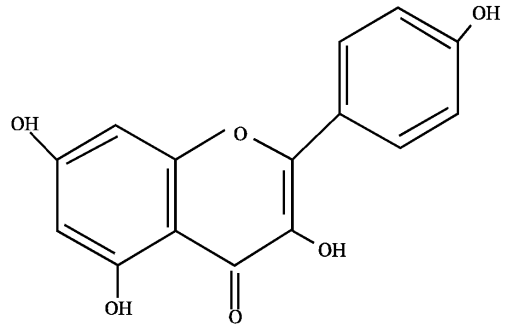

Fig. 1: Structure of kaempferol

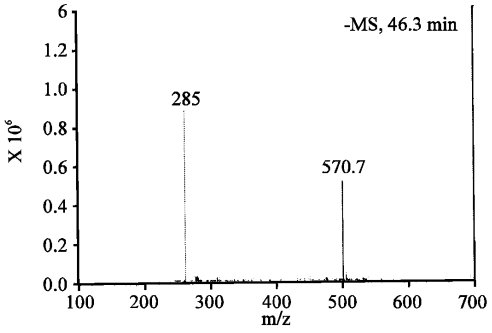

Fig. 2: ESI Mass spectra of kaempferol

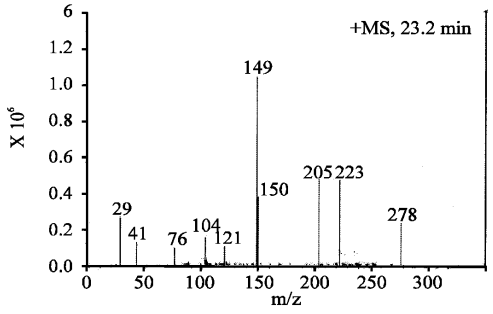

Fig. 3: ESI Mass spectra of dibutyl phthalate

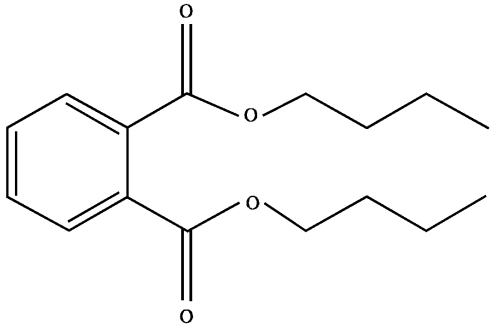

Fig. 4: Structure of dibutyl phthalate

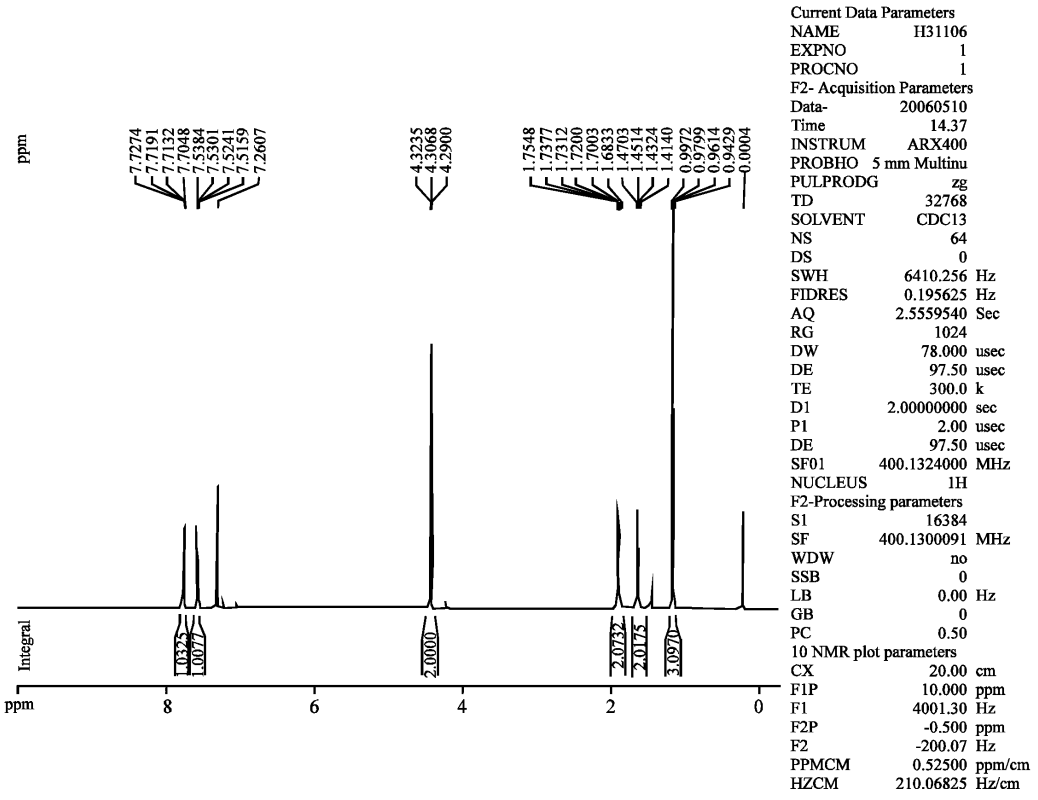

Fig. 5: 1H NMR spectra of dibutylphthalate

EI-MS spectral analysis exhibited spectra for the compound-5 fragmentation at m/z 29, 41, 76, 104, 121, 149, 205, 223, [M-H]+ at m/z 278 (*R*t 23.2 min); (Fig. 3). The chemical formula for dibutyl phthalate is $C_{16}H_{22}O_4$ (Fig. 4)

1H-NMR (400 MHz) were recorded on a Bruker, ARX-400 spectrometer with TMS as internal standard and CDC_{13} as solvent (Fig. 5). By comparison EI-MS and NMR spectra from given literature as narrated by Yong-Lai *et al.* (2005) and Marin *et al.* (1996), it is identified as Dibutyl phthalate (DBP), synonym *n*-Butyl phthalate/Phthalic acid dibutyl ester. It is an odorless and colorless o faint yellow oily liquid, mp; 35°C, bp; 340°C, soluble in alcohol, ether and benzene (Mehmet *et al.*, 2006; ATSDR, 1990).

CONCLUSIONS

Natural products are naturally derived metabolites and/or by-products from microorganisms, plants, or animals. These products have been exploited for human use for thousands of years and plants have been the chief source of compounds used for medicine. The phytochemical analysis of *Torreya grandis* leaves led to the isolation of five compounds by using different chromatographic techniques. Among these, compounds 1 and 5 have not been reported from *T. grandis* and are first time reported from this plant. Compound-1 (kaempferol) has several biological activities including antioxidant, anti-bactrial, monoamine oxidase inhibitor and potential neuroprotectant. Dibutyl phthalate (DBP) had a pharmacological activity in eliminating tumor cells, induce apoptosis and also use as plasticizer.

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REFERENCES

Agency for Toxic Substances and Disease Registry (ATSDR), 1990. Toxicological Profile for Di-n- Butyl Phthalate. Public Health Service, U.S. Department of Health and Human Services.

Baker, D., U. Mocek and C. Garr, 2000. Natural Products vs. Combinatorials: A Case Study, The Royal Society of Chemistry, Cambridge, United Kingdom, pp: 66-72.

Guo, D., Y. Su, J. Dai and H. Guo, 2002. Lecture on the 23rd IUPAC International Symposium on the Chemistry of Natural Products, Florence.

Hans, G.W., H. Andreas, H. Hans-Ulrich and H.M. Liebich, 1999. Identification of plasticizers in medical products by a combined direct thermo-desorption-cooled injection system and gas chromatography-mass spectrometry. J. Chromatog., A, 847: 1-7.

Huang, Y.J., J.F. Wang, G.L. Li, Z.H. Zheng and W.J. Su, 2001. Antitumor and antifungal activities in endophytic fungi isolated from pharmaceutical plants, *Taxus mairei*, *Cephalataxus fortunei* and *Torreya grandis*. FEMS Immunol. Med. Microbiol., 31: 163-167.

Jing, Xu., Li. Xian, Peng, Zhang., Li. Zhan-Lin and Yi Wang, 2005. Antiinflammatory Constituents from the Roots of *Smilax bockii* Warb. Arch. Pharm. Res., 28: 395-399.

Li-Sheng Wang., Hong-Jun Liu., Jin-Hui Zhang and Wu. Chu-Tse, 2002. Purging effect of dibutyl phthalate on leukemia cells involves fas independent activation of caspase-3/CPP32 protease. Cancer Lett., 186: 177-182.

Marin, M.L., A. Jimrnez, J. Lepez and J. Vilaplana, 1996. Analysis of poly (vinyl chloride) additives by supercritical fluid extraction and gas chromatography J. Chromatogr., A, 750:183-190.

Mehmet Bilgin, Sü. Ismail Kırbasülar, Onder Ozcan and Umur Dramur, 2006. Distribution of Butyric Acid between Water and Several Solvents. J. Chem. Eng., 51: 1546-1550.

Miean, K.H. and S. Mohamed, 2001. Flavonoid (myricetin, quercetin, kaempferol, luteolin and apigenin) content of edible tropical plants. J. Agric. Food Chem., 49: 3106-3112.

Ortuno, A., A. Baidez, P. Gomez, M.C. Arcas, I. Porras, A. Garcıa-Lidon and J.A. Del Rıo, 2006. *Citrus paradisi* and *Citrus sinensis* flavonoids: Their influence in the defense mechanism against *Penicillium digitatum*. Food Chem., 98: 351-358.

Rafael, L., Angel, G., Federico, F. and F. T. S. Barberaan, 2003. HPLC-DAD-MS/MS ESI Characterization of Unusual Highly Glycosylated Acylated Flavonoids from Cauliflower (*Brassica oleracea* L. var. *botrytis*) Agro-industrial Byproducts. J. Agric. Food Chem., 51: 3895-3899

Saeed, M.K., Y. Deng, Z. Parveen, R. Dai, W. Ahmad and Y. Yu, 2006. Wseas Transactions on Biology and Biomedicine, 3: 484-491.

Sevda, S., H. Ali Mericlia and J.H.B. Peter Cubukcua, 2005. Flavonoids of *Helichrysum compactum* and their antioxidant and antibacterial activity. Fitoterapia, 76: 269-272.

Slavica, B., S.S.K. Ilic, B. Zoran and Todorovic, 2004. Flavonoids from flower of *Linum capitatum* kit udc 547.972.2. Facta Universitatis Series: Physics, Chem. Technol., 3: 67-71.

Sloley, B.D., L.J. Urichuk, P. Morley, J. Durkin, J.J. Shan, P.K. Pang and R.T. Coutts, 2000. Identification of kaempferol as a monoamine oxidase inhibitor and potential Neuroprotectant in extracts of *Ginkgo biloba* leaves. J. Pharm. Pharmacol., 52: 451-459.

Strobel, G. and B. Daisy 2003. Bioprospecting for Microbial endophytes and their natural products microbiol. Mol. Biol. Rev., 67: 491-502.

Subhashinee, S.K., W. Mamdouh, M. Abou-Zaid and F. Shahidi, 2006. Antioxidant polyphenols in almond and Its coproducts. J. Agric. Food Chem., 54: 312-318.

Tina, T.X. Dong., J. Kui, Zhao, G. Qiut, N.J. Zhao, T. Ting, Zhu. Junli, D. Ran, W.H.C. Anna and W.K.K. Karl, 2006. Chemical and biological assessment of a chinese herbal decoction containing radix astragali and radix angelicae sinensis: Determination of drug ratio in having optimized properties. J. Agric. Food Chem., 54: 2767-2774.

Yong-Lai, F., J. Zhua and R. Sensenstein, 2005. Development of a headspace solid-phase microextraction method combined with gas chromatography mass spectrometry for the determination of phthalate esters in cow milk. Anal. Chim. Acta, 538: 41-48.