NPC Natural Product Communications

Composition and *in-vitro* CytotoxicActivities of the Leaf Essential Oil of *Beilschmiedia erythrophloia* from Taiwan

Yu-Chang Su^a and Chen-Lung Ho^{b*}

^aDepartment of Forestry, National Chung Hsing University, 250 Kuo Kuang Rd., Taichung, Taiwan 402 ^bDivision of Wood Cellulose, Taiwan Forestry Research Institute. 53, Nanhai Rd., Taipei, Taiwan 100

chenlung@tfri.gov.tw

Received: October 9th, 2012; Accepted: October 14th, 2012

This study investigated the chemical composition and *in-vitro* cytotoxic activities of the essential oil isolated from the leaf of *Beilschmiedia erythrophloia*. The essential oil was isolated using hydrodistillation in a Clevenger-type apparatus, and characterized by GC–FID and GC–MS. Fifty-five compounds were identified, representing 100% of the oil. The main components identified were β -caryophyllene (22.6%), α -humulene (21.9%), terpinen-4-ol (5.3%), *cis*- β -ocimene (5.1%), sabinene (5.0%) and limonene (4.5%). The anticancer activities of oil were evaluated. The results showed that the oil exhibited cytotoxic activity against human oral, liver, lung, colon, melanoma, and leukemic cancer cells.

Keywords: Beilschmiedia erythrophloia, Essential oil, Cytotoxic activity, β -Caryophyllene, α -Humulene.

Beilschmiedia erythrophloia Hayata (Lauraceae) is a large plant widely distributed in Taiwan [1]. No studies have investigated the chemical composition and biological activities of the essential oils or other extracts from this species. Thus, we used hydrodistillation to collect the leaf oil, which was analyzed its chemical composition by GC–FID and GC–MS. In the second part of the study, we examined the *in vitro* anticancer activities of the leaf oil. The purpose of this study was to establish a chemical basis for effective multipurpose utilization of the tree species.

Hydrodistillation of *B. erythrophloia* leaves gave a yellow oil with a yield of 1.56 ± 0.02 mL/100 g, based on the dry weight of leaves. The identified constituents are presented in Table 1, where all compounds are listed in order of their elution from the DB-5 column. Fifty-five compounds were identified (Table 1), representing 100.0% of the oil. Among the groups, sesquiterpene hydrocarbons were predominant (51.7%), followed by monoterpene hydrocarbons (31.0%), oxygenated monoterpenes (13.9%), oxygenated sesquiterpenes (3.1%), and others (0.3%). Among the sesquiterpene hydrocarbons, β-caryophyllene (22.6%) and α-humulene (21.9%) were the major compounds. Of the monoterpene (4.5%), γ -terpinene (3.7%) and α -pinene (3.5%) were the chief compounds, whereas of the oxygenated monoterpenes, terpinen-4-ol (5.3%), and camphor (3.6%) was the major component.

Although the leaf oil constituents of *B. erythrophloia* was primarily sesquiterpenoids, like those of *B. pendula* [2], *B. brenesii*, *B. costaricensis*, *B. tilaranensis* [3], and *B. miersii* [4], their main components differed. Further comparison with the leaf oil of *B. alloiophylla* [3] and *B. tarairie* [4] were predominantly monoterpenoids and differed from the leaf oil of *B. erythrophloia*.

To evaluate the anticancer activities of leaf essential oil of *B. erythrophloia* from Taiwan, we tested the effect of essential oil on the viability of six human cancer cell lines: human oral squamous cancer OEC-M1 cells, human hepatocellular carcinoma J5 cells,

Table 1: Chemical composition of the leaf essential oil of B. erythrophloia.

Compounds	KI ^a	Content(%)	Identification ^b
n-Hexanol	871	0.1	MS, KI, ST
α-Thujene	930	0.3	MS, KI, ST
α-Pinene	939	3.5	MS, KI, ST
Camphene	954	1.1	MS, KI, ST
Sabinene	975	5.0	MS, KI, ST
β-Pinene	979	1.8	MS, KI, ST

γ-Eudesmol	1632	0.1	MS, KI
Humulene epoxide II 1- <i>epi</i> -Cubenol	1608 1629	0.3 0.1	MS, KI MS, KI
Viridiflorol	1583	1.6 0.1	MS, KI, ST MS, KI, ST
Spathulenol Carvonhyllene oxide	1578	0.2	MS, KI, ST
trans-Nerolidol	1563	0.7	MS, KI, ST
Elemol	1550	0.1	MS, KI, ST
δ-Cadinene	1523	1.9	MS, KI
y-Cadinene	1514	0.2	MS, KI
(Z,E) - α -Farnesene	1506	0.5	MS, KI
α-Muurolene	1500	0.2	MS. KI
Bicyclogermacrene	1500	1.2	MS, KI
β-Selinene	1490	0.1	MS, KI
Germadrene D	1485	1.8	MS. KI. ST
v-Muurolene	1480	0.3	MS, KI
allo-Aromadendrene	1460	0.1	MS KI
a-Humulene	1455	21.9	MS KI ST
Aromadendrene	1441	0.1	MS KI ST
B-Conaene	1412	0.1	MS KI ST
β-Carvonhyllene	1412	22.6	MS KL ST
a-Cedrene	1410	0.2	MS KI
g-Guriunene	1391	0.4	MS KI ST
B-Flemene	1301	0.1	MS KI ST
Geranyl acetate	1331	0.1	MS, KI, SI MS KI
g Cubabana	1289	0.8	MS, KI, SI MS VI ST
Bornyl acetate	1208	0.1	MS, KI, SI MS VI ST
trans Piperitol	1202	0.2	MS, KI, SI MS VI ST
n Decemel	1196	0.1	MS, KI, SI
a-1 erpineol	1189	0.7	MS, KI, SI
Terpinen-4-01	11//	5.3	MS, KI, SI
Borneol	1169	0.1	MS, KI, ST
Camphor	1146	3.6	MS, KI, ST
trans-p-Menth-2-en-1-ol	1141	0.2	MS, KI
allo-Ocimene	1132	0.1	MS, KI
cis-p-Menth-2-en-1-ol	1122	0.2	MS, KI
Perillene	1101	1.2	MS, KI, ST
Linalool	1097	0.6	MS, KI, ST
Terpinolene	1089	1.2	MS, KI, ST
cis-Sabinene hydrate	1070	0.1	MS, KI, ST
γ-Terpinene	1060	3.7	MS. KI. ST
cis-β-Ocimene	1037	5.1	MS, KI, ST
1,8-Cineole	1031	1.0	MS, KI, ST
Limonene	1029	4.5	MS, KI, ST
<i>p</i> -Cymene	1025	1.5	MS KI ST
a-Terninene	1017	1.8	MS KI ST
a-Phellandrene	1003	0.3	MS KI ST

^a Kovats index on a DB-5 column with reference to *n*-alkanes [5]. ^b MS, NIST and Wiley library spectra and the literature; KI, Kovats index; ST, authentic standard compounds.

human lung adenocarcinoma A549 cells, human colon cancer HT-29 cells, human melanoma UACC-62 cells, and human leukemic ell K562 cells. Cells were incubated with various concentration of essential oils for 48 h, and then the cell viabilities were measured by the alamarBlue[®] proliferation assay. The results showed that oil treatment for 48 h reduced the viability of OEC-M1 cells, J5 cells, A549 cells, HT-29 cells, UACC-62 cells, and K562 cells, with IC₅₀ around 32.6, 48.6, 38.8, 18.9, 5.8, and 6.8 µg/mL, respectively (Table 2). This is the first report on the anticancer activities of B. erythrophloia leaf essential oil against human oral, liver, lung, colon, melanoma, and leukemic cancer cells.

However, in order to ascertain the source compounds of anticancer activities from *B. erythrophloia*, the main components, α -pinene, sabinene, limonene, cis- β -ocimene, γ -terpinene, camphor, terpinen-4-ol, β -caryophyllene, and α -humulene were individually tested for their anticancer activities. The results showed that the active compounds were β -caryophyllene, and α -humulene. The IC₅₀ values of the two compounds against the six cancer cells were 24.0, and 14.7 µg/mL against OEC-M1 cells; 111.2, and 57.1 µg/mL against J5 cells; 31.3, and 16.7 µg/mL against A549 cells; 9.8, and 6.8 µg/mL against HT-29 cells; 3.2, and 2.8 µg/mL against UACC-62 cells; and 4.6 and 3.6 µg/mL against K562 cells, respectively (Table 2). β-Caryophyllene is reported to be cytotoxic against a number of human cancer cell lines including MCF-7, MDA-MB-468, UACC-257, A549, Hela, and HT-29 [6,7]. α -Humulene has been reported to be cytotoxic to MCF-7, DLD-1, L-929, A549, Hela, HT-29, and UACC-257 [7,8]. The presence of β -caryophyllene, and α humulene significantly contributed to the anticancer activities of B. erythrophloia leaf oil.

Experimental

Plant materials: Fresh leaves of *B. erythrophloia* were collected in May 2012 from from Chilan Mt in northeast Taiwan (Yilan County, elevation 950 m, N 24° 38' 58", 121° 39' 13"). The samples were compared with specimen no. ou 9856 from the Herbarium of National Chung-Hsing University and positively identified by Prof. Yen-Hsueh Tseng of NCHU. The voucher specimen (CLH-025) was deposited in the NCHU herbarium. Leaves of the species were collected for subsequent extraction and analysis.

Isolation of the leaf essential oil: Leaves of B. erythrophloia (1 Kg) was hydrodistilled for 8 h with 3 L of distilled water. The Su & Ho

essential oil obtained was dried with anhydrous sodium sulfate. The oil yield and all test data are the average of triplicate analyses.

Essential oil analysis: A Hewlett-Packard HP 6890 gas chromatograph equipped with a DB-5 fused silica capillary column (30 m x 0.25 mm x 0.25 µm film thickness, J&W Scientific) and a FID detector was used for the quantitative determination of oil components. Oven temperature was programmed as follows: 50°C for 2 min, rising to 250°C at 5°C/min. Injector temperature: 270°C. Carrier gas: He with a flow rate of 1 mL/min. Detector temperature: 250°C, split ratio: 1:10. Diluted samples (1.0 µL, 1/100, v/v, in ethyl acetate) were injected manually in the split mode. Identification of the oil components was based on their retention indices and mass spectra, obtained from GC/MS analysis on a Hewlett-Packard HP 6890/HP5973 equipped with a DB-5 fused silica capillary column (30 m x 0.25 mm x 0.25 µm film thickness, J&W Scientific). The GC analysis parameters are listed above and the MS were obtained (full scan mode: scan time: 0.3 s, mass range was m/z 30-500) in the EI mode at 70 eV. All data were the average of triplicate analyses.

Component identification: Identification of the leaf essential oil constituents was based on comparisons of Kovats index (KI) [5], retention times (RT), and mass spectra with those obtained from authentic standards and/or the NIST and Wiley libraries spectra, and literature [5,9].

Cell culture: Human oral squamous cancer OEC-M1 cells, human hepatocellular carcinoma J5 cells, human lung adenocarcinoma A549 cells, human colon cancer HT-29 cells, human melanoma UACC-62 cells, and human leukemic cell K562 cells were obtained from ATCC (Rockville, MD, USA) and multiplied in RPMI-1640 medium supplemented with 10% heated-inactivated FCS and 2 mM L-glutamine (Life Technologies, Inc., MD), and cultured in a 37°C, 5% CO₂ incubator.

Cell viability assay: The cytotoxicity of the essential oil was assessed using the alamarBlue® proliferation assay according to a protocol from AbD Serotec. Cells (3000 cells/well) were incubated with either essential oils (dissolved in DMSO, final 0.1% DMSO in medium) or vehicle control (0.1% DMSO) for 24 h and 48 h, followed by replacing with fresh medium containing 10% alamarBlue® reagent for an additional 6 h. The absorbances at 570 nm and 600 nm were measured by a microplate reader. All data were the average of triplicate analyses.

Table 2: IC₅₀ values of *B. erythrophloia* leaf oil and it's main constituents against cancer cell lines.

	$IC_{so}(\mu g/mL)$									
Cell lines ^a	Essential oil	Compounds ^o								
		α-Pinene	Sabinene	Limonene	cis-β-Ocimene	γ-Terpinene	Camphor	Terpinen-4-ol	β-Caryophyllene	α-Humulene
OEC-M1	32.6	>200	>200	>200	>200	>200	>200	>200	24	14.7
J5	48.6	>200	>200	>200	>200	>200	>200	>200	111.2	57.1
A549	38.8	>200	>200	>200	>200	>200	>200	>200	31.3	16.7
HT-29	18.9	>200	>200	>200	>200	>200	>200	>200	9.8	6.8
UACC-62	5.8	>200	>200	>200	>200	>200	>200	>200	3.2	2.8
K562	6.8	>200	>200	>200	>200	>200	>200	>200	4.6	3.6

^a Cell lines: OEC-M1 (human oral squamous); J5 (human hepatocellular carcinoma); A549 (human lung adenocarcinoma); HT-29 (human colon), UACC-62 (*human* melanoma); K562 (*human* leukemic).^b α-Pinene (98%), sabinene (99%), limonene (97%), *cis*-β-ocimene (95%), γ-terpinene (98%), camphor (96%), terpinen-4-ol (95%), β-caryophyllene (98.5%), α-humulene (96%). All compounds were purchased from Sigma-Aldrich (Milwaukee, USA).

References

- Kuo PC. (1995) The Precious 5 Conifers of Taiwan. Chinese Forestry Association, Taipei, Taiwan.
- [1] [2] Chaverri C, Ciccio JF. (2010) Essential oils from Beilschmiedia pendula (Sw.) Hemsl. (Lauraceae) from Costa Rica. Journal of Essential Oil Research, 22, 259-262
- [3] Setzer WN, Haber WA. (2007) Leaf essential oil composition of five species of Beilsehmiedia from Monteverde, Costa Rica. Natural Product Communications, 2, 79-83
- Scora RW, Scora PE. (2001) Essential leaf oil of Persea subgenus Eriodaphne and closely related Perseoid genera. Journal of Essential Oil [4] Research, 13, 37-42.
- Massada Y. (1976) Analysis of Essential Oil by Gas Chromatography and Spectrometry, Wiley, New York. Cole RA, Bansal A, Moriarity DM, Haber WA, Setzer WN. (2007) Chemical composition and cytotoxic activity of the leaf essential oil of Eugenia [6] zuchowskiae from Monteverde, Costa Rica. Journal of Natural Medicines, 61, 414-417
- [7] Silva SL, Chaar JS, Figueiredo PMS, Yano T. (2008) Cytotoxic evaluation of essential oil from Casearia sylvestris Sw on human cancer cells and erythrocytes. Acta Amazonica, 38, 107-112.
- Legault J, Pichette A. (2007) Potentiating effect of beta-caryophyllene on anticancer activity of alpha-humulene, isocaryophyllene and paclitaxel. [8] Journal of Pharmacy and Pharmacology, 59, 1643-1647.
- [9] Adams RP. (2001) Identification of Essential Oil Components by Gas Chromatography/Quadruple Mass Spectroscopy, Allured, Carol Stream, IL.

Fusaodavinvin, a Novel Metabolite Containing Sulfur from the Endophytic Fungus <i>Fusarium sp.</i> (CTGU-ZL-34) Liang Zhang, Yanhui Liu, Zhangshuang Deng, Zhiyong Guo, Jianfeng Chen, Xuan Tu and Kun Zou	83
Summation Solute Hydrogen Bonding Acidity Values for Hydroxyl Substituted Flavones Determined by NMR Spectroscopy William L. Whaley, Ekua M. Okoso-amaa, Cody L. Womack, Anna Vladimirova, Laura B. Rogers, Margaret J. Risher and Michael H. Abraham	85
Phenolic Content and DPPH Radical Scavenging Activity of the Flowers and Leaves of Trifolium repens Agnieszka Kicel and Maria Wolbiś	99
Kenganthranol F, a new Anthranol from <i>Psorospermum aurantiacum</i> Gesquiere M. Tiani, Ishtiaq Ahmed, Karsten Krohn, Ivan R. Green and Augustin E. Nkengfack	103
Inhibition of <i>In Vitro</i> Leukotriene B ₄ Biosynthesis in Human Neutrophil Granulocytes and Docking Studies of Natural Quinones Premysl Landa, Zsofia Kutil, Veronika Temml, Jan Malik, Ladislav Kokoska, Ute Widowitz, Marie Pribylova, Marcela Dvorakova, Petr Marsik, Daniela Schuster, Rudolf Bauer and Tomas Vanek	105
Effect of <i>Hypogymnia physodes</i> Extracts and their Depsidones on Micronucleus Distribution in Human Lymphocytes Igor Ž. Stojanović, Miroslava Stanković, Olga Jovanović, Goran Petrović, Andrija Šmelcerović and Gordana S. Stojanović	109
A New Lyoniresinol Derivative from <i>Smilax microphylla</i> Li-Sha Liu, Hui-Lian Huang, Rong-Hua Liu, Gang Ren, Feng Shao, Yao-Hui Ye and Tao Lin	113
Anti-metastatic Activities of Bibenzyls from <i>Dendrobium pulchellum</i> Pithi Chanvorachote, Akkarawut Kowitdamrong, Thidarat Ruanghirun, Boonchoo Sritularak, Chutichot Mungmee and Kittisak Likhitwitayawuid	115
Synthesis of 3,5,3',4'-Tetrahydroxy- <i>trans</i> -stilbene-4'- <i>O</i> -β-D-glucopyranoside by Glucosyltransferases from <i>Phytolacca americana</i> Tomoya Iwakiri, Hiroya Imai, Hiroki Hamada, Toru Nakayama and Shin-ichi Ozaki	119
Challenges of Curcumin Bioavailability: Novel Aerosol Remedies Parasuraman Aiya Subramani and Venkata R. Narala	121
Molecular Analysis of <i>Vitex</i> Species Using Candidate DNA Barcoding and PCR-RFLP of the <i>mat</i> K Gene for Authentication of <i>Vitex glabrata</i>	
Waranyoo Phoolcharoen and Suchada Sukrong	125
Volatiles from <i>Syzygium paniculatum</i> Fruit Clara E. Quijano-Célis, Daniel Echeverri-Gil, Yinet Ruiz and Jorge A. Pino	129
Seasonal Variations in the Composition of the Essential Oils of Rosemary (<i>Rosmarinus officinalis</i> , Lamiaceae) Dmitar Lakušić, Mihailo Ristić, Violeta Slavkovska and Branislava Lakušić	131
Chemical Compositions and Antimicrobial and Antioxidant Activities of the Essential Oils from Magnolia grandiflora, Chrysactinia mexicana, and Schinus molle Found in Northeast Mexico Laura Guerra-Boone, Rocío Álvarez-Román, Ricardo Salazar-Aranda, Anabel Torres-Cirio, Verónica Mayela Rivas-Galindo, Noemí Waksman de Torres, Gloria María González González and Luis Alejandro Pérez-López	135
Chemical Composition and Biological Activities of the Essential Oil from <i>Calamintha nepeta</i> Plants from the Wild in Southern Italy	
Emilia Mancini, Laura De Martino, Hanna Malova and Vincenzo De Feo	139
Composition and <i>in-vitro</i> Cytotoxic Activities of the Leaf Essential Oil of <i>Beilschmiedia erythrophloia</i> from Taiwan Yu-Chang Su and Chen-Lung Ho	143

Natural Product Communications 2013

Volume 8, Number 1

Contents

Argan Tree Metabolites and Argan Oil

(Guest Editor: Dom Guillaume)

<u>Original Paper</u>	<u>Page</u>
The Social and Environmental Context of Argan Oil Production Yann le Polain de Waroux	1
Climatic Conditions and Herbivory Effects on Morphological Plasticity of Argania spinosa Fatima Ain-Lhout, María Zunzunegui, Mari Cruz Díaz Barradas, Juan Jáuregui, Tarik Tagma and Said Boutaleb	5
Some Secrets of Argania spinosa Water Economy in a Semiarid Climate Mari Cruz Díaz Barradas, María Zunzunegui, Mari Paz Esquivias, Said Boutaleb, Javier Valera-Burgos, Tarek Tagma and Fátima Ain-Lhout	11
Germination Success and Seedling Development of <i>Argania spinosa</i> under Different Climatic Conditions and Browsing Intensity María Zunzunegui, Juan Jáuregui, Fatima Ain-Lhout, Said Boutaled, Leonor Álvarez-Cansino and MariPaz Esquivias	15
Modeling of the Distribution of Heavy Metals and Trace Elements in Argan Forest Soil and Parts of Argan Tree Faez A. E. Mohammed, Rahma Bchitou, Mohamed Boulmane, Ahmed Bouhaouss and Dominique Guillaume	21
Can Fruit-form be a Marker for Argan Oil Production? Saïd Gharby, Hicham Harhar, Badr Eddine Kartah, Hanae El Monfalouti, Clément Denhez, Miloudi Hilali, Dom Guillaume and Zoubida Charrouf	25
Chemical Changes in Extra Virgin Argan Oil after Thermal Treatment Saïd Gharby, Hicham Harhar, Badr Eddine Kartah, Dom Guillaume and Zoubida Charrouf	29
Volatile Compound Formation During Argan Kernel Roasting Hanae El Monfalouti, Zoubida Charrouf, Manuela Giordano, Dominique Guillaume, Badreddine Kartah, Hicham Harhar, Saïd Gharby, Clément Denhez and Giuseppe Zeppa	33
Quality Parameters for Cold Pressed Edible Argan Oils Bertrand Matthäus	37
Triterpenoids from Argania spinosa: 20 Years of Research Imane Chafchaouni-Moussaoui, Zoubida Charrouf and Dom Guillaume	43
Argan Oil-contained Antioxidants for Human Mitochondria Luis C. López, Carmen Cabrera-Vique, Carmen Venegas, Laura García-Corzo, Marta Luna-Sánchez, Darío Acuña-Castroviejo and Germaine Escames	47
Effect of Argan and Olive Oil Consumption on the Hormonal Profile of Androgens Among Healthy Adult Moroccan Men Abdelfettah Derouiche, Ali Jafri, Issam Driouch, Mohammed El Khasmi, Ahmed Adlouni, Nada Benajiba, Youssef Bamou, Rachid Saile and Mohammed Benouhoud	S56
Argan Oil and Postmenopausal Moroccan Women: Impact on the Vitamin E Profile Hanae El Monfalouti, Zoubida Charrouf, Asma El Hamdouchi, Hanane Labraimi, Imane Chafchaouni-Moussaoui, Badreddine Kartah, Khalid El Kari, Yahya Bensouda, Abdelfettah Derouich, Sylvie Dodin, Clément Denhez, Dom Guillaume and Hassan Agnaou	55
Microbial Transformations of Isophorone by Alternaria alternata and Neurospora crassa Ismail Kiran, Özge Özşen, Turgay Çelik, Semra İlhan, Bükay Yenice Gürsu and Fatih Demirci	59
Headspace, Solid-phase Micro-extraction, Gas Chromatographic-Mass Spectrometric Analysis of Terpenoids in the Latex of Euphorbia Species	
Asmita V. Patel, Stephen Sumner, H. Leslie Thompson, Gerald Blunden, David Wright, Liu Jun-feng and Zan Jun-feng Trichodermaerin, a New Diterpenoid Lactone from the Marine Fungus <i>Trichoderma erinaceum</i> Associated with the Sea Star	63
Acanthaster planci Zhong-Liang Xie, Hou-Jin Li, Lai-You Wang, Wan-Ling Liang, Wei Liu and Wen-Jian Lan	67
Chemical Composition and Biological Activities of Soldiers of the Brazilian Termite Species, <i>Nasutitermes macrocephalus</i> (Isoptera: Natutitermitinae)	
Daniela S. Alviano and Claudia M. Rezende	69
Evaluation of the Quality of Chinese and Vietnamese Cassia Using LC-MS and Multivariate Analysis Ken Tanaka, Feng Li, Yasuhiro Tezuka, Shiro Watanabe, Nobuo Kawahara and Hiroaki Kida	75
Norcucurbitane Triterpenoids from the Fruits of <i>Momordica charantia</i> var. abbreviata Yun-Wen Liao, Chiy-Rong Chen, Jue-Liang Hsu, Yun-Sheng Lin, Hsueh-Ling Cheng, Wen-Ling Shih, Yueh-Hsiung Kuo and Chi-I Chang	79

Continued inside backcover