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

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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 hydrocarbons, *cis*-β-ocimene (5.1%), sabinene (5.0%), limonene (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
β-Myrcene	991	1.2	MS, KI, ST
α-Phellandrene	1003	0.3	MS, KI, ST
α-Terpinene	1017	1.8	MS, KI, ST
p-Cymene	1025	1.5	MS, KI, ST
Limonene	1029	4.5	MS, KI, ST
1,8-Cineole	1031	1.0	MS, KI, ST
cis-β-Ocimene	1037	5.1	MS, KI, ST
γ-Terpinene	1060	3.7	MS, KI, ST
cis-Sabinene hydrate	1070	0.1	MS, KI, ST
Terpinolene	1089	1.2	MS, KI, ST
Linalool	1097	0.6	MS, KI, ST
Perillene	1101	1.2	MS, KI, ST
cis-*p*-Menth-2-en-1-ol	1122	0.2	MS, KI
allo-Ocimene	1132	0.1	MS, KI
trans-*p*-Menth-2-en-1-ol	1141	0.2	MS, KI
Camphor	1146	3.6	MS, KI, ST
Borneol	1169	0.1	MS, KI, ST
Terpinen-4-ol	1177	5.3	MS, KI, ST
α-Terpineol	1189	0.7	MS, KI, ST
cis-Piperitol	1196	0.1	MS, KI, ST
n-Decanal	1202	0.2	MS, KI, ST
trans-Piperitol	1208	0.1	MS, KI, ST
Bornyl acetate	1289	0.8	MS, KI, ST
α-Cubebene	1351	0.1	MS, KI, ST
Geranyl acetate	1381	0.1	MS, KI
β-Elemene	1391	0.4	MS, KI, ST
α-Gurjunene	1410	0.2	MS, KI, ST
α-Cedrene	1412	0.2	MS, KI
β-Caryophyllene	1419	22.6	MS, KI, ST
β-Copaene	1432	0.1	MS, KI, ST
Aromadendrene	1441	0.1	MS, KI, ST
α-Humulene	1455	21.9	MS, KI, ST
allo-Aromadendrene	1460	0.1	MS, KI
γ-Muurolene	1480	0.3	MS, KI
Germadrene D	1485	1.8	MS, KI, ST
β-Selinene	1490	0.1	MS, KI
Bicyclogermacrene	1500	1.2	MS, KI
α-Muurolene	1500	0.2	MS, KI
(*Z*,*E*)-α-Farnesene	1506	0.5	MS, KI
γ-Cadinene	1514	0.2	MS, KI
δ-Cadinene	1523	1.9	MS, KI
Elemol	1550	0.1	MS, KI, ST
trans-Nerolidol	1563	0.7	MS, KI, ST
Spathulenol	1578	0.2	MS, KI, ST
Caryophyllene oxide	1583	1.6	MS, KI, ST
Viridiflorol	1593	0.1	MS, KI, ST
Humulene epoxide II	1608	0.3	MS, KI
1-*epi*-Cubenol	1629	0.1	MS, KI
γ-Eudesmol	1632	0.1	MS, KI
Monoterpene hydrocarbons		**31.0**	
Oxygenated monoterpenes		**13.9**	
Sesquiterpene hydrocarbons		**51.7**	
Oxygenated sesquiterpenes		**3.1**	
Others		**0.3**	
Yield (mL/100g)		**1.56 ± 0.02**	

[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 IC_{50} 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 IC_{50} 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 24º 38′ 58″, 121º 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 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: 50ºC for 2 min, rising to 250ºC at 5ºC/min. Injector temperature: 270ºC. Carrier gas: He with a flow rate of 1 mL/min. Detector temperature: 250ºC, 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% CO_2 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: IC_{50} values of *B. erythrophloia* leaf oil and it's main constituents against cancer cell lines.

Cell lines [a]	Essential oil	IC_{50} (μg/mL) Compounds[a]								
		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).

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