NOTE

Chemistry and Cytotoxic Activity of Essential Oil from the Stem Bark of *Calophyllum soulattri*

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GCMS analysis of the essential oil from the stem bark of *Calophyllum soulattri* detected twenty components. Allo-aromadendrene was present as the most abundant component. Two other major constituents are α -gurjunene and β -eudesmene. The volatile oil exhibited moderate cytotoxicity against SNU-1, Hep G2, NCI-H23, K562, Raji, IMR-32 and SK-MEL-28 cells.

Key Words: *Calophyllum soulattri*, Clusiaceae, Cytotoxic, Essential oil.

Calophyllum soulattri belongs to a big family of Guttiferae or Clusiaceae which has 40 genera and over 1600 species of trees and shrubs. This species is widely distributed in South-east Asia, Australasia, Pacific Island and Latin America. *Calophyllum soulattri* has a variety of common names such as mintak, bintangor labu, malang-malang and pamintaogon. Previous studies on *Calophyllum* species have revealed it to be rich in potential biologically active secondary metabolites¹⁻⁴. A number of these compounds have been reported to have good medicinal properties and have shown important biological effects in anti HIV^{5,6}, antifungal and antimicrobial⁷ tests. Some of these compounds are cancer chemo-preventive agents⁸. As part of our continuing interest in the chemotaxonomy of medicinally important flora of Malaysia, we report here our findings on the chemical composition of the essential oil from the stem bark of *Calophyllum soulattri* and its cytotoxicity towards nine human cancer cell lines.

The stem bark of *Calophyllum soulattri* was collected from the Sri Aman Division, Sarawak, Malaysia.

Extraction and isolation: About 1 kg of air-dried stem bark of *Calophyllum soulattri* was ground into fine powder and extracted successively with 2 L of *n*-hexane using a soxhlet apparatus for 24 h. The hot hexane extract was evaporated to dryness under vacuum condition to give 101.2 g of dry hexane extract. Part of the hexane extract was subjected to a vacuum column chromatography over silica gel and eluted with *n*-hexane followed by dichloromethane, ethyl acetate and methanol to give 12 fractions. The first three fractions were combined and further subjected to column chromatography

over silica gel by eluting with hexane-dichloromethane, dichloromethane-ethyl acetate and ethyl acetate-methanol to yield 10 fractions. A pale yellowish oil was obtained as the first fraction.

GC-MS analysis: The essential oil was analyzed by Shimadzu GC-MS model QP2010 Plus spectrophotometer equipped with HP5MS (5 % phenyl methylsiloxane) capillary column of dimension 30.0 m $\times$ 250 μ m $\times$ 0.25 μ m with helium as the carrier gas. The GC oven temperature was programmed from 50-320 °C at a rate of 10 °C min⁻¹ with an initial hold of 3 min and a final hold of 15 min. The essential oil was dissolved in chloroform and 0.1 μ L of the dilute sample was injected into the GC. Identification of compounds was accomplished by direct comparison with retention times of the peaks and corresponding data in literature and computer mass spectral libraries of Wiley 275 and PMW TOX2.

Cytotoxicity (MTT assay): The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed according to the reported method⁹. The tests were performed in the sterile 96-well flat bottom plate. A six-point serial dilution was developed to obtain six different sub-stocks with different concentrations. The essential oil was tested in triplicate together with the controls.

After 72 h incubation at 37 °C with 5 % of CO₂, 20 μ L of MTT solution was added into all the filled wells and incubated again for 3 h. The plate was spun at 1500 rpm for 10 min followed by discarding *ca.* 80 % of the supernatant carefully. The volume of the supernatant discarded was the same as the volume of DMSO added into the wells. The absorbance of

each well was determined by a microplate reader at 550 nm after the purple formazan crystals fully dissolved in DMSO. Three independent experiments for both suspension and anchorage-dependant cell lines were conducted. The average absorbance value was used in the calculation of percentage of cell viability. The cytotoxicity index used was IC_{50} , which is the concentration that yields 50 % inhibition of the cells compared with the untreated control.

Purification process by column chromatography techniques on the hexane extract of *Calophyllum soulattri* resulted in a mixture of essential oils. There is no previous report on the constituents of the essential oils of *Calophyllum soulattri* obtained either from hydro-distillation or the isolation and purification process. Analysis of the essential oil by GC and GC-MS resolved the oil constituents into 20 components. Details of each component which included retention time and molecular mass are presented in Table-1.

TABLE-1
CHEMICAL COMPOSITION OF THE ESSENTIAL
OIL FROM STEM BARK OF *Calophyllum soulattri*

Retention time (min)	Compound	Retention indices	Area (%)	Molecular mass
14.484	α -Cubebene	1344	0.23	204
14.955	α -Gurjunene	1419	12.30	204
15.703	allo-Aromadendrene	1386	36.79	204
16.083	β -Eudesmene	1469	2.42	204
16.150	Isocaryophyllene	1494	0.73	204
16.200	β -Cyperene	1481	0.21	204
16.337	γ -Cadinene	1435	1.42	204
17.956	Aromadendrene oxide	1462	1.21	220
18.350	Cadalene	1706	0.55	198
20.200	Tridecyl ester	1846	0.59	268
21.220	Pentadecyl ester	2044	0.45	296
22.191	Heptadecyl ester	2243	0.16	296
23.968	Muscalure	2315	0.15	322
24.167	<i>n</i> -Tricosane	2307	0.07	324
25.007	<i>n</i> -Tetracosane	2407	0.10	338
25.637	<i>Z</i> -12-Pentacosene	2514	0.38	350
25.814	<i>n</i> -Eicosane	2009	0.15	282
26.589	<i>n</i> -Octacosane	2804	0.08	394
27.183	1-Octacosanol	3047	0.20	410
28.210	Spinacene	2914	0.29	410

Allo-aromadendrene (36.79 %) was present as the major constituent followed by α -gurjunene (12.30 %) and β -eudesmene (2.42 %). The other 17 components present in the essential oils were minor constituents. These were made up of γ -cadinene (1.42 %), aromadendrene oxide (1.21 %), isocaryophyllene (0.73 %), tridecyl ester (0.59 %), cadalene (0.55 %), pentadecyl ester (0.45 %), *Z*-12-pentacosene (0.38 %), spinacene (0.29 %), α -cubebene (0.23 %), β -cyperene (0.21 %), 1-octacosanol (0.20 %), heptadecyl ester (0.16 %), muscalure (0.15 %), *n*-eicosane (0.15 %), *n*-tetracosane (0.10 %), *n*-octacosane (0.08 %) and *n*-tricosane (0.07 %).

The essential oil was evaluated for its cytotoxic activity against several human cancer cell lines, *in vitro*, using the MTT method. The cell lines are SNU-1 (stomach), HeLa (cervix), Hep G2 (liver), NCI-H23 (lung), K562 (leukemia), Raji

(lymphoma), LS174T (colon), IMR-32 (neuroblastoma) and SK-MEL-28 (skin) cells. The oil showed moderate cytotoxic activity against all the cell lines except for HeLa and LS174T cells. Kaempferol and quercetin were used as positive controls for all the cell lines. The IC_{50} values of the essential oil against the human cancer cell lines are summarized in Table-2. It was observed that the essential oil mixture gave moderate activity against the SNU-1 (stomach), K562 (leukemia), IMR-32 (neuroblastoma) and Raji (lymphoma) cell lines with IC_{50} values of 21.87, 23.75, 27.50 and 29.16 μ g/mL.

TABLE-2
CYTOTOXICITY (IC_{50} , μ g/mL) OF ESSENTIAL OIL
AGAINST SNU-1, HeLa, NCI-H23, Hep G2, K562, Raji,
LS174T, SK-MEL-28 AND IMR-32 CELLS

Human cancer cells	IC_{50} (μ g/mL)*		
	Essential oil	Kaempferol**	Quercetin**
SNU-1 (stomach)	21.87 $\pm$ 0.58	10.93 $\pm$ 0.08	6.30 $\pm$ 1.01
HeLa (cervix)	>35.00	5.00 $\pm$ 1.13	8.00 $\pm$ 1.00
NCI-H23 (lung)	31.25 $\pm$ 1.10	18.75 $\pm$ 0.21	17.50 $\pm$ 0.31
Hep G2 (liver)	31.25 $\pm$ 1.22	33.33 $\pm$ 0.51	5.21 $\pm$ 0.95
K562 (leukemia)	23.75 $\pm$ 0.20	>35.00	9.89 $\pm$ 1.20
Raji (lymphoma)	29.16 $\pm$ 1.10	12.50 $\pm$ 0.80	2.08 $\pm$ 0.40
LS174T (colon)	>35.00	>35.00	>35.00
SK-MEL-28 (skin)	33.33 $\pm$ 0.32	21.87 $\pm$ 0.86	21.88 $\pm$ 0.84
IMR-32 (neuroblastoma)	27.50 $\pm$ 0.07	>35.00	31.25 $\pm$ 0.41

*The data shown are means $\pm$ SEM of three independent experiments.

** Positive control.

Conclusion

The stem bark of *Calophyllum soulattri* afforded an essential oil mixture which exhibited moderate cytotoxicity towards seven human cancer cell lines, SNU-1, NCI-H23, Hep G2, K562, Raji, SK-MEL-28 and IMR-32 cells. The major constituent of the non-volatile oil was allo-Aromadendrene (36.79 %) followed by α -gurjunene (12.30 %) and β -eudesmene (2.42 %).

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