Indonesian Journal of Cancer Chemoprevention, October 2011
ISSN: 2088–0197
e-ISSN: 2355-8989

Anticancer Evaluation of Plants from Indonesian Tropical Rain Forests

Jamilah Abbas*

Research Centre for Chemistry, Indonesian Institute of Sciences
PUSPIPTEK. Serpong. 15314. Indonesia

Abstract

The anticancer activities of medicinal plants from Indonesia's tropical rainforests were investigated against Hela cell line. Maytenfoliol from leaves of *Calophyllumtetrapterum* 3-epibetulinic acid from stem bark of *C. tomentosum* Wight and D.A-friedo-oleanan-3-on from stem bark of *C. moonii* showed anticancer activity. The compound 3-epibetulinic acid showed the more potent anticancer activity than maytenfoliol and D.A-friedo-oleanan-3- with thean IC_{50} value were 3.17 μg/mL, 4.89 μg/mL and 5.63 μg/mL, respectively.

Keywords: Anticancer evaluation, Hela cell line, *Calophyllumtetrapterum. C. tomentosum* Wight and *C. moonii*

INTRODUCTION

Cancer is the greatest human killer after cardiovascular disease. According to the World Health Organization (WHO) there are about 6 million people who suffer from disease each year, and resulting up to 15 deaths/100.000 patients. Cancer is caused by cell proliferation. Some factors such as carcinogenic chemicals, radioactive substances (UV ray, X ray), mutagenic reagents, virus and fast food cause have increasingly contributed to cancer cases every year. Until now, the treatment of cancer remains increasingly problematic and apparently the battle has not been successful (Marcus A.K *et al*., 1979 and Ross R., 1993). A currently popular approach to cancer therapy is through utilizing natural resources.

The genus *Calophyllum* is a member of Guttiferae (Clusiaceae) family. The Guttiferae family is well known to contain xanthones, coumarins, flavonoids and benzophenones. *C. monii* is a small tree and grown in lownlawn land. *C. tetrapterum* and *tomentosum* Wight are big trees reaching a heightof 33 m and 250 cm girth. The most popular species from the genus is the *C. lanigerum* since from it calanolida as an anti HIV-1 have been successfully been isolated (Taher M *et al., 2005*).

The compound 4-phenylcoumarin have successfully been isolated from *C. inophyllum* L. (guttiferae), and have activity as an antitumor agent, by examining their possibility of inhibitory effects on Epstein-Barr virus early antigen (EBV-EA), activation induced by 12-O-tetradecanoylphorbol-13-acetate in raji Cell (Itogawa *et al*., 2001). Herein we wish to report the isolation and structural elucidation of Maytenfoliol, 3-epibetulinic acid and D.A-friedo-oleanan-3-on as anticancer from the ethyl acetate extract of *C. tetrapterum*, *C. tomentosum* Wight and *C. moonii* respectively have been conducted. The genus *Calophyllum* which comprises 200 species is are widely distributed in the tropical rain forest where several species are used in folk medicine (Guilet D *et al* 2001). *C. tetrapterum*, *C. tomentosum* Wight and *C. moonii* are from the Tropical Rain Forest of the Indonesian continent (Soerianaga and Lemmens, 1994).

*Corresponding author e-mail : jamilahabbas@yahoo.com

MATERIALS AND METHODS

General experimental materials and equipment

^{1}H-NMR, ^{13}C-NMR one 1D,and two dimensional spectrum 2D type JNM-ECA-400 were observed using NMR-spectrophotometer type JNM-ECA-400, from Jeol with 500 MHz in, $CDCl_3$ was used as solvent : ∂ (ppm).LC-MS Mariner Biored (70 eV). FTIR type Prestige 21 Shimadzu (KBr pellets), spectrophotometer UV-VIS prepared by using Hitachi model 2000 U with MeOH and or $CHCl_3$ as a solvent. Fisher Scientific serial 903N 0056 was used for determined of melting point instrument. The following adsorbents were used for purification. TLC was conducted using Merck Kieselgel 60 F254. Column chromatography was carried out using: Merck Kieselgel 60 also usedas stationary phase and H_2SO_4 in MeOH as mobile phase and $FeCl_3$ as spraying reagent.

Plant material

The stem bark of *C. monni*were collected from Jayapura, Papua, *C. tomentosum* Wight from Bogor, West Java and leaves of *C. tetrapterum* fromKerinci district, Riau-Jambi Province, West Sumatra. The samples were then authenticated by Ismail Rahman. The voucher specimen of plant material were deposited at the Herbarium of Botanical Research Institute, Indonesian Institute of Science, Bogor, Indonesia.

Cell line and reagents

Hela cell line was obtained from Japan, Eangle's medium (Nissui), foetal serum (Flow laboratories), Glutamin (Nissui/Sigma). Other chemical reagents were of analytical grade. $NaHCO_3$, methanol, ethanol, *n*-hexane, ethyl acetate, chloroform was purchased from local market.

Bioasssay anticancer

Preparation of medium

Medium formula prepared as below:

- 47 g Eangles MEM medium (Nissui) are dissolved in 475 mL H_2O (solvent A)
- 13 g $NaHCO_3$ (E Merck) are dissolved in 50 mL H_2O and added 0.3g glutamine (Nissui) (solvent B)
- 25 mL solvent B was added to solvent A, and filtered by Millipore, these medium stored until used

For bioassay purpose: 15 ml foetal serum (flow laboratories) was added to 85ml medium. The medium containing serum was used for bioassay test and initial cell amount 100 x 10^4 cell/mL.

Bioasssay test (Yoo, T.J (1999)

The anticancer assays were carried out in against Hela cell lines. The bioassays were performed in the multi-well plate tissue culture (1 mL cell/well). Five various doses of the samples (0 ; 0.8; 1.6; 3.2 and 6.4 µg/mL) were diluted in methanol and 1 ml methanol was used as control. The samples and control were added to the cells and were incubated during 48 h in CO_2 incubator at 37^o C. After incubation, cell growth was calculated by microscope haemocytometer Fuch Rosentral. The cultured cells were treated at five concentrations of pure test compounds ranging (0; 0.8; 1.6; 3.2 and 6.4 µg/mL). Initial cell amount of 100 x 10^4 cell/mL. Percentage of inhibition cancer cell lines by three compounds were calculated as:

$$\% \text{ inhibition} = \frac{\text{cell in control} - \text{cell in sample}}{\text{cell in control}} \times 100\%$$

IC_{50} value was defined as the concentration of sample necessary needed to inhibit the cell growth to 50% of the control.

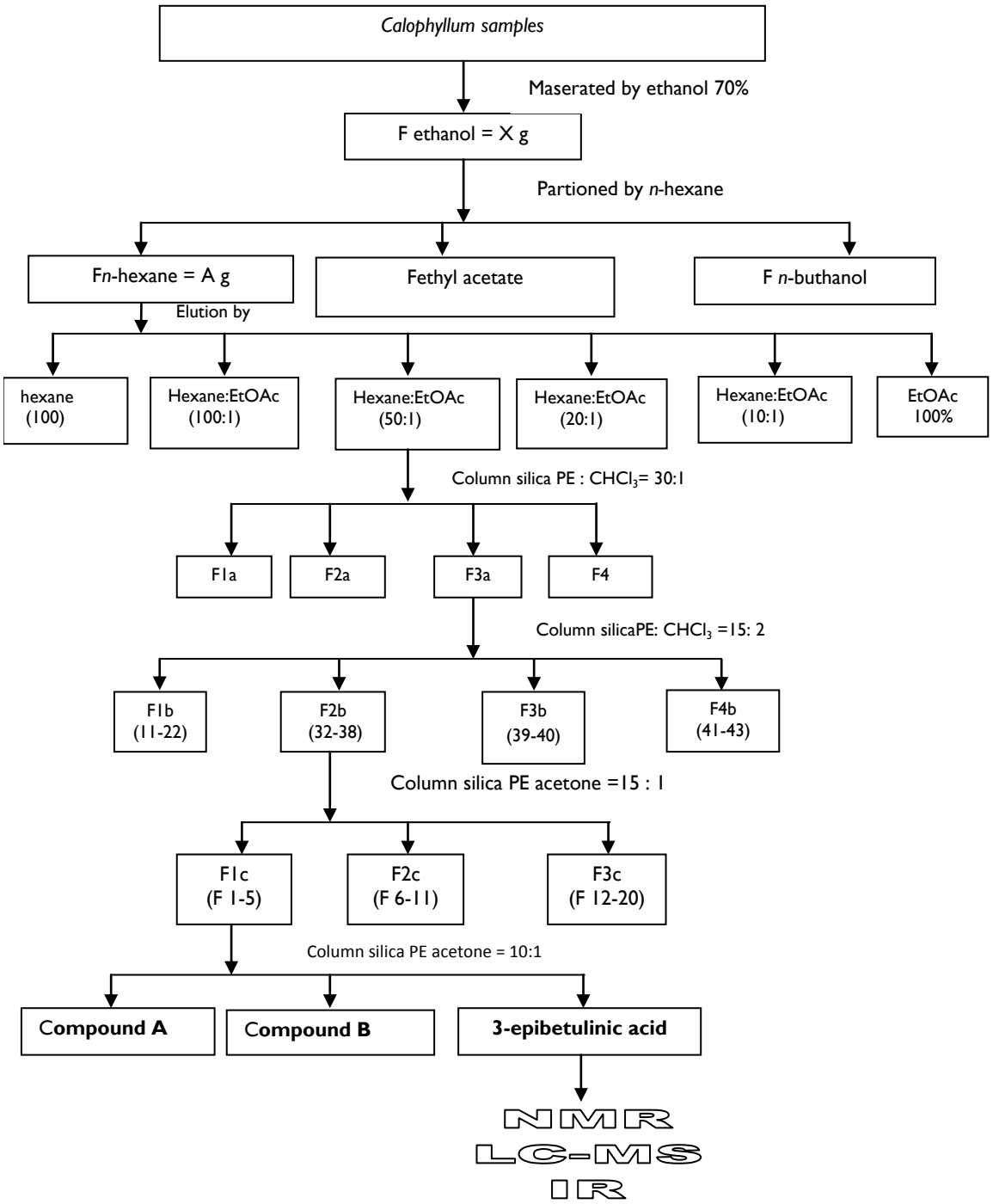


Figure 1. Scheme of extraction and isolation of plant materials

RESULTS AND DISCUSSION

Compound 1 (maytenfoliol)

Compound 1 (maytenfoliol) was obtained from leaves of *C. tetrapterum* as white needles with melting point at 300-302°C. The infrared spectrum (KBr) showed absorption bands for carbonyls group at 1700 cm^{-1}. The band at 1707cm^{-1} assigned to carbonyl (C=O), for two alcohol at C-17 & C-20 and one ketone. For C-3 and the band at 2920, 2850 cm^{-1} were assigned to C-H aliphatic stretching (C-3). The band at 3547 cm^{-1} was assigned as hydroxyl group. The presence of C-H bending can be found at 1445, 1380, 1040 and 1010 (cm^{-1}). Based on the ^{1}H-NMR spectrum of compound 1 ($CDCl_3$ Table I) of compound 1, it can be suggested that the compound 1 is maytenfoliol, $C_{30}H_{50}O_3$Mol weight 458.2.

^{13}C-NMR ($CDCl_3$): ∂ 6.8 (q), 14.6 (q), 18.0 (q), 18.3 (t) 19.0 (q), 19.2 (q), 22.2 (t), 28.1. (t), 29.1 (q), 30.1(t), 31.2 (t) ,31.4 (t), 32.8 (t), 33.3 (t), 34.2 (s) 34.5 (s) 35.1 (t); 35,39 (s); 37,4 (d); 38,1(s); 39,3(t); 41,2(t); 41,5(s);42,1(d); 52,4(d); 58,2(d); 59,4(t); 68.0(t); 213.2 (s, C=O)

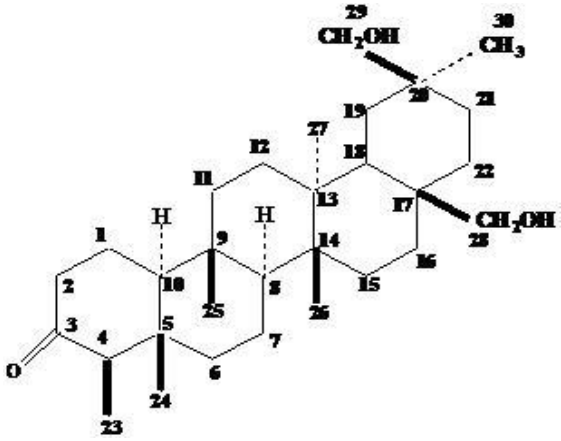


Figure 2. Structure of the Maytenfoliolmaytenfoliol

Compound 2 (3-epibetulinic acid)

Compound 2 (3-epibetulinic acid) was obtained from the stem bark of *C. tomentosum* Weigh as fine white crystal. Melting point 280-281^{o}C, MW: 456.711, Molecule formula $C_{30}H_{48}O_3$ (calc 456.3603)$[M]^+$ = 456.711. The infrared spectrum with KBr pellet showed absorption bands for hydroxyl (OH) at υ 3481 cm^{-1} indicating the presence of hydroxyl group (OH). In addition, the spectrum showed the signal and for ($C=CH_2$) at 2926, 1384, 780 indicating the presence of ($C=CH_2$), and the signal for (CO_2H) at 1707 indicating the presence of (CO_2H). It is similar with 3-epibetulinic acid from reference (Sung, T.V *et al.*, 1991): e.i 3440 (OH), 3070, 1644, 890 ($C=CH_2$), 1700 (CO_2H).

The band at υ 2926, 2868 cm^{-1} were assigned to C-H aliphatic stretching and the signals at C-H bending at υ 1450 1384, 1361 (cm^{-1}) indicating the presence of C-H bending absorptions. The complete data of NMR spectrum can be found in the Table I In the ^{1}H-NMR spectrum ($CDCl_3$) Table I ^{1}HNMR and ^{13}C NMR spectra data of compound 2 (3-epibetulinic acid).

Table 1. ^{1}HNMR and ^{13}CNMR spectra data of 3-epibetulinic acid. From From *C. tomentosum* Wight compared to reference

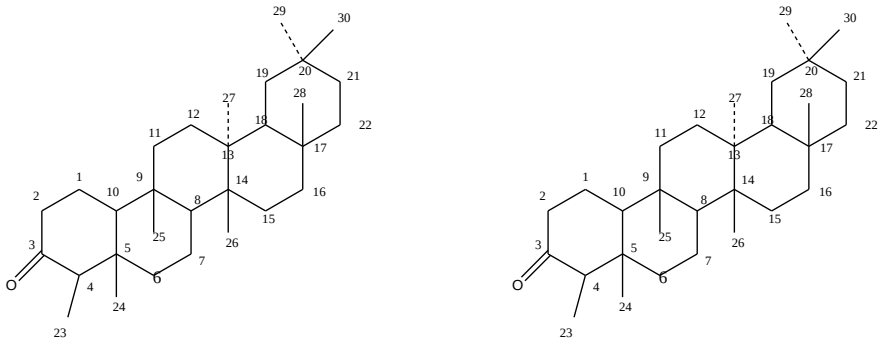
	^{13}C NMR 3-epibetulinic		^{1}H NMR 3-epibetulinic (m, *J* in Hz)	
Carbon No	Sample	Reference	Sample	Reference
1	30,1 (t)	30,5 (t)		
2	29,2 (t)	29,0 (t)		
3	76,7 (C-OH) (d)	76,9 (C-OH) (d)	3,33 (s) 2,94 (1 H, *d*, 2,4)	3,39 (*d*,2)
4	36,7 (s)	36,0 (s)	-	-
5	49,9 (d)	49,9 (d)	2,21 (1 H, *d*, 11, 8:12, 2:12)	
6	17,9 (t)	17,9 (t)		
7	36,3 (t)	36,3 (t)		
8	38,5 (s)	38,5 (s)		
9	48,5 (d)	48,5 (d)	2,11 (1H, *d*, 9, 8)	
10	41,9 (s)	41,9 (s)		
11	25,0 (t)	25,0 (t)		
12	33,9 (t)	33,7 (t)		
13	46,6 (d)	46,8 (d)	2,97 (1H, *d*, 6, 4)	2, 32, (1H, *s*)
14	38,5 (s)	38,4 (s)		
15	38,2 (t)	38,2 (t)		
16	27,1 (t)	27,0 (t)		
17	55,4 (s)	55,4 (s)		
18	37,6 (d)	37,6 (d)	1, 77 (1H, *d*, 6, 8)	
19	54,8 (d)	54,8 (d)	1,80 (1H, *d*, 4)	1, 80 (1H, *m*)
20	150,3 (s)	150,3 (s)		
21	20,4 (t)	20,4 (t)		
22	31,6 (t)	31,6 (t)		
23	15,7 (q)	16,1 (q)	0,76 (3H, *s*)	0,82 (3H, *s*)
24	15,8 (q)	15,1 (q)	0,64 (3H, *s*)	0,80 (3H, *s*)
25	15,9 (q)	16,2 (q)	0,88 (3H, *s*)	0,95 (3H, *s*)
26	14,4 (q)	14,8 (q)	0,88 (3H, *s*)	0,96 (3H, *s*)
27	18,9 (q)	18,1 (q)	0,93 (3H, *s*)	0,97 (3H, *s*)
28	177,2 (COOH)	178,2 (COOH)		
29a, 29b	109,6 (t)	107,1 (t)	4,56 (1H) 4,68 (1H)	4,60 (1H) 4,77 (1H)
30	28,2 (q)	27,4 (q)	1,64 (3H, *s*)	1,71 (3H, *s*)

Compound 3 (D.A-friedo-oleanan-3-on)

Compound 3 (D.A-friedo-oleanan-3-on) was obtained from the stem bark of *C. moonii*.

Table II. ^{1}HNMR and ^{13}CNMR spectra data of D.A-friedo-oleanan-3-on from sample was compare compared to references (Garmen, Lucia, 2000)

	^{13}C NMR D.A-friedo-olenan-3-on		^{1}H NMR
Carbon No	Reference δc (ppm)	Sample δc (ppm)	Sample δ_H (ppm)
1	21,9 (t)	22,5 (t)	1,94 – 1,97
2	41,2 (t)	41,7 (t)	
3	212,7 (s)	213,5 (s)	
4	57,9 (d)	58,4 (d)	2,25
5	41,8 (s)	42,3 (s)	
6	41,0 (t)	41,5 (t)	
7	17,9 (t)	18,4 (t)	
8	52,8 (d)	53,3 (d)	
9	37,1 (s)	37,6 (s)	
10	59,2 (d)	59,6 (d)	
11	35,7 (t)	35,8 (t)	
12	29,3 (t)	30,7 (t)	
13	30,0 (s)	39,9 (s)	
14	39,4 (s)	38,5 (s)	
15	32,5 (t)	32,6 (t)	
16	35,3 (t)	36,2 (t)	
17	29,7 (s)	30,2 (s)	
18	42,5 (d)	42,9 (d)	
19	35,0 (t)	35,5 (t)	
20	27,8 (4)	28,4 (4)	
21	32,1 (t)	32,9 (t)	
22	38,9 (t)	39,4 (t)	
23	4,6 (q)	7,0 (q)	0,88
24	14,3 (q)	14,8 (q)	0,72
25	17,6 (q)	18,1 (q)	0,86
26	18,3 (q)	20,5 (q)	0,99
27	19,9 (q)	18,8 (q)	1,04
28	31,8 (q)	32,0 (q)	1,0
29	34,7 (q)	35,2 (q)	0,95
30	31,5 (q)	31,9 (q)	1,18



A **B**

Figure 3. Structure of (A) 3-*epi*-betulinic acid, (B) D.A-friedo-oleanan-3-on

The chemical shifts in the ^{1}H-NMR and ^{13}C-NMR spectra of compound 1, 2 and 3 closely resembled those of triterpene derivatives. On the basis of this evidence the structure of compound 1 was established as maytenfoliol, compound 2 as 3-*epi*-betulinic acid and compound 3 as D.A-friedo-oleanan-3-on, which were found to be known compounds.

The present anticancer evaluation principles from *C. tetrapterum* has led to the finding of maytenfoliol, and from *C. tomentosum* has led to the finding of 3-epibetulinic acid and from *C. moonii* has led to D.A-friedo-oleanan-3-on derivates. Among the triterpene which had moderate-to-highest anticancer activity on Hela cell line cancer.

Anticancer activity assay was measured using Yoo. T.J (1999) method. The result can be displayed in the Tables III, IV and V. 3-epibetulinic acid exhibited a significant best anticancer activity against Hela cell lines compared to maytenfoliol and D.A-friedo-oleanan-3-on compounds. 3-epibetulinic acid, maytenfoliol and of D.A-friedo-oleanan-3-on tested exhibited a significant activity in this assay since these compound inhibited 50% of the cellular growth at concentration from 4 to 8 µg/mL, According to IC_{50} values showed that all of compounds most potential as cancer drug candidate.

Table III. Anticancer activity of maytenfoliol (TL8-9) by Hela cell line

Dosage (µg/mL)	Cell x 10^4			% inhibition	IC_{50} (µg/mL)
	I	II	Mean		
6.4	41	43	42.0	58.21	4.89
3.2	60	59	59.5	40.80	
1.6	70	73	71.5	28.86	
0.8	90	92	91.0	9.45	

Table IV. Anticancer activity of 3-epibetulinic acid by Hela cell line

Dosage (μg/mL)	Cell x 10^4			% inhibition	IC_{50} (μg/mL)
	I	II	Mean		
6.4	24	27	25.5	75.12	
3.2	49	53	51.0	50.24	
1.6	67	65	66.0	35.61	3.17
0.8	80	83	81.5	20.49	
0	102	103	102.5		

Table V. Anticancer activity of D.A-friedo-oleanan-3-on by Hela cell line

Dosage (μg/mL)	Cell x 10^4			% inhibition	IC_{50} (μg/mL)
	I	II	Mean		
6.4	49	47	48.0	53.40	
3.2	62	63	62.5	39.32	
1.6	81	83	82.0	20.39	5.63
0.8	91	94	92.5	10.19	
0	102	104	103		

Note: IC_{50} < 4 μg/mL is highly cytotoxic, IC_{50} of 4-30 μg/mL is moderately cytotoxic, while IC_{50}> 40 μg/mL is weakly cytotoxic

CONCLUSION

All of compounds have anticancer activity, epibetulinic acid obtained from the *C. tomentosum* Weigh is more active than maytenfoliol and D.A-friedo-oleanan-3-on, with an IC_{50} value were 3.,17 μg/mL, 4.89 μg/mL and 5.63 μg/mL, respectively. Conclusion that all of compounds most potential as cancer drug candidate.

ACKNOWLEDGEMENT

The authors are grateful to Mr Suhandafor bioassay and Sofa Fajriah for the NMR measurement and Puspa Dewi for MS measurement and thanks are due to Mr. Ismail Rahman at the Herbarium Bogor Research Institute - LIPI Bogor for identifying the plant material, and also to Reseach Centre for Chemistry – LIPI for a research support.

REFERENCES

Krupp, M.A.K. and Chatton, M. J., 1979, Current Medical Diagnosis Treatment Lange *Medicinal* Publication Los Altos, Calofornia 94022, 184-199.

Ross, R., 1993, The Pathogenesis of Atherosclerosis: a Perspective for the 1990s, *Nature*, **362,** 801-808.

Taher., Idris, S., Ahmad and Arbain, D., 2005, A polyisoprenylated ketone from *Calophyllum enervosum*, *Phytochemistry,* **66**,

723-726.

Itoigawa, M., Ito, C., Tan, H.T.W., Kuchide, M., Tokuda, H., Nishino, H. and Furukawa, H., 2001, Cancer Chemopreventive agents, 4-phenylcoumarins from *Calophyllum inophyllum*, *Cancer letters*., **169**(1), 15-19.

Guilet, D., Seraphin, D., Rondeau, D., Richomme, P. and Bruneton., 2001, Cytotoxic coumarins from *Calophyllum dispar*. *Phytochemistry*., **58**, 571-575.

Soerianegara and Lemmens, R.H.M.J., 1994, Plant Resources of South-East Asia, Timber trees mayor commercial timbers, Bogor. Indonesia, *Prosea*., **1**(5), 114-132.

Sung, T.V., Steglich, W., and Adam, G., 1991, Triterpene glycosides from Schefflera octophylla, *Phytochemistry.,* **7**(30), 2349-2356.

Herz, Santhanam, and Wahlberg, 1972, 3-*epi*-betulinic acid, a new triterpenoid from *Picramnia pentandra*, *Phytochemistry*, 11, 3061-3063.

Garmen, Lucia, Faria, G., Correa, P., Possenti, A. and Ernesto, J., 2000, Evaluation of the antiulcerogenic activity of friedelin-3ß-ol and friedelin isolated from Maytenus ilicifolia (Celastraceae), *Journal Ehno Pharmacology*, 72, 465-468.

Yoo, T.J., Yoo, Y.C., Kang, T.B., Shimazaki, K., Song, S.K., Lee, K.H, Kim, S.H., Park, C.H., Azuma, I. and Kim, J.B., 1999, Lectins Isolated from Korean mistletoe (*Viscum album coloratum*) induce apoptosis in tumor cells, *Cancer Letter*, **136**(1), 33-40.