CHAPTER 4

RESULTS

4.1 Total composition Analysis of *Vanilla siamensis* Rolfe ex Downie

The GC-MS Conditions for total composition analysis of the extraction of *V. siamensis* are shown in Table 4 and total ion chromatograms of the separation are presented in Figure 18 (for mass spectra, see Appendix A). On the basis of the mass fragmentation pattern of each component the Wiley library indicated that the extraction of *Vanilla siamensis* Rolfe ex Downie is shown in Table 5.

Table 4 The GC-MS Conditions

Operation	Condition
1. Column	DB-Wax, 30 m x 0.25 mm I.D. and 0.25 μM film thickness
2. Temperature program	50 °C (hold 3 min) to 230 °C with program rate at 10°C/min hold 10 min
3. Injector temperature	230 °C
4. Injection	Split (Split ratios 100:1) , 0.2 μL
5. Carrier gas	Helium at 1.0 mL/min
6. Transfer-line temperature	230 °C
7. Ion source temperature	230 °C
8. Ionization mode	EI, 70 eV
9. Scan parameter	30 – 500 amu.
10 Solvent delay	14 min

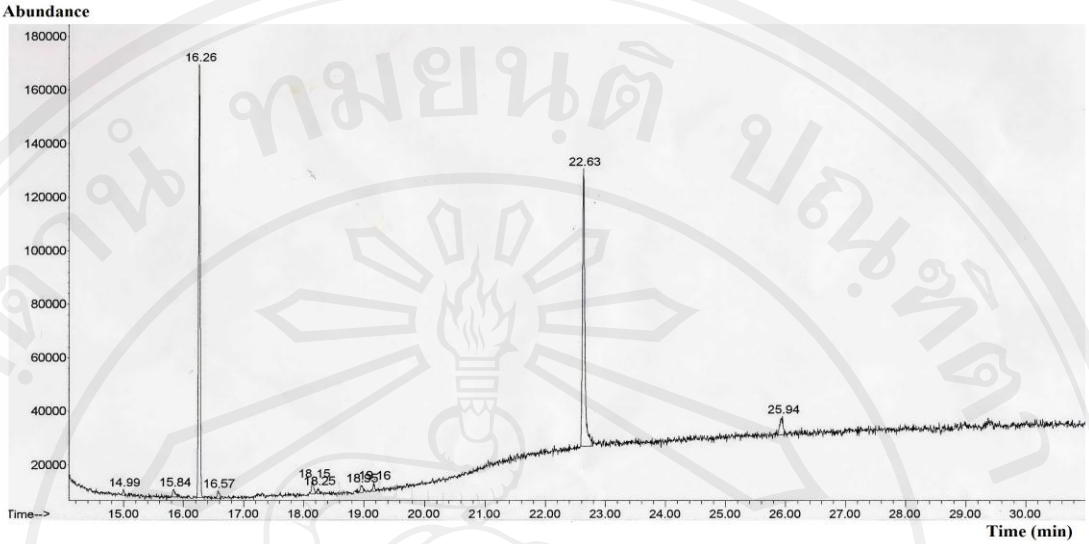


Figure 18 Total ion chromatograms of the extraction of *V. siamensis* obtained by GC-MS in the Full-mode Scan at 70 eV. The numbers labeling the peaks correspond to those in the Peak column of Table 5.

Table 5 Chemical compounds of *Vanilla siamensis* Rolfe ex Downie

Peak No.	Retention time (min)	% Relative peak area	Possible compounds
-	12.80	-	Dimethyl sulfoxide
1	14.992	0.567	Ethyl oxamate
2	15.839	1.260	Phenol 2-methoxy
3	16.260	45.856	Dimethyl sulfone
4	16.573	1.013	Cyclohexanol 2-(Methylaminomethyl)-,trans.
5	18.153	1.771	2-Amino-4-hydroxypteridine-6-carboxylic acid
6	18.245	0.569	Isobutyric acid 2-D1
7	18.952	1.097	1,3-Dioxolane,4-methyl
8	19.162	0.855	2-Methoxy-4-vinylphenol
9	22.632	43.400	Vanillin
10	25.934	3.611	Vanillyl alcohol

4.2 Estrogenic Activity of *Vanilla siamensis* extract

4.2.1 Standardization of YES-hERα and YES-hERβ systems for evaluation of estrogenic activity

To standardize the YES-hERα and YES-hERβ systems for evaluation of estrogenic activity in standard compounds: 17β-estradiol (E_2), at various dosed ranging from 10^{-12} M to 10^{-3} M were incubated with yeast cells of both systems and then β-galactosidase activity was assayed. The sigmoidal dose response curves for standard compounds plotted by β-galactosidase unit against log concentration of standard compounds were show in Figure 20 and EC_{50} (50 percent effective concentration) value was calculated. As shown in Table 4.3, EC_{50} of E_2 was 3.22 $x10^{-11}$M by using YES-hERα. On the other hand when using YES-hER β, the EC_{50} value of $E_2$was of $2.14x10^{-7}$ M. The result suggested that the estrogenic activity of the E_2 tested in YES-hERα was higher than YES-hERβ system**.**

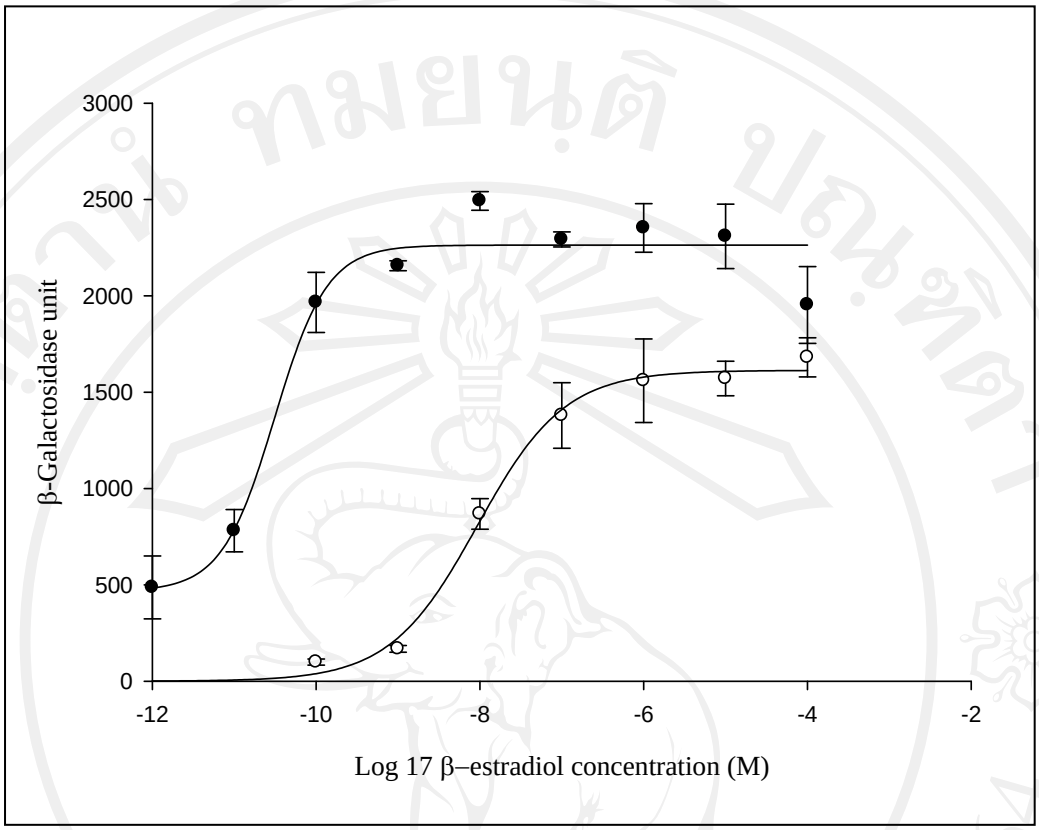


Figure 20 Dose response curves of17β-estradiol (E_2) determined by YES-hERα (•) and YES-hERβ (o) systems. Each value represents the mean ± SEM of 3 independent experiments.

4.3.2 Evaluation of the estrogenic activity of *V. siamensis* plant extract

To evaluate of estrogenic activity of *V. siamensis* plant extract, YES-hERα and YES-hERβ were applied in this study. Crude plant extract of *V. siamensis* in the dose ranging from 10 mg/mL to 10,000 mg/mL were incubated with yeast cell and β-galactosidase activity was assayed. Sigmoidal dose-response curves for plant extracts were plotted by β-galactosidase unit against log concentration were show in Figure 21. EC_{50} and relative potency value that represented estrogenic activity as compare to E_2 were calculated and summarized in Table 6. In order to compare the estrogenic activity of *V. siamensis* plant extracts between YES-hERα and YES-hERβ, the relative potency values presented in Table 6 were compared. The maximum relative potency value of *V. siamensis* plant extracts in YES-hERα was high than the relative potency of YES-hERα.

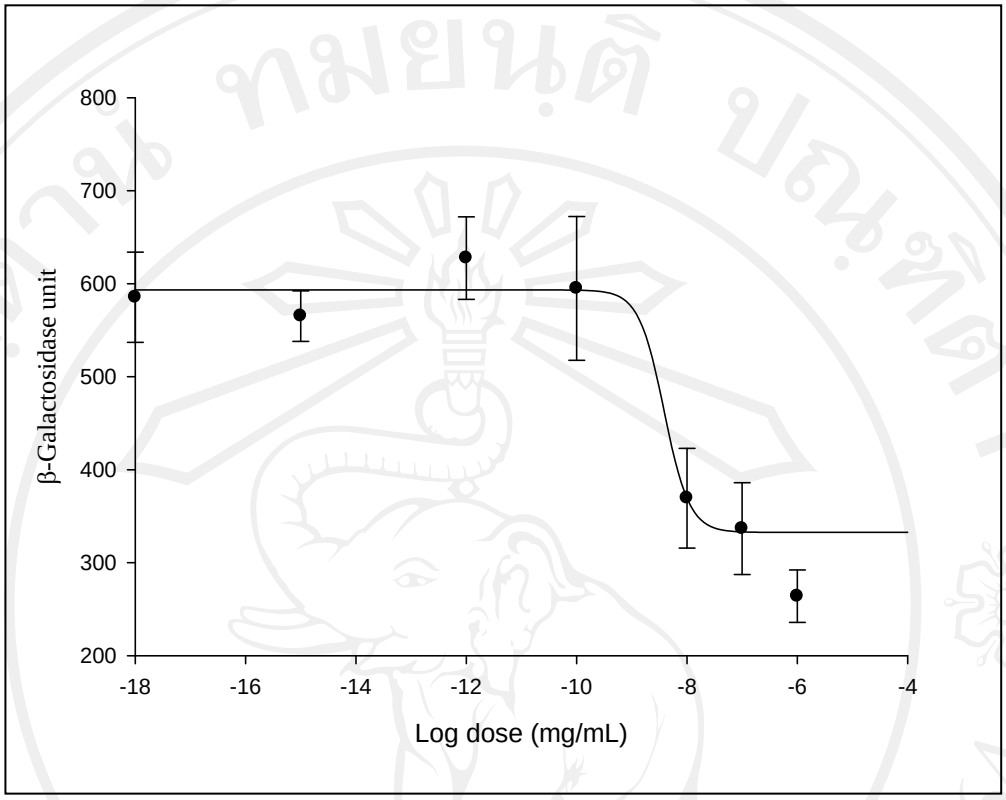


Figure 21 Dose response curves of *V. siamensis* plant extract determined by YES-hERα (•) systems. Each value represents the mean ± SEM of 3 independent experiments.

Table 6 The estrogenic activity of *V. siamensis* plant extracts determined by YES systems

Compounds	**YES-hERα**		**YES-hERβ**	
	EC_{50} (mg/l)	Relative potency[a]	EC_{50} (mg/l)	Relative potency[a]
V. siamensis	$3.87x10^{-6}$	227	N.D	N.D
17β-estradiol (E_2)	$8.77x10^{-6}$ ($3.22x10^{-11}$M)	100	$5.83x10^{-2}$ ($2.14x10^{-7}$M)	100

Note: Each value represents the mean ± SEM of 3 independent experiments.

[a]Relative potency = (EC_{50} of 17β-estradiol (E_2)/ EC_{50} of test compound) x 100

4.3 Investigation of effects on bone matrix maturation phase

4.3.1 Study on the cytotoxic effect of *Vanilla siamensis* extract in hFOB1.19 osteoblast cell

To assess whether *V. siamensis* plant extract was toxic to hFOB1.19 cells, the 0 -10 μg/mL *V. siamensis* plant extract were used to detect its cytotoxic effect on cells using MTT assay. As shown in Figure 22, treatment of hFOB cells with *V. siamensis* plant extract for 48 hours increased the number of viable hFOB cells in the cultures in a dose dependent manner compared with vehicle-treated cells for 24 hours. It was found that 5 - 10 μg/mL *V. siamensis* plant extract increased cell survival in the dose-dependent manner. However, 8 and 10 μg/mL *V. siamensis* plant extract also increased cell survival but did not in the dose dependent manner. All used concentration of *V. siamensis* plant extract had no toxic effect to hFOB1.19 cell. The *V. siamensis* plant extract concentrations of 8 -10 μg/mL had been used in the next study.

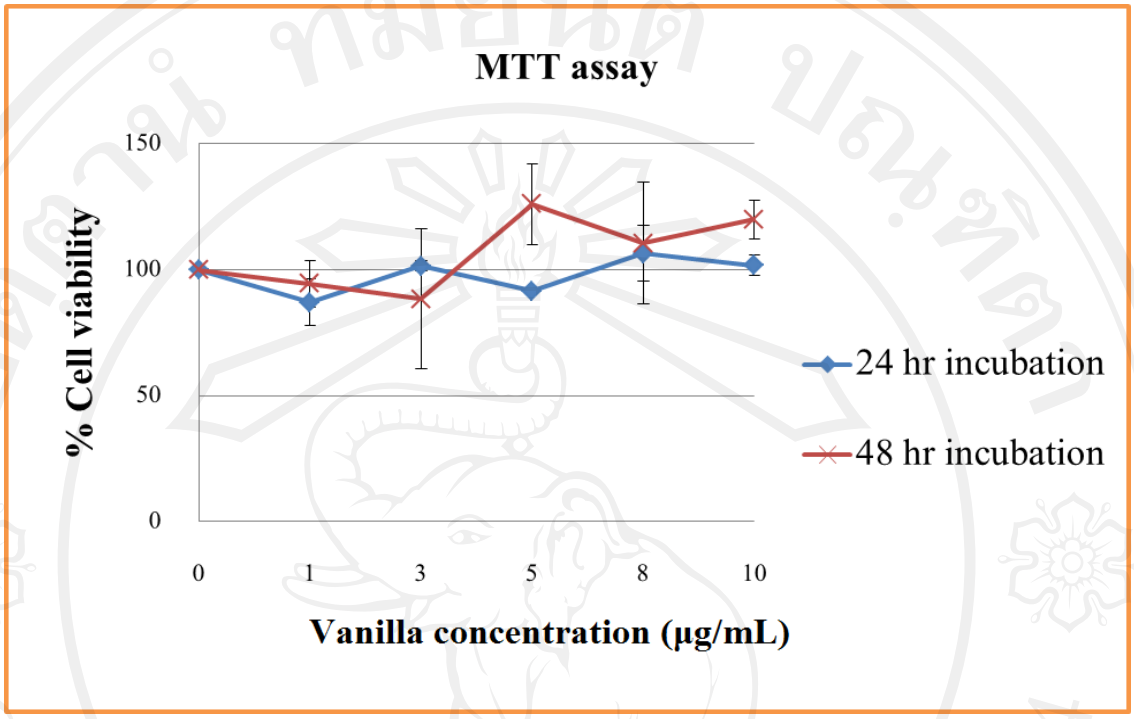
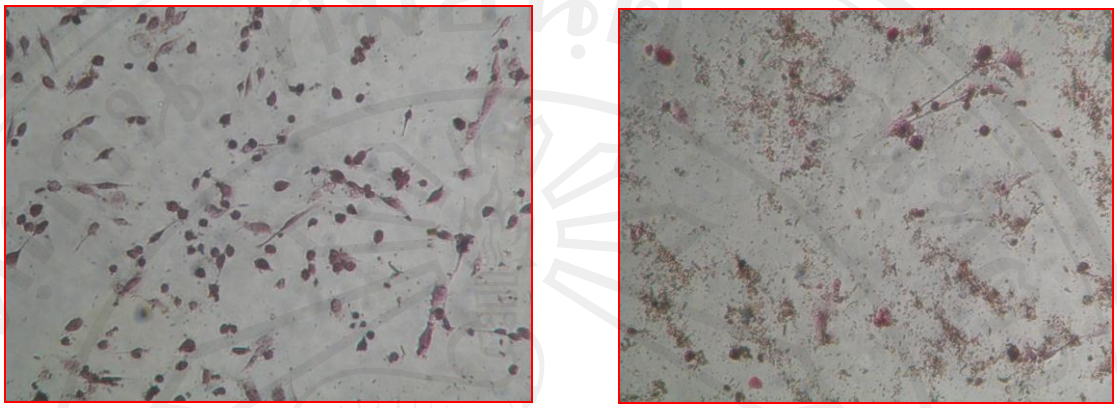


Figure 22 Effect of *V. siamensis* plant extract on the growth of hFOB1.19 cells by the MTT assay. Human FOB1.19 cells were cultured with various concentrations of *V. siamensis* plant extract or DMEM control for 24 h and 48 h.

4.3.2 Investigation of the effect of *Vanilla siamensis* plant extract on mineralization phase

Formation of a mineralized matrix is a definitive halkmark of osteoblastic differentiation. To examine the effects of *V. siamensis* plant extract on bone formation, hFOB cells were stained for calcium in corporation using Alizarin Red.

As shown in figure 23, Human FOB1.19 cells were treated with 8µg/mL of *V. siamensis* plant extract in DMEM/F12 while hFOB1.19 cells were cultured in DMEM/F12 media used as a control. On day 11, cell layers were stained with Alizarin red-S and investigated the nodule formation using the inverted light microscope. The qualitative measurement of calcium deposition was examined by histochemical staining, Alizarin red-S, and shown in figure 24.



A B

Figure 23 Effect of *V. siamensis* plant extract on hFOB cell mineralization. hFOB cells were seeded in dishs and cultured in DMEM/F12 medium with(A) or without 8µg/mL *V. siamensis* plant extract (B) . Alizarin red-S staining revealed the calcium nodule under the inverted light microscope.

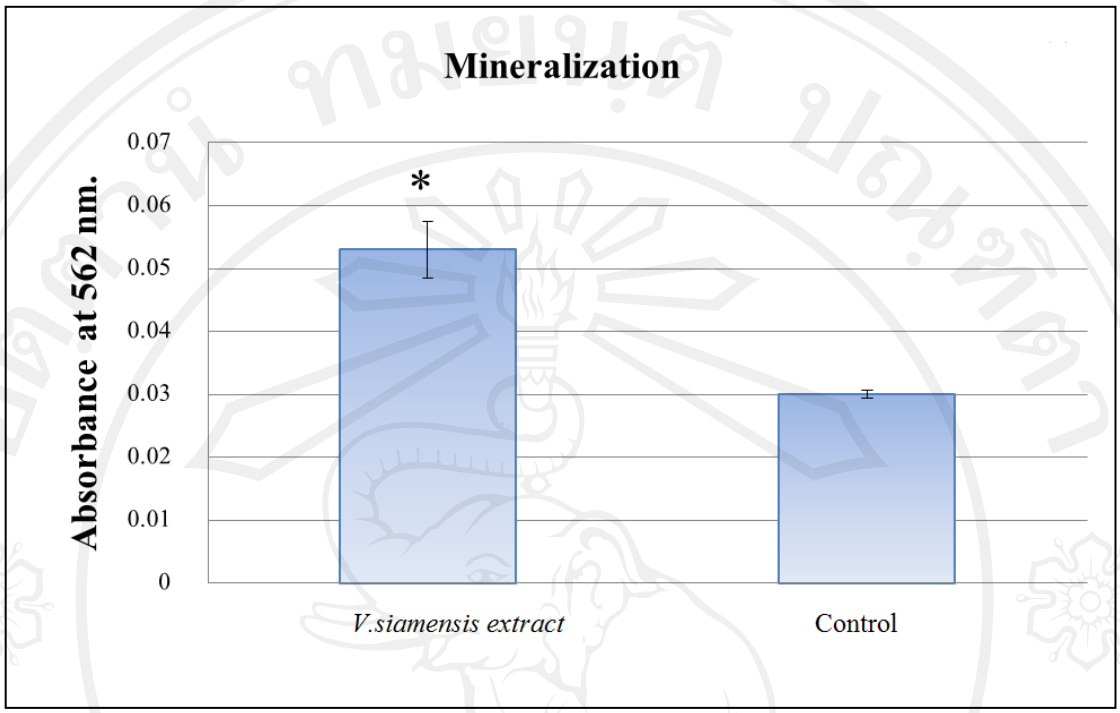


Figure 24 The degree of *V. siamensis* plant extract on hFOB cell mineralization was determined in dish using Alizarin Red staining and compared between DMEM/F12 medium with 8µg/mL *V. siamensis* plant extract and DMEM/F12 medium as a control . Data shown are mean value standard deviation of two experiments done in duplicate, *Denoted values that were significantly different from control (p = 0.001).