CHAPTER 4

RESULTS

4.1 SCREENING OF ABILITY OF FUNGI IN BIOTRANSFORMATION

Five fungal strains of *Aspergillus* sp., *A. oryzae* (Ozykat-1), *A. niger* TISTR 3254, *A. usamii* TISTR 3258, *A. terricola* TISTR 3109 and *A. melleus* TISTR 3128, were examined for their abilities to transform artemisinin to its derivatives. The TLC analysis of crude extracts from fungal cultures was shown in Figure 4.1.

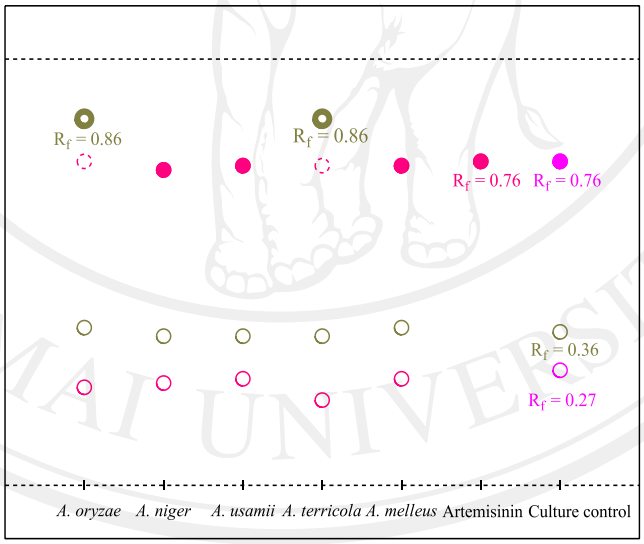


Figure 4.1 TLC Chromatogram of EtOAc extracts from fungal cultures after 4 days of artemisinin addition, the culture control is the medium added with artemisinin. The mobile phase is 1: 1 v/v of EtOAc and hexane.

TLC chromatogram showed that the transformed product (R_f = 0.86) was found in the cultures of *A. oryzae* (Ozykat-1) and *A. terricola* TISTR 3109 (Figure 4.1). No transformed product was observed in the controls which were the mixture of artemisinin and the transformed medium without cells and the fungal cultures without artemisinin adding.

4.2 OPTIMIZATION CONDITIONS

Biotransformation of artemisinin by *A. oryzae* (Ozykat-1) and *A. terricola* TISTR 3109 were conducted to determine the effect of temperature, transformed medium, artemisinin concentration, fungal pre-incubated period and transformation time on the growth and the production of the transformed product.

4.2.1 Effect of temperature on the growth of fungi and the production of the transformed product

The study was found that the optimum temperature for *A. oryzae* (Ozykat-1) and *A. terricola* TISTR 3109 growth was 37^{o}C. When *A. oryzae* (Ozykat-1) and *A. terricola* TISTR 3109 were cultured on SDA and incubated at 30^{o}C and 37^{o}C, both of fungi were grown within 24 hours. On the other hand, when the fungi were cultured and incubated at room temperature (26^{o}C), they were able to grow after 48 hours (Table 4.1).

Table 4.1 The growth of *A. oryzae* (Ozykat-1) and *A. terricola* TISTR 3109 at room temperature (26°C), 30°C and 37°C after 48 hours.

Fungi	**Temperature (°C)**		
	Room temperature (26°C)	**30°C**	**37°C**
A. oryzae (Ozykat-1)			
A. terricola TISTR 3109			

The effect of temperature on the biotransformation of artemisinin by *A. oryzae* (Ozykat-1) and *A. terricola* TISTR 3109 was studied at 30°C and 37°C. The transformed product was extracted and then purified using chromatography. The yields of the crude extracts and the purified transformed product were shown in Table 4.2. The biotransformation at 37°C showed higher yields in the crude extract and the transformed product when compared with that of 30°C in both fungal systems. Therefore, the temperature 37°C was used as the suitable temperature in this research.

Table 4.2 The yields of the crude extracts and the purified transformed product when artemisinin was transformed by *A. oryzae* (Ozykat-1) or *A. terricola* TISTR 3109 at 30°C and 37°C after 4 days of artemisinin addition.

Fungi	Temperature (°C)	% Yield	
		Crude extract*	Purified transformed product
A. oryzae (Ozykat-1)	30	70	8
	37	80	18
A. terricola TISTR 3109	30	78	9
	37	95	21

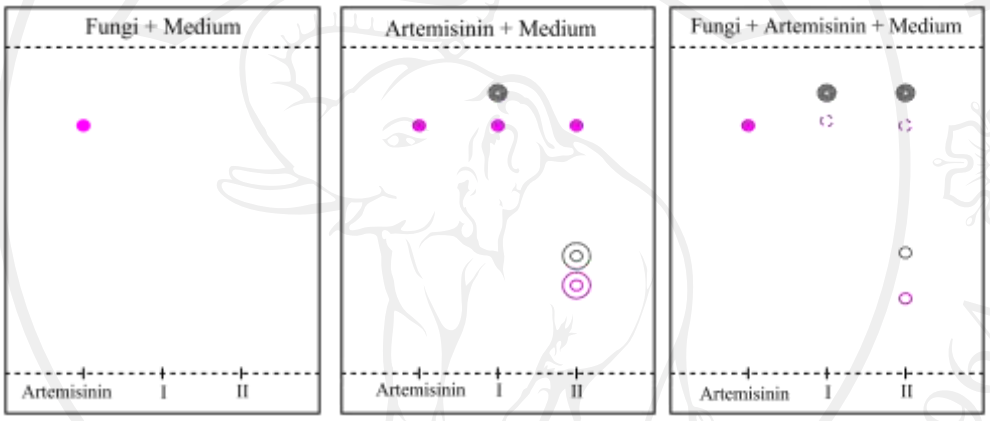
* Crude extracts are EtOAc extracted from the culture fungi added with 0.5 mg/ml of artemisinin and incubation for 4 days

4.2.2 Effect of the transformed medium on the production of the transformed product

The effect of different transformed medium on biotransformation of artemisinin by *A. oryzae* (Ozykat-1) and *A. terricola* TISTR 3109 were showed in Figure 4.2 and 4.3. After artemisinin was transformed, the culture extract in each medium was subjected to TLC. Both fungi showed the similar TLC chromatogram for each transformed medium.

In single-stage biotransformation system, the transformed medium I (PDB) and II (SDB) were used in this system. *A. oryzae* (Ozykat-1) and *A. terricola* TISTR

3109 did not react with the transformed medium I and II (Figure 4.2A). Figure 4.2B showed that the crude extract of the transformed medium I showed the spot which had R_f similar to the obtained transformed product. The transformed medium II reacted with artemisinin but did not give any compound which overlapped with the transformed product. In addition, both fungi showed abilities to transform artemisinin into one derivative, as showed in Figure 4.2C.

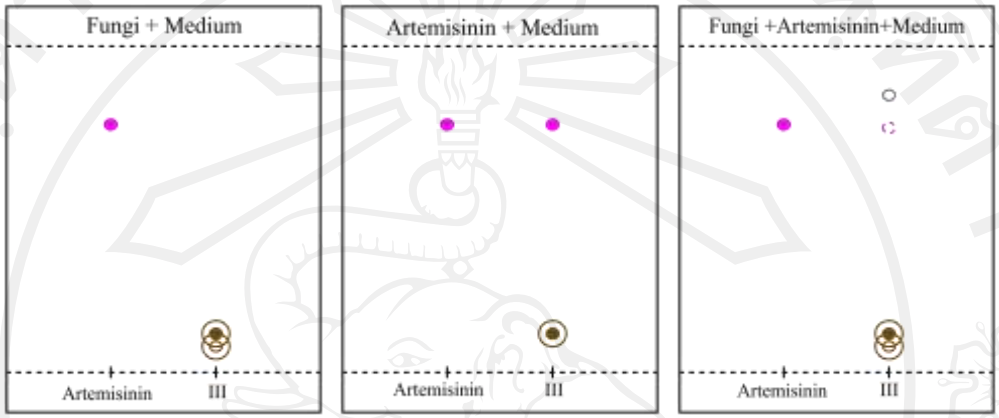


(A) (B) (C)

Figure 4.2 TLC chromatogram of the transformed medium I and II of EtOAc extracts in single-stage biotransformation when using 1: 1 v/v of EtOAc and hexane as a mobile phase. The fungi represent *A. oryzae* (Ozykat-1) or *A. terricola* TISTR 3109.

The transformed medium III (0.5M phosphate buffer pH 7.0) was used in two-stage biotransformation system. In Figure 4.3A, *A. oryzae* (Ozykat-1) or *A. terricola* TISTR 3109 did not produce the metabolites which reacted with TLC reagent (*p*-anisaldehyde). The Figure 4.3B indicated the medium could be reacted with artemisinin and then gave compounds which showed R_f less than artemisinin in 1:1

v/v of EtOAc and hexane system. However, the transformed medium III was found to generate a transformed product as same as the product from the single-stage biotransformation system (Figure 4.3C).



(A) (B) (C)

Figure 4.3 TLC chromatogram of the transformed medium III of EtOAc extracts in two-stage biotransformation when using 1: 1 v/v of EtOAc and hexane as a mobile phase. The fungi represent *A. oryzae* (Ozykat-1) or *A. terricola* TISTR 3109.

In this study, the transformed medium II and III appeared to be the suitable transformed mediums in biotransformation of artemisinin. However, two-stage biotransformation required longer time in biotransformation and provided lower yield. In addition, the transformed product was not reproducible in each batch of biotransformation. Therefore, the single-stage biotransformation of artemisinin in the transformed medium II was used as preferable medium in this research.

4.2.3 Effect of artemisinin concentration on the growth of fungi and the production of the transformed product

The growth of *A. oryzae* (Ozykat-1) and *A. terricola* TISTR 3109 after addition of artemisinin caused a significant decrease in growth of both fungi. Artemisinin addition at the beginning of the culture cycle led to the reduction in the cells dry weight of *A. oryzae* (Ozykat-1) when comparing to the fungal control (Figure 4.4). The reductions in growth were observed after 2, 3 and 5 days after 1.0, 0.75 and 0.5 mg/ml of artemisinin addition, as showed in Figure 4.4B, 4.4C and 4.4D, respectively, The culture containing 1.0 mg/ml of artemisinin showed the greatest decrease in growth rate (Figure 4.4D). The highest dry weights of *A. oryzae* (Ozykat-1) cultures added with artemisinin at the concentration of 0.5, 0.75 and 1.0 mg/ml were 41.03 ± 0.22, 38.99 ± 0.70 and 36.94 ± 0.43 mg/ml, respectively. On the contrary, the growth of non-artemisinin culture was raised until the third day and then it had been decreasing afterward until the eighth of cultivation.

The pH profile of control *A. oryzae* (Ozykat-1) cultures was eventually decreased from the pH value of 5.61 and remained steady after 4 days of incubation at the pH value of 4.81 ± 0.03(Figure 4.4A). However, the pH of the culture adding of artemisinin to *A. oryzae* (Ozykat-1) culture was slightly lower than non-artemisinin treatment (Figure 4.4B, 4.4C and 4.4 D). The pH of the culture containing artemisinin at the concentration of 0.5 mg/ml was found to increase after 5 days of addition (Figure 4.4B). In comparison, the pH profiles of *A. oryzae* (Ozykat-1) after adding artemisinin at the concentration of 0.75 mg/ml showed no significant difference in pH

profile when comparing to the culture containing artemisinin at the concentration of 1.0 mg/ml Figure 4.4C and 4.4D).

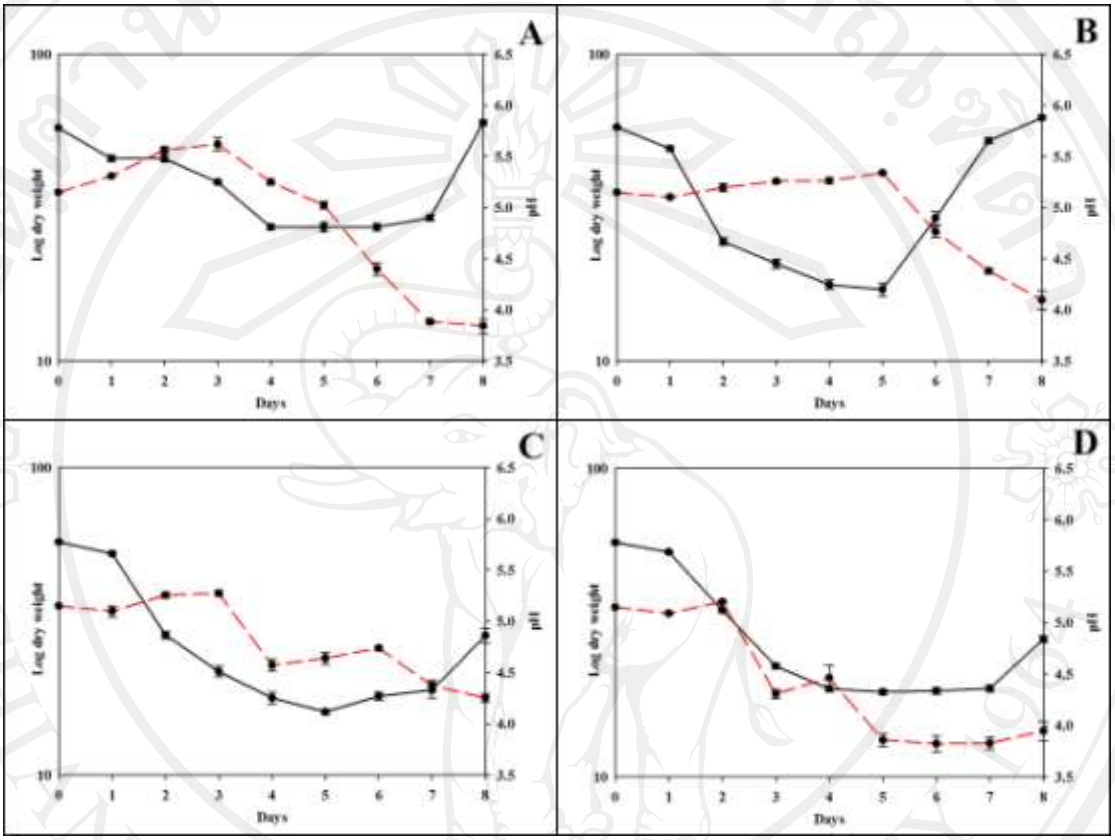


Figure 4.4 Time course of growth and pH by *A. oryzae* (Ozykat-1) during the transformation at lag phase. The symbol (---●---) and (—●—) represented experimental values of the dry weight and the pH value of the culture growth without additional of artemisinin (A) and the cultures growth with addition of artemisinin at the concentration of 0.5 (B), 0.75 (C) and 1.0 (D) mg/ml, respectively.

The effect of concentration of artemisinin on growth of *A. terricola* TISTR 3109 when artemisinin was introduced at the beginning of the culture cycle caused a significant decrease in dry weight of *A. terricola* TISTR 3109 when comparing to

non-treated fungal culture. After 2 days, the reduction in growth of *A. terricola* TISTR 3109 culture containing artemisinin concentration of 0.5 and 0.75 mg/ml were observed (Figure 4.5B and 4.5C). At the concentration of 1.0 mg/ml of artemisinin, the decrease in growth was also observed after 5 days of artemisinin introduction (Figure 4.5D). The highest growths of fungal culture containing artemisinin were 48.67 ± 0.45, 46.38 ± 4.83 and 39.36 ± 1.16 mg/ml for the cultures added with artemisinin 0.5, 0.75 and 1.0 mg/ml, respectively. However, the highest growth of non-treated culture was 57.41 ± 0.97 mg/ml after 3 days (Figure 4.5A).

The pH of *A. terricola* TISTR 3109 contained artemisinin culture was slightly higher than non-artemisinin added culture. The pH values of fungal cultures containing artemisinin at all tested concentration were continued to decrease after 1 day from pH 5.61 to pH 4.48 ± 0.02, 4.47 ± 0.03 and 4.60 ± 0.02 on the third day of cultivation for the fungal cultures added with artemisinin 0.5, 0.75 and 1.0 mg/ml, respectively (Figure 4.5B, 4.5C and 4.5D). The pH of non-artemisinin added culture eventually decreased and remained steady after 2 days and also immediately increased after 6 days (Figure 4.5A). The increase in the pH was observed after the introduction of artemisinin for 7 days in the culture added with 0.5 mg/ml of artemisinin (Figure 4.5B). In contrast, the pH of the culture added with artemisinin 0.75 mg/ml decreased continually after the addition of artemisinin (Figure 4.5C) which was the same as the concentration 1.0 mg/ml (Figure 4.5D).

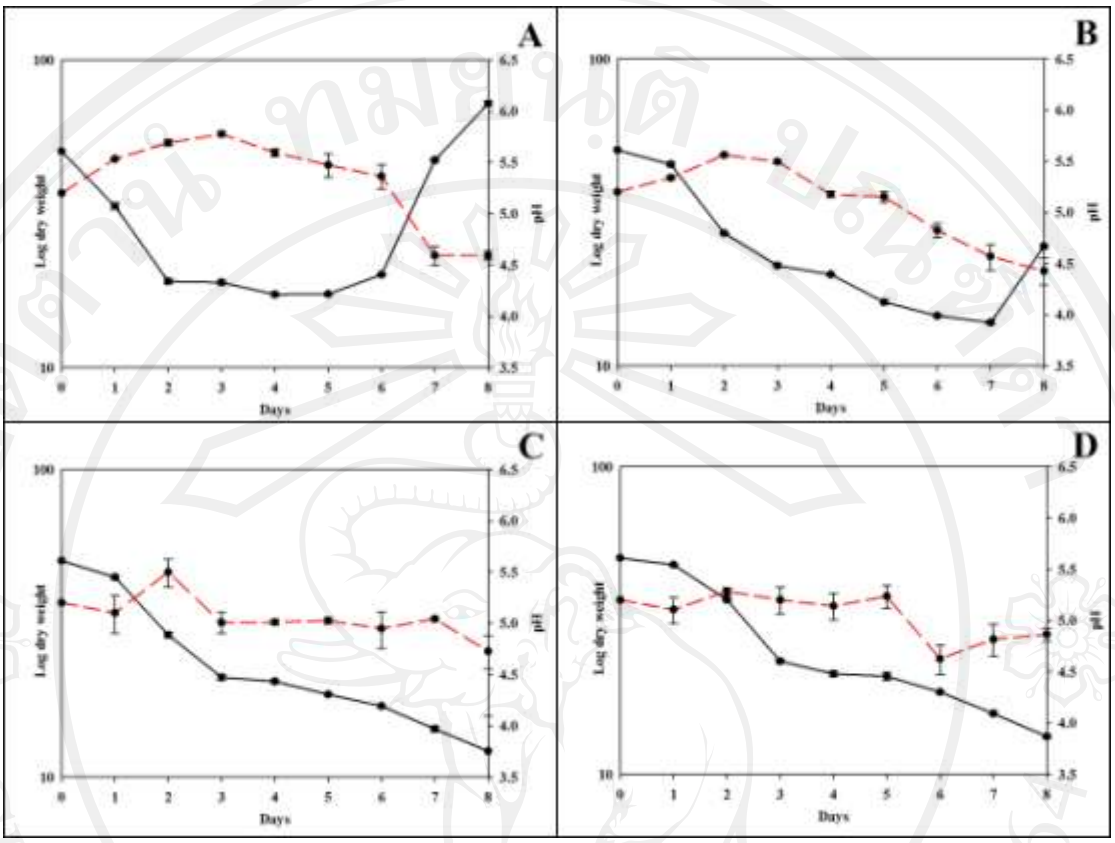


Figure 4.5 Time course of growth and pH by *A. terricola* TISTR 3109 during the transformation at lag phase. The symbol (---●---) and (—●—) represented experimental values of the dry weight and the pH value of the culture growth without additional of artemisinin (A) and the cultures growth with addition of artemisinin at the concentration of 0.5 (B), 0.75 (C) and 1.0 (D) mg/ml, respectively.

The fungal pellets of *A. oryzae* (Ozykat-1) and *A. terricola* TISTR 3109 became loose and formed the hairy structure in the outer layer, as shown in Figure 4.6. In addition, the effect of artemisinin concentration on production of the transformed product was performed at the concentration of 0.5, 0.75 and 1.0 mg/ml. Artemisinin was added to the cultures after incubation for 2 days. The transformed

product was extracted and purified after 4 days of artemisinin addition. The yields of crude extracts and the purified transformed product were shown in Table 4.3. The highest yield in the transformed product was achieved when artemisinin at the concentration of 0.5 and 0.75 mg/ml were added into the cultures of *A. oryzae* (Ozykat-1) and *A. terricola* TISTR 3109, respectively. However, the yield of the transformed product after the addition of 0.5 mg/ml of artemisinin to the culture of *A. terricola* TISTR 3109 was not significantly different compared to the addition of 0.75 mg/ml of artemisinin. Therefore, artemisinin with the concentration of 0.5 mg/ml were used in favor of artemisinin conversion by both fungal systems.

Table 4.3 The yields of the purified transformed product when the artemisinin concentrations of 0.5, 0.75 and 1.0 mg/ml were transformed by *A. oryzae* (Ozykat-1) or *A. terricola* TISTR 3109

Fungi	Artemisinin concentration (mg/ml)	% Yield of the purified transformed product
A. oryzae (Ozykat-1)	0.5	29
	0.75	13
	1.0	4
A. terricola TISTR 3109	0.5	19
	0.75	20
	1.0	8

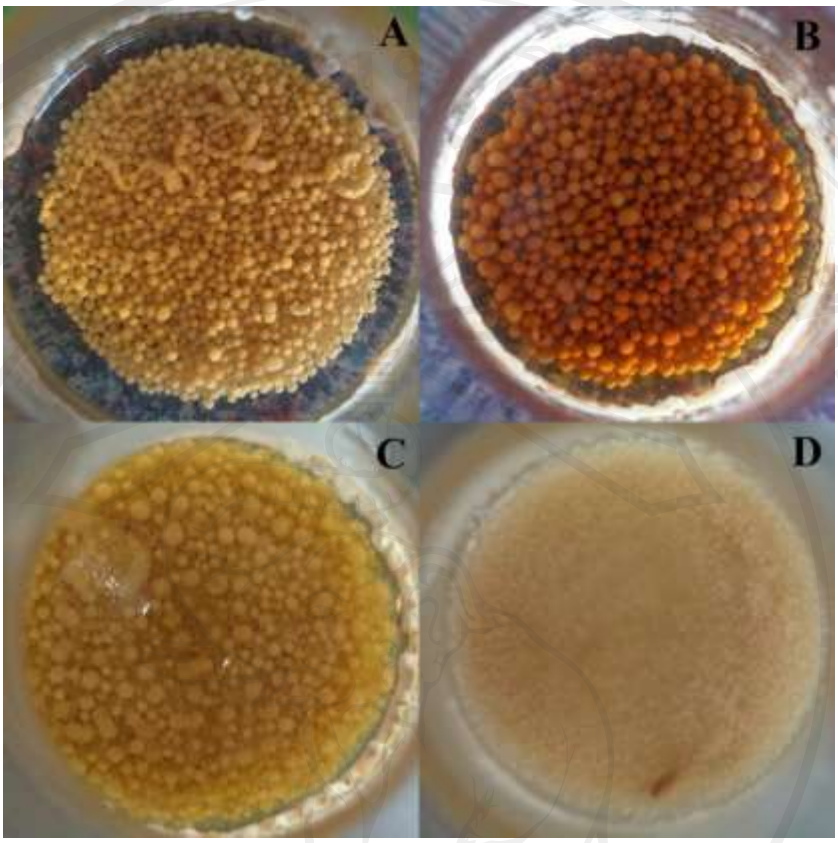


Figure 4.6 Fungal pellets of *A. oryzae* (Ozykat-1) and *A. terricola* TISTR 3109 without the addition of artemisinin (A and B, respectively) and *A. oryzae* (Ozykat-1) and *A. terricola* TISTR 3109 adding with 0.5 mg/ml of artemisinin at lag phase (C and D, respectively). All cultures were incubated for 4 days.

4.2.4 Effect of pre-incubation period on the production of the transformed product

The highest yield of the transformed product, 12%, was obtained when *A. oryzae* (Ozykat-1) was pre-incubated to log phase prior to the addition of artemisinin and incubated for 6 day, as showed in Table 4.4. When the fungal culture was pre-incubated to lag phase, the maximum yield of the transformed product was 9% on the

sixth day of artemisinin addition. Addition of artemisinin at stationary phase of *A. oryzae* (Ozykat-1) resulted in the highest production of the transformed product, which was 10% after the addition of artemisinin for 6 days.

A. terricola TISTR 3109 was able to transform artemisinin into the transformed product that could provide the highest yield when the culture was pre-incubated to log phase following 6 days incubation after the introduction of artemisinin. The maximum yield was 11%, as reported in Table 4.4. The addition artemisinin to the lag and stationary phase period of the cultures induced the maximum conversion after the cultures were 6 days incubations at 9% and 10%, respectively. It was clear that the introduction of artemisinin at the different pre-incubation period had no effect on the production of the transformed product.

In order to investigate the activities of the enzymes in artemisinin transformation by *A. oryzae* (Ozykat-1) and *A. terricola* TISTR 3109, the fungi was cultured for 2 days in the transformed medium and the free-cell cultures were separated from fungal pellets using centrifugation and filtration. Artemisinin was added to the free-cell culture and further incubated for 4 days. The TLC chromatogram showed the free-cell cultures extracts of both fungi did not have the ability to transform artemisinin (Figure 4.7). This result demonstrated that the activities of the enzymes in artemisinin transformation presented in the cells and the transformed product leached out into medium.

Table 4.4 Comparative efficiency of *A. oryzae* (Ozykat-1) and *A. terricola* TISTR 3109 cultures for biotransformation of artemisinin to the transformed product as a function of pre-cultured period and incubation time**.**

Fungal system	Concentration of artemisinin addition (mg/ml)	Pre-cultured period	Incubation time (Days)*	% Yield of the transformed product
A. oryzae (Ozykat-1)	0.5	Lag phase	2	0
			4	3
			6	9
		Log phase	2	4
			4	6
			6	12
		Stationary phase	2	5
			4	9
			6	10
A. terricola TISTR 3109	0.75	Lag phase	2	0
			4	4
			6	9
		Log phase	2	5
			4	9
			6	11
		Stationary	2	5
			4	7
			6	10

* The days after artemisinin addition

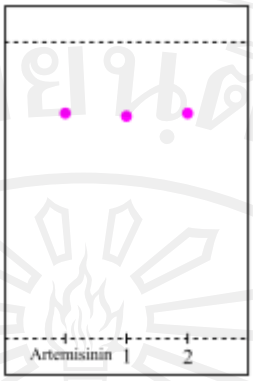


Figure 4.7 TLC chromatogram of the EtOAc extracts performed by the free-cell culture broth of *A. oryzae* (Ozykat-1) (1) and *A. terricola* TISTR 3109 (2) after artemisinin addition for 4 days. The mobile phase is 1: 1 v/v of EtOAc and hexane.

4.3 CHEMICAL CHARACTERIZATION OF THE TRANSFORMED PRODUCT

The structure of the purified transformed product was identified by IR, ESIMS, ^{1}H and ^{13}C –NMR spectroscopy, as well as by comparing to the previous reported spectral data.

4.3.1 Nuclear Magnetic Resonance (NMR) Spectra of the purified transformed product

The transformed product was obtained as colorless needle solid. The ^{1}H-NMR ($CDCl_3$, 400 MHz) of the transformed product showed the proton signal of three methyl protons at δ 1.55 (3H, s, H-15), 1.22 (3H, d, J = 7.2 Hz, H-13) and 0.96 (3H, J = 5.6 Hz, H-14), as well as the spectrum of methylene proton signals was observed at δ 1.95 (1H, m, H-8b), 1.92 (1H, m, H-2b), 1.78 (1H, m, H-9b), 1.77 (1H, m, H-3b),

1.65 (1H, m, H-3a), 1.27 (1H, m, H-2a), 1.15 (1H, m, H-9a), 1.03 (1H, m, H-8a) and the signals of methine proton showed at δ 1.30 (1H, m, H-1), 5.71 (1H, s, H-5), 2.03 (1H, m, H-7), 1.29 (1H, m, H-10) and 3.20 (2H, dq, H-11) (Figure 4.8). Comparison of ^{1}H-NMR spectra of the transformed product with that of artemisinin indicated the difference in chemical shift at δ 5.71 (1H, s) of the transformed product instead of δ 5.85 (1H, s) in artemisinin which indicated the absence of endoperoxide bridge (Figure 4.9).

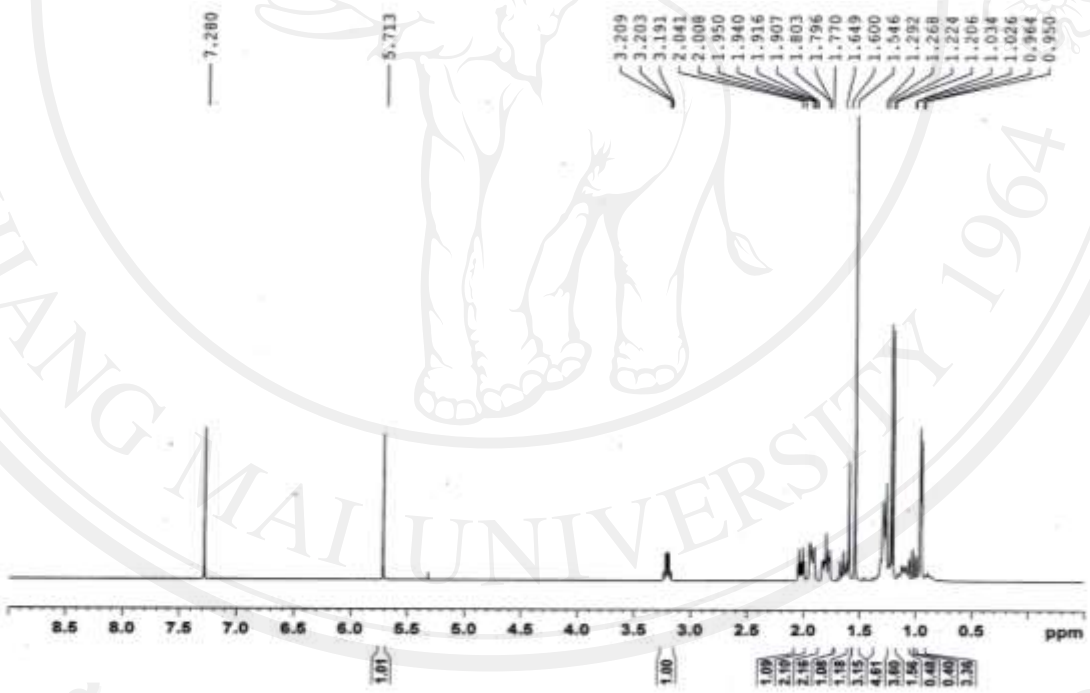


Figure 4.8 The ^{1}H-NMR spectra of the purified transformed product

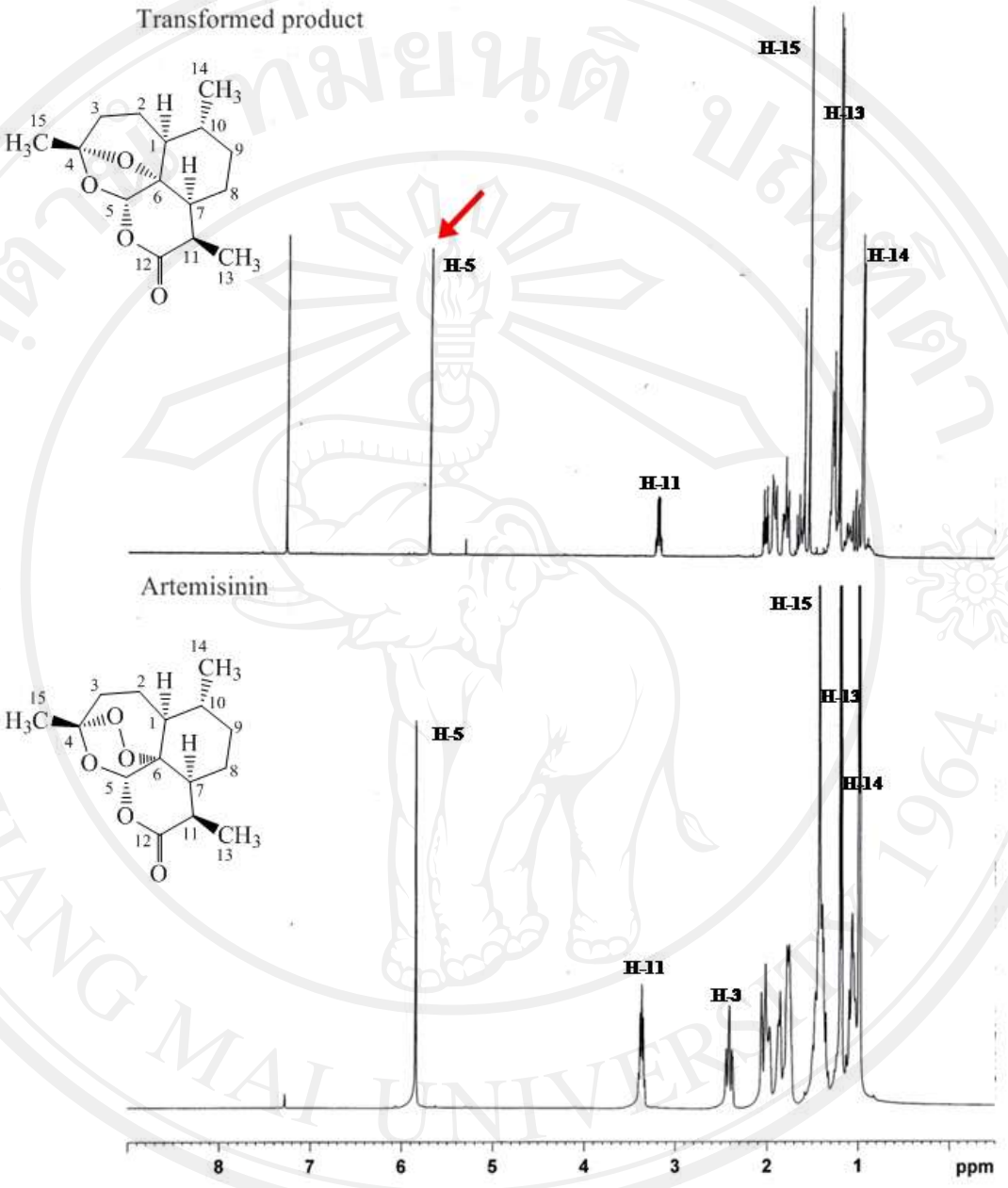


Figure 4.9 Comparison of the ^{1}H-NMR spectra of the transformed product and artemisinin

The ^{13}C-NMR (Figure 4.10) and DEPT 135 (Figure 4.11) experiments displayed 15 carbons including three methyl (δ 24.2 (C-15), 18.8 (C-14) and 12.8 (C-13)), four methylene (δ 22.3 (C-2), 34.2 (C-3), 23.7 (C-8) and 33.7 (C-9)), five methine (δ44.9 (C-1), 99.9 (C-5), 42.7 (C-7), 35.6 (C-10) and 33.0 (C-11)) and three quaternary carbons (δ 109.4 (C-4), 82.6 (C-6) and 172.1 (C-12),).

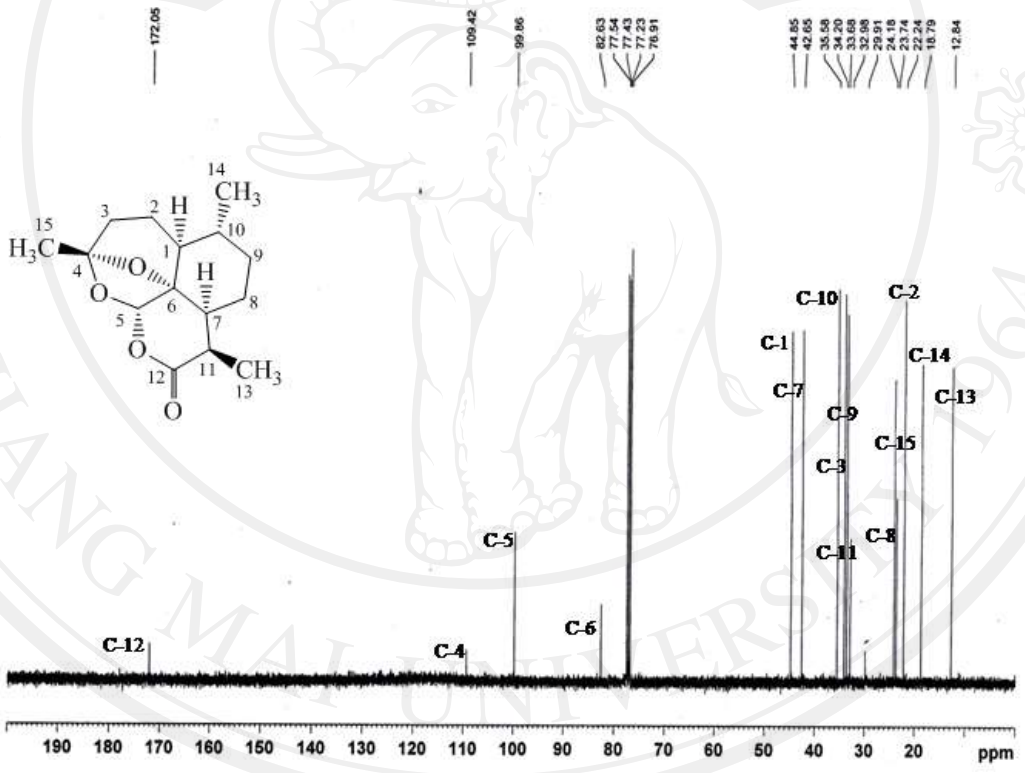


Figure 4.10 The ^{13}C-NMR spectra of the purified transformed product

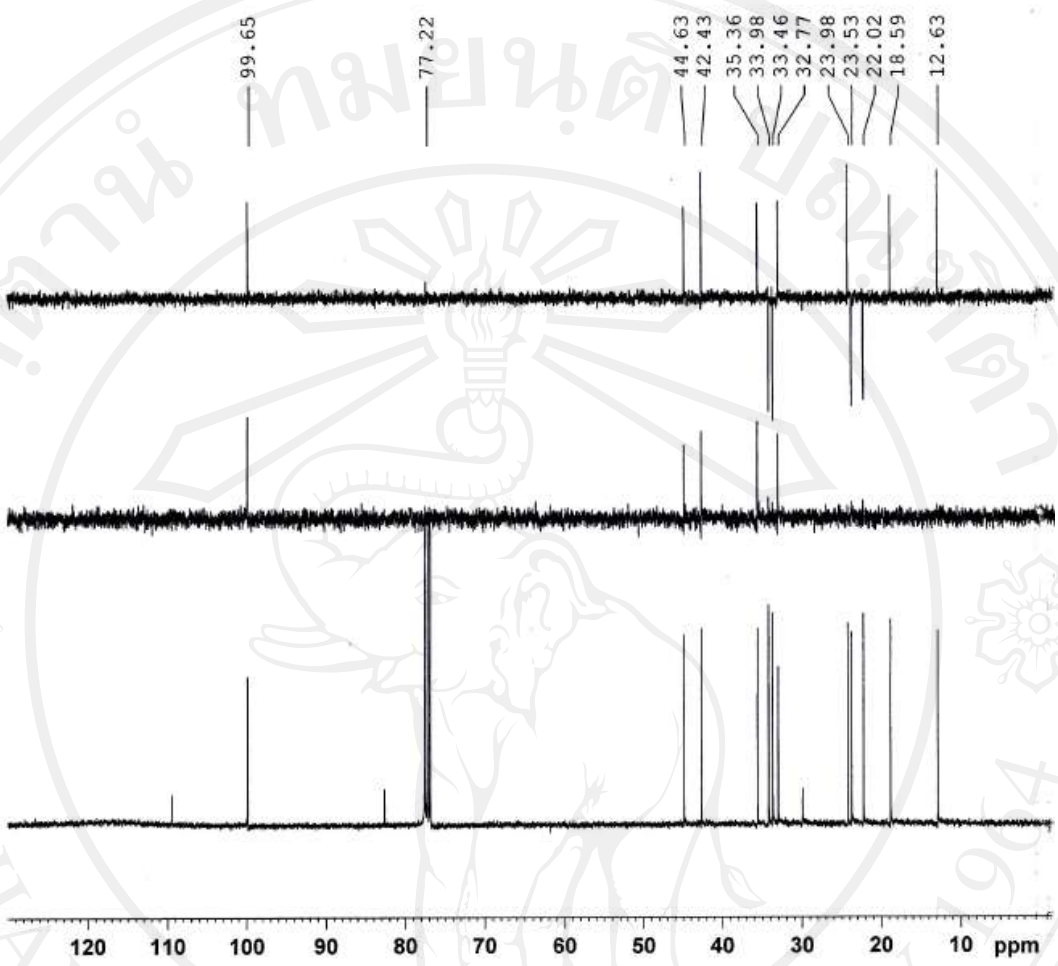


Figure 4.11 The DEPT 135 spectra of the purified transformed product

According to the examination of ^{1}H-, ^{13}C-NMR, DEPT 135 spectra suggested a presence of sesquiterpene skeleton with three methyls and one lactone group. Further analysis was carried out using 2D-NMR, COSY (Figure 4.12), NOESY (Figure 4.13) and HMQC (Figure 4.14) spectra, and by comparing to the spectroscopic data with those of the literature values, this transformed product was assigned as deoxyartemisinin.

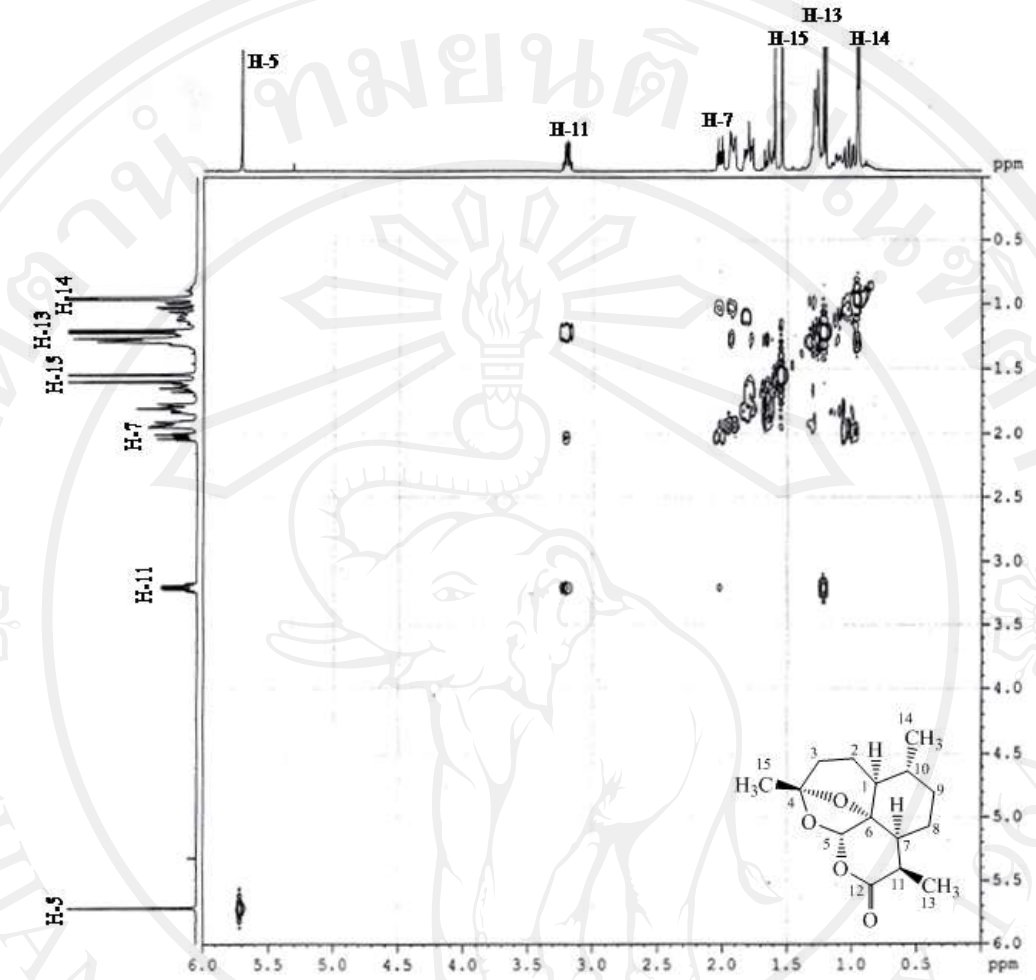


Figure 4.12 The COSY spectra of the purified transformed product

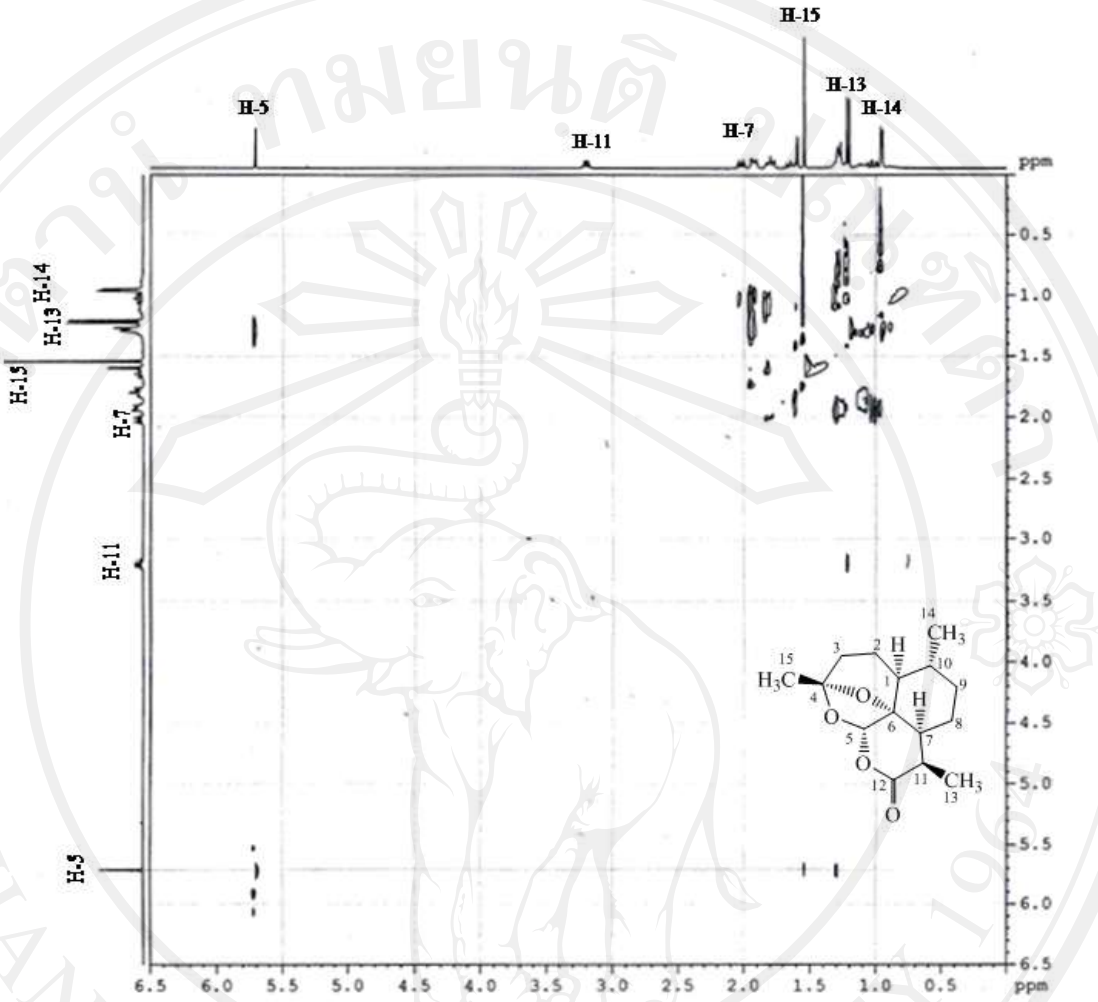


Figure 4.13 The NOESY spectra of the purified transformed product

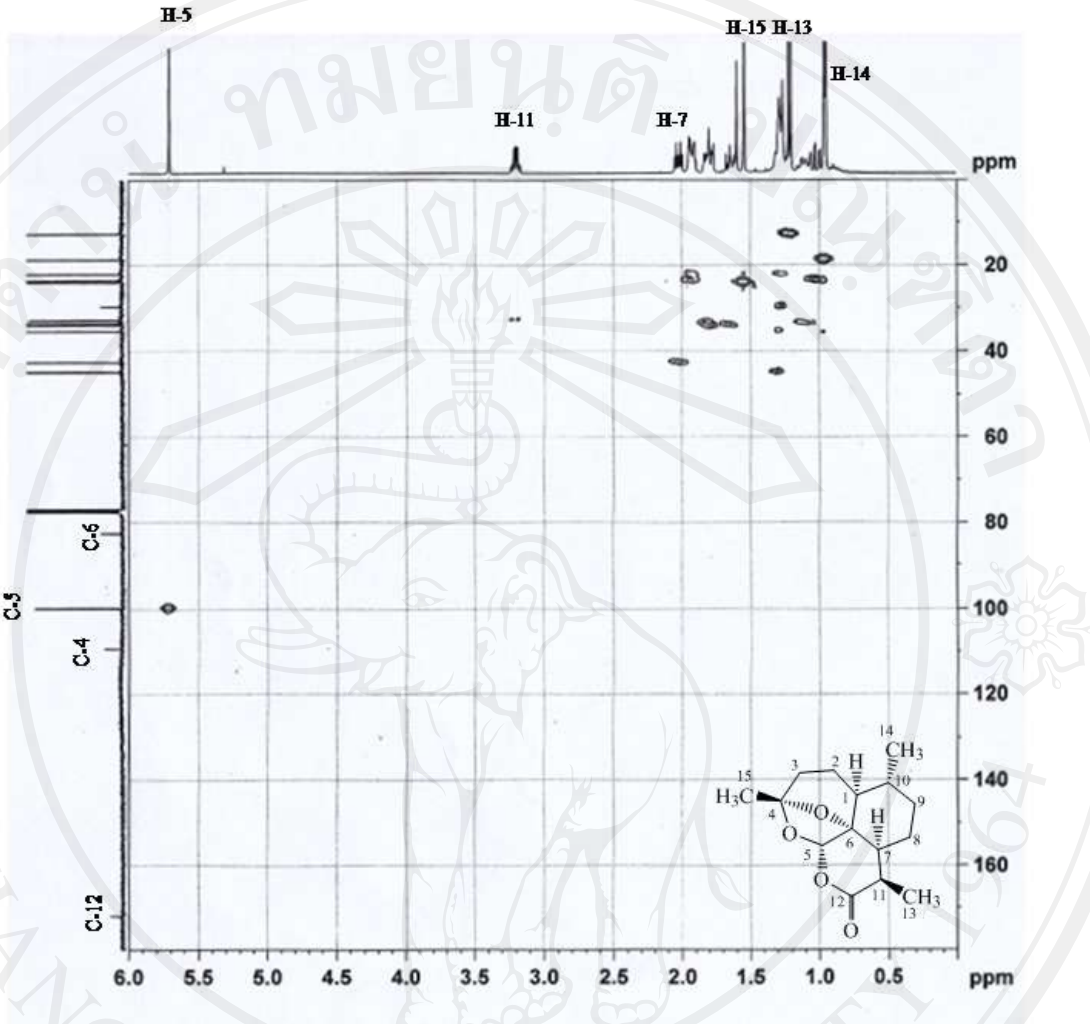


Figure 4.14 The HMQC spectra of the purified transformed product

4.3.2 Infrared (IR) Spectra of the purified transformed product

In the FT-TR spectrum of the transformed product and artemisinin indicated a presence of lactone carbonyl group at υ_{max} 1747 cm^{-1}. The transformed product had no absorption peak at 1117 cm^{-1}, which was presented in artemisinin (C-O-O-C). The important absorptions band of this transformed product were 2941 (C-H), 1748 (C=O), 1385 (C-H), 1140 and 1017(C-O-C) cm^{-1}, as shown in Figure 4.15.

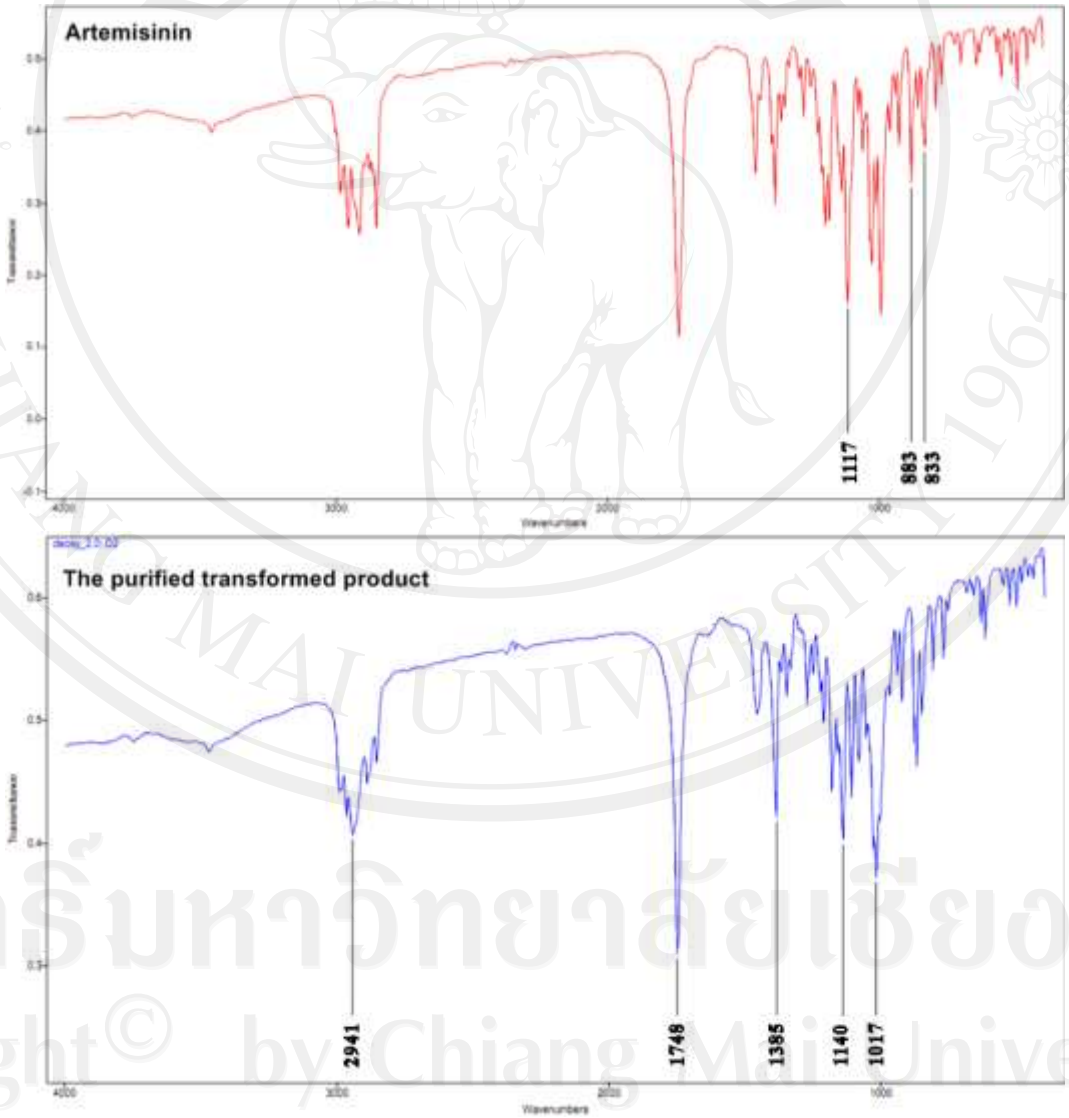
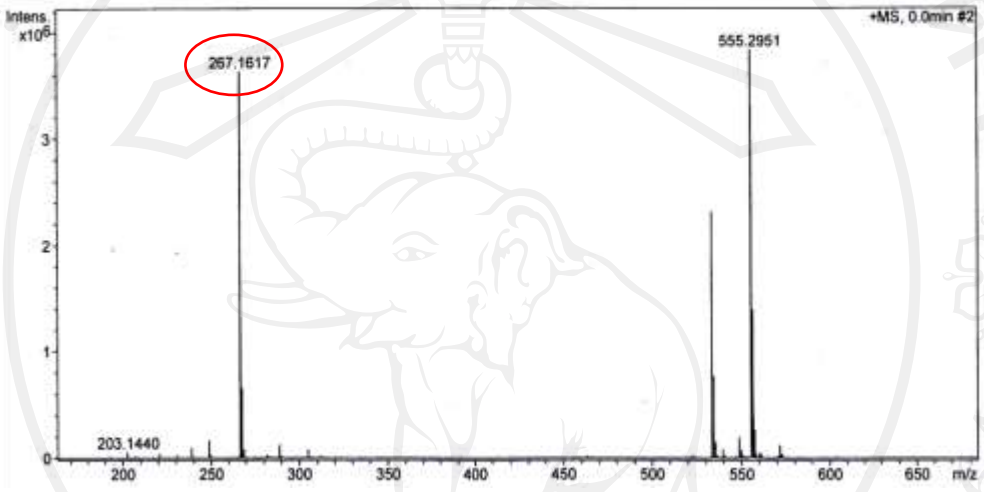


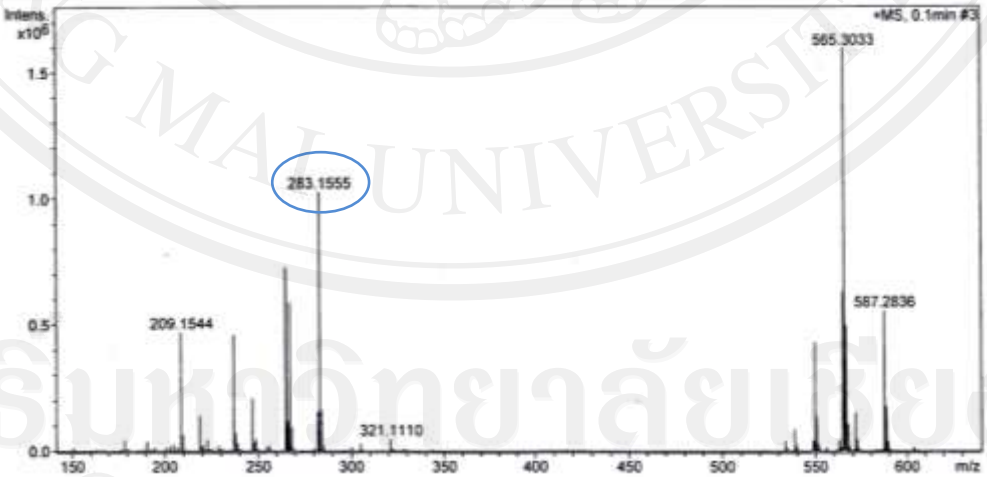
Figure 4.15 IR spectrums of artemisinin and the purified transformed product

4.3.3 Mass Spectra (MS) of the purified transformed product

The molecular formula (Figure 4.16) was established as $C_{15}H_{22}O_4$ based on the molecular ion peak at 267.1617 *m/z* in the high-resolution positive ESIMS, calculation for $C_{15}H_{23}O_4$, 267.3407 m/z.



Mass spectrum of the transformed product



Mass spectrum of artemisinin

Figure 4.16 Mass spectrums of artemisinin and the purified transformed product

4.3.4 Melting points of the purified transformed product

The melting point of the purified transformed product was between 110 and 111^{o}C.

4.4 BIOLOGICAL ACTIVITY OF DEOXYARTEMISININ

The transformed product of artemisinin, deoxyartemisinin, by *A. oryzae* (Ozykat-1) and *A. terricola* TISTR 3109 was assessed for antimalarial, antimicrobial and cytotoxic activities in order to evaluate the effect of structural modification of artemisinin on its biological activities.

4.4.1 Antimalarial activity of deoxyartemisinin

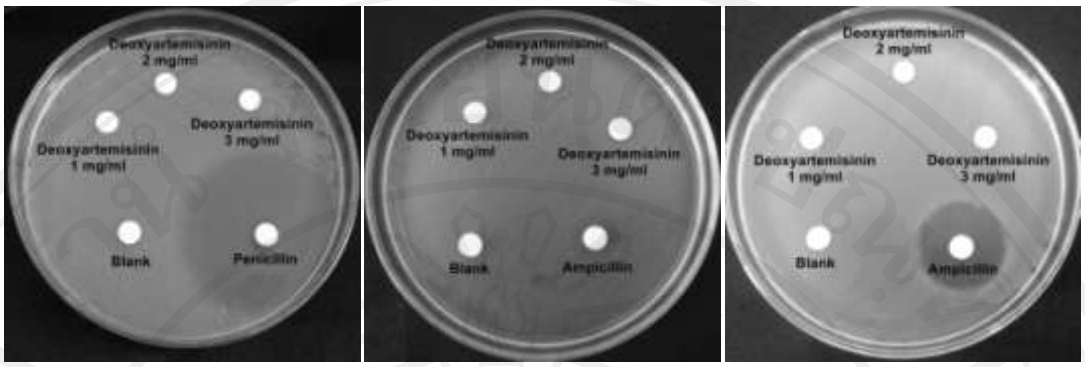
The antimalarial activity of deoxyartemisinin and artemisinin towards the chloroquine-resistant *Plasmodium falciparum* K1 was showed in Table 4.5. Artemisinin exhibited approximately 100-fold greater antimalarial activity when comparing to deoxyartemisinin. Dihydroartemisinin and mefloquine were used as positive controls which showed IC_{50} values of 0.00146 ± 0.006 and 0.0290 ± 0.002 µM, respectively. The negative control was 0.1% DMSO.

Table 4.5 The antimalarial activity of deoxyartemisinin and artemisinin against *P. falciparum* K1

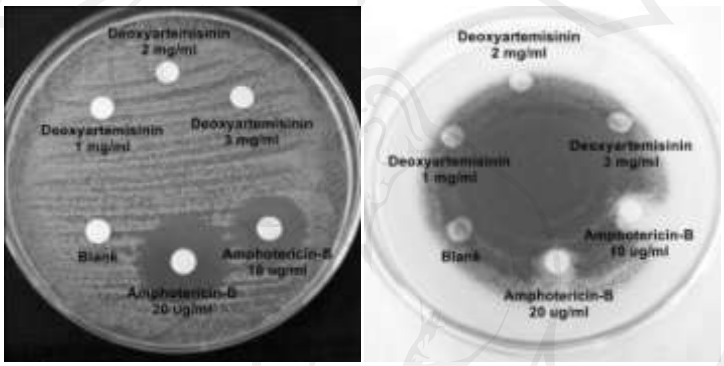
Compound	IC_{50} values (μM)			
	1	**2**	**3**	**Average**
Deoxyartemisinin	1.120	1.105	1.252	1.158 ± 0.081
Artemisinin	0.0106	0.0103	0.0117	0.0110 ± 0.001
Dihydroartemisinin	0.00179	0.00135	0.0123	0.00146 ± 0.006
Mefloquine	0.0263	0.0302	0.0306	0.0290 ± 0.002

4.4.2 Antimicrobial activity of deoxyartemisinin

The *in vitro* antimicrobial activities of deoxyartemisinin against Gram-positive and Gram-negative microorganisms, pathogenic fungus and yeast by agar disc and well diffusion assays were investigated compared to parental compound. The results of agar disc and well diffusion methods were presented in Figure 4.17 and 4.18, respectively. The results indicated that deoxyartemisinin and artemisinin did not exhibited antimicrobial activity against *S. aureus* TISTR 1466, *S. typhimurium* TISTR 292, *E.coli* TISTR 780, *A. niger* TISTR3254 and *C. albicans* BCC 5390.

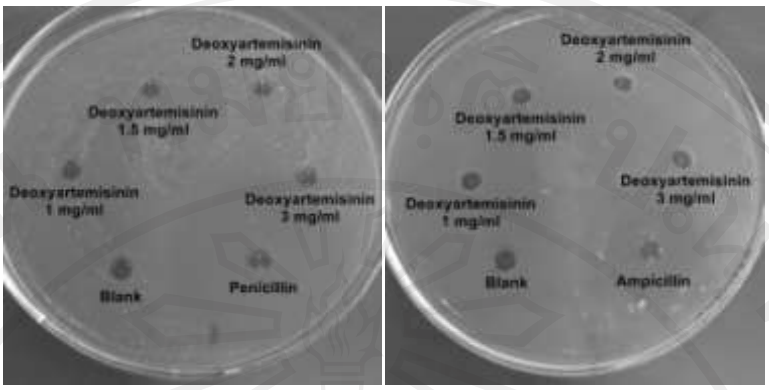


(A) (B) (C)



(D) (E)

Figure 4.17 Antimicrobial activity of deoxyartemisinin determined by agar disc diffusion assay against *S. aureus* TISTR 1466 (A), *S. typhimurium* TISTR 292 (B), *E.coli* TISTR 780 (C), *A. niger* TISTR3254 (D) and *C. albicans* BCC 5390 (E)



(A) (B)

Figure 4.18 Antimicrobial activity of deoxyartemisinin determined by agar well diffusion assay against *S. aureus* TISTR 1466 (A) and *E.coli* TISTR 780 (B)

4.4.3 Cytotoxicity of deoxyartemisinin

The cytotoxicity of deoxyartemisinin was measured using MTT assay. The results of the *in vitro* cytotoxicity were shown in Table 4.6. Deoxyartemisinin showed the inhibitory effects against all tested cell lines, normal mouse fibroblast L929 (ATCC-CCL-1), mouse melanoma B10F16 (ATCC-CRL-6475), human lung adenocarcinoma A549 (ATCC-CCL-185), human colorectal adenocarcinoma HT-29 and human colorectal adenocarcinoma Caco-2 cell lines. Nevertheless, artemisinin inhibited growth of normal mouse fibroblast L929, mouse melanoma B16F10, human lung adenocarcinoma A549 and human colorectal adenocarcinoma HT-29 cell lines (Table 4.6).There were significant differences ($p < 0.05$) between the value of IC_{50} (LD_{50}) of deoxyartemisinin and artemisinin or doxorubicin in all tested cell lines. The control, doxorubicin, showed significantly ($p < 0.05$) decreased IC_{50} (LD_{50}) when compared to the other compounds. Post hoc tests (Scheffe for L929, B16F10 and HT-

29 cells; Tamhane's T2 for A549 cells) were used to determine statistical differences between cells means of the IC_{50} (LD_{50}) values, as showed in Table 4.7. Post hoc pairwise comparison by Scheffe multiple comparison test indicated that significant differences ($p < 0.05$) between deoxyartemisinin and both artemisinin and the control doxorubicin against L929, B16F10 and HT-29 cell lines. No significant difference ($p > 0.05$) of growth inhibition was observed between artemisinin and doxorubicin on HT-29 cell lines. The results from Tamhane statistical analysis indicated that deoxyartemisinin showed a significant ($p < 0.05$) decrease in IC_{50} compared to doxorubicin in the inhibition of cells growth. There was no significant ($p > 0.05$) increase in inhibitory of A549 growth when compared to artemisinin. Thus, Deoxyartemisinin was slightly more effective than artemisinin in inhibitory of growth toward B16F10 and Caco-2 cell lines.

Table 4.6 The *in vitro* cytotoxicity of deoxyartemisinin, artemisinin and doxorubicin against normal mouse fibroblast L929, mouse melanoma B16F10, human lung carcinoma A549, human colorectal adenocarcinoma HT-29 and Caco-2 cell lines

Cell lines	Compounds	IC_{50} (LD_{50})* (mg/ml)			*p* value
		Mean	SD	Activity	
L929	Deoxyartemisinin	1.621	0.095	-	< 0.05**
	Artemisinin	1.108	0.130	-	
	Doxorubicin	0.00169	0.00011	-	
B16F10	Deoxyartemisinin	0.585	0.058	Inactive	< 0.05**
	Artemisinin	0.871	0.070	Inactive	
	Doxorubicin	0.00066	0.00006	Active	
A549	Deoxyartemisinin	0.394	0.049	Inactive	< 0.05**
	Artemisinin	0.589	0.102	Inactive	
	Doxorubicin	0.00190	0.00052	Active	
HT-29	Deoxyartemisinin	1.292	0.213	Inactive	< 0.05**
	Artemisinin	0.080	0.002	Inactive	
	Doxorubicin	0.00216	0.00057	Active	
Caco-2	Deoxyartemisinin	0.940	0.087	Inactive	-
	Artemisinin	Not determine			
	Doxorubicin	Not determine			

***** LD_{50} values are used for L929 (Normal cells)

** Significant (ANOVA test)

Table 4.7 Post hoc test results of IC_{50} (LD_{50}) between compounds in each cell lines.

Cells	Compounds	*p* value
L929	Deoxyartemisinin - Artemisinin	0.000*
	Deoxyartemisinin - Doxorubicin	0.000*
	Artemisinin - Doxorubicin	0.000*
B16F10	Deoxyartemisinin - Artemisinin	0.002*
	Deoxyartemisinin - Doxorubicin	0.000*
	Artemisinin - Doxorubicin	0.000*
A549	Deoxyartemisinin - Artemisinin	0.174
	Deoxyartemisinin - Doxorubicin	0.015*
	Artemisinin - Doxorubicin	0.030*
HT-29	Deoxyartemisinin - Artemisinin	0.000*
	Deoxyartemisinin - Doxorubicin	0.000*
	Artemisinin - Doxorubicin	0.821

* The mean difference is significant at $p < 0.05$.