CHAPTER 3

Results

3.1 Extraction of 26 medicinal plants and Ya-Mud Mahoog

The medicinal plants and Ya-Mud Mahoog were extracted with 95 % Ethanol**,** and water by using Soxhlet's extraction and decoction method, respectively. The results of the extraction of 26 medicinal plants were shown in Table 3.1. The yields of water and ethanol extract of 26 medicinal plants were shown in Table 3.1. Ya-Mud Mahoog gave 5.43 % w/w and 15.45 % w/w of the ethanol extract and water extract, respectively.

Table 3.1 Percentage yield of water and ethanol extracts of 26 medicinal plants

Family name	**Scientific name**	**Part used**	**% Yield (w/w)**	
			Water extract	**Ethanol extract**
Acanthaceae	*T. laurifolia*	stem	8.99	7.11
Annonaceae	*A. dulcis*	stem	9.40	16.31
Apocynaceaee	*A. marginata*	stem	13.68	12.99
Bignoniaceae	*M. hortensis*	stem	6.40	14.80
Caesalpineaceae	*C. sappan*	heartwood	5.25	15.05
	S. siamensis	stem	9.12	22.25
Celastraceae	*C. paniculatus*	stem	8.47	10.70
Combretaceae	*C. quadrangulare*	stem	10.36	9.65
	T. bellerica	stem	7.10	12.99
	C. deciduum	stem	11.21	13.72

Table 3.1 Percentage yield of water and ethanol extracts of 26 medicinal plants (continued)

Family name	Scientific name	Part used	% Yield (w/w)	
			Water extract	Ethanol extract
Dipterocarpusceae	*S. obtusa*	stem	10.80	14.60
Erythroxylaceae	*E. cuneatum*	root	5.45	0.78
Euphorbiaceae	*T. reidioides*	root	16.65	7.67
	C. crassifolius	root	9.81	8.15
Leeaceae	*L. rubra*	stem	9.76	11.80
	L. rubra	root	10.44	21.95
	L. indica	stem	6.68	7.02
	L. indica	root	10.22	13.80
Papilionaceae	*P. macrocarpus*	stem	3.81	4.41
	D. scandens	stem	9.06	10.23
Piperaceae	*Piper sp.*	stem	11.76	22.59
Rhamnaceae	*V. denticulata*	stem	7.30	5.14
	Z. mauritiana	stem	3.05	3.47
	Z. oenoplia	stem	8.93	8.78
	Z. cambodiana	stem	5.33	2.51
Rubiaceae	*O. horridus*	stem	8.53	10.57
	A. dulcis	stem	9.40	16.31
Sapindaceae	*S. oleosa*	stem	2.95	12.90
Ulmaceae	*H. integrifolia*	stem	2.77	4.22

3.2 Antibacterial activity of 26 medicinal plants and Ya-Mud Mahoog Extract

Table 3.2 Antibacterial activity of 26 medicinal plants and Ya-Mud Mahoog Extract

Extracts	Inhibition zone diameter (mm) against							
	B. subtilis		***S. aureus***		***E. coli***		***P. aeruginosa***	
	EE	**WE**	**EE**	**WE**	**EE**	**WE**	**EE**	**WE**
Family Acanthaceae								
T. laurifolia	8.5±0.5	8.0±0	8.5±0.5	8.0±0	-	-	-	-
Family Annonaceae								
A. dulcis	13.0±0.9	9.2±0.8	10.8±0.8	11.0±0.5	-	-	-	-
Family Apocynaceae								
A. marginata	7.5±0.5	7.0±0	8.5±0.5	-	-	-	-	-
Family Bignoniaceae								
M. hortensis	9.0±1.0	7.3±0.6	-	-	-	-	-	-
Family Caesalpineaceae								
C. sappan	32.3±0.6	33.3±1.5	36.0±1.0	36.3±0.6	13.3±0.3	14.0±0.5	12.8±0.3	11.2±0.3
S. siamensis	16.2±0.3	13.5±0.5	17.7±0.3	16.5±0.5	-	-	-	-
Family Celastraceae								
C. paniculatus	10.8±0.8	8.2±0.3	9.2±0.6	-	-	-	-	-
Family Combretaceae								
C. deciduum	13.0±0.9	9.3±0.8	18.0±0.5	15.8±0.3	-	-	-	-
C. quadrangulare	10.7±0.3	10.0±1.0	8.2±0.3	14.6±0.3	-	-	-	-
T. bellerica	14.2±0.8	8.2±0.3	20.1±0.8	16.3±0.6	-	-	-	-
Family Dipterocarpusceae								
S. obtusa	16.2±0.6	13.5±0.5	15.6±0.3	13.8±0.6	-	-	-	-
Family Erythroxylaceae								
E. cuneatum	10.0±0.5	7.0±0.5	-	7.7±0.6	-	-	-	-
Family Euphorbiaceae								
C. crassifolius	9.3±0.6	8.0±1.0	7.5±0.5	7.5±0.5	-	-	-	-
T. reidioides	9.2±0.3	7.0±0.5	13.5±0.9	10.6±1.0	-	-	-	-
Family Leeaceae								
L. indica (stem)	9.5±0.5	7.0±0	7.5±0.5	-	-	-	-	-
L. indica (root)	13.0±0	13.0±0	11.5±0.5	11.5±0.5	-	-	-	-
L. rubra (stem)	12.5±0.5	7.7±0.6	8.5±0.9	-	-	-	-	-

Table 3.2 Antibacterial activity of 26 medicinal plants and Ya-Mud Mahoog (continued)

Extracts	Inhibition zone diameter (mm) against							
	B. subtilis		***S. aureus***		***E. coli***		***P. aeruginosa***	
	EE	**WE**	**EE**	**WE**	**EE**	**WE**	**EE**	**WE**
L. rubra (root)	14.0±0	9.5±0.5	16.0±0	12.0±1.0	-	-	-	-
Family Papilionaceae								
D. scandens	12.5±0.5	8.0±0	10.5±0.5	7.5±0.5	9.0±0	-	-	-
P. macrocarpus	9.7±0.3	7.0±0	12.5±0.5	12.2±0.8	-	-	-	-
Family Piperaceae								
Piper sp.	8.5±0.5	8.0±0	8.5±0.5	8.0±0	-	-	-	-
Family Rhamnaceae								
V. denticulata	15.0±1.0	10.3±0.6	15.0±0.5	8.0±0.5	-	-	-	-
Z. cambodiana	9.0±0.5	7.0±0	7.0±0	-	-	-	-	-
Z. mauritiana	13.3±0.6	10.0±0.5	13.5±0.9	10.0±0.5	-	-	-	-
Z. oenoplia	10.5±0	9.0±0	10.5±0	9.0±0	-	-	-	-
Family Rubiaceae								
O. horridus	10.0±1.0	8.0±1.3	-	8.0±1.0	-	-	-	-
Family Sapindaceae								
S. oleosa	13.0±0.5	12.0±0.5	17.0±0.9	17.5±0.5	-	-	-	-
Family Ulmaceae								
H. integrifolia	8.0±0	7.0±0	8.5±0.5	7.0±0	-	-	-	-
Mahoog Formula	22.0±0	16.0±0	30.5±0	23.5±0.5	8.5±0	10.0±0	9.5±0	8.0±0

Diameter of well 6 mm, (-) no inhibition (values are mean ± S.D. of three replicates). WE: Water extract, EE: Ethanol extract. The inhibition zone diameter of standard chloramphenicol against *B. subtilis* and *S. aureus* were 27.5±0.5 and 25.0±0.5 mm. The inhibition zone diameter of standard gentamicin against *E.coli* and *P.aeruginosa* were 31.5±0.5 and 28.5±0.5 mm.

3.3 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The results of the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were shown in Table 3.3.

Table 3.3 The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Scientific name	MIC (mg/mL)		MBC (mg/mL)	
	S. aureus	***B. subtilis***	***S. aureus***	***B. subtilis***
C. sappan	0.0488	0.390	0.098	0.390
S. siamensis	0.0488	12.500	0.098	12.500
T. bellerica	3.125	12.500	6.250	12.500
L. rubra	6.250	12.500	12.500	12.500
S. oleosa	0.098	6.25	0.195	12.500

3.4 Antioxidant activity of 26 medicinal plants and Ya-Mud Mahoog

Table 3.4 Antioxidant activity of 26 medicinal plants and Ya-Mud Mahoog

Extracts	Antioxidant activity (TEAC, g of Trolox/g of sample)					
	ABTS		DPPH		FRAP	
	EE	WE	EE	WE	EE	WE
Family Acanthaceae						
T. laurifolia	0.134	0.097	0.077	0.068	0.310	0.109
Family Annonaceae						
A. dulcis	0.444	0.194	0.319	0.141	0.104	0.101
Family Apocynaceae						
A. marginata	0.363	0.180	0.183	0.144	0.192	0.139
Family Bignoniaceae						
M. hortensis	0.193	0.093	0.133	0.040	0.081	0.050

Table 3.4 Antioxidant activity of 26 medicinal plants and Ya-Mud Mahoog (continued)

Extracts	Antioxidant activity (TEAC, g of Trolox/g of sample)					
	ABTS		DPPH		FRAP	
	EE	WE	EE	WE	EE	WE
Family Caesalpineaceae						
C. sappan	1.359	0.903	1.004	0.695	1.280	1.148
S. siamensis	0.976	0.650	0.652	0.554	0.916	0.538
Family Celastraceae						
C. paniculatus	0.096	0.123	0.055	0.108	0.051	0.103
Family Combretaceae						
C. deciduum	0.445	0.726	0.510	0.442	0.418	0.558
C. quadrangulare	0.169	0.505	0.111	0.455	0.189	0.270
T. bellerica	0.506	0.562	0.369	0.425	0.633	0.401
Family Dipterocarpusceae						
S. obtusa	0.869	0.861	0.666	0.703	1.116	0.597
Family Erythroxylaceae						
E. cuneatum	0.331	0.107	0.244	0.136	0.190	0.066
Family Euphorbiaceae						
C. crassifolius	0.092	0.049	0.076	0.040	0.096	0.035
T. reidioides	0.362	0.032	0.076	0.023	0.176	0.034
Family Leeaceae						
L. indica (stem)	0.162	0.105	0.114	0.121	0.051	0.078
L. indica (root)	0.280	0.437	0.290	0.183	0.217	0.295
L. rubra (stem)	0.213	0.228	0.140	0.218	0.134	0.104
L. rubra (root)	0.579	0.319	0.427	0.154	0.279	0.243

Table 3.4 Antioxidant activity of 26 medicinal plants and Ya-Mud Mahoog (continued)

Extracts	Antioxidant activity (TEAC, g of Trolox/g of sample)					
	ABTS		DPPH		FRAP	
	EE	WE	EE	WE	EE	WE
Family Papilionaceae						
D. scandens	0.398	0.156	0.188	0.156	0.312	0.120
P. macrocarpus	0.421	0.321	0.203	0.244	0.172	0.156
Family Piperaceae						
Piper sp.	0.248	0.150	0.126	0.092	0.108	0.111
Family Rhamnaceae						
V. denticulata	0.262	0.114	0.159	0.092	0.117	0.072
Z. cambodiana	0.172	0.122	0.108	0.114	0.070	0.082
Z. mauritiana	0.831	0.351	0.412	0.295	0.223	0.229
Z. oenoplia	0.252	0.204	0.144	0.171	0.070	0.198
Family Rubiaceae						
O. horridus	0.116	0.140	0.110	0.104	0.068	0.111
Family Sapindaceae						
S. oleosa	0.210	0.244	0.326	0.238	0.106	0.286
Family Ulmaceae						
H. integrifolia	0.179	0.055	0.031	0.032	0.101	0.053
Family Euphorbiaceae						
C. crassifolius	0.092	0.049	0.076	0.040	0.096	0.035
T. reidioides	0.362	0.032	0.076	0.023	0.176	0.034
Mahoog Formula	1.018	0.560	0.975	0.767	0.689	0.544

WE : Water extract, EE : Ethanol extract

3.5 Phytochemical screening of plants species in Ya-Mud Mahoog

Table 3.5 Phytochemical screening of plants species in Ya-Mud Mahoog

Tests	*C. sappan*		*E. cuneatum*		*T. reidioides*		*L. rubra*		*S. oleosa*	
	EE	**WE**	**EE**	**WE**	**EE**	**WE**	**EE**	**WE**	**EE**	**WE**
Alkaloids	-	-	-	-	-	-	-	-	-	-
Tannin	+	+	+	-	-	-	+	+	+	+
Coumarins	-	-	-	-	-	-	-	-	-	-
Anthraqui-nones	+	+	+	+	+	-	+	+	+	+
Saponins	-	-	-	-	-	-	-	-	-	-
Flavonoids	+	+	-	-	-	-	-	-	-	-
Terpenoids	+	+	-	-	-	-	+	+	-	-
Steroids	+	+	+	+	+	+	+	-	+	+
Cardiac glycoside	-	-	-	-	-	-	-	-	-	-
Anthocyanin	-	-	-	-	-	-	-	-	-	-

WE : Water extract, EE : Ethanol extract; + : positive, - : negative

3.6 Bioassay guided isolation of bioactive compounds from *Leea rubra* (stem)

The stems of *L. rubra* were selected to study the chemical constituents related to antimicrobial and antioxidant activities. Overall of the isolation of bioactive compounds were shown in Fig.3.1

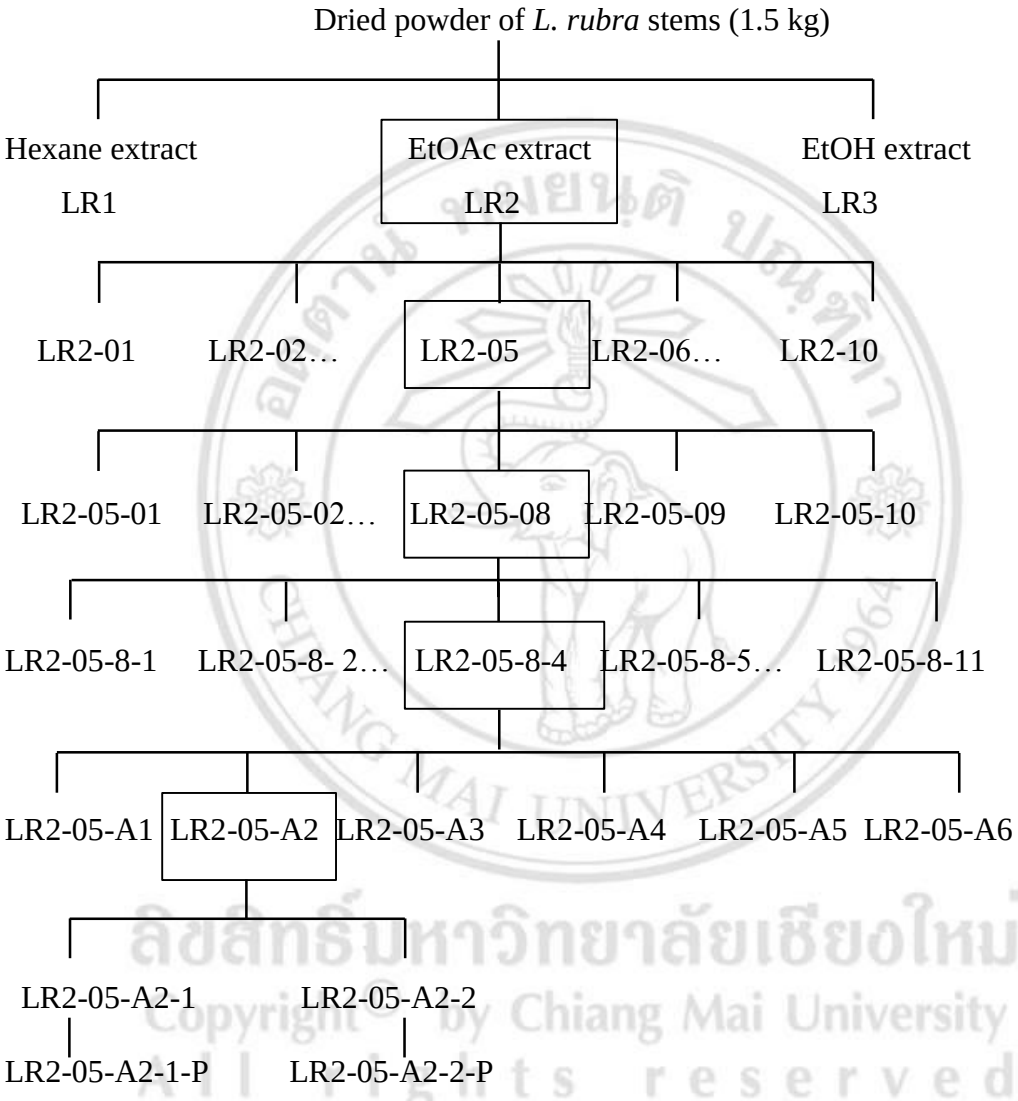

Figure 3.1 Isolation of chemical compounds of *Leea rubra stem*

The detail of each step in isolation process is as follows;

3.6.1 Extraction of *L. rubra*

The dried powder of stems (1.5 kg) were successively extracted with *n*-hexane, ethyl acetate and 95 % ethanol by soxhlet's apparatus, respectively. The solutions were filtered through Whatman filter paper No. 1 and then the solvents were removed by using rotary evaporator to obtain *n*-hexane extract (0.19 % w/w), ethylacetate extract (3.32 % w/w) and ethanol extract (10.12 % w/w). Then, each crude extract was evaluated antibacterial and antioxidant activity. The extraction procedure was shown in Figure 3.7.

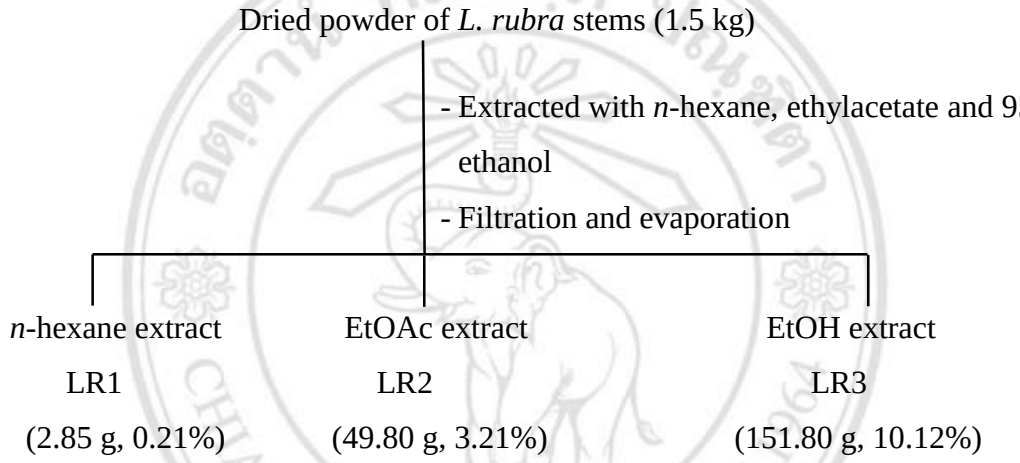

Figure 3.2 Extraction of dried powder of *L. rubra* stems

3.6.1.1 Antibacterial activity of *L. rubra* extracts

Table 3.6 Antibacterial activity of *L. rubra* extracts

Fraction	Inhibition zone diameter (mm) against			
	B. subtilis	***S. aureus***	***E. coli***	***P. aeruginosa***
n-hexane	-	-	-	-
Ethyl acetate	9.0±0.0	9.0±0.0	-	-
Ethanol	-	-	-	-

Diameter of well 6 mm, (-) no inhibition (values are mean ± S.D. of three replicates).

3.6.1.2 Antioxidant activity of *L. rubra* extracts

Table 3.7 Antioxidant activity of *L. rubra* extracts

Fraction	Antioxidant activity (TEAC, g of Trolox/g of sample)		
	ABTS	DPPH	FRAP
n-hexane	0.060	0.026	0.036
Ethyl acetate	0.136	0.149	0.124
Ethanol	0.110	0.123	0.086

The ethyl acetate extract (LR2) gave the highest activity followed by ethanol extract (LR3) and *n*-hexane extract (LR1). Therefore the ethyl acetate extract (LR2) was selected to isolate the active component.

3.6.2 Isolation of the ethyl acetate extract

The ethyl acetate extract, LR2 (40.00 g) was fractionated on column chromatography of silica gel (500 g, 70-230 mesh, cat. No. 7734, Merck), with diameter 9.5 cm, using *n*-hexane, ethyl acetate and methanol as eluting solvent. Forty seven fractions (500 mL each) were obtained according to their TLC analysis patterns giving 10 fractions, LR2-01 to LR2-10.

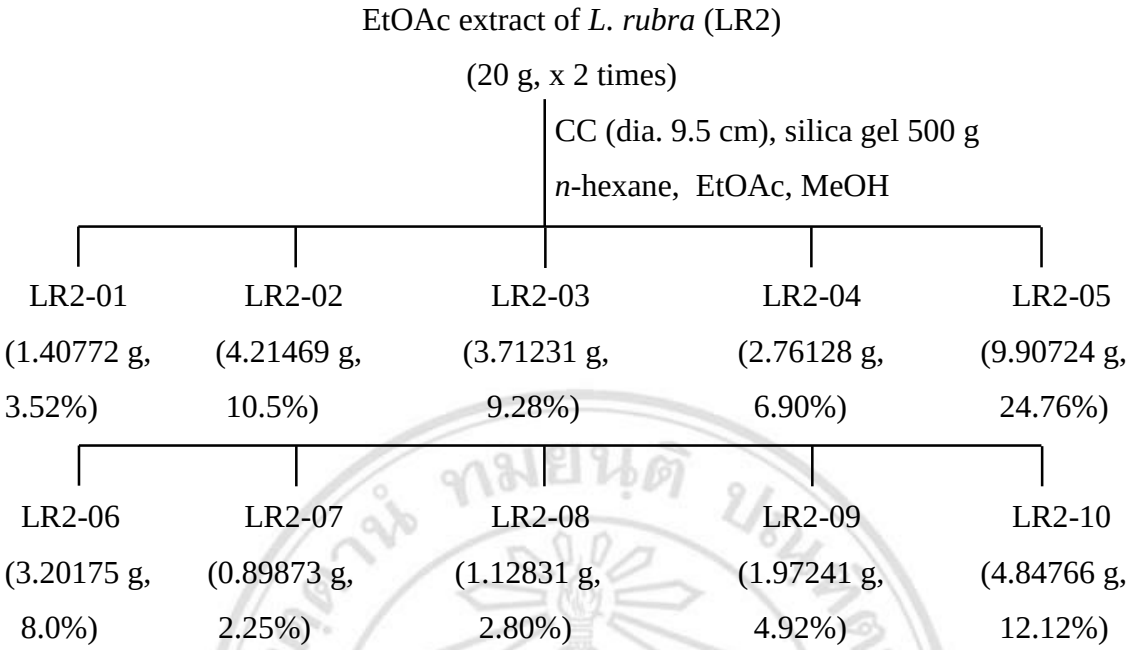

Figure 3.3 Isolation of EtOAc ext. of *L. rubra* extracted by EtOAc (%yield)

Then, all fractions, LR2-01 to LR2-10 were assessed for antibacterial and antioxidant activities.

3.6.2.1 Antibacterial activity of LR2-01 to LR2-10 fraction

Table 3.8 Antibacterial activity of LR2-01 to LR2-10 fraction

Fractions	**Inhibition zone diameter (mm) against**			
	B. subtilis	***S. aureus***	***E. coli***	***P. aeruginosa***
LR2	9.0±0.0	9.0±0.0	-	-
LR2-01	-	-	-	-
LR2-02	-	-	-	-
LR2-03	12.5±0.5	-	-	-
LR2-04	9.0±0.0	9.0±0.0	-	-
LR2-05	9.5±0.0	11.0±0.0	-	-
LR2-06	9.0±0.0	8.0±0.0	-	-
LR2-07	-	-	-	-

Table 3.8 Antibacterial activity of LR2-01 to LR2-10 fraction (continued)

Fractions	Inhibition zone diameter (mm) against			
	B. subtilis	***S. aureus***	***E. coli***	***P. aeruginosa***
LR2-08	-	-	-	-
LR2-09	-	-	-	-
LR2-10	-	-	-	-

Diameter of well 6 mm, (-) no inhibition (values are mean ± S.D. of three replicates).

3.6.2.2 Antioxidant activity of LR2-01 to LR2-10 fraction

Table 3.9 Antioxidant activity of LR2-01 to LR2-10 fraction

Fractions	Antioxidant activity (TEAC, g of Trolox/g of sample)		
	ABTS	**DPPH**	**FRAP**
LR2	0.136	0.149	0.124
LR2-01	0.047	0.018	0.017
LR2-02	0.044	0.016	0.020
LR2-03	0.112	0.034	0.034
LR2-04	0.114	0.084	0.109
LR2-05	0.163	0.157	0.143
LR2-06	0.112	0.105	0.095
LR2-07	0.137	0.113	0.108
LR2-08	0.111	0.060	0.070
LR2-09	0.110	0.100	0.094
LR2-10	0.090	0.045	0.052

The results of antibacterial activity and antioxidant activity were shown in Table 3.9 and 3.10. The fraction LR2-05 gave good activity and then was selected to isolate its active component.

3.6.3 Isolation of LR2-05 fraction

The fraction LR2-05 (9.00 g) was rechromatographed with silica gel (280 g, 70-230 mesh, cat. No. 7734, Merck), with diameter 4.5 cm, using *n*-hexane, ethyl acetate and methanol as eluting solvent with gradient composition. One hundred fractions (100 mL each) were obtained according to their TLC analysis patterns giving 10 fractions, LR2-05-1 to LR2-05-10.

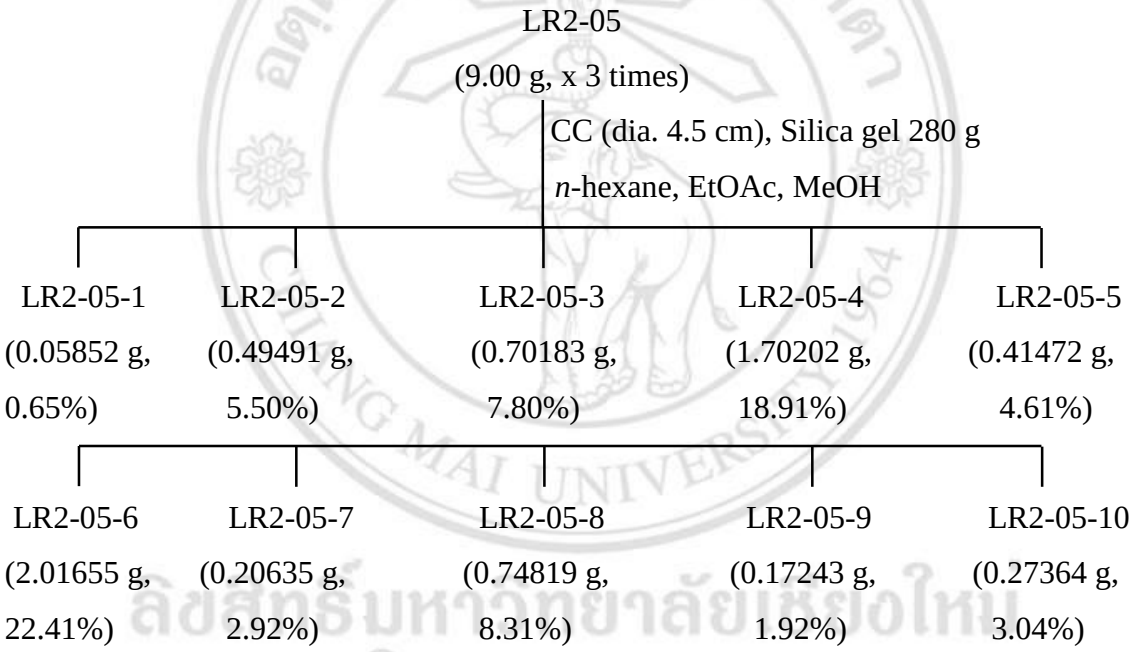

Figure 3.4 Isolation of LR2-05 fraction

Then, all fractions, LR2-05-1 to LR2-05-10 were assessed for antibacterial and antioxidant activities.

3.6.3.1 Antibacterial activity of LR2-05-1 to LR2-05-10 fraction

Table 3.10 Antibacterial activity of LR2-05-1 to LR2-05-10 fraction

Fractions	Inhibition zone diameter (mm) against			
	B. subtilis	***S. aureus***	***E. coli***	***P. aeruginosa***
LR2-05	9.0±0.0	9.0±0.0	-	-
LR2-05-1	-	-	-	-
LR2-05-2	10.0±0.0	8.0±0.0	-	-
LR2-05-3	9.0±0.0	8.0±0.5	-	-
LR2-05-4	9.0±0.5	9.0±0.0	-	-
LR2-05-5	9.0±0.0	-	-	-
LR2-05-6	9.0±0.0	10.0±0.0	-	-
LR2-05-7	9.0±0.5	9.0±0.0	-	-
LR2-05-8	9.0±0.0	9.0±0.0	-	-
LR2-05-9	9.0±0.0	9.0±0.0	-	-
LR2-05-10	-	-	-	-

Diameter of well 6 mm, (-) no inhibition (values are mean ± S.D. of three replicates).

3.6.3.2 Antioxidant activity of LR2-05-1 to LR2-05-10 fraction

Table 3.11 Antioxidant activity of LR2-05-1 to LR2-05-10 fraction

Fractions	Antioxidant activity (TEAC, g of Trolox/g of sample)		
	ABTS	DPPH	FRAP
LR2-05	0.163	0.157	0.143
LR2-05-1	0.007	0.004	0.037
LR2-05-2	0.103	0.022	0.177
LR2-05-3	0.094	0.096	0.040
LR2-05-4	0.162	0.100	0.107
LR2-05-5	0.131	0.064	0.059
LR2-05-6	0.095	0.112	0.074
LR2-05-7	0.089	0.001	0.036
LR2-05-8	0.198	0.170	0.145
LR2-05-9	0.096	0.010	0.056
LR2-05-10	0.079	-0.009	0.016

The results of antibacterial activity and antioxidant activity were shown in Table 3.11 and 3.12. The fraction LR2-05-8 gave good activity and then was selected to isolate its active component.

3.6.4 Isolation of LR2-05-8 fraction

The fraction LR2-05-8 (600.00 mg) was fractionated on column chromatography of silica gel (60 g, 70-230 mesh, cat. No. 7734, Merck), with diameter 2.0 cm, using *n*-hexane, ethyl acetate and methanol as eluting solvent with gradient solvents. Sixty four fractions (50 mL each) were obtained according to their TLC analysis patterns giving 11 fractions, LR2-05-8-1 to LR2-05-8-11.

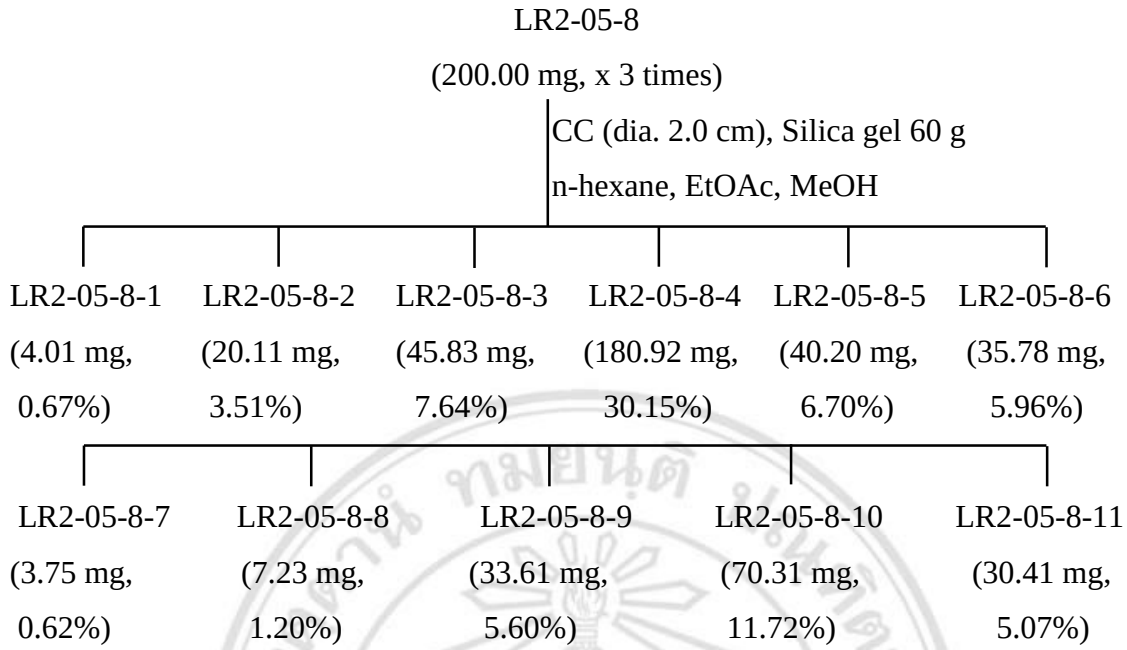

Figure 3.5 Isolation LR2-05-8 fraction

Then, all fractions, LR2-05-8-1 to LR2-05-8-11 were assessed for antioxidant activity because of the limitation of their quantity.

3.6.4.1 Antioxidant activity of LR2-05-8-1 to LR2-05-8-11 fractions

Table 3.12 Antioxidant activity of LR2-05-8-1 to LR2-05-8-11 fractions

Fractions	**Antioxidant activity (TEAC, g of Trolox/g of sample)**		
	ABTS	**DPPH**	**FRAP**
LR2-05-8	0.198	0.170	0.145
LR2-05-8-1	0.011	0.008	0.037
LR2-05-8-2	0.091	0.087	0.072
LR2-05-8-3	0.104	0.095	0.078
LR2-05-8-4	0.259	0.232	0.225
LR2-05-8-5	0.227	0.210	0.204
LR2-05-8-6	0.145	0.124	0.116

Table 3.12 Antioxidant activity of LR2-05-8-1 to LR2-05-8-11 fractions (continued)

Fractions	Antioxidant activity (TEAC, g of Trolox/g of sample)		
	ABTS	DPPH	FRAP
LR2-05-8-7	0.088	0.076	0.054
LR2-05-8-8	0.079	0.008	0.043
LR2-05-8-9	0.067	0.011	0.049
LR2-05-8-10	0.033	0.007	0.026
LR2-05-8-11	0.064	0.003	0.025

The results of antioxidant activity were shown in Table 3.13. The fraction LR2-05-8-4 , and LR2-05-8-5 showed the highest activity compared to the other. LR2-05-8-4 was selected to isolate its active component.

3.6.5 Isolation of LR2-05-8-4 fraction

The fraction LR2-05-8-4 (160.00 mg) was fractionated on column chromatography of silica gel (20 g, 230-400 mesh, cat. No. 9385, Merck), with diameter 1.5 cm, using *n*-hexane, ethyl acetate and methanol as eluting solvent with increasing amount of the more polar solvent. Forty fractions (20 mL each) were obtained according to their TLC analysis patterns giving 6 fractions, LR-1 to LR-6.

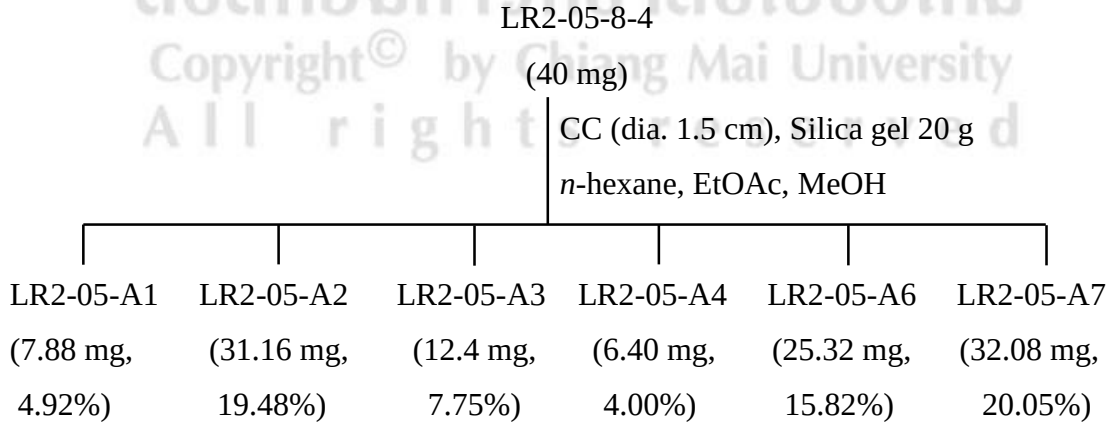

Figure 3.6 Isolation of LR2-05-8-4 fraction (%yield)

Then, all fractions, LR2-05-A1to LR2-05-A6 were assessed for antioxidant activity by spraying with DPPH reagent on TLC to isolate bioactive antioxidant compounds in this fraction.

3.6.6 Isolation of LR2-05-A2 fraction

The fraction LR2-05-A2 (30 mg) was further purified by preparative thin layer chromatography (prep TLC) by using *n*-hexane : ethyl acetate (70:30) as mobile phase. Two bands on prep TLC were isolated and obtained to be fractional.

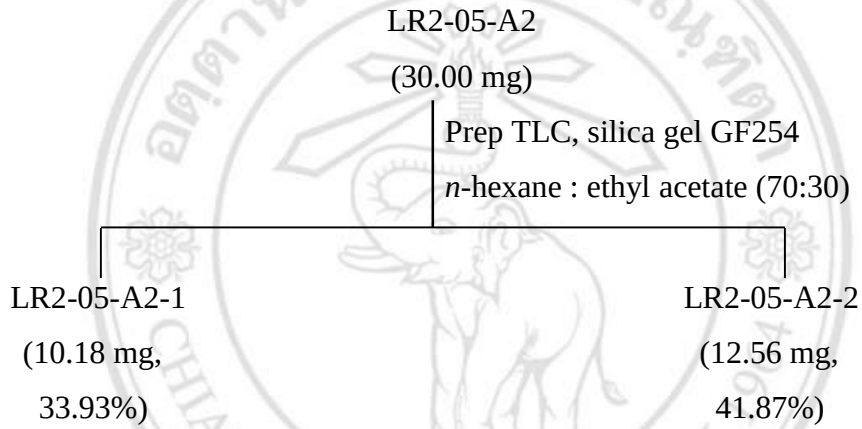

Figure 3.7 Isolation of LR2-05-A2 fraction (%yield)

3.6.7 Isolation of LR2-05-A2-1 fraction

The fraction LR2-05-A2-1 (10.18 mg) was further purified by preparative thin layer chromatography (silica gel GF254) and using mobile phase as chloroform to afford LR2-05-A2-1-P (6.05 mg) as yellow powder.

3.6.8 Isolation of LR2-05-A2-2 fraction

The fraction LR2-05-A2-2 (12.56 mg) was further purified by prep TLC (silica gel GF254) and using mobile phase as chloroform to afford LR2-05-A2-2-P (7.54 mg) as yellow powder.

3.7 Structure elucidation of the isolated compound

3.7.1 LR2-05-A2-1-P

Yellow powder; UV (MeOH) λmax nm: 291; EIMS m/z: 420, 405, 377, 365, 202; IR (KBR disc) cm^{-1}: 3331.77, 2923.78, 1644.07; ^{1}H, ^{13}C NMR, HMQC and HMBC data: Table 3.15 – 3.17.

3.7.2 LR2-05-A2-2-P

Yellow powder; UV (MeOH) λmax nm: 274; EIMS m/z: 420, 405, 377, 365, 202; IR (KBR disc) cm^{-1}: 3285.85, 2923.34, 1643.80; ^{1}H, ^{13}C NMR, HMQC and HMBC data: Table 3.18 – 3.20.

Table 3.13 ^{1}H and ^{13}C NMR spectral data of compound LR2-05-A2-1-P compared with reference

Position	**δ_H**	**δ_C**	**HMBC correlation**
2	7.89 (1H, s)	152.91	C-3, C-4, C-5
3	-	123.32	-
4	-	181.47	-
5	-	154.79	-
6	-	107.57	-
7	-	157.05	-
8	-	105.49	-
9	-	152.91	-
10	-	105.86	-
1´	-	123.08	-
2´	7.05 (1H,*d J*=1.5 Hz)	116.27	C-3, C-3´, C-6´
3´	-	144.53	-
4´	-	143.79	-
5’	6.88 (1H, *d*, *J*=8.2 Hz)	115.38	C-1´, C-2´, C-3´
6´	6.85 (1H, *dd*, *J*=1.5, 8.2 Hz)	121.37	C-2´, C-3´
1´´	3.40 (2H, *d*, *J*=7.3 Hz)	21.28	C-8, C-2´´
2´´	5.17 (1H, *t*, *J*=7.3 Hz)	121.89	-
3´´	-	131.74	-
4´´	1.81 (3H, *s*)	17.88	C-2´´, C-3´´, C-5´´
5´´	1.68 (3H, *s*)	25.71	C-2´´, C-3´´, C-4´´
2´´´	-	77.87	-
3´´´	5.63 (1H, *d*, *J*=10 Hz)	128.11	C-2´´´
4´´´	6.74 (1H, *d*, *J*=9.9 Hz)	115.81	C-5, C-7, C-2´´´
5´´´	1.47 (3H, *s*)	28.21	C-3´´´, C-6´´´
6´´´	1.47 (3H, *s*)	28.21	C-3´´´, C-5´´´

In $CDCl_3$, TMS as internal standard

Table 3.14 Carbon-proton correlations of compound LR2-05-A2-1-P observed in the HMQC spectrum

Carbon	**δ_C (ppm)**	**Correlation with proton at δ_H (ppm)**
C-2	152.91	7.89
C-2′	116.27	7.05
C-5′	115.38	6.88
C-6′	121.37	6.85
C-1′′	21.28	3.40
C-2′′	121.89	5.17
C-4′′	17.88	1.81
C-5′′	25.71	1.68
C-3′′′	128.11	5.63
C-4′′′	115.81	6.74
C-5′′′, C-6′′′	28.21	1.47

Table 3.15 ^{1}H and ^{13}C NMR spectral data of compound LR2-05-A2-1-P compared with reference

Position	LR2-05-A2-1-P		Reference	
	Chemical shift[a]		Chemical shift	
	^{1}H	^{13}C	^{1}H[159, a]	^{13}C[160, b]
2	7.89 (1H, s)	152.91	7.90 (1H, *s*)	154.4
3	-	123.32	-	123.8
4	-	181.47	-	182.1
5	-	154.79	-	155.8
6	-	105.49	-	108.0
7	-	157.05	-	157.4
8	-	105.86	-	105.9
9	-	152.91	-	155.4
10	-	107.57	-	106.5
1´	-	123.08	-	123.6
2´	7.05 (1H,*d* *J*=1.5 Hz)	116.27	7.00 (1H,*d*, *J*=1.4Hz)	117.2
3´	-	144.53	-	146.2
4´	-	143.79	-	145.6
5’	6.88 (1H, *d*, *J*=8.2 Hz)	115.38	6.85 (1H, *d*, *J*=8.2 Hz)	115.9
6´	6.85 (1H, *dd*, *J*=1.5, 8.2 Hz)	121.37	6.83(1H, *dd*, *J*=1.4, 8.2 Hz)	121.5
1´´	3.40 (2H, *d*, *J*=7.3 Hz)	21.28	3.40 (2H, *d*, *J*=7.2 Hz)	21.8
2´´	5.17 (1H, *t*, *J*=7.3 Hz)	121.89	5.17 (1H, *t*, *J*=7.2 Hz)	122.9
3´´	-	131.74	-	132.0
4´´	1.81 (3H, *s*)	17.88	1.81 (3H, *s*)	18.0
5´´	1.68 (3H, *s*)	25.71	1.68 (3H, *s*)	25.9
2´´´	-	77.87	-	78.6
3´´´	5.63 (1H, *d*, *J*=10 Hz)	128.11	5.65 (1H, *d*, *J*=10 Hz)	129.2
4´´´	6.74 (1H, *d*, *J*=9.9 Hz)	115.81	6.73 (1H, *d*, *J*= 10 Hz)	116.2
5´´´	1.47 (3H, *s*)	28.21	1.43 (3H, *s*)	28.3
6´´´	1.47 (3H, *s*)	28.21	1.43 (3H, *s*)	28.3

[a]In $CDCl_3$, TMS as internal standard; [b]In Me_2CO-d_6, TMS as internal standard

Table 3.16 ^{1}H and ^{13}C NMR spectral data of compound LR2-05-A2-2-P compared with reference

Position	δ_H	δ_C	HMBC correlation
2	7.86 (1H, s)	152.83	C-3, C-4, C-9
3	-	123.78	-
4	-	181.44	-
5	-	159.15	-
6	-	113.10	-
7	-	157.58	-
8	-	100.86	-
9	-	150.66	-
10	-	105.33	-
1´	-	122.87	-
2´	6.97 (1H,*d J*=1.5) Hz)	116.40	C-3, C-3´, C-6´
3´	-	144.59	-
4´	-	143.84	-
5´	6.82 (1H, *d*, *J*=8.1 Hz)	115.42	C-1´, C-4´
6´	6.76 (1H, *dd*, *J*=1.8, 8.1 Hz)	121.43	C-3, C-2´, C-3´
1´´	3.34 (2H, *d*, *J*=7.2 Hz)	21.31	C-5, C-6, C-7, C-2´´, C-3´´
2´´	5.23 (1H, *t*, *J*=7.2 Hz)	121.86	-
3´´	-	131.65	-
4´´	1.80 (3H, *s*)	25.75	C-2´´, C-3´´
5´´	1.68 (3H, *s*)	17.89	C-2´´, C-3´´
2´´´	-	78.03	-
3´´´	5.60 (1H, *d*, *J*=9.9 Hz)	127.26	C-8
4´´´	6.70 (1H, *d*, *J*=9.9 Hz)	114.88	C-7, C-8, C-9
5´´´	1.48 (3H, *s*)	28.15	C-2´´´, C-3´´´, C-6´´´
6´´´	1.48 (3H, *s*)	28.15	C-2´´´, C-3´´´, C-5´´´

In $CDCl_3$, TMS as internal standard

Table 3.17 Carbon-proton correlations of compound LR2-05-A2-2-P observed in the HMQC spectrum

Carbon	**δ_C (ppm)**	**Correlation with proton at δ_H (ppm)**
C-2	152.83	7.86
C-2´	116.40	6.97
C-5´	115.42	6.82
C-6´	121.43	6.76
C-1´´	21.31	3.34
C-2´´	121.86	5.23
C-4´´	25.75	1.80
C-5´´	17.89	1.68
C-3´´´	127.26	5.60
C-4´´´	114.88	6.70
C-5´´´, C-6´´´	28.15	1.48

Table 3.18 ^{1}H and ^{13}C NMR spectral data of compound LR2-05-A2-2-P compared with reference

Position	LR2-05-A2-2-P		Reference[160]	
	Chemical shift[a]		Chemical shift[b]	
	^{1}H	^{13}C	^{1}H	^{13}C
2	7.86 (1H, s)	152.83	8.16	154.1
3	-	123.78	-	124.0
4	-	181.44	-	181.8
5	-	159.15	-	160.1
6	-	113.10	-	113.0
7	-	157.58	-	157.7
8	-	100.86	-	101.5
9	-	150.66	-	151.2
10	-	105.33	-	106.1
1´	-	122.87	-	123.6
2´	6.97 (1H,*d* *J*=1.5) Hz)	116.40	7.17	117.2
3´	-	144.59	-	146.2
4´	-	143.84	-	145.5
5´	6.82 (1H, *d*, *J*=8.1 Hz)	115.42	6.89	115.9
6´	6.76 (1H, *dd*, *J*=1.8, 8.1 Hz)	121.43	6.94	121.5
1´´	3.34 (2H, *d*, *J*=7.2 Hz)	21.31	3.31	21.8
2´´	5.23 (1H, *t*, *J*=7.2 Hz)	121.86	5.22	122.9
3´´	-	131.65	-	131.6
4´´	1.80 (3H, *s*)	25.75	1.80	25.9
5´´	1.68 (3H, *s*)	17.89	1.65	18.0
2´´´	-	78.03	-	78.7
3´´´	5.60 (1H, *d*, *J*=9.9 Hz)	127.26	5.71	128.2
4´´´	6.70 (1H, *d*, *J*=9.9 Hz)	114.88	6.69	115.4
5´´´	1.48 (3H, *s*)	28.15	1.47	28.2
6´´´	1.48 (3H, *s*)	28.15	1.47	28.2

[a]In $CDCl_3$, TMS as internal standard; [b]In Me_2CO-d_6, TMS as internal standard

3.8 Antibacterial and antioxidant activities of isolated compounds

The LR2-05-A2-1-P and LR2-05-A2-2-P were assessed for antibacterial and antioxidant activities by ABTS, DPPH and FRAP method. The results of antibacterial and antioxidant activities were shown in Table 3.21 and 3.22, respectively.

Table 3.19 Antibacterial activity of LR2-05-A2-1-P and LR2-05-A2-2-P

compound (1 mg/well)	**Inhibition zone diameter (mm) against**			
	B. subtilis	***S. aureus***	***E. coli***	***P. aeruginosa***
LR2-05-A2-1-P	9.5±0.0	8.5±0.0	-	-
LR2-05-A2-2-P	9.5±0.0	8.5±0.0	-	-

Table 3.20 Antioxidant activity of LR2-05-A2-1-P and LR2-05-A2-2-P

compound	**Antioxidant activity (TEAC, g of Trolox/g of sample)**		
	ABTS	**DPPH**	**FRAP**
LR2-05-A2-1-P	0.401	0.386	0.586
LR2-05-A2-2-P	0.537	0.472	0.884