CHAPTER 4

RESULTS AND DISCUSSION

4.1 Yield of crude extracts from plants

In this study, 42 samples from 26 plant species were investigated for their antioxidant activity. The crude ethanol extracts of these samples were used for comparison of their antioxidant power. The percentage yield of these ethanol crude extracts is presented in Table 4.1. It ranged from 0.46 – 17.52 (w/w) %. The peels of *Citrus hystrix* gave the highest percentage yield whereas the fresh leaves of *Cymbopogon citratus* gave the lowest. Several methods have been used for evaluation of the antioxidant activity from the plants. Those are DPPH scavenging assay (Gamez et al., 1998), ABTS decolorization (Re et al., 1999), FRAP method (Benzie and Strain, 1996), and beta-carotene bleaching model (Jayaprakasha et al., 2001). Free radical is a major cause on the propagation stage of oxidation process. The high potential on scavenging free radical could inhibit spreading of oxidation. Hence the comparative study to seek for the highest potential antioxidant from the ethanol crude extracts in this study was carried out by free radical scavenging method using ABTS as free radical. This is an excellent method for determining the antioxidant activity of a broad diversity of substances, such as hydrogen-donating antioxidants or scavengers of aqueous phase radicals and of chain-breaking antioxidants or scavengers of lipid peroxyl radicals[66, 67, 68].

Table 4.1 Percentage of yield of samples (ordering)

Scientific name	Used parts	Fresh weight (g)	Dry weight (g)	Extract weight (g)	%Yield
Citrus hystrix	Peels	90.0	27.0	4.73	17.52
Cymbopogon citratus	Stems	550.0	100.0	16.37	16.37
Musa sapientum	Ripe peels	130.2	14.8	2.14	14.44
Dregea volubilis	Leaves and stems	190.7	32.3	4.00	12.38
Gymnema inodorum	Leaves	500.0	150.0	17.88	11.92
Marsdenia glabra	Leaves	64.6	15.2	1.76	11.56
Mentha cordifolia	Leaves	62.8	11.5	1.28	11.13
Nephelium lappaceum	Peels	300.0	100.0	10.68	10.68
Cymbopogon citratus	Leaves	325.0	80.0	8.04	10.05
Ocimum basilicum	Leaves	128.2	22.5	2.21	9.82
Euphoria longan	Seeds	265.0	132.1	12.66	9.59
Psidium guajava	Leaves	400.0	200.0	17.40	8.70

Table 4.1 (continued)

Scientific name	Used parts	Fresh weight (g)	Dry weight (g)	Extract weight (g)	%Yield
Clausena lansium	Leaves	900.0	400.0	34.64	8.66
Musa sapientum	Green peels	220.0	50.0	3.83	7.66
Oroxylum indicum	Pods	2800.0	400.0	29.99	7.50
Citrus hystrix	Leaves	700.0	350.0	25.97	7.42
Garcinia mangostana	Peels	620.0	400.0	28.85	7.21
Mentha cordifolia	Stems	27.4	5.0	0.37	7.04
Andrographis paniculata	Leaves	800.0	300.0	19.45	6.48
Punica granatum	Peels	500.0	150.0	9.31	6.21
Ocimum gratissimum	Leaves	157.9	52.0	3.21	6.17
Leucaena leucocephala	Leaves	1400.0	300.0	18.26	6.09
Ocimum sanctum	Leaves	173.4	42.9	2.59	6.04
Cocos nucifera	White peels	670.0	250.0	14.51	5.80
Lansium domesticum	Peels	126.5	50.3	2.87	5.71
Ocimum basilicum	Stems	51.4	20.0	0.97	4.85
Cymbopogon citratus	Fresh stems	550.0	25.64	-	4.66

Table 4.1 (continued)

Scientific name	Used parts	Fresh weight (g)	Dry weight (g)	Extract weight (g)	%Yield
Psidium guajava	Fruits	1100.0	200.0	9.29	4.65
Thunbergia laurifolia	Leaves	1000.0	200.0	8.93	4.47
Punica granatum	Seeds	400.0	100.0	4.46	4.46
Hyptis suaveolens	Leaves and stems	700.0	100.0	4.34	4.34
Gymnema inodorum	Stems	800.0	250.0	9.77	3.91
Ocimum sanctum	Stems	80.8	22.8	0.83	3.65
Piper sarmentosum	Leaves	200.0	80.0	2.45	3.06
Passiflora foetida	Peels	668.7	105.7	2.75	2.60
Citrus hystrix D.C.	Stems	450.0	250.0	4.92	1.97
Andrographis paniculata	Stems	650.0	200.0	3.07	1.54
Hylocereus undatus	Peels	552.6	58.6	0.82	1.40
Leucaena leucocephala	Pods	800.0	150.0	1.80	1.20
Psidium guajava	Stems	100.0	50.0	0.56	1.12
Cymbopogon citratus	Fresh leaves	325.0	1.50	-	0.46

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Thailand, is a tropical global country shows on amazing diversity of plants species. Some of them have long been used as traditional medicines. Many of them were reported to have various desirable activities[69, 70, 71]. The antioxidant activity of plants is mainly contributed by the active compounds present in them. The amount of such compounds deposited in each part of the plant is usually different.

4.2 Antioxidant activities and cytotoxicity capacities of the ethanolic crude extracts

The ethanolic crude extracts from the various parts of plants such as leaf, stem, fruit pulp and fruit peel were evaluated and compared for their antioxidant activity. The plant whose ethanol crude extract showed the highest antioxidant potential was further evaluated on the mechanism of antioxidant activity and phenolic content. The results of preliminary screening antioxidant activity of all ethanol crude extracts were expressed as TEAC, $IC_{50,}$ percentage of antioxidant activity and EC values as shown in Tables 4.2, 4.3, 4.4 and 4.5, respectively. The results of primary screening antioxidant activity of all ethanol crude extracts were expressed as TEAC value as shown in Table 4.2. This value represented the mM trolox equivalent/mg extract. The antioxidant activity of the samples demonstrated widely ranged from 0.207 – 4.908 mM trolox equivalent/mg extract. The leaves of guava (*Psidium guajava*) showed the highest antioxidant activity with the TEAC value of 4.908 ± 0.050 mM trolox equivalent/mg extract, followed by the fruit peels of *Punica granatum* and *Nephelium lappaceum* with the TEAC values of 4.066 ± 0.009 and 3.074 ± 0.003 mM trolox equivalent/mg extract, respectively. The peels of *Lansium domesticum* showed the lowest antioxidant activity among the plant samples included in this study with the TEAC value of 0.207 ± 0.002 mM trolox equivalent/mg extract.

When the leaf and the stem of each plant samples were compared, it was found that all plant extracts from the leaf exhibited higher antioxidant activity than that from the stem. The extract from fruit peels also demonstrated wide range antioxidant activity of 0.507 – 3.074 mM trolox equivalent/mg extract, depending on plant species. Among the fruit peel samples, the pericarp of rambutan showed the highest antioxidant capacity whereas that of *Lansium domesticum* showed the lowest. Of the 42 samples we analyzed, 4 showed extremely high antioxidant activity (TEAC values were higher than 3.0), 16 showed high antioxidant activity (TEAC values were lower than 3.0 but higher than 1.0), 15 showed moderate antioxidant activity (TEAC values were lower than 1.0 but higher than 0.5), and 7 showed low antioxidant activity (TEAC values were lower than 0.5). Among the extremely high antioxidant activity group, the leaves of guava exhibited the highest potential. According to the results from ABTS assay, we could expect that one of the antioxidant mechanisms of guava leaf extract was via free radical scavenging action. In our study, three parts of the guava plant were examined for their antioxidant activity. The results showed that the antioxidant activity of each part is obviously different. Guava leaves showed the extremely highest activity with the TEAC value of 4.908 ± 0.050 mM trolox equivalent/mg extract, whereas the stem and the fruit pulp showed deep lower with the TEAC value of 1.955 ± 0.016 and 0.898 ± 0.008 mM trolox equivalent/mg extract, respectively. Therefore, the dried powder of guava leaves was selected for further investigation.

Table 4.2 Antioxidant activity of ethanolic crude extracts from plants by ABTS assay

Order	Plants Name (Parts)		TEAC (mM)
1	*Psidium guajava*	(Leaves)	4.908 ± 0.050
2	*Punica granatum*	(Peels)	4.066 ± 0.009
3	*Nephelium lappaceum*	(Peels)	3.074 ± 0.003
4	*Garcinia mangostana*	(Peels)	3.001 ± 0.016
5	*Euphoria longan*	(Seeds)	2.585 ± 0.002
6	*Cymbopogon citratus*	(Rhizomes)	2.307 ± 0.012
7	*Psidium guajava*	(Stems)	1.955 ± 0.016
8	*Mentha cordifolia*	(Leaves)	1.844 ± 0.030
9	*Musa sapientum*	(Green peels)	1.795 ± 0.038
10	*Leucaena leucocephala*	(Pods)	1.713 ± 0.033
11	*Thunbergia laurifolia*	(Leaves)	1.663 ± 0.011
12	*Cocos nucifera*	(White peels)	1.530 ± 0.044
13	*Ocimum sanctum*	(Leaves)	1.483 ± 0.030
14	*Piper sarmentosum*	(Leaves)	1.464 ± 0.020
15	*Leucaena leucocephala*	(Leaves)	1.430 ± 0.007
16	*Citrus hystrix*	(Stems)	1.374 ± 0.009
17	*Ocimum gratissimum*	(Leaves)	1.346 ± 0.014
18	*Citrus hystrix*	(Peels)	1.202 ± 0.034
19	*Punica granatum*	(Seeds)	1.184 ± 0.003
20	*Dregea volubilis*	(Leaves and stems)	1.062 ± 0.087
22	*Psidium guajava*	(Fruits)	0.898 ± 0.008

Table 4.2 (continued)

Order	Plants Name (Parts)		TEAC (mM)
23	*Musa sapientum*	(Ripe peels)	0.880 ± 0.048
24	*Ocimum basilicum*	(Leaves)	0.877 ± 0.010
25	*Ocimum sanctum*	(Stems)	0.877 ± 0.004
26	*Hyptis suaveolens*	(Leaves and stems)	0.850 ± 0.022
27	*Ocimum basilicum*	(Stems)	0.783 ± 0.022
28	*Citrus hystrix*	(Leaves)	0.781 ± 0.013
29	*Gymnema inodorum*	(Stems)	0.718 ± 0.027
30	*Hylocereus undatus*	(Peels)	0.685 ± 0.021
31	*Marsdenia glabra*	(Leaves)	0.673 ± 0.044
32	*Andrographis paniculata*	(Stems)	0.648 ± 0.047
33	*Cymbopogon citratus*	(Dried leaves)	0.631 ± 0.057
34	*Passiflora foetida*	(Peels)	0.591 ± 0.023
35	*Oroxylum indicum*	(Pods)	0.506 ± 0.009
36	*Ciausena lansium*	(Leaves)	0.491 ± 0.011
37	*Andrographis paniculata*	(Leaves)	0.397 ± 0.022
38	*Mentha cordifolia*	(Stems)	0.364 ± 0.006
39	*Cymbopogon citratus*	(Dried stems)	0.324 ± 0.014
40	*Cymbopogon citratus*	(Fresh leaves)	0.301 ± 0.016
41	*Cymbopogon citratus*	(Fresh stems)	0.260 ± 0.020
42	*Lansium domesticum*	(Peels)	0.207 ± 0.002

Table 4.3 Antioxidant activity of ethanolic crude extracts from plants by DPPH assay

Order	Plants Name (Parts)		IC_{50} (mg/ml)
1	*Punica granatum*	(Peels)	0.003 ± 0.002
2	*Nephelium lappaceum*	(Peels)	0.006 ± 0.003
3	*Euphoria longan*	(Seeds)	0.010 ± 0.001
4	*Psidium guajava*	(Leaves)	0.013 ± 0.006
5	*Garcinia mangostana*	(Peels)	0.023 ± 0.007
6	*Piper sarmentosum*	(Leaves)	0.031 ± 0.004
7	*Musa sapientum*	(Green peels)	0.031 ± 0.009
8	*Psidium guajava*	(Stems)	0.044 ± 0.008
9	*Cocos nucifera*	(White peels)	0.047 ± 0.005
10	*Mentha cordifolia*	(Leaves)	0.049 ± 0.002
11	*Thunbergia laurifolia*	(Leaves)	0.051 ± 0.001
12	*Hyptis suaveolens*	(Leaves and stems)	0.055 ± 0.010
13	*Leucaena leucocephala*	(Leaves)	0.057 ± 0.019
14	*Leucaena leucocephala*	(Pods)	0.063 ± 0.002
15	*Cymbopogon citratus*	(Fresh leaves)	0.073 ± 0.024
16	*Dregea volubilis*	(Leaves and stems)	0.074 ± 0.010
17	*Ocimum sanctum*	(Leaves)	0.078 ± 0.011
18	*Hylocereus undatus*	(Peels)	0.084 ± 0.036
19	*Cymbopogon citratus*	(Dried leaves)	0.089 ± 0.008
20	*Ocimum sanctum*	(Stems)	0.094 ± 0.018
21	*Psidium guajava*	(Fruits)	0.098 ± 0.012

Table 4.3 (continued)

Order	Plants Name (Parts)		IC_{50} (mg/ml)
22	*Mentha cordifolia*	(Stems)	0.102 ± 0.002
23	*Passiflora foetida*	(Peels)	0.104 ± 0.014
24	*Ocimum gratissimum*	(Leaves)	0.104 ± 0.017
25	*Musa sapientum*	(Ripe peels)	0.105 ± 0.005
26	*Ocimum basilicum*	(Leaves)	0.118 ± 0.013
27	*Andrographis paniculata*	(Leaves)	0.119 ± 0.007
28	*Cymbopogon citratus*	(Rhizomes)	0.120 ± 0.012
29	*Citrus hystrix*	(Leaves)	0.133 ± 0.016
30	*Andrographis paniculata*	(Stems)	0.135 ± 0.015
31	*Gymnema inodorum*	(Leaves)	0.146 ± 0.031
32	*Ciausena lansium*	(Leaves)	0.170 ± 0.026
33	*Ocimum basilicum*	(Stems)	0.177 ± 0.020
34	*Citrus hystrix*	(Stems)	0.185 ± 0.022
35	*Oroxylum indicum*	(Pods)	0.241 ± 0.012
36	*Marsdenia glabra*	(Leaves)	0.251 ± 0.014
37	*Citrus hystrix*	(Peels)	0.256 ± 0.011
38	*Punica granatum*	(Seeds)	0.307 ± 0.013
39	*Gymnema inodorum*	(Stems)	0.349 ± 0.038
40	*Cymbopogon citratus*	(Dried stems)	0.423 ± 0.015
41	*Cymbopogon citratus*	(Fresh stems)	0.439 ± 0.016
42	*Lansium domesticum*	(Peels)	1.291 ± 0.001

The radical scavenging activity on DPPH expressed as IC_{50}. This value was the concentration of the extract required to inhibit 50% DPPH free radical. The IC_{50} of all extracts was shown in Table 4.3. The IC_{50} values ranged from 0.003–1.291 mg/ml. The extract of pomegranate peel showed the highest antioxidant activity with the IC_{50} of 0.003 mg/ml, followed by the peels extracts of *Nephelium lappaceum* and seeds of *Euphoria longan* with the IC_{50} values of 0.006 and 0.010 mg/ml, respectively. The weakest antioxidant activity was obtained from the extract of *Lansium domesticum* peel with the IC_{50} of 1.291 mg/ml.

Membrane lipids are rich in unsaturated fatty acids that are most susceptible to oxidative processes. Specially, linoleic acid and arachidonic acid are targets of lipid peroxidation[72]. The inhibition of lipid peroxidation by antioxidants may be due to their free radical-scavenging activities. Superoxide indirectly initiates lipid peroxidation because superoxide anion acts as a precursor of singlet oxygen and hydroxyl radical[73]. Hydroxyl radicals eliminate hydrogen atoms from the membrane lipids, which results in lipid peroxidation. The inhibitory capacity of plants extracts against the coupled oxidation of β-carotene and linoleic acid was tested. The antioxidant activity of ethanolic extracts exhibited values from 2.70% to 94.44% (Table 4.4). The highest antioxidant activity was obtained with alcoholic leaves extracts of *Mentha cordifolia* as 94.44%. Stem extracts of *Mentha cordifolia* showed the lowest antioxidant activities as 2.70 % AA.

Table 4.4 Anti-β-carotene bleaching activity of ethanolic crude extracts from plants

Order	Plants Name (Parts)		%AA
1	*Mentha cordifolia*	(Leaves)	94.44 ± 0.10
2	*Andrographis paniculata*	(Stems)	93.33 ± 0.07
3	*Ocimum sanctum*	(Leaves)	91.74 ± 0.11
4	*Leucaena leucocephala*	(Pods)	90.82 ± 0.22
5	*Psidium guajava*	(Leaves)	90.00 ± 0.14
6	*Cymbopogon citratus*	(Rhizomes)	88.99 ± 0.10
7	*Citrus hystrix*	(Peels)	88.07 ± 0.22
8	*Ocimum gratissimum*	(Leaves)	87.84 ± 0.16
9	*Garcinia mangostana*	(Peels)	87.84 ± 0.10
10	*Thunbergia laurifolia*	(Leaves)	87.50 ± 0.13
11	*Cymbopogon citratus*	(Dried leaves)	80.73 ± 0.09
12	*Citrus hystrix*	(Stems)	78.90 ± 0.17
13	*Piper sarmentosum*	(Leaves)	78.90 ± 0.21
14	*Gymnema inodorum*	(Leaves)	77.78 ± 0.25
15	*Punica granatum*	(Peels)	75.00 ± 0.21
16	*Gymnema inodorum*	(Stems)	73.33 ± 0.30
17	*Ciausena lansium*	(Leaves)	71.62 ± 0.08
18	*Punica granatum*	(Seeds)	62.22 ± 0.18
19	*Nephelium lappaceum*	(Peels)	62.16 ± 0.22
20	*Psidium guajava*	(Stems)	56.88 ± 0.28

Table 4.4 (continued)

Order	Plants Name (Parts)		%AA
21	*Marsdenia glabra*	(Leaves)	55.56 ± 0.24
22	*Leucaena leucocephala*	(Leaves)	54.55 ± 0.32
23	*Cymbopogon citratus*	(Dried stems)	54.13 ± 0.38
24	*Citrus hystrix*	(Leaves)	50.00 ± 0.19
25	*Dregea volubilis*	(Leaves and stems)	47.78 ± 0.11
26	*Cymbopogon citratus*	(Fresh stems)	47.71 ± 0.22
27	*Cocos nucifera*	(White peels)	45.95 ± 0.21
28	*Hylocereus undatus*	(Peels)	44.44 ± 0.20
29	*Ocimum basilicum*	(Leaves)	41.89 ± 0.19
30	*Euphoria longan*	(Seeds)	37.62 ± 0.16
31	*Hyptis suaveolens*	(Leaves and stems)	33.03 ± 0.18
32	*Passiflora foetida*	(Peels)	32.43 ± 0.22
33	*Psidium guajava*	(Fruits)	27.78 ± 0.22
34	*Ocimum sanctum*	(Stems)	25.69 ± 0.20
35	*Oroxylum indicum*	(Pods)	20.18 ± 0.10
36	*Andrographis paniculata*	(Leaves)	18.89 ± 0.10
37	*Cymbopogon citratus*	(Fresh leaves)	18.35 ± 0.21
38	*Musa sapientum*	(Ripe peels)	9.46 ± 0.14
39	*Lansium domesticum*	(Peels)	8.11 ± 0.17
40	*Musa sapientum*	(Green peels)	4.05 ± 0.11
41	*Ocimum basilicum*	(Stems)	4.05 ± 0.20
42	*Mentha cordifolia*	(Stems)	2.70 ± 0.17

The principle of the FRAP method is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous colored form in the presence of antioxidants. The reducing power property indicates that the antioxidant compounds are electron donors and can reduce the oxidized intermediates of lipid peroxidation process, so that they can act as primary and secondary antioxidants[74]. Table 4.5 showed the reducing power of plants extracts from different species. It was shown that all extracts possessed the reducing power but not on the same level. The result indicated that the peels extract of *Garcinia mangostana* possessed the highest reducing power with the EC value of 5.308 ± 0.129 mM/mg extract. The fresh stems of *Cymbopogon citratus* extracts showed lower activity with the EC values of 0.323 ± 0.009 mM/mg extract. According to its high EC value, it could be considered that compounds were good electron donors and could terminate oxidation chain reaction by reducing the oxidized intermediates into the stable form.

The Caco-2 cell line and PBMC were used for the cytotoxicity test in this study. The Caco-2 cell line is derived from a human colon adenoma and has been used routinely in drug absorption screening, since the Caco-2 monolayer displays several features of the small intestinal epithelial barrier[75]. The cytotoxicity against the Caco-2 cell line could provide the preliminary information for the toxicity on the intestinal cancer cell type and for the selection of appropriate concentrations required in the future permeability study of active components. The potential toxicity of the extract on normal cells was assessed by the cytotoxicity test against human PBMC. Many studies had utilized the PBMC to assess the effects of chemicals or extracts on the proliferation of normal cells[76, 77].

Table 4.5 Antioxidant activity of ethanolic crude extracts by FRAP assay

Order	Plants Name (Parts)		EC (mM)
1	*Garcinia mangostana*	(Peels)	5.308 ± 0.129
2	*Punica granatum*	(Peels)	4.740 ± 0.113
3	*Euphoria longan*	(Seeds)	4.288 ± 0.105
4	*Piper sarmentosum*	(Leaves)	4.194 ± 0.279
5	*Nephelium lappaceum*	(Peels)	4.139 ± 0.100
6	*Cocos nucifera*	(White peels)	3.574 ± 0.085
7	*Mentha cordifolia*	(Stems)	2.860 ± 0.098
8	*Ocimum basilicum*	(Stems)	2.604 ± 0.105
9	*Hyptis suaveolens*	(Leaves and stems)	2.496 ± 0.197
10	*Cymbopogon citratus*	(Dried leaves)	2.282 ± 0.070
11	*Andrographis paniculata*	(Leaves)	2.134 ± 0.043
12	*Lansium domesticum*	(Peels)	1.902 ± 0.096
13	*Ocimum sanctum*	(Leaves)	1.860 ± 0.200
14	*Psidium guajava*	(Leaves)	1.694 ± 0.117
15	*Ciausena lansium*	(Leaves)	1.596 ± 0.113
16	*Psidium guajava*	(Stems)	1.531 ± 0.113
17	*Ocimum gratissimum*	(Leaves)	1.493 ± 0.019
18	*Musa sapientum*	(Ripe peels)	1.477 ± 0.108
19	*Leucaena leucocephala*	(Leaves)	1.197 ± 0.029
20	*Leucaena leucocephala*	(Pods)	1.177 ± 0.025

Table 4.5 (continued)

Order	Plants Name (Parts)		EC (mM)
21	*Mentha cordifolia*	(Leaves)	1.060 ± 0.025
22	*Ocimum sanctum*	(Stems)	1.040 ± 0.041
23	*Musa sapientum*	(Green peels)	1.040 ± 0.108
24	*Citrus hystrix*	(Leaves)	0.967 ± 0.167
25	*Citrus hystrix*	(Peels)	0.936 ± 0.035
26	*Citrus hystrix*	(Stems)	0.872 ± 0.090
27	*Cymbopogon citratus*	(Rhizomes)	0.801 ± 0.070
28	*Marsdenia glabra*	(Leaves)	0.748 ± 0.025
29	*Ocimum basilicum*	(Leaves)	0.720 ± 0.031
30	*Thunbergia laurifolia*	(Leaves)	0.718 ± 0.038
31	*Cymbopogon citratus*	(Fresh leaves)	0.660 ± 0.045
32	*Dregea volubilis*	(Leaves and stems)	0.581 ± 0.004
33	*Punica granatum*	(Seeds)	0.510 ± 0.009
34	*Andrographis paniculata*	(Stems)	0.500 ± 0.006
35	*Psidium guajava*	(Fruits)	0.481 ± 0.050
36	*Oroxylum indicum*	(Pods)	0.477 ± 0.033
37	*Cymbopogon citratus*	(Dried stems)	0.455 ± 0.009
38	*Gymnema inodorum*	(Leaves)	0.427 ± 0.001
39	*Gymnema inodorum*	(Stems)	0.395 ± 0.004
40	*Hylocereus undatus*	(Peels)	0.387 ± 0.010
41	*Passiflora foetida*	(Peels)	0.384 ± 0.021
42	*Cymbopogon citratus*	(Fresh stems)	0.323 ± 0.009

The cytotoxicity against Caco-2 cell line and human PBMC of all tested crude extracts was summarized and shown in Table 4.6. From this table, the inhibition or stimulation to the cells could be observed. The ID_{50} was obtained when the inhibition activity whereas the ED_{50} was obtained when the stimulation activity. The results showed that most of the crude extracts included in this study had no cytotoxic activity against both cell types, except those from *Garcinia mangostana* (mangosteen) and *Punica granatum* (pomegranate) peels. This mangosteen peel extract exhibited potential toxicity against Caco-2 cells and PBMC with the ED_{50} and ID_{50} of 32.0 and 4.9 µg/ml, respectively. This indicated that the extract of mangosteen peel contained potential cytotoxic agent(s). Therefore, further purification to eliminate the toxic agent(s) might be beneficial for products contained the extract of the mangosteen peel. The extract of pomegranate peel showed stimulating activity of cell proliferation in both Caco-2 cells and PBMC with the ED_{50} and ID_{50} of 4.7 and 44.4 µg/ml, respectively. This suggested that the application of this extract as natural antioxidant for food or drug to human should be used with caution when exposed to living cells. Among all of the crude extracts which possessed high antioxidative activity, *Psidium guajava* (guava) leaves extract exhibited the highest value of ID_{50} (> 100 µg/ml) against PBMC cell and no inhibitory with caco-2 cell types indicating the least toxicity. In this study, we found that the ethanol extract from leaves of guava expressed high potential of antioxidant activity with no toxic to normal cells. The leaves of this plant will be promising source for good antioxidative agents. However, investigation of the activity associated with further purification, the cultivated conditions and the active constituents of this plant may provide useful comparative information in the future.

Table 4.6 Cytotoxicity of crude extracts with PBMC and Caco-2 cell (Safety ordering)

Ordering	Plant (Used parts)		ID_{50} (mcg/ml)* (PBMC)	ED_{50} (mcg/ml)* (Caco-2 cell)
1	*Cymbopogon citratus*	(Fresh stems)	> 100	-
2	*Cymbopogon citratus*	(Dried stems)	> 100	-
3	*Psidium guajava*	(Stems)	> 100	-
4	*Andrographis paniculata*	(Stems)	> 100	-
5	*Mentha cordifolia*	(Stems)	> 100	ND
6	*Ocimum basilicum*	(Stems)	> 100	-
7	*Cymbopogon citratus*	(Dried leaves)	> 100	-
8	*Psidium guajava*	(Leaves)	> 100	-
9	*Musa sapientum*	(Green peels)	> 100	-
10	*Musa sapientum*	(Ripe peels)	> 100	-
11	*Hylocereus undatus*	(Peels)	> 100	> 100
12	*Nephelium lappaceum*	(Peels)	> 100	> 100
13	*Cocos nucifera*	(Peels)	> 100	-
14	*Lansium domesticum*	(Peels)	> 100	-
15	*Passiflora foetida*	(Peels)	> 100	-
16	*Psidium guajava*	(Fruits)	> 100	-
17	*Oroxylum indicum*	(Pods)	> 100	-

Table 4.6 (continued)

Ordering	Plant (Used parts)		ID_{50} (mcg/ml)* (PBMC)	ED_{50} (mcg/ml)* (Caco-2 cell)
18	*Punica granatum*	(Seeds)	> 100	> 100
19	*Hyptis suaveolens*	(Leaves and stems)	> 100	> 100
20	*Cymbopogon citratus*	(Rhizomes)	> 100	-
21	*Gymnema inodorum*	(Stems)	> 100	> 100
22	*Leucaena leucocephala*	(Leaves)	100	-
23	*Euphoria longan*	(Seeds)	100	-
24	*Marsdenia glabra*	(Leaves)	100	> 100
25	*Leucaena leucocephala*	(Pods)	80	> 100
26	*Ocimum basilicum*	(Leaves)	55	-
27	*Citrus hystrix*	(Stems)	51	-
28	*Ocimum sanctum*	(Leaves)	50	-
29	*Citrus hystrix*	(Peels)	47.5	-
30	*Punica granatum*	(Peels)	44.4	4.7
31	*Ocimum sanctum*	(Stems)	40	-
32	*Ocimum gratissimum*	(Leaves)	38.5	-
33	*Dregea volubilis*	(Leaves and stems)	37.5	> 100
34	*Gymnema inodorum*	(Leaves)	36.8	-
35	*Piper sarmentosum*	(Leaves)	25	> 100
36	*Cymbopogon citratus*	(Fresh leaves)	25	-
37	*Clausena lansium*	(Leaves)	25	-

Table 4.6 (continued)

Ordering	Plant (Used parts)		ID_{50} (mcg/ml)* (PBMC)	ED_{50} (mcg/ml)* (Caco-2 cell)
38	*Mentha cordifolia*	(Leaves)	21.9	-
39	*Citrus hystrix*	(Leaves)	12.5	-
40	*Andrographis paniculata*	(Leaves)	8.6	> 100
41	*Thunbergia laurifolia*	(Leaves)	5.3	-
42	*Garcinia mangostana*	(Peels)	4.9	32
43	Standard Tamoxifen		ND	<5
44	Standard vinblastine sulfate		ND	> 100

*Note: - = no inhibition

ND = not determined

4.3 Antioxidant activity of guava leaves extracts

Qian and Nihorimbere (2004)[78] reported that the extracts of guava leaves of aqueous ethanol 50% (1:1) ratio showed a much higher antioxidant activity than that of water. This suggested that the polarity of the active components in guava leaves was lower than water. As the polarity of methanol was slightly lower than water, this was considered as one of the most suitable solvents for this extraction. On the other hand, the antioxidant activity of the guava leaves extracts from other lower polarity solvents such as *n*-hexane, ethyl acetate and *n*-butanol has not yet been reported elsewhere. Therefore, in this experiment the antioxidant activity of guava leaves was further studied by using several kinds of solvents for maceration from higher to lower

non-polar; *n*-hexane, ethyl acetate, *n*-butanol, and methanol, respectively. The extracts from each solvent were subjected to ABTS, DPPH, β-carotene bleaching model and FRAP assays. These methods represented different mechanisms of antioxidant action. A sample possessed ABTS or DPPH free radical scavenging property indicated that its mechanism of action was hydrogen donor and termination the oxidation process by converting free radicals to more stable products. Hydroxyl radicals eliminate hydrogen atoms from the membrane lipids, which results in lipid peroxidation. The inhibitory capacity of plants extracts against the coupled oxidation of β-carotene and linoleic acid was tested. Whereas a compound exhibited positive result in FRAP assay indicated its mechanism of action was electron donor and termination the oxidation chain reaction by reducing the oxidized intermediates into the stable form. The percentage yield of guava leaves crude extracts in different solvents was ranged from 1.49 – 18.94 %. The methanol extraction gave the highest percentage yield (18.94%) followed by hexane and ethyl acetate extracts, respectively (1.87, 1.74%) whereas the butanol extraction gave the lowest (1.49%). The antioxidant activities from guava leaves by four methods were shown in Table 4.7.

Table 4.7 Comparison of antioxidant activity of guava leaves fractions by DPPH, ABTS, β-carotene bleaching model and FRAP assays

Solvent extraction	IC_{50} (mg/ml)	TEAC (mM)	%AA	EC (mM)
Methanol	0.002 ± 0.001	3.785 ± 0.003	64.67 ± 0.095	3.647 ± 0.038
n-Butanol	0.056 ± 0.004	2.901 ± 0.023	24.21 ± 0.101	1.358 ± 0.032
Ethyl acetate	0.022 ± 0.001	2.647 ± 0.065	81.46 ± 0.153	1.146 ± 0.132
Hexane	0.121 ± 0.027	1.061 ± 0.017	87.74 ± 0.141	0.713 ± 0.003

4.3.1 ABTS scavenging activity

The TEAC values of guava leaves extracted from different solvents were shown in Figure 4.1. It was observed that all extracts possessed free radical scavenging activity but on different levels. The highest activity was obtained from the methanol extract with the TEAC value of 3.785 ± 0.003 mM trolox equivalent/mg extract, followed by the butanol extract and ethyl acetate extract with the TEAC values of 2.901 ± 0.023 and 2.647 ± 0.065 mM trolox equivalent/mg extract, respectively. The activity of the later two fractions was above 70 % of the methanol extract. The hexane extract exhibited the lowest scavenging action with the TEAC value of 1.061 ± 0.017 mM trolox equivalent/mg extract which was lower than 30 % of the methanol extract. It was observed that the antioxidant activity of the methanol extract was higher than that of the two positive controls, BHT and QCT, whereas the other two high activity fractions showed a little lower value. It was considered that methanol fraction of guava leaves was a good source for potent natural antioxidant activity. Regarding to its high TEAC value, it indicated that the mechanism of antioxidant action of this fraction was hydrogen donor and could terminate the oxidation process by converting free radicals to the stable forms.

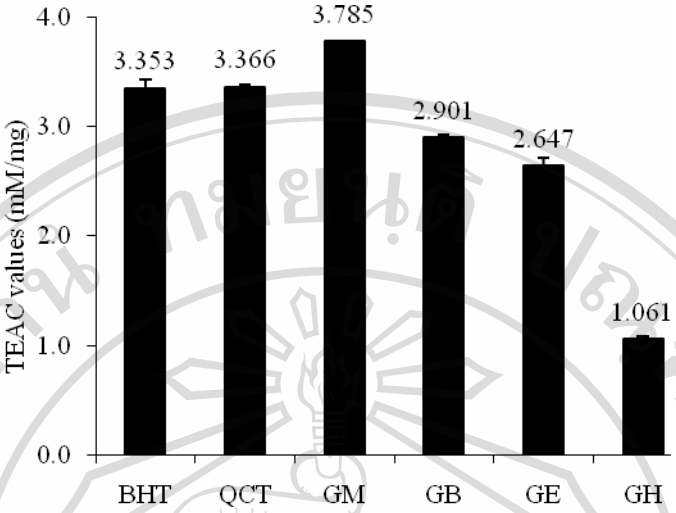

Figure 4.1 Free radical scavenging activity of guava leaves extracts from methanol (GM), butanol (GB), ethyl acetate (GE), and hexane (GH) in comparison with that of butylated hydroxyl toluene (BHT) and quercetin (QCT)

4.3.2 FRAP reducing power

Figure 4.2 showed the reducing power of guava leaves extracts from different solvents. It was shown that all extracts possessed the reducing power but not on the same level. The result clearly indicated that the methanol extract of guava leaves possessed the highest reducing power with the EC value of 3.647 ± 0.038 mM/mg extract. It was much higher than that of BHT but slightly lower than QCT. The ethyl acetate and butanol extracts showed lower activity with the EC values of 1.146 ± 0.132 and 1.358 ± 0.032 mM/mg extract respectively, which was about 31-37 % of methanol extract. The lowest reducing property was obtained from hexane fraction. From this point of view, it was confirmed that the methanol fraction of guava leaves possessed the potent antioxidant compounds. According to its high EC value, it could

be considered that compounds in methanol fraction were good electron donors and could terminate oxidation chain reaction by reducing the oxidized intermediates into the stable form.

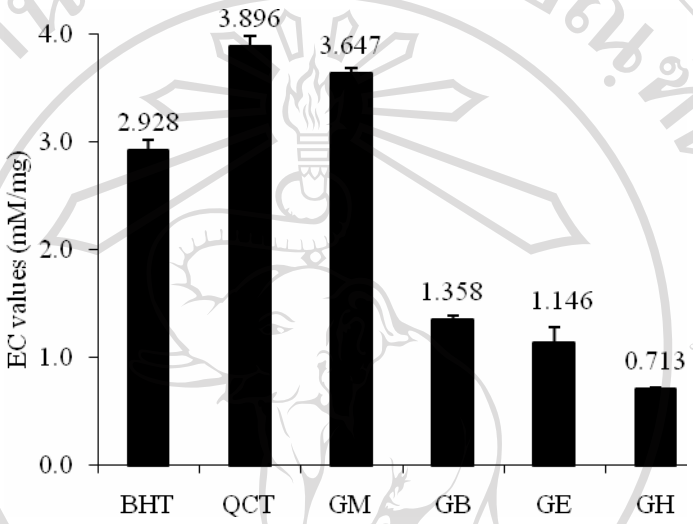

Figure 4.2 Reducing powers of guava leaves extracts from methanol (GM), butanol (GB), ethyl acetate (GE), and hexane (GH) in comparison with that of butylated hydroxyl toluene (BHT) and quercetin (QCT)

4.3.3 DPPH scavenging activity

The IC_{50} values of guava leaves extracted from different solvents were shown in Figure 4.3. It was observed that all extracts possessed free radical scavenging activity but on different levels. The highest activity was obtained from the methanol extract with the IC_{50} value of 0.002 ± 0.001 mg/ml, followed by the ethyl acetate extract and butanol extract with the IC_{50} values of 0.022 ± 0.001 and 0.056 ± 0.004 mg/ml, respectively. The hexane extract exhibited the lowest scavenging action with

the IC_{50} value of 0.121 ± 0.027 mg/ml. It was observed that the antioxidant activity of the methanol extract was higher than that of the two positive controls, BHT and QCT, whereas the other two high activity fractions were a little lower. It was considered that methanol fraction of guava leaves was a good source for potent natural antioxidant activity. Regarding its high TEAC value, it indicated that the mechanism of antioxidant action of this fraction was hydrogen donor and could terminate the oxidation process by converting free radicals to the stable forms.

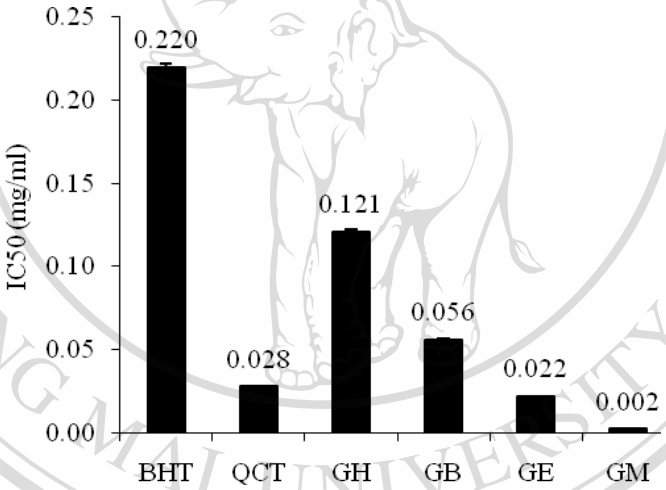

Figure 4.3 The IC_{50} of guava leaves extracts from methanol (GM), butanol (GB), ethyl acetate (GE), and hexane (GH) in comparison with that of butylated hydroxyl toluene (BHT) and quercetin (QCT)

4.3.4 β-Carotene bleaching model

The antioxidant activity of hexane extract exhibited the highest activity with the %AA value of 87.74% (Figure 4.4). The highest antioxidant activity was obtained with non-polar fraction from guava leaves extracts because the chemical compounds

in non-polar fractions could possibly inhibited lipid peroxidation better than polar fractions. Moreover, all of fraction from guava leaves showed activity in lipid peroxidation higher than BHT and QCT.

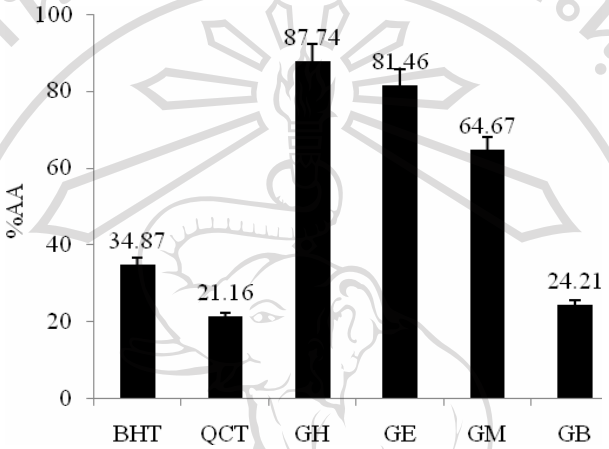

Figure 4.4 The percentage of antioxidant activity of guava leaves extracts from methanol (GM), butanol (GB), ethyl acetate (GE), and hexane (GH) in comparison with that of butylated hydroxyl toluene (BHT) and quercetin (QCT)

However, %AA of hexane fraction showed the highest activity than ethyl acetate, butanol and methanol in beta-carotene bleaching model but in ABTS, DPPH and FRAP tests the methanol fraction showed the highest activity. So, the methanol fraction from guava leaves was selected for further study.

4.3.5 Total phenolic content

The total phenolic content of guava leaves fractions was reported as gallic acid equivalent concentration (mg/ml). The results showed that guava leaves fractions contained a mixture of phenolic compounds in different levels according to the polarity of solvent used in the extraction process, in the following order; methanol > butanol > ethyl acetate > hexane as shown in Figure 4.5.

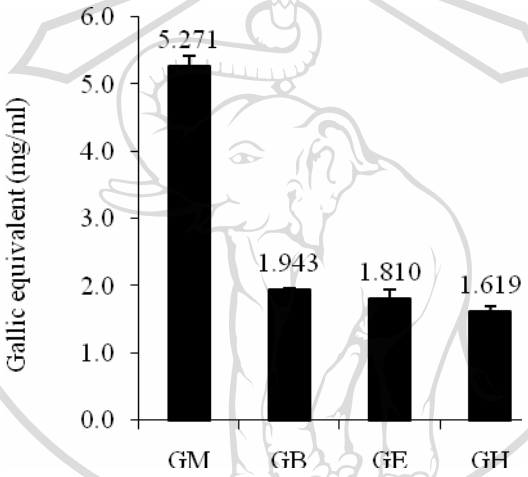

Figure 4.5 Total phenolic content of guava leaves extracts from methanol (GM), butanol (GB), ethyl acetate (GE), and hexane (GH)

From antioxidant and total phenolic content studied in this work, we checked the chemical compound in all fraction from guava leaves by chemical test. The results are showed in Table 4.8. First, from phenolic and tannin test, it was found that the methanol fraction showed positive with gelatin and ferric chloride. It means methanol fraction contained tannin. The other fraction showed negative results in this test. Second, the results of flavonoids test demonstrated that. The methanol fraction may possibly contained flavanonol, flavonol, flavone or leucocyanidin compounds.

Table 4.8 Chemical test results of guava leaf extracts from methanol, butanol, ethyl acetate, and hexane

1. Phenolic and tannin data

Solvent extraction	Gelatin test	Ferric chloride test	Bromine water test	Lime water test	Vanillin-HCl test	Interpretation
Methanol	+	+	-	-	-	Tannin
n-Butanol	-	-	-	-	-	
Ethyl acetate	-	-	-	-	-	
Hexane	-	-	-	-	-	

Note : - = negative

\+ = positive

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2. Flavonoids data

Solvent extraction	Cyanidin test	Pew test	Base reaction test	Anthocyanins test	Leucoanthocyanidin test	Interpretation
Methanol	Orange	Red	Orange-brown	No reaction	Red	Flavanonol, Flavonol, Flavone, Leucocyanidin
n-Butanol	Yellow	Red	Yellow	No reaction	Yellow	Flavonol, Flavone
Ethyl acetate	Green	Yellow	Yellow	No reaction	Green	Flavonol, Flavone
Hexane	Yellow	Yellow	Colorless	No reaction	Green	Flavanone

The aim of this study was to identify the active principles of guava leaves and to evaluate the antioxidant potential in comparatively among them. The most potent crude extract of guava leaves was undertaken for purification along with biological study. The antioxidant power assay by DPPH was employed to each fraction in order to make a systematic comparison among their antioxidant activity (Table 4.9). Only the top three compounds that showed the highest antioxidant activity to DPPH assay were selected for chemical identification by means of spectroscopic analysis. Their antioxidant potential was confirmed with the other two antioxidant assays, free radical scavenging ABTS and reducing power FRAP methods. The antioxidant values; IC_{50}, TEAC, and EC from DPPH, ABTS, and FRAP assays respectively of the active principles were comparatively evaluated.

Table 4.9 The yield and antioxidant activity (IC_{50}) of fractions from column chromatography

Fraction	Yield (g)	IC_{50} (µg/ml)
A	4.997	31.0
B	0.995	5.8
C	1.529	4.2
D	2.144	4.9
E	1.133	5.3
F	4.028	12.9

4.4 Isolation of antioxidant compounds

Solvent extraction, followed by column chromatography, of dry powder of guava leaves, yielded a potential antioxidant compound. The crude extracts obtained from n-hexane fraction, EtOAc fraction, and n-butanol fraction, yield of which is negligible (1.88, 1.74 and 1.49 %, respectively). The percent yielded of MeOH fraction was the highest (18.94 %) which probably due to the solvent could extract both low and high molecular weight phenolic compounds[79]. Table 4.7 summarized the extraction yield of different solvents and their antioxidant activity. Antioxidant activity of methanol extract was higher than the other extracts. Hence, the methanol extract was selected for further isolation of antioxidant compound. Each fraction from column chromatography was subjected to antioxidant activity DPPH guided repeated fractionation. Results revealed that three active compounds, Compound 1 (14.9 mg), Compound 2 (33.7 mg), and Compound 3 (4.0 mg) could be isolated from the chromatographic active fractions C (1.5 g), Fraction E (1.2 g), and fraction D (2.2 g), respectively. The three isolated compounds were of similar appearance as pale yellow fine powder.

4.5 Identification of bioactive compounds

The structures of the isolated bioactive compounds were determined by various spectroscopic techniques; ^{13}C-NMR, ^{1}H-NMR, MS analyses in comparison with the data of authentic quercetin[63], quercetin-3-*O*-glucopyranoside[64] and morin[65].

Compound **1** appeared as a pale yellow powder, mp 300°C (decomposed) and EI-MS m/z: 302 $[M]^+$, showed the IR absorption band at 3293.82, 1616.06, 1511.92 and 1166.72 cm^{-1} which were consistent with the presence of hydroxyl, carbonyl, aromatic

ring and ether groups respectively. The ^{1}H-NMR (400MHz, CD_3OD) and ^{13}C-NMR were shown in Table 4.10. It was considered to be quercetin.

Compound **2** appeared as a yellow powder, mp 220-225 °C and EI-MS m/z: 464 $[M]^+$, showed the IR absorption bands at 3739.30, 1648.84, 1562.06, 1492.63, 1295.93, 1054.87, and 622.89 cm^{-1} which were consistent with the presence of hydroxyl, carbonyl, aromatic ring and ether groups respectively. The ^{1}H-NMR (600MHz, CD_3OD) and ^{13}C-NMR (150MHz, CD_3OD) were shown in Table 4.11. It was considered to be quercetin-3-*O*-glucopyranoside.

Compound **3** appeared as a yellow powder, mp 300°C (decomposed) and EI-MS m/z: 302 $[M]^+$, showed the IR absorption bands at 3484.74, 1604.48, 1526.31, 1052.94cm^{-1} which were consistent with the presence of hydroxyl, carbonyl, aromatic ring and ether groups respectively. The ^{1}H-NMR (400MHz, CD_3OD) and ^{13}C-NMR (100MHz, CD_3OD) were shown in Table 4.12. It was considered to be morin.

Table 4.10 ^{13}C and ^{1}H NMR data of Compound 1 and authentic quercetin[63]

Attribution	Authentic quercetin (δ in ppm)		Compound 1 (δ in ppm)	
	^{13}C	^{1}H	^{13}C	^{1}H
2	146.9		148.9	
3	135.5		137.3	
4	175.8		177.5	
5	160.7		162.6	
6	98.2	6.16 s	99.4	6.17 s
7	163.9		165.7	
8	93.3	6.36 s	94.5	6.37 s
9	156.2		158.4	
10	103.1		104.6	
1′	122.1		124.3	
2′	115.3	7.72 s	116.1	7.72 s
3′	145.0		146.3	
4′	147.6		148.1	
5′	115.6	6.87 d (J=8.5)	116.3	6.86 d (J=8.4)
6′	120.0	7.62 d (J=8.5)	121.8	7.62 d (J=8.6)

Table 4.11 ^{13}C and ^{1}H NMR data of Compound 2 and authentic quercetin-3-*O*-glucopyranoside[64]

Attribution	Authentic quercetin-3-*O*-glucopyranoside (δ in ppm)		Compound 2 (δ in ppm)	
	^{13}C	^{1}H	^{13}C	^{1}H
2	156.5		156.4	
3	133.7		134.4	
4	177.6		177.7	
5	161.3		161.4	
6	98.8	6.20 d (J=2.2)	99.8	6.13 d (J=2.0)
7	164.2		165.5	
8	93.6	6.39 d (J=2.2)	94.2	6.30 d (J=2.0)
9	156.5		157.4	
10	104.2		104.3	

Table 4.11 (continued)

Attribution	Authentic quercetin-3-*O*-glucopyranoside (δ in ppm)		Compound 2 (δ in ppm)	
	^{13}C	^{1}H	^{13}C	^{1}H
1′	121.4		121.7	
2′	115.3	7.83 s	116.3	7.83 d (J=2.2)
3′	144.8		144.5	
4′	148.5		148.6	
5′	116.5	6.85 d (J=8.5)	114.7	6.86 d (J=8.5)
6′	121.6	7.58 dd (J=8.5)	121.5	7.58 dd (J=8.5)
1″	101.4	5.16 d (J=7.8)	103.1	5.09 d (J=7.7)
2″	74.3	3.63 m	73.8	3.55 m
3″	76.8	3.56 m	75.8	3.48 m
4″	70.3	3.84 m	68.6	3.85 m
5″	77.5	3.46 dt (J=6.4)	77.0	3.42 t (J=8.7)
6″	61.3	3.53 m	60.5	3.54 m
				3.64 d

Table 4.12 ^{13}C and ^{1}H NMR data of Compound 3 and authentic morin[65]

Attribution	Authentic morin (δ in ppm)		Compound 3 (δ in ppm)	
	^{13}C	^{1}H	^{13}C	^{1}H
2	157.4		157.0	
3	134.5		132.1	
4	178.7		177.9	
5	157.8		161.8	
6	98.5	6.20 s (J=2.0)	98.5	6.19 s (J=2.0)
7	162.1		164.7	
8	93.7	6.39 s (J=2.0)	93.4	6.39 s (J=2.0)
9	165.0		158.0	
10	104.9		104.4	
1′	122.0		121.9	
2′	145.1		144.7	
3′	116.2	7.20 s	115.8	7.68 s
4′	149.0		148.7	
5′	114.5	6.85 d (J=8.0)	114.9	6.87 d (J=8.4)
6′	122.3	7.58 d (J=8.0)	121.7	7.63 d (J=8.4)

4.6 Antioxidant activity of the isolated compounds

The antioxidant activity can be evaluated with different methods depended on the mechanism of action. The mechanism of antioxidant action might be free radical scavenging or reducing the oxidized intermediated compounds or chelating the oxidative catalytic metal ions. In this study, we used the DPPH free radical scavenging method to screen the antioxidant properties of the crude extracts in order to select the best three potent fractions and finally for the isolated pure compounds. Results for the three isolated pure compounds indicated that Compound 1 (quercetin) was the most active compound with the IC_{50} value of 1.20 ± 0.02 µg/ml as shown in Figure 4.6, followed by compound 2 (quercetin-3-*O*-glucopyranoside) and 3 (morin) with the IC_{50} values of 3.48 ± 0.05 and 5.41 ± 0.20 µg/ml, respectively.

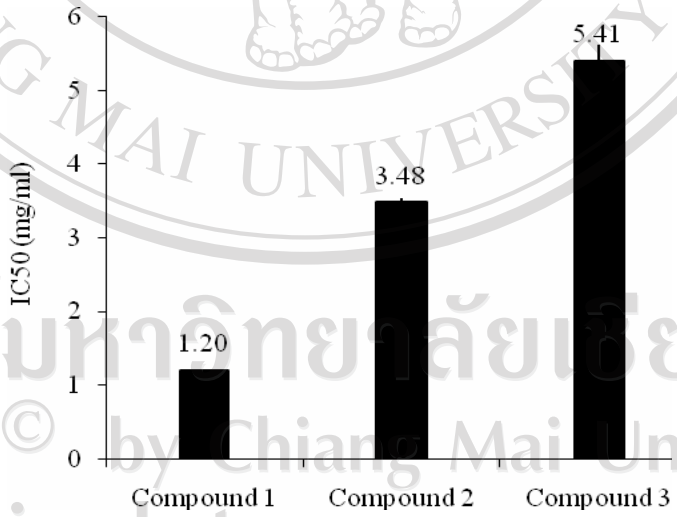

Figure 4.6 Antioxidant activity of isolated and purified compounds by DPPH method

The ABTS method was used to confirm the free radical potential of the three isolated bioactive compounds. The results were shown in Figure 4.7. It was confirmed that the Compound 1 possessed excellent free radical scavenging activity with the TEAC value of 57.54 ± 0.07 mM/mg, followed by Compounds 2 and 3 with the TEAC values of 32.35 ± 0.12 and 14.59 ± 0.62 mM/mg, respectively. It was noticed that the TEAC value of Compound 1 was significantly higher than the other two compounds. The DPPH and ABTS assays represent the antioxidant activity main mechanism is free radical scavenging action whereas FRAP method reveals as reducing power to the oxidized intermediate species.

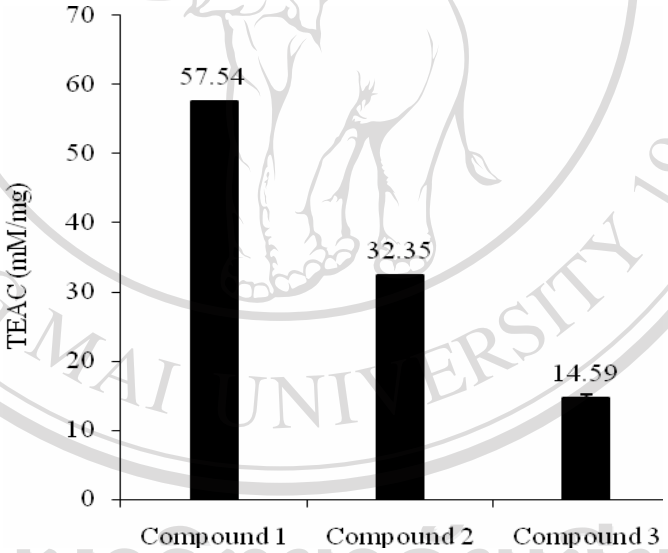

Figure 4.7 Antioxidant activity of isolated and purified compounds by ABTS method

The reducing power property indicates that the antioxidant compounds are electron donors and can reduce the oxidized intermediates of the lipid peroxidation process, so they can act as primary and secondary antioxidants. The reducing power of Compounds 1, 2, and 3 is shown in Figure 4.8. It exhibited that all compounds had reducing power but not at the same level. The result clearly indicated that Compound 1 possessed the highest reducing power with the EC value of 72.69 ± 1.06 mM/mg. This value was significantly higher than that of Compounds 2 and 3 which showed the EC values of 42.51 ± 1.08 and 8.56 ± 0.33 mM/mg, respectively. From this point of view, it was confirmed that the Compound 1 was the major principle that possessed the most potent antioxidant activity. According to its high EC value, it could be considered that this compound was good electron donors and could terminate oxidation chain reactions by reducing the oxidized intermediates into the stable form. Compounds 2 and 3 also had the antioxidant power but much lower than Compound 1.

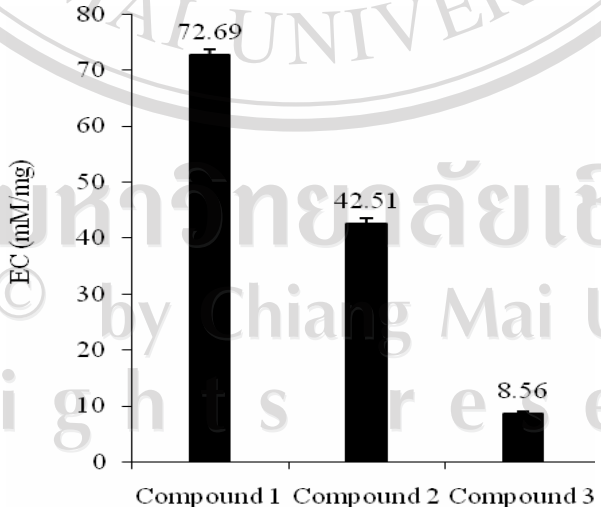

Figure 4.8 Antioxidant activity of isolated and purified compounds by FRAP method

Bors et al., 1990[80] have proposed that three structural determinants should be responsible for effective radical scavenging by flavonoids: 1) the ortho-dihydroxy or catechol group in the B-ring, which confers a high stability to the radical formed; 2) the conjugation of the B-ring to the 4-oxo group via the 2, 3-double bond, which ensures the electron delocalization from the B-ring and 3) the 3- and 5-OH groups with the 4-oxo group, which allows electron delocalization from the 4-oxo group to both substituents. The combination of all of these structural features enables a higher electron delocalization conferring, therefore, a higher stability to aroxyl radicals. The ortho-catechol group confers a high stability to the resulting radical since when the OH bond is broken a strong H-bond is formed between the radical and the other OH group, which stabilizes the radical and decreases the O–H bond dissociation enthalpy. The flavonoids quercetin in this study was expected to be the most efficient flavonoid antioxidant which mention above reason. The glycosylation of flavonoids reduces their activity when compared to corresponding aglycone. Blocking the 3-hydroxyl group in the C ring of quercetin as in quercetin-3-*O*-glucopyranoside decreases the antioxidant activity. Thus, the maximum effectiveness for radical scavenging apparently requires the 3-OH group attached to the 2, 3-double bond and adjacent to the 4-carbonyl in the C ring. The importance of the adjacence of the two hydroxyl groups in the ortho-diphenolic arrangement in the B ring of quercetin to high antioxidant activity was revealed from a study of morin in which the dihydroxy groups are arranged meta in the B ring (no catechol structure), decreasing the antioxidant activity also presents a lower activity than quercetin and and quercetin-3-*O*-glucopyranoside, respectively.