CHAPTER 4

Antioxidant Activity of Medicinal Plant Extracts

4.1 Introduction

Free radicals are a chemical compounds which contain an unpaired electron spinning on the peripheral layer around the nucleus. Free radical can generate by normal cellular metabolism such as inflammation, the respiratory burst and many exogenous factors or environmental interaction including UV, stress, pesticides, environmental pollutants, smoking and radiation. Oxidative stress may be produced by pathological process which caused of the oxidation of bimolecular such as DNA, proteins, carbohydrates, and lipids leading to cell death, cellular compound destruction, DNA breaks, mutagenesis, protein inactivation and membrane disruption (Halliwell *et al.,* 1992; Pradhan *et al.,* 2004). Moreover, the effects of free radical are reported to be associated with pathological process such as cardiovascular diseases, cancer, diabetes, Parkinson's disease, Alzheimer's disease, cancerogenesis and the aging process (Bagchi *et al.,* 2000). The protein oxidation results in a deleterious loss of protein function and loss of critical enzymatic activity or protein signaling (Dean *et al.,* 1997). However, antioxidant supplement can help human body to reduce oxidative damage by free radical and oxygen species. Several studies have indicated that Thai medicinal plants contain a wide variety of natural antioxidant such as phenolic acids, flavonoids and tannins. The numbers of methods and variations in methods to measure antioxidant in botanicals were compared. The antioxidant capacities are influenced by many factors. Single method does not accurately reflect all of the radical sources or all antioxidants in a mixed or complex system. Therefore, it is necessary to perform more than one type of antioxidant capacity measurement to determine various mechanism of action of antioxidant in plant extracts (Schlesier *et al.,* 2002).

4.2 Materials and methods

4.2.1 Antioxidant activity

1) ABTS radical anion scavenging assay

The ABTS radical scavenging assay was performed according to the method of Re *et al.* (1999) with some modification. Briefly, the ABTS radical was generated by oxidation of ABTS with potassium persulfate ($K_2S_2O_8$). ABTS (7mM), 10 ml was mixed with potassium persulfate (2.45 mM), 176 µl and kept in dark condition at room temperature for 12-16 hours before use. ABTS working solution was diluted with 95% ethanol to obtain absorbance of 0.700 ± 0.020 at 734 nm. Then, twenty milliliter of crude extract was mixed with ABTS working solution and left in room temperature for 6 minutes. The absorbance was measured at 734 nm using 95% ethanol for standard blank. The percentage of free radical inhibition by extract was calculated by the following equation:

$$\% \text{ Inhibition} = [(A_{734\text{control}} - A_{734\text{test sample}})/A_{734\text{control}}] \times 100$$

The antioxidant activity of extracts was expressed as trolox equivalent antioxidant capacity (TEAC), which was calculated from standard curve of trolox.

$$\text{Antioxidant activity (mg TE/g extract)} = \frac{IC_{50} \text{ trolox (mg/ml)}}{IC_{50} \text{ plant extracts (mg/ml)}} \text{ X1000}$$

2) DPPH radical scavenging

The DPPH radical scavenging assay was conducted according to the modified method (Brand-Williams *et al*., 1995; Ho *et al.*, 2010). The extracts were dissolved with methanol to prepare various concentrations. Each concentration (0.5 ml) was incubated with 1.5 ml of 0.1 mM methanolic solution of DPPH in the dark at room temperature for 20 minutes. The absorbance was determined at 517 nm. Methanol was used as a blank solution, and DPPH without extract was used as a control. The percentage of free radical inhibition by the extract was calculated by the following equation:

$$\% \text{ Inhibition} = [(A_{517\text{control}} - A_{517\text{test sample}})/A_{517\text{control}}] \times 100$$

The antioxidant activity of the extracts was expressed as gallic acid equivalent (GAE) antioxidant capacity, which was determined from standard curve of gallic acid.

$$\text{Antioxidant activity (mg GAE/g extract)} = \frac{IC_{50} \text{ gallic acid (mg/ml)}}{IC_{50} \text{ plants extract (mg/ml)}} \text{ X1000}$$

3) Ferric reducing assay (FRAP)

The reducing power was determined using a ferric reducing antioxidant power (FRAP) assay described by Benzie and Strain (1996) with some modification. Briefly, the extracts were dissolved in ethanol to a concentration of 1 mg/ml. Then, an aliquot of 500 µl of extract in ethanol was mixed with FRAP reagent (1.5 ml). The mixture was left in the dark for 15 minutes and then the absorption was measured at 593 nm. The reducing power was expressed as equivalent concentration (EC) using $FeCl_3$ as a standard curve.

4) Total phenolic contents

The total phenolic contents were determined by Folin- Ciocalteau method (Chandler and Dodds, 1983). Briefly, 0.25 ml of extract (1 mg/ml) was mixed with 1.25 ml of water. Then, 0.25 ml of 95% ethanol and 0.125 ml of 50% Folin-Ciocalteau were added and mixed thoroughly. The mixture was incubated for 5 minutes. After that, 0.25 ml of 5% Na_2CO_3 was added and incubated for 1 hour. The absorbance was measured at 725 nm. The standard curve was prepared by 10-100 µg/ml of gallic acid solutions. The concentration of phenolic compounds of extract was calculated from standard curve and expressed as gallic acid equivalent antioxidant capacity (GAE). Moreover, Pearson's correlation test was used to assess correlation between means. P-values<0.05 were regarded as significant.

4.2.2 Inhibition of oxidative protein damage by medicinal plant extracts (Modified from Mayo *et al.*, 2003; Tit-oon and Chotpadiwetkul, 2004)

1) Metal-catalyzed oxidation of BSA

Hydroxyl radical mediated oxidation was carried out using a metal-catalyzed reaction. BSA (Fraction V, 98% electrophoresis grade) was dissolved in 150 mM phosphate buffer (pH=7.3) to the final concentration of 5 mg/ml. BSA was incubated with or without 1 mM $Cu^{2+}$and 25 mM H_2O_2 in the presence or absence of crude plant extracts (5, 10, 15, 20 and 25 mg/ml) as shown in Table 4.1. The reaction was performed in 37°C for 30 minutes. DMSO was used as vehicle control and glutathione was served as a positive control.

Table 4.1 Amount of sample used in oxidative protein damage model

Test	**Volume (µl)**						
	BSA	**H_2O_2**	**Cu^{2+}**	**DMSO**	**GSH**	**Plant extracts**	**ddH_2O**
blank	10	-	-	-	-	-	24
control	10	4	4	-	-	-	16
DMSO control	10	4	4	16	-	-	-
positive control	10	4	4	-	16	-	-
test	10	4	4	-	-	16	-

2) SDS – Polyacrylamide gel electrophoresis (SDS-PAGE)

After treatment, the samples were mixed with loading dye (20% glycerol, 8% SDS, 0.125 M Tris HCl (pH=6.8), 0.02 % bromophenol blue, 0.5% 2- mercaptoethanol) and heated at 100 °C for 4 minutes. Protein samples were subjected to 12% SDS-polyacrylamide gel electrophoresis using the Bio-Rad Mini-PROTEIN. After 1 hour, the gels were stained with 0.025% coomassie brilliant blue R250 for 1 hour and destained until the background was clear. The gels were scan with scanner and measured the band intensity with Gene tool program.

4.2.3 Preparation of crude plant extracts

E. prostrata and *Hiptage* sp. powder (2.5 kg) were separately macerated using ethanol as a solvent with the ratio of 1:4 (w/v) for 72 hours at room temperature with frequent agitation. The ethanol extracts of each was concentrated to dryness under reduced pressure at 45ºC using rotary evaporator.

1) Isolation of *E. prostrata*

For preliminary screening, the crude ethanolic extract *E. prostrata* was separated using partition technique. Four kinds of solvents, *n*- hexane, ethyl acetate, *n*-butanol and water, were selected to separate their chemical constituents based on their polarity. The crude ethanolic extract (25g) was initially dissolved in 56 ml of water and then partitioned with 50 ml of *n*-hexane four times to obtain the *n*-hexane extract. After that, the water layer was partitioned with 50 ml of ethyl acetate four times to obtain ethyl acetate extract. Next, the water layer was partitioned with 50 ml of *n*-butanol four times to obtain *n*-butanol extract. Supernatants were collected and evaporated to obtain each extract from *n*-hexane, ethyl acetate, *n*-butanol and aqueous fractions. The extracts were subsequently tested for their antioxidant activity.

Moreover, the ethanolic extract (100 g) was also isolated by celite column chromatography. The crude extract was mixed with celite® 545 (Fluka, Switzerland) thoroughly. After that, celite: crude extract mixture was dried packed into glass column (7 cm diameter) to 12 cm high and then eluted with *n*-hexane, *n*-hexane: ethyl acetate, 95% ethanol, respectively. The eluted samples were collected in 12 fractions, dried under evaporator and then antioxidant activity was tested. The fraction with high antioxidant activity was further separated using column chromatography on siliga gel (Merk Ltd., Lutterworth, UK) using gradient solvent system of chloroform: acetone: methanol (90: 5: 5), chloroform: acetone: methanol (85: 5: 10), chloroform: ethanol (50: 50) and finally eluted with ethanol. Fractions were collected and combined on the basis of their TLC results after visualization with ultraviolet light (254 and 366 nm) and anisaldehyde sulfuric acid spraying reagent. The eluted samples were collected in 16 fractions, dried under evaporator and then antioxidant activity was tested. The sample with high antioxidant activity was further determined

phytochemical constituents. The sample with high antioxidant activity was further determined phytochemical constituents.

2) Isolation of *Hiptage* sp.

For preliminary screening, the crude ethanolic extract *Hiptage* sp. was isolated using partition method. Four kinds of solvents, *n*- hexane, ethyl acetate, *n*-butanol and water, were selected to separate their chemical constituents based on their polarity. The crude ethanolic extract (25g) was initially dissolved in 56 ml of water and then partitioned with 50 ml of *n*-hexane four times to obtain the *n*-hexane extract. After that, the water layer was partitioned with 50 ml of ethyl acetate four times to obtain ethyl acetate extract. Next, the water layer was partitioned with 50 ml of *n*-butanol four times to obtain *n*-butanol extract. Supernatant was collected and evaporated to obtain each extract from *n*-hexane, ethyl acetate, *n*-butanol and aqueous fractions. The extracts were subsequently tested for their antioxidant activity.

Furthermore, the ethanolic extract of *Hiptage* sp. (150 g) was isolated by celite column chromatography. The crude extract was mixed with celite® 545 (Fluka, Switzerland) thoroughly. After that, celite: crude extract mixture was dried packed into glass column (7 cm diameter) to 12 cm high and then eluted with hexane: ethyl acetate (50: 50), ethyl acetate, ethyl acetate: ethanol (50: 50) and ethanol, respectively. The eluted samples were collected in 8 fractions, dried under evaporator and then antioxidant activity was tested. The fraction with high antioxidant activity was further separated using a Sephadex LH-20 column chromatography and then eluted using ethanol: water (50: 75), ethanol: water (75: 25), acetone: water (25: 75), acetone: water (50: 50), acetone: water (75: 25) and acetone as a mobile phase. The eluted samples were collected in 3 fractions, dried under evaporator and then antioxidant activity was tested. The sample with high antioxidant activity was further determined phytochemical constituents.

3) Phytochemical screening of plant extracts

The plant extracts were evaluated for phytochemicals constituents including alkaloids, glycosides, tannins and phenolics. The methods of phytochemical screening had been previously described in chapter 3.

4.3 Results and discussion

4.3.1 Antioxidant activity

The antioxidant activity is influenced by many factors that cannot be assessed by a single method, hence at least two test models have been recommended for the evaluation of antioxidant activity (Schlesier *et al.*, 2002). In this study, aqueous and ethanolic extracts of 22 medicinal plants were investigated for antioxidant activity by various methods including ABTS, DPPH and FRAP methods. The total phenolic content was also measured by Folin-Ciocalteau method.

1) ABTS decolorization assay

The ABTS radical scavenging activity is widely used to evaluate total radical scavenging capacity from natural products. The ABTS radical cation (ABTS•+) can be generated through the reaction between ABTS radical with strong oxidizing agents such as potassium permanganate ($KMnO_4$) or potassium persulfate ($K_2S_2O_8$) and these molecules can be detected at absorbance of 734 nm. This assay is principle based on antioxidant in plant extracts that can scavenge ABTS•+ by electron transferring (Miller and Rice-Evans, 1996). The ABTS radical is soluble in both aqueous and organic solvents and did not affect by ionic strength. The ABTS assay can be used to measure the antioxidant capacity of hydrophilic and lipophilic compounds in tested samples (Arno *et al.*, 2000). The results of ABTS•+ radical scavenging activity was expressed in terms of the concentration of the extract that required to inhibition 50% of initial ABTS radical (IC_{50}). Trolox equivalent antioxidant activity (TEAC) was also defined as milligram of trolox equivalents per 1 g of extract (Figure 4.1). In our result, it was found that the IC_{50} of trolox which was used as standard antioxidant was 0.249 mg/ml, the representative regression coefficient (R^2) was 0.9998 and the linear regression equation was $y = 193.9742x + 1.6930$.

The results of ABTS scavenging ability of 22 plant extracts in terms of IC_{50} and TEAC value were represented in Table 4.2 - 4.3. The result showed that the IC_{50} of aqueous extracts was ranging from 1.168 - 28.962 mg/ml while the IC_{50} of ethanolic extracts was ranging from 1.247 - 59.613 mg/ml. For aqueous extract, *S. alata* had the highest antioxidant activity with TEAC value of 214.128 mg TE/g extracts,

followed by the aqueous extract of *R. nasatus, T. crispa, Z. montanum* and *C. fenestratum* with TEAC values of 203.843, 156.906, 151.002 and 73.714 mg TE/g extracts, respectively. On the other hand, the aqueous extract of *S. leucantha* showed the lowest antioxidant activity with TEAC value of 3.195 mg TE/ g extract. For considering in ethanolic extract, *Sphenodeama* sp. had the highest antioxidant activity with TAEC values of 199.898 mg TE/ g extract followed by *P. amarus, E. prostrata, Z. montanum* and *G. pentaphylum* with TEAC values of 145.073, 90.447, 89.688 and 84.963 mg TE/g extract while *M. charantia* gave the lowest antioxidant activity with TEAC values of 4.177 mg TE/g extract. Therefore, it was clearly indicated that the aqueous extract of *S. alata* had the greatest ABTS free radical scavenging activity.

Many researchers have been conducted to search for antioxidant activity in natural products especially medicinal plants. Previous study reported that the *S. alata* extract had high potential source of antioxidant activity. Panichayupakaranant and Kaewsuwan (2003) reported the methanolic extract of leaf had higher antioxidant activity than flower and pod powders after measuring the antioxidant activity by DPPH assay. From their spectroscopic data, including IR, 1H NMR and 13C NMR showed a flavonol compound of kaempferol, which was a major active compounds and also gave antioxidant activity stronger than standard compounds butylated hydroxytoluene (BHT) and emodin. Moreover the methanolic extract of this plant also showed good values for *in vitro* enzymatic antioxidant activity such as superoxide, hydrogen peroxide inhibition and β-carotene bleaching inhibition (Deepika and Sujatha, 2013).

Table 4.2 IC_{50} values of plant extracts after determination of free radical inhibition by ABTS decolorization assay

Plant extracts	**IC_{50} value (mg/ml) ± SD**	
	Water	**Ethanol**
Andrographis paniculata	9.998 ± 0.041	30.063 ± 0.241
Cissus quadrangularis	12.237 ± 0.189	6.486 ± 0.042
Coscinium fenestratum	3.382 ± 0.144	6.994 ± 0.053
Derris scandens	5.396 ± 0.082	4.272 ± 0.036
Eclipta prostrata	28.962 ± 0.373	2.753 ± 0.004
Glycyrrhiza glabra	6.850 ± 0.483	8.678 ± 0.076
Gynostemma pentaphyllum	9.133 ± 0.175	2.931 ± 0.006
Hiptage sp.	4.968 ± 0.012	1.247 ± 0.049
Houttuynia cordata	7.539 ± 0.061	4.900 ± 0.019
Momordica charantia	27.816 ± 0.160	59.613 ± 0.687
Phyllanthus amarus	3.633 ± 0.071	1.716 ± 0.015
Pluchea indica	10.702 ± 0.379	3.912 ± 0.029
Pseuderanthemum palatiferum	5.647 ± 0.009	4.634 ± 0.071
Rhinacanthus nasutus	1.222 ± 0.021	5.978 ± 0.113
Schefflera leucantha	77.965 ± 2.254	14.483 ± 0.114
Senna alata	1.168 ± 0.098*	7.992 ± 0.134
Stemona sp.	23.227 ± 0.066	10.091± 0.159
Stephania venosa	18.349 ± 0.318	5.850 ± 0.100
Thunbergia laurifolia	8.110 ± 0.110	7.381 ± 0.038
Tinospora crispa	1.625 ± 0.320	4.402 ± 0.086
Vernonia cinerea	7.578 ± 0.080	7.246 ± 0.065
Zingiber montanum	1.650 ± 0.055	2.776 ± 0.025

Data in table are given as mean ± standard deviation (SD) of triplicate experiments. Statistical comparison between groups applied using Post hoc Duncan test. (*) represent significantly difference values from other plant samples ($P<0.05$)

Table 4.3 Antioxidant activity of plant extracts assessed by ABTS decolorization assay

Plant extracts	**Antioxidant activity TEAC (mg TE/g of extract)± SD**	
	Water	**Ethanol**
Andrographis paniculata	24.905 ± 0.102	8.283 ±0.066
Cissus quadrangularis	20.351 ± 0.311	38.390 ± 0.247
Coscinium fenestratum	73.714 ± 3.187	35.601 ± 0.269
Derris scandens	46.155 ± 0.709	58.295 ± 0.486
Eclipta prostrata	8.598 ± 0.111	90.447 ± 0.122
Glycyrrhiza glabra	36.477 ± 2.660	28.696 ± 0.253
Gynostemma pentaphyllum	27.271 ± 0.526	84.963 ± 0.161
Hiptage sp.	50.123 ± 0.125	199.898 ±7.742
Houttuynia cordata	33.032 ± 0.267	50.821 ± 0.197
Momordica charantia	8.952 ± 0.051	4.177 ± 0.048
Phyllanthus amarus	68.555 ± 1.342	145.073 ± 1.234
Pluchea indica	23.286 ± 0.811	63.651 ± 0.479
Pseuderanthemum palatiferum	44.093 ± 0.068	53.738 ± 0.818
Rhinacanthus nasutus	203.843 ± 3.553	41.665 ± 0.791
Schefflera leucantha	3.195 ± 0.091	17.193 ± 0.134
Senna alata	214.128 ± 17.210*	31.164 ± 0.517
Stemona sp.	10.720 ± 0.030	24.679 ±0.386
Stephania venosa	13.573 ± 0.234	42.569 ± 0.719
Thunbergia laurifolia	30.708 ±0.418	33.737 ± 0.175
Tinospora crispa	156.906 ± 28.377	56.579 ± 1.094
Vernonia cinerea	32.863 ± 0.348	34.366 ± 0.307
Zingiber montanum	151.002 ± 5.026	89.688 ± 0.811

Data in table are given as mean ± standard deviation (SD) of triplicate experiments. Statistical comparison between samples applied using Post hoc Duncan test. (*) represent significantly difference values from other plant samples ($P<0.05$)

2) DPPH radical scavenging activity assay

DPPH assay is another method, which is widely used to determine the free radical scavenging ability in plant extracts. DPPH is a free radical compound that stable in room temperature and produces a violet solution in organic solvents with maximum absorbance at 517 nm. Antioxidant compound scavenges free radical by hydrogen donation and reduction of DPPH• (violet color) to DPPH-H (yellowish color) (Yamaguchi *et al.,* 1998; Jadhav *et al.,* 2009). In the present finding, the radical scavenging on DPPH was expressed as the concentration of the extract required to inhibition 50% of initial DPPH radical (IC_{50}) and gallic acid equivalent antioxidant activity (GAE) which defined as milligram of gallic acid equivalents per 1 g of extract. Gallic acid was used as a standard antioxidant and it was found that the IC_{50} of gallic acid was 0.005 mg/ml. The representative regression coefficient (R^2) was 0.9861 and the linear regression equation was y = 9,187.97x + 3.86 (Figure 4.2). The results of DPPH radical scavenging activity which expressed as IC_{50} and gallic acid equivalent (GAE) values was shown in Table 4.4 - 4.5. In our present study, the IC_{50} of aqueous extract of tested medicinal plants was ranging from 0.326 - 15.376 mg/ml while the IC_{50} of ethanolic extracts were ranging from 0.064 - 5.856 mg/ml, respectively. The ethanolic extract of *S. venosa* showed the lowest IC_{50} with 0.064 mg/ml, followed by the ethanolic extracts of *P. amarus*, *H. cordata*, *E. prostrata* and *P. palatiferum* with IC_{50} values of 0.069, 0.102, 0.213 and 0.226 mg/ml, respectively. The aqueous extract of *A. paniculata* had the highest IC_{50} with 15.376 mg/ml. Percentage of inhibition of radical was compared to gallic acid and expressed as gallic acid equivalent (GAE). In aqueous extract, *S. alata* gave the highest DPPH radical scavenging activity with GAE value of 22.114 mg GAE/g extract followed by *E. prostrata*, *C. fenestratum*, *D. scandens* and *P. amarus* with GAE values of 15.355, 13.048, 10.874 and 10.051 mg GAE/ g extract, respectively. Moreover, the result of ethanolic extract showed that *Hiptage* sp. had the highest antioxidant activity with GAE value of 77.913 mg GAE/ g extract followed by *P. amarus*, *H. cordata*, *E. prostrata* and *P. pentaphylum* with GAE values of 72.619, 48.938, 23.4462 and 22.118 mg GAE/ g extract, respectively. Therefore, it was concluded that the ethanolic extract of *Hiptage* sp. gave the highest antioxidant potential when using DPPH radical scavenging assay. Nevertheless, the pharmacological potential and chemical constituents of this plant had

been unexplored. *Hiptage* sp. is a traditional folk medicine to provide energy, relieve back pain, relieve flank pain, and help in case of exhaustion (Wiart, 2006). In our study, *P. amarus* extract also gave high antioxidant activity. *P. amarus* widespread throughout the tropical and subtropical countries of the world and have been widely used in folk medicine in India and other tropical countries (Patel *et al.*, 2011). Previous study showed that the methanol extract of *P. amarus* contained highest amount of phenolic compounds and exhibits the greatest antioxidant activity in comparison to other extracts when testing with DPPH assay (Sen and Batra, 2013). In addition, other research showed that *P. amarus* gave high DPPH, hydroxyl, superoxide and nitric oxide radical scavenging and reducing power activity, and the majority of the compounds were found to exist as glycosides and gallic acid derivatives (Maity *et al.*, 2013).

However, the antioxidant testing by DPPH assay had some limitation that might be disturbed to the experiment. DPPH is long lived nitrogen radical unlike radicals present in living organisms and has no similarity to the highly reactive and transient peroxyl radicals those are involved in lipid peroxidation (Huang *et al.*, 2005). Another weakness is interference of carotenoids to this assay because they have an absorbance spectrum that overlaps with DPPH at 515 nm (Pérez-Jiménez *et al.*, 2008). However, DPPH is always a broad solvent solubility in aqueous, polar and non polar solvents. Thus, hydrophilic and lipophillic antioxidants were evaluated by DPPH assay (Cao *et al.*, 1997).

Table 4.4 IC_{50} values of plant extracts after determination of free radical inhibition by DPPH radical scavenging assay

Plant extracts	**IC_{50} value (mg/ml) ± SD**	
	Water	**Ethanol**
Andrographis paniculata	15.376± 0.181	5.856 ± 0.096
Cissus quadrangularis	2.754 ± 0.029	0.832 ± 0.005
Coscinium fenestratum	0.383 ± 0.003	0.591 ± 0.003
Derris scandens	0.460 ± 0.006	0.700 ± 0.010
Eclipta prostrata	0.326 ± 0.006	0.213 ± 0.007
Glycyrrhiza glabra	2.567 ± 0.040	2.925 ± 0.057
Gynostemma pentaphyllum	10.727 ± 0.061	0.974 ± 0.009
Hiptage sp.	0.641 ± 0.011	0.064 ± 0.001**
Houttuynia cordata	0.686 ± 0.015	0.102 ± 0.002
Momordica charantia	2.658 ± 0.187	2.445 ± 0.017
Phyllanthus amarus	0.498 ± 0.009	0.069 ± 0.001
Pluchea indica	2.818 ± 0.016	0.538 ± 0.015
Pseuderanthemum palatiferum	2.809 ± 0.034	0.226 ± 0.003
Rhinacanthus nasutus	0.550 ± 0.014	0.303 ± 0.013
Schefflera leucantha	0.857 ± 0.003	3.693 ± 0.062
Senna alata	0.226 ± 0.003	0.550 ± 0.007
Stemona sp.	0.968 ± 0.025	2.747 ± 0.018
Stephania venosa	4.552 ± 0.031	0.793 ± 0.011
Thunbergia laurifolia	0.745 ± 0.013	0.519 ± 0.006
Tinospora crispa	3.829 ± 0.035	0.649 ± 0.001
Vernonia cinerea	0.668 ± 0.009	0.303 ± 0.003
Zingiber montanum	6.585 ± 0.222	0.445 ± 0.014

Data in table are given as mean ± standard deviation (SD) of triplicate experiments. Statistical comparison between groups applied using Post hoc Duncan test. (**) represent significantly difference values from other plant samples ($P<0.01$)

Table 4.5 Antioxidant activity of plant extracts assessed by DPPH radical scavenging assay

Plant extracts	**Antioxidant activity GAE (mg GAE/g of extract) ± SD**	
	Water	**Ethanol**
Andrographis paniculata	0.325 ± 0.004	0.854 ± 0.014
Cissus quadrangularis	1.816 ± 0.019	6.009 ± 0.033
Coscinium fenestratum	13.048 ± 0.086	8.467 ± 0.040
Derris scandens	10.874 ± 0.152	7.140 ± 0.100
Eclipta prostrata	15.355 ± 0.263	23.462 ± 0.806
Glycyrrhiza glabra	1.948 ± 0.030	1.710 ± 0.033
Gynostemma pentaphyllum	0.466 ± 0.003	5.134 ± 0.048
Hiptage sp.	7.804 ± 0.140	77.913 ±1.477**
Houttuynia cordata	7.293 ± 0.162	48.938 ± 0.974
Momordica charantia	1.887 ± 0.148	2.045 ± 0.014
Phyllanthus amarus	10.051 ± 0.179	72.169 ± 1.086
Pluchea indica	1.774 ± 0.010	9.297 ± 0.255
Pseuderanthemum palatiferum	1.780 ± 0.021	22.118 ± 0.256
Rhinacanthus nasutus	9.087 ± 0.236	16.534 ± 0.704
Schefflera leucantha	5.838 ± 0.022	1.354 ± 0.023
Senna alata	22.114 ± 0.324	9.090 ± 0.111
Stemona sp.	5.167 ± 0.133	1.820 ± 0.012
Stephania venosa	1.098 ± 0.007	6.308 ± 0.086
Thunbergia laurifolia	6.711 ± 0.121	9.637 ± 0.108
Tinospora crispa	1.306 ± 0.012	7.707 ± 0.011
Vernonia cinerea	7.481 ± 0.104	16.478 ± 0.137
Zingiber montanum	0.760 ± 0.025	11.239 ± 0.354

Data in table are given as mean ± standard deviation (SD) of triplicate experiments. Statistical comparison between groups applied using Post hoc Duncan test. (**) represent significantly difference values from other plant samples ($P<0.01$)

3) Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power (FRAP) assay is another method for measuring reducing power in natural extract. The principle of FRAP method is based on the reduction of 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 (Benzie and Strain, 1996). The ferric reducing ability of the tested medicinal plant extracts were determined using FRAP assay by manipulation of the regression equation of $FeSO_4$ calibration curve ($y = 2.529x - 0.008$, $R_2 = 0.9960$) (Figure 4.3). It was hypothesized that the plant which had high equivalent capacity (EC) value could be considered as the good electron donors and could terminate oxidation reactions by reducing the oxidized intermediates into the stable form. The result of FRAP assay was shown in Table 4.6. For aqueous extract, *Stemona* sp. gave the highest reducing activity with EC value of 433.900 mg $FeSO_4$/ g extract followed by the aqueous extracts of *P. amarus*, *S. alata*, *E. prostrat*a and *Hiptage* sp. with EC values of 232.239, 226.176, 183.208 and 132.332 mg $FeSO_4$/ g extract, respectively. Moreover, in ethanolic extract, *R. nasatus* had the highest ferric reducing capacity with EC value of 201.529 mg $FeSO_4$/ g extract followed by *Hiptage* sp., *P. palatiferum*, *E. prostrata* and *V. cinerea* with EC values of 196.520, 172.796, 168.709 and 160.406 mg $FeSO_4$/ g extract, respectively. Thus, it was summarized that the aqueous extract of *Stemona* sp. gave the highest ferric reducing antioxidant power activity. By contrast, the aqueous extract of *G. pentaphyllum* showed the lowest reducing ability with EC value of 6.590 ± 1.812 mg $FeSO_4$/g extract. FRAP assay was conducted to examine in both hydrophilic and lipophilic antioxidants. However, measurement of protein and thiol antioxidant such as glutathione and some carotenoids were not performed by this assay (Pérez-Jiménez *et al.*, 2008).

Table 4.6 Antioxidant activity of extracts assessed by FRAP assay

Plant extract	EC (mg $FeSO_4$/g of extract) ± SD	
	Water	**Ethanol**
Andrographis paniculata	10.676 ± 1.951	27.415 ± 7.951
Cissus quadrangularis	40.069 ± 5.949	90.813 ± 7.512
Coscinium fenestratum	79.083 ± 6.807	96.481 ± 7.951
Derris scandens	60.894 ± 4.229	136.286 ± 2.092
Eclipta prostrata	183.208 ± 10.583	168.709 ± 9.120
Glycyrrhiza glabra	38.751 ± 1.827	23.198 ± 2.202
Gynostemma pentaphyllum	6.590 ± 1.812	61.025 ± 1.783
Hiptage sp.	132.332 ± 7.718	196.520 ± 0.823
Houttuynia cordata	80.928 ± 4.560	113.220 ± 9.477
Momordica charantia	41.123 ± 3.841	152.498 ± 16.299
Phyllanthus amarus	232.239 ± 7.912	24.120 ± 4.373
Pluchea indica	26.888 ± 3.221	123.632 ± 1.812
Pseuderanthemum palatiferum	43.232 ± 7.732	172.796 ± 4.265
Rhinacanthus nasutus	67.484 ± 11.440	201.529 ± 1.582
Schefflera leucantha	40.859 ± 2.416	26.493 ± 3.980
Senna alata	226.176 ± 19.796	48.109 ± 8.452
Stemona sp.	433.900 ± 35.204**	18.584 ± 0.604
Stephania venosa	16.344 ± 6.001	62.212 ± 1.646
Thunbergia laurifolia	80.532 ± 8.672	156.056 ± 7.627
Tinospora crispa	20.957 ± 0.913	73.020 ± 8.369
Vernonia cinerea	34.401 ± 7.359	160.406 ± 7.323
Zingiber montanum	21.352 ± 2.178	73.415 ± 5.578

Data in table are given as mean ± standard deviation (SD) of triplicate experiments. Statistical comparison between groups applied using Post hoc Duncan test. (**) represent significantly difference values from other plant samples ($P<0.01$)

4) Total phenolic content assay

Phenolic compounds were commonly found in plants and had been reported to have several biological activities such as antibacterial and antioxidant activities. The phenolic compounds act as antioxidant due to scavenge free radicals, donate hydrogen atoms or electrons and chelate metal cations (Javanraedi *et al.*, 2003). In this study, the total phenolic compounds of Thai medicinal plant extracts were shown. The total phenolic contents of the tested medicinal plant extracts were determined using the Folin-Ciocalteau colorimetric method. Gallic acid is the commonly used as a standard for total phenolic content determination and the results are expressed as milligram gallic acid equivalent per gram sample. The regression equation of gallic acid calibration curve ($y = 8.3373x - 0.0616$, $R^2 = 0.9945$) was shown in Figure 4.4. The result showed that various plant extracts gave varieties in the results (Table 4.7). For aqueous extracts, *S. alata* gave the highest total phenolic content with GAE value of 70.903 mg GAE/ g extract followed by *D. scandens*, *H. cordata*, *C. fenestratum* and *Hiptage* sp. with GAE values of 48.122, 41.567, 39.329 and 33.773 mg GAE / g extract, respectively. For ethanolic extract, *Hiptage* sp. gave the highest total phenolic content with GAE value of 132.454 mg GAE/ g extract followed by *P. amarus*, *P. palatiferum*, *S. alata* and *D. scandens* with GAE values of 93.046, 61.950, 50.799 and 46.563 mg GAE/ g extract. Therefore, it was summarized that the ethanolic extract of *Hiptage* sp. significantly gave the highest total phenolic content.

The previous study demonstrated the water extract of *P. amarus* showed the great amount of total phenolic content with 106.26 mg/g (Chunthorng-Orn *et al.*, 2012). Moreover, *P. amarus* extract also showed high phenolic compounds content which had strong correlated with free radical scavenging potential, lipid peroxidation inhibition capacity and cytoprotective efficiency against Chromium (VI) - induced oxidative cellular damage in MDA-MB-435S cells (Guha *et al.*, 2010). Although, the total phenolic content determined by Folin-Ciocalteau method has some disadvantages such as a possible interference by other reducing agents but it still remained a popular method since it was simple, rapid and inexpensive.

Table 4.7 Total phenolic content of extracts assessed by Folin-Ciocalteau assay

Plant extract	**Total phenolic GAE (mg GAE/g of extract)± SD**	
	Water	**Ethanol**
Andrographis paniculata	7.994 ± 0.302	10.671 ± 0.240
Cissus quadrangularis	12.950 ± 0.240	0.208 ± 0.523
Coscinium fenestratum	39.329 ± 0.432	28.377 ± 0.900
Derris scandens	48.122 ± 1.201	46.563 ± 1.816
Eclipta prostrata	29.337 ± 0.962	36.731 ± 0.485
Glycyrrhiza glabra	28.857 ± 1.583	15.068 ± 0.733
Gynostemma pentaphyllum	7.514 ± 0.250	23.781 ± 1.843
Hiptage sp.	33.773 ± 1.207	132.454 ± 1.971**
Houttuynia cordata	41.567 ± 0.302	39.249 ± 0.302
Momordica charantia	11.631 ± 0.360	22.022 ± 1.390
Phyllanthus amarus	31.375 ± 1.088	93.046 ± 0.549
Pluchea indica	13.829 ± 0.366	27.498 ± 0.660
Pseuderanthemum palatiferum	19.345 ± 0.541	61.950 ± 0.366
Rhinacanthus nasutus	22.382 ± 0.485	33.133 ± 1.445
Schefflera leucantha	19.824 ± 0.977	16.267 ± 0.998
Senna alata	70.903 ± 1.048	50.799 ± 0.421
Stemona sp.	27.698 ± 0.623	15.907 ± 2.105
Stephania venosa	8.153 ± 0.120	38.489 ± 1.559
Thunbergia laurifolia	22.182 ± 1.269	33.133 ± 1.133
Tinospora crispa	11.191 ± 1.088	42.126 ± 0.421
Vernonia cinerea	21.942 ± 1.066	29.057 ± 0.591
Zingiber montanum	11.351 ± 0.138	43.165 ± 0.668

Data in table are given as mean ± standard deviation (SD) of triplicate experiments. Statistical comparison between groups applied using Post hoc Duncan test. (**) represent significantly difference values from other plant samples ($P<0.01$)

5) Correlations among antioxidant activity assay and total phenolic contents

Generally, antioxidant activity from different methods have been determined based on the different reaction mechanism that resulting in different results. Therefore, antioxidant capacities testing could be predicted from one assay to another by determined the correlation. The correlation analyses were conducted in this research to determine the relationship between the phenolic content and three antioxidant activities from ABTS, DPPH and FRAP assays. In the present study, Pearson's correlation coefficients were used to evaluate the correlation between various antioxidant activities testing models in 22 medicinal plant extracts. The results in Table 4.8 showed that there were high correlation in total phenolic content with other antioxidant testing model including ABTS (R = 0.543, $P<0.01$), DPPH (R = 0.857, $P<0.01$) and FRAP (R = 0.371, $P<0.05$) methods. It was suggested that phenolics in tested medicinal plants might contributed antioxidant activity to scavenge both ABTS•+ and DPPH radicals and also had ability to reduce ferric ion. Previous reported by Fidrianny *et al*. (2013) revealed that there were positively high correlation between total phenolic content in various extracts of four varieties of mangoes and antioxidant capacities using two methods ABTS and DPPH assays. Moreover, Othman *et al.* (2007) found a strong correlation between total phenolic content and FRAP assay. Furthermore, strong correlation coefficients between the phenolic content and antioxidant activities had been reported for various food such as sorghum (R = 0.971) and cactus pear (R = 0.970) (Rabah *et al*., 2004; Stintzing *et al.*, 2005).

In this study, ABTS method also showed strong correlation with DPPH method (R = 0.507, $P<0.01$). This result was in agreement with Leong and Shui (2001) who found a strong correlation (R = 0.90) between ABTS and DPPH values for various fruit extracts. On the other hand, FRAP method did not correlate with other antioxidant models; ABTS (R = 0.106) and DPPH (R = 0.269) methods. However, previous resulted reported that high correlation (R>0.9, $P<0.05$) between antioxidant activities as determined by DPPH or FRAP assays and total phenolic assay in nectarines, peaches and plums (Gil *et al*., 2002). Other studies, there were not significant correlation between antioxidant activity using FRAP assay and other assay; total phenolic content, β-carotene, ABTS and DPPH assay (Thaipong *et al.*, 2006).

Therefore, it was suggested that compounds which scavenge the peroxyl radical were mostly unable to reduce ferric ions or chelate metals. Some antioxidant might act as free radical quenching such as thiol and some carotenoids were determined by FRAP assay (Pérez-Jiménez *et al.*, 2008). From the present finding, it was be concluded that the correlation of the antioxidant activity using various methods depended on the reaction mechanism of each test and the chemical compound found in various plant species might interfere to some test.

Table 4.8 Pearson's correlation coefficients of antioxidant activities by ABTS, DPPH, FRAP and total phenolic content

Trait [a]	**DPPH**	**FRAP**	**TPC**
ABTS	0.507^{**}	0.106^{ns}	0.543^{**}
DPPH	-	0.269^{ns}	0.857^{**}
FRAP		-	0.371^{*}
TPC			-

[a] TPC = total phenolic content, ns = non significant and * = significant at $P< 0.05$ or ** = significant at $P<0.01$, respectively

4.3.2 Protection of oxidative protein damage

Hydroxyl ($^{\bullet}OH$) radical is probably the final mediator of most free radical induced tissue damage (Lloyd *et al.*, 1997). It reacts with all macromolecules with extremely high rate constant. By far the most common mechanism, $^{\bullet}OH$ radical formation in *vivo* is likely to be the transition metal catalyzed decomposition of superoxide and hydrogen peroxide. $^{\bullet}OH$ radical is generated from the reaction of Cu^{2+} and H_2O_2 leading to protein structural modification (Mark and Chevion, 1985). The attacking of proteins by $^{\bullet}OH$ radical results in protein fragmentation, cross linking and more sensitive to proteolysis enzyme. Moreover, $^{\bullet}OH$ radical can cause site specific damage and induction of immidazole ring in histidine residues resulting in protein inactivation or degradation (Stadtman, 1993).

In the present study, the effect of plant extracts on oxidative protein damage protection was accessed using Bovine serum albumin (BSA) as a protein standard. BSA was catalyzed by metal catalyzed oxidation using the reaction between H_2O_2

and Cu^{2+} and determined by SDS gel electrophoresis. The plants which had high antioxidant activity were selected to determine protective oxidative protein damage ability. Five aqueous extracts; *D. scandens* , *P. amarus* , *R. nasatus*, *S. alata*, *Hiptage* sp. and five ethanolic extracts; *E. prostrata*, *H. cordata*, *P. amarus*, *P. platiflorum*, *Hiptage* sp. at concentration of 5, 10, 15 and 20 mg/ml were reflected for further study. Figure 4.1-4.10 showed the protective effect of medicinal plant extracts against oxidative protein damage. The result showed that there was rapid protein degradation when BSA was incubated in the presence of Cu^{2+} /H_2O_2. This $^{\bullet}OH$ radical decreased the amount of protein by reduction of band intensity between 26.37-37.18 %. DMSO was used as a solvent control for the antioxidant tested in this study and it was found that the band intensity after incubation with DMSO was slightly reduced when compared to the untreated proteins with band intensity ranging between 81.31-95.31 %, however, when compared with the plant extract treated protein, it was not significant interfere to the system. Moreover, glutathione (GSH) at the concentration 10 mg/ml was used as a positive antioxidant compound. The result showed that the aqueous extracts of *P. amarus* at different concentration (5, 10, 15, 20 mg/ml) showed the highest ability to prevent oxidative protein damage by dose dependent manner with BSA band intensity of 56.79, 58.78, 69.50 and 87.06 %, respectively while glutathione protected against protein damage only 67.10 % (Figure 4.2). Moreover, the ethanolic extract of *P. palatiferum* also showed high ability to prevent protein degradation with the protein amount of 64.20, 66.31, 66.81 and 83.20 %, respectively while glutathione could protect 57.19 % (Figure 4.9). Furthermore, BSA protection of the ethanolic extract of *P. amarus* also showed high ability to prevent oxidative protein damage with 60.06, 65.75, 72.58 and 75.01 % of band intensity when compared to glutathione by 68.60 % (Figure 4.8). In addition, the aqueous extract of *R. nasatus* had the percentage of protein damage prevention by 55.30, 50.73, 66.87 and 73.11 %, respectively as compared to glutathione by 55.69 % (Figure 4.3). The prevention of protein damage by the aqueous extract of *Hiptage* sp. was 56.75, 57.09, 64.31 and 64.45 % while glutathione had only 64.31% (Figure 4.5). The aqueous extract of *D. scandens* showed ability to protect BSA by 44.35, 56.49, 54.67, and 64.14 %, respectively while glutathione showed with protein protection of 57.68 % (Figure 4.1). Additionally, BSA protection by the ethanolic extract of *E. prostrata* was 51.94,

57.91, 62.69 and 63.23 % as compared to positive control, glutathione with protein protection of 68.86 % (Figure 4.6). Moreover, the ethanolic extract of *H. cordata* showed the percentage of oxidative protein damage prevention with 46.43, 48.79, 53.09 and 57.60 % when compared to glutathione protein protection of 53.47 % (Figure 4.7). The BSA damage of the aqueous extract of *S. alata* was 45.54, 49.81, 50.12 and 53.06 % when compared to glutathione with protein protection of 57.09 % (Figure 4.4). However, the ethanolic extract of *Hiptage* sp. gave the lowest ability to prevent oxidative protein damage with 37.60, 37.31, 37.93 and 38.17 % when compared to glutathione protein protection of 66.82 % (Figure 4.10). Interestingly, almost plant extract at concentration of 15 mg/ml gave high ability to protect protein damage caused by oxidation more than glutathione.

Similarly, the previous studies demonstrated that basil, celery, Thai copper pod, red sorrel and star fruit possessed antioxidant activity as revealed by inhibition of oxidative damage. Their result showed that celery gave the highest inhibition of metal catalyzed of BSA whereas basil, Thai copper pod, star fruit and red sorrel extract did not protect oxidative protein damage (Tit-oon and Chotpadiwetkul, 2004). Other researches also studied the effect of some synthesis antioxidants including melatonin, glutathione, ascorbic acid, trolox, reveratrol and D-mannitol on the oxidative protein damage protection. Melatonin is the most effective than other antioxidant compounds for protection against the structural damage caused by Cu^{2+}/H_2O_2 and completely inhibited at the low concentration of 50 µM while glutathione also had an effective to inhibit protein damage at the highest concentration (100 µM). However, ascorbic acid did not protect against protein damage when increased the Cu^{2+}/H_2O_2 to induce BSA degradation. The reason was described that ascorbic acid is known to be a strong reducing agents and it had been used to reduce transition metals such as Fe^{3+} or Cu^{2+} to generate •OH. Therefore, the combination between ascorbic acid and Cu^{2+} could cause molecular damage in both protein and DNA. Moreover, at the concentration used in the current study, D-mannitol and reveratrol did not protect against the metal catalyzed oxidation in this model (Mayo *et al.,* 2003). From the present finding, even the ethanolic extract of *Hiptage* sp. had high antioxidant activity in ABTS and DPPH method but in this study this plant did not protect BSA from hydroxyl radical mediated oxidation. Thus, it might be had some biochemical compound that reacted with Cu^{2+}.

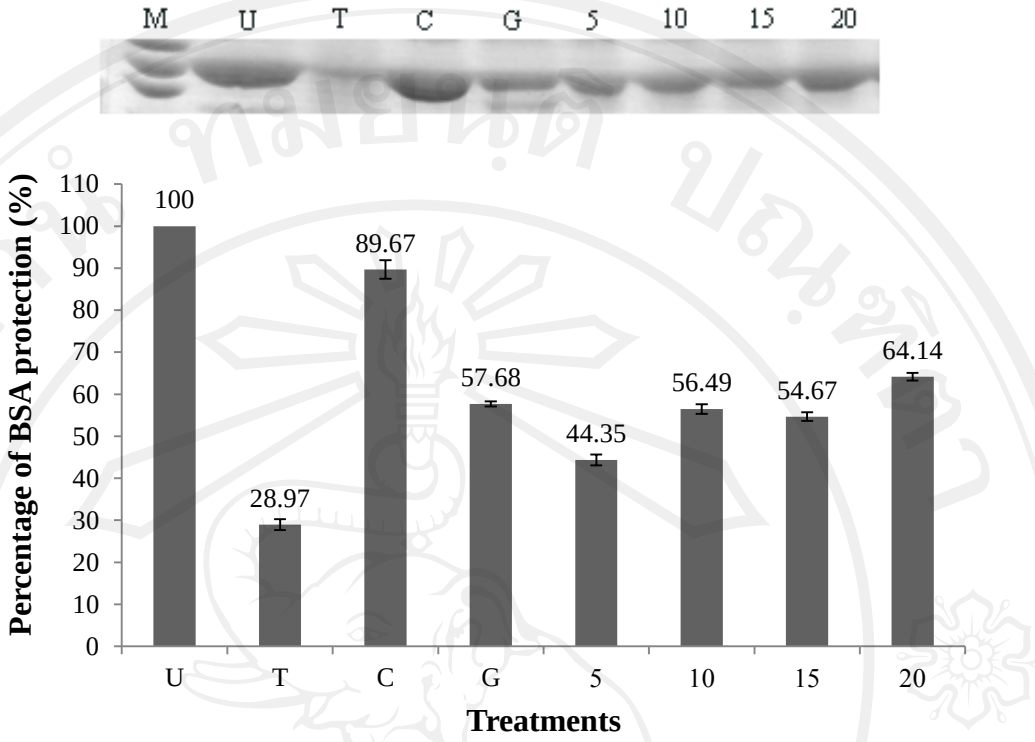

Figure 4.1 PAGE profile of BSA protein and percent of BSA protein protection after treatment with aqueous extract of *D. scandens*; M = marker, U = untreated BSA, T = $Cu^{2+}$and H_2O_2 treated BSA, C = control DMSO, G = Glutathione treated BSA, 5 = 5 mg/ml of extract treated BSA, 10 = 10 mg/ml of extract treated BSA, 15 = 15 mg/ml of extract treated BSA, 20 = 20 mg/ml of extract treated BSA

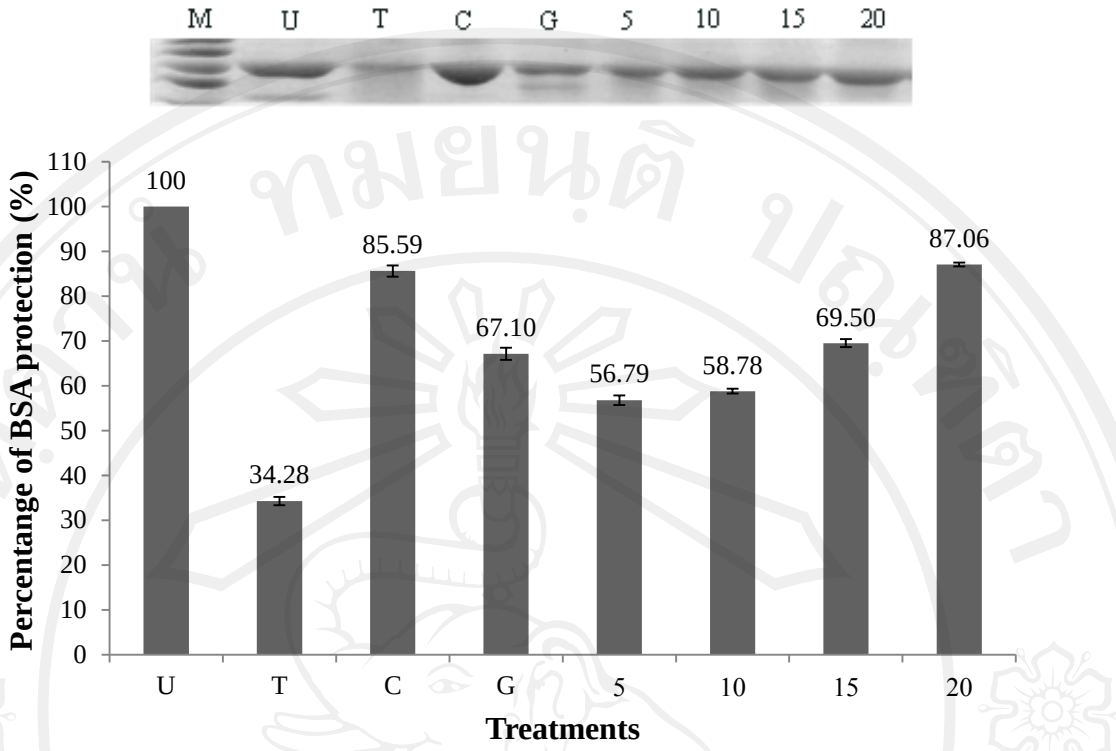

Figure 4.2 PAGE profile of BSA protein and percent of BSA protein protection after treatment with aqueous extract of *P. amarus*; M = marker, U = untreated BSA, T = Cu^{2+} and H_2O_2 treated BSA, C = control DMSO, G = Glutathione treated BSA, 5 = 5 mg/ml of extract treated BSA, 10 = 10 mg/ml of extract treated BSA, 15 = 15mg/ml of extract treated BSA, 20 = 20 mg/ml of extract treated BSA

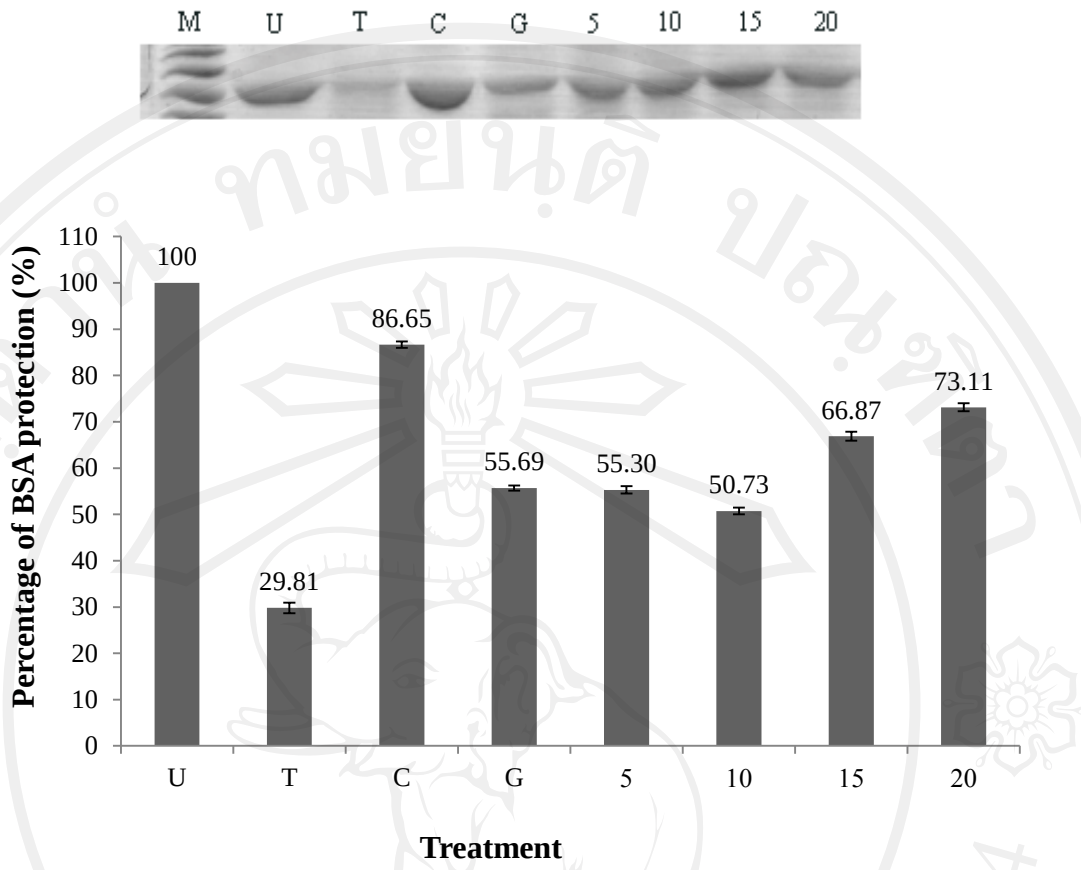

Figure 4.3 PAGE profile of BSA protein and percent of BSA protein protection after treatment with aqueous extract of *R. nasatus*; M = marker, U = untreated BSA, T = $Cu^{2+}$and H_2O_2 treated BSA, C = control DMSO, G = Glutathione treated BSA, 5 = 5 mg/ml of extract treated BSA, 10 = 10 mg/ml of extract treated BSA, 15 = 15 mg/ml of extract treated BSA, 20 = 20 mg/ml of extract treated BSA

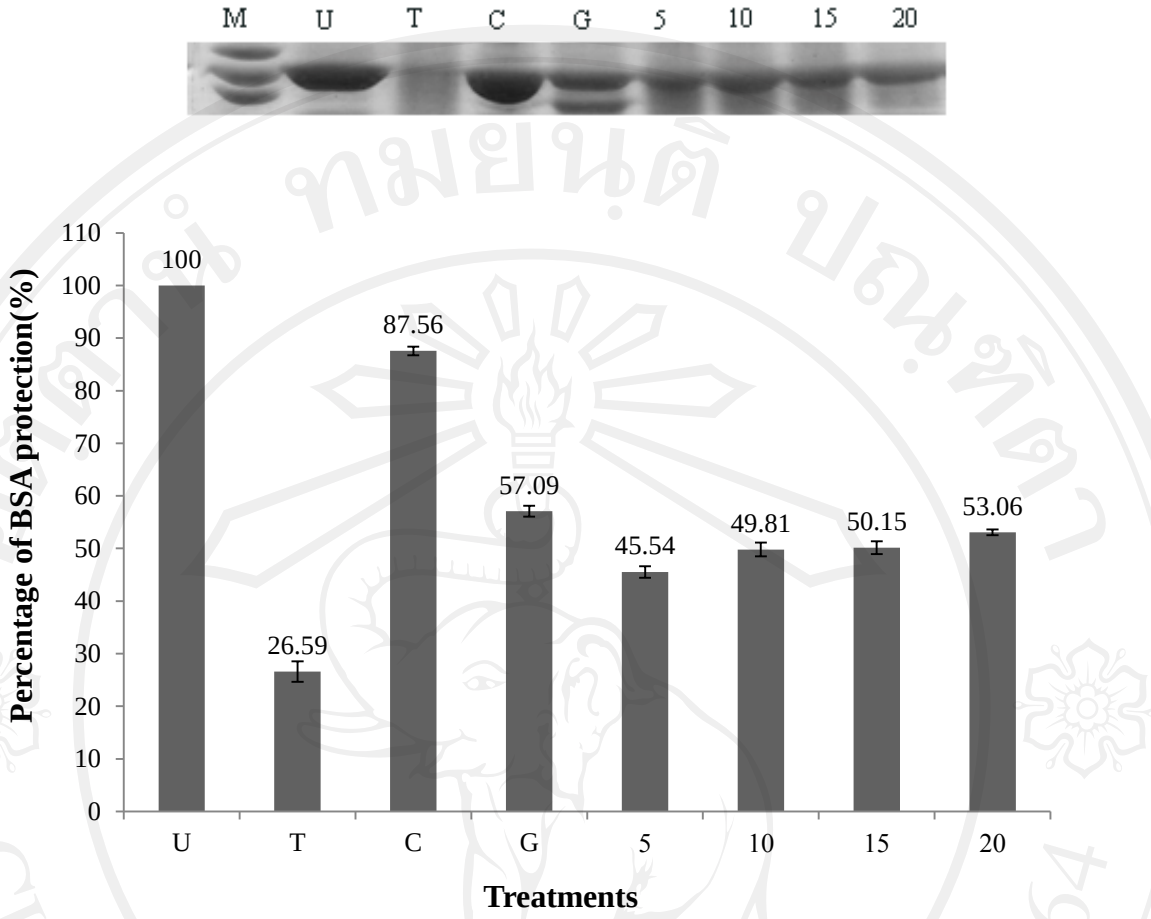

Figure 4.4 PAGE profile of BSA protein and percent of BSA protein protection after treatment with aqueous extract of *S. alata*; M = marker, U= untreated BSA, T = $Cu^{2+}$and H_2O_2 treated BSA, C = control DMSO, G = Glutathione treated BSA, 5 = 5 mg/ml of extract treated BSA, 10 =10 mg/ml of extract treated BSA, 15 = 15 mg/ml of extract treated BSA, 20 = 20 mg/ml of extract treated BSA

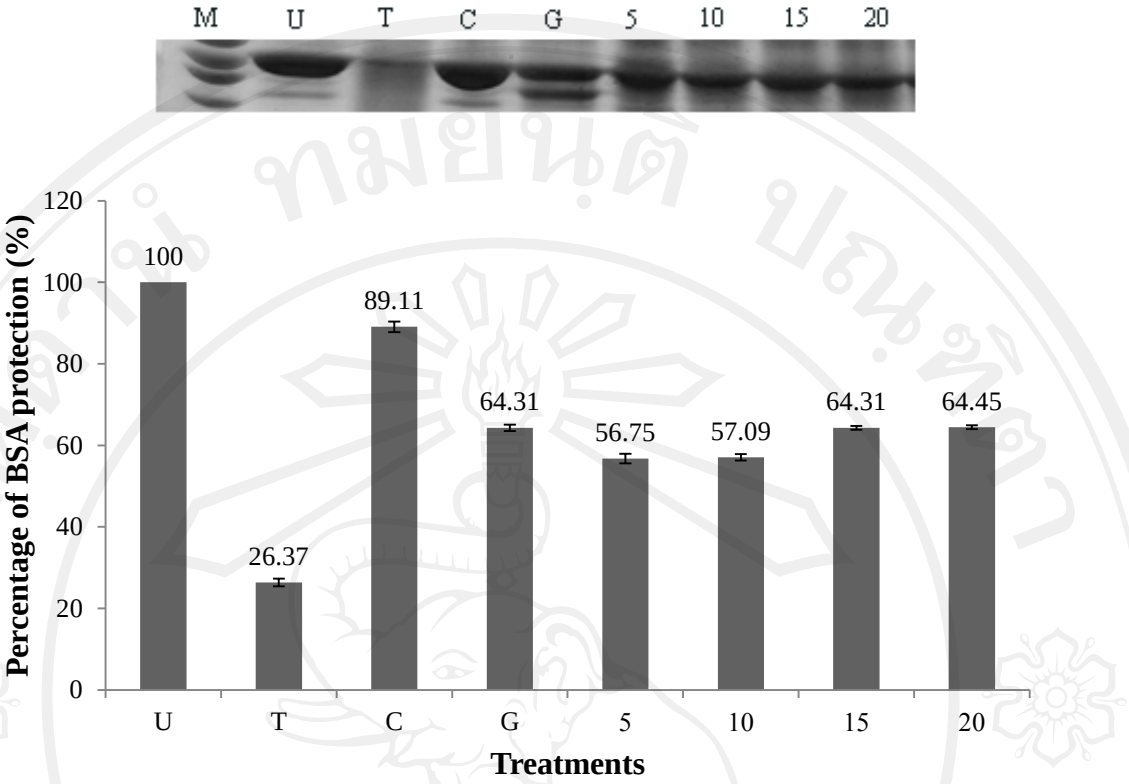

Figure 4.5 PAGE profile of BSA protein and percent of BSA protein protection after treatment with aqueous extract of *Hiptage* sp.; M = marker, U= untreated BSA, T = $Cu^{2+}$and H_2O_2 treated BSA, C = control DMSO, G = Glutathione treated BSA, 5 = 5 mg/ml of extract treated BSA, 10 = 10 mg/ml of extract treated BSA, 15 = 15 mg/ml of extract treated BSA, 20 = 20 mg/ml of extract treated BSA

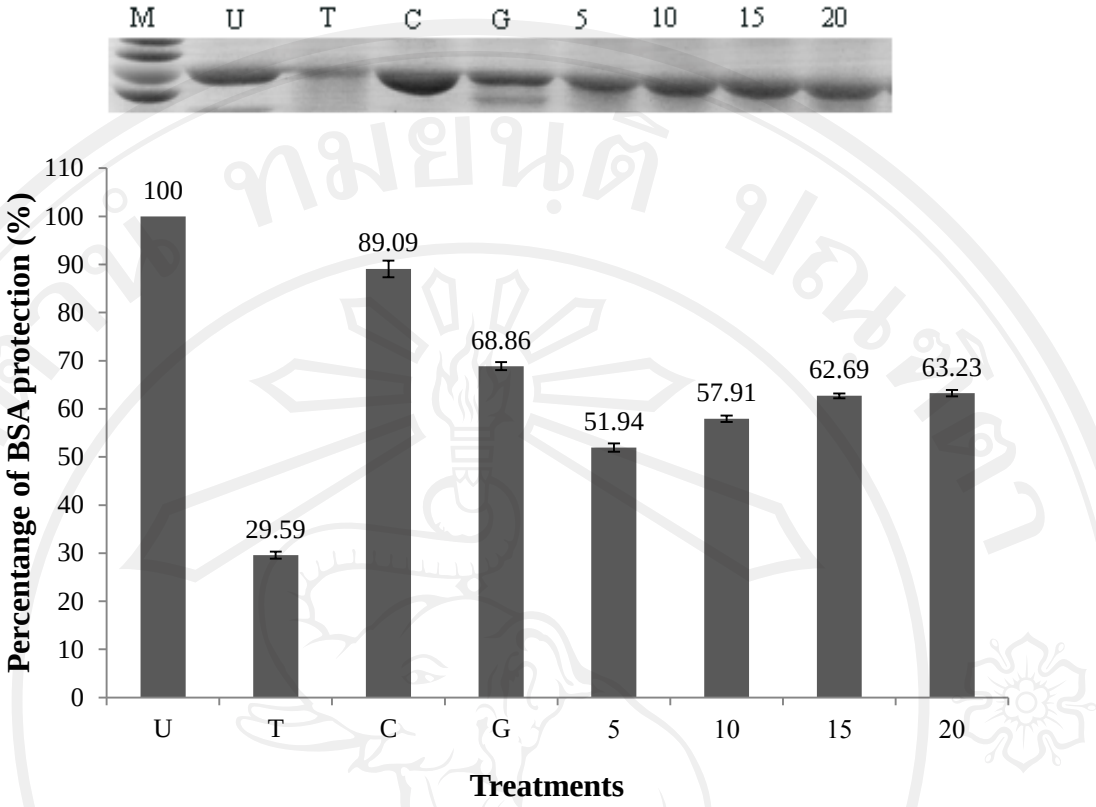

Figure 4.6 PAGE profile of BSA protein and percent of BSA protein protection after treatment with ethanolic extract of *E. prostrata*; M = marker, U= untreated BSA, T = $Cu^{2+}$and H_2O_2 treated BSA, C = control DMSO, G = Glutathione treated BSA, 5 = 5 mg/ml of extract treated BSA, 10 = 10 mg/ml of extract treated BSA, 15 = 15 mg/ml of extract treated BSA, 20 = 20 mg/ml of extract treated BSA

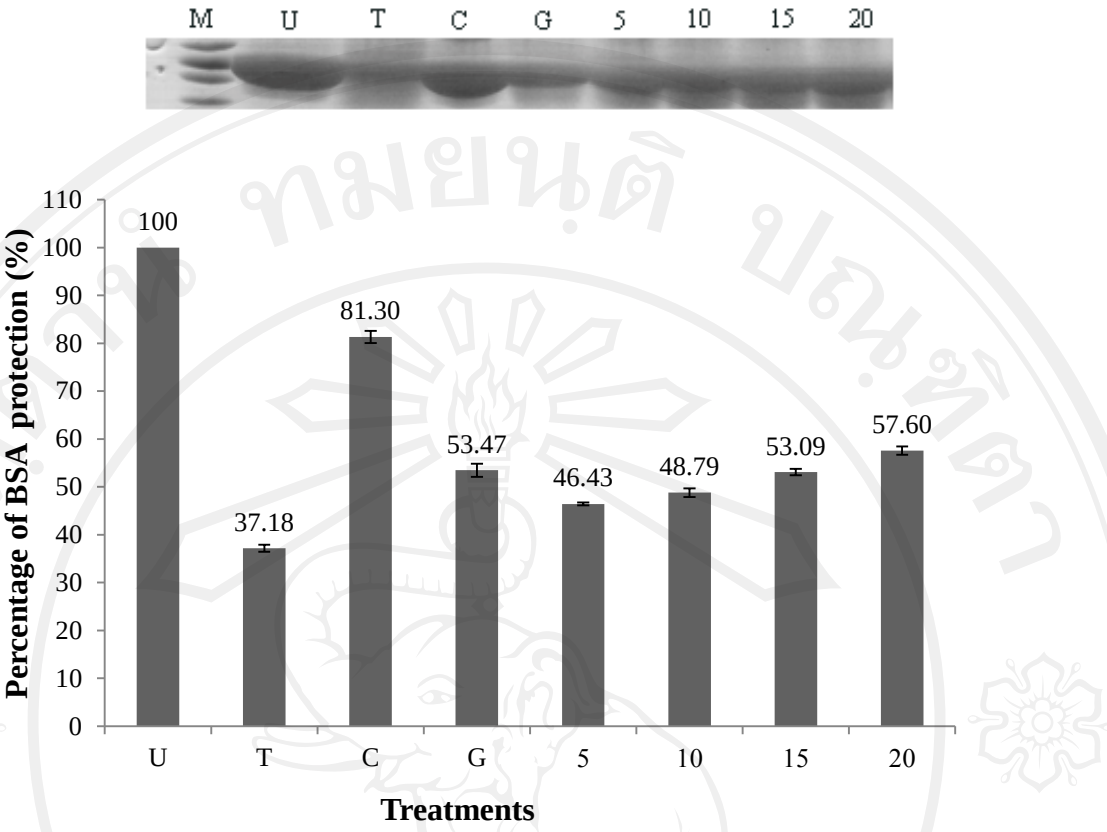

Figure 4.7 PAGE profile of BSA protein and percent of BSA protein protection after treatment with ethanolic extract of *H. cordata*; M = marker, U = untreated BSA, T = $Cu^{2+}$and H_2O_2 treated BSA, C = control DMSO, G = Glutathione treated BSA, 5 = 5 mg/ml of extract treated BSA, 10 = 10 mg/ml of extract treated BSA, 15 = 15 mg/ml of extract treated BSA, 20 = 20 mg/ml of extract treated BSA

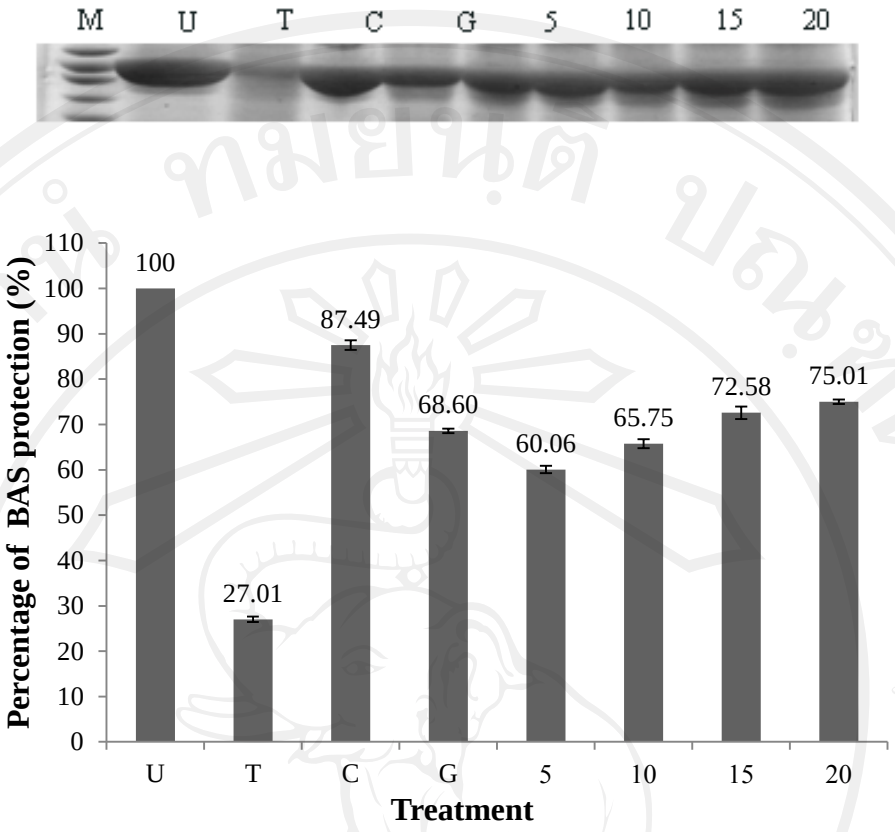

Figure 4.8 PAGE profile of BSA protein and percent of BSA protein protection after treatment with ethanolic extract of *P. amarus*; M = marker, U = untreated BSA, T = $Cu^{2+}$and H_2O_2 treated BSA, C = control DMSO, G = Glutathione treated BSA, 5 = 5 mg/ml of extract treated BSA, 10 = 10 mg/ml of extract treated BSA, 15 = 15 mg/ml of extract treated BSA, 20 = 20 mg/ml of extract treated BSA

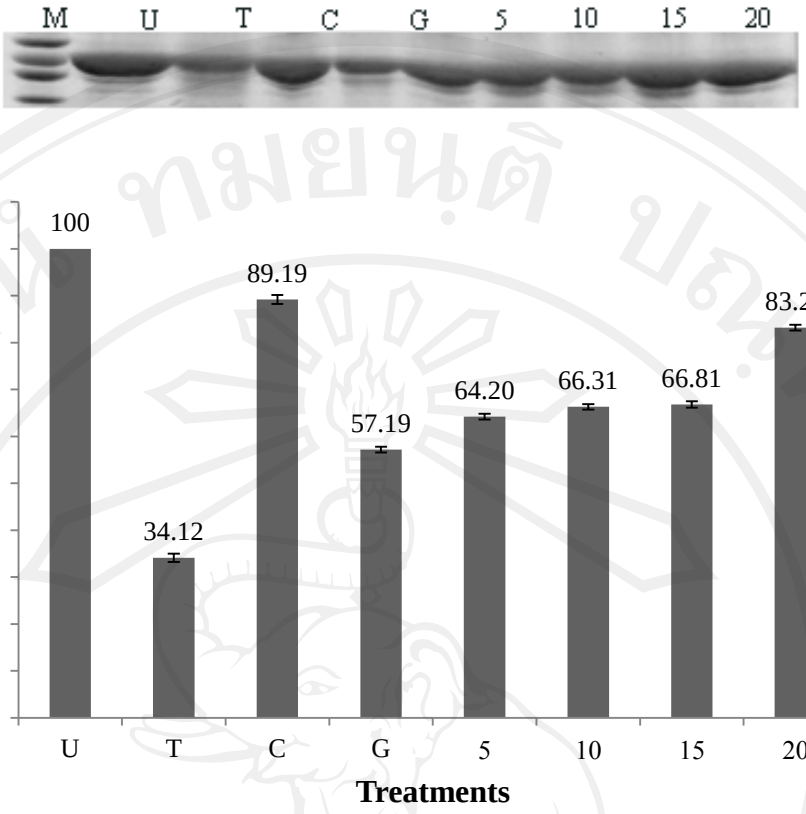

Figure 4.9 PAGE profile of BSA protein and percent of BSA protein protection after treatment with ethanolic extract of *P. palatiferum*; M = marker, U = untreated BSA, T = $Cu_{2+}$and H_2O_2 treated BSA, C = control DMSO, G = Glutathione treated BSA, 5 = 5 mg/ml of extract treated BSA, 10 = 10 mg/ml of extract treated BSA, 15 = 15 mg/ml of extract treated BSA, 20 = 20 mg/ml of extract treated BSA

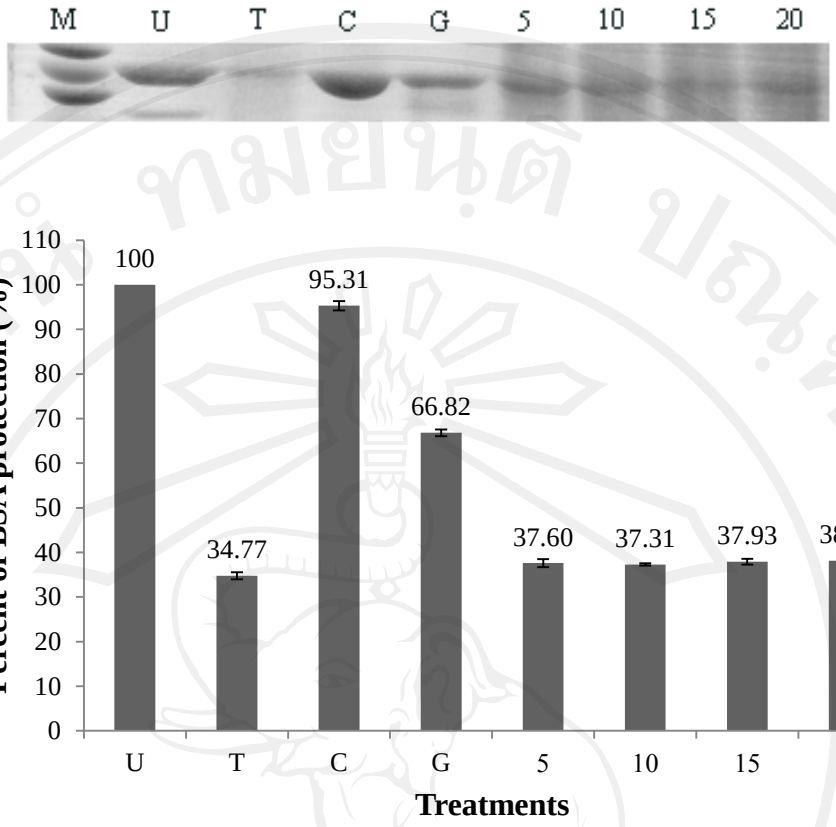

Figure 4.10 PAGE profile of BSA protein and percent of BSA protein protection after treatment with ethanolic extract of *Hiptage* sp.; M = marker, U = untreated BSA, T = $Cu^{2+}$and H_2O_2 treated BSA, C = control DMSO, G = Glutathione treated BSA, 5 = 5 mg/ml of extract treated BSA, 10 = 10 mg/ml of extract treated BSA, 15 = 15 mg/ml of extract treated BSA, 20 = 20 mg/ml of extract treated BSA

4.3.3 Plant isolation and their antioxidant activity

From preliminary study, two medicinal plants; *E. prostrata* and *Hiptage* sp. which gave high percentage yield and high antioxidant activity, were selected for partition purification using partition technique and column chromatography. Then, an effective fraction was further screened for phytochemical constituents including alkaloids, flavonoids, coumarins, saponins, cardiac glycosides, antraquinone glycosides, tannins and phenolics.

1) Fractionation of bioactive constituents of *E. prostrata*

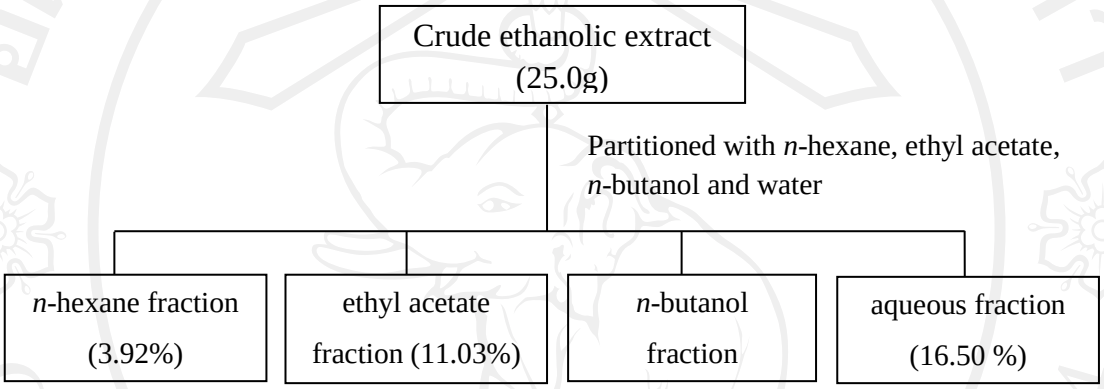

Figure 4.11 Schematic diagram shows the isolation procedure of *E. prostrata* by partition technique

In preliminary screening, the crude ethanolic extract of *E. prostrata* was fractionated by partition technique to obtain four fractions including *n*-hexane, ethyl acetate, *n*-butanol and aqueous fractions. For percentage yield recovery, the aqueous fraction gave the highest percentage yield with 16.50% followed by ethyl acetate, *n*-hexane and *n*-butanol fractions with 11.03, 3.92 and 2.18%, respectively (Figure 4.11). Four fractions were determined for antioxidant activity and evaluated total phenolic content as shown in Table 4.9-4.10.

For ABTS radical scavenging activity, *n*-butanol fraction had the highest antioxidant activity with TEAC values 105.808 mg TE/ g extract followed by ethyl acetate, aqueous and *n*-hexane fractions with TEAC values 95.295, 40.954 and 12.008 mg TE/ g extract, respectively. For DPPH radical scavenging activity, *n*-butanol fraction also showed the highest DPPH radical scavenging activities with 94.769 mg GAE/g extract followed by ethyl acetate, aqueous and *n*-hexane fractions with 67.210,

13.387 and 5.614 mg GAE/g extract, respectively (Table 4.9). Moreover, *n*-butanol fraction also had the highest total phenolic compound with 90.288 mg GAE/ g extract, followed by ethyl acetate, aqueous and *n*-hexane fractions with 74.820, 18.945 and 16.627 mg GAE/g extract, respectively (Table 4.10). Therefore, it was concluded that the *n*-butanol fraction was active fractions that had the highest antioxidant activity in ABTS and DPPH methods. Moreover, *n*-butanol fraction also had the highest total phenolic content.

Table 4.9 Antioxidant activity of *E. prostrata* fractions by ABTS and DPPH method

Fractions	**ABTS TEAC (mg TE/g of extract)*±SD**	**DPPH GAE (mg GAE/g of extract)*±SD**
n-hexane	12.008 ±0.077a	5.614 ±0.151a
ethyl acetate	95.295 ± 0.644d	67.210 ± 0.056d
n- butanol	105.808 ± 0.575e	94.769 ± 1.800e
aqueous	40.954 ± 0.197b	13.387 ± 0.262b
crude extract	90.447 ± 0.122c	23.462 ± 0.806c

(*) Data in table are given as mean ± standard deviation (SD) of triplicate experiments. Statistical comparison between groups applied using Post hoc Duncan test. Values with different alphabets within each column are significantly different (*P<0.05*)

Table 4.10 Total phenolic content of *E. prostrata* fractions

Fractions	**Total phenolic content GAE (mg GAE/g of extract)*±SD**
n-hexane	16.627 ± 0.366^{a}
ethyl acetate	74.820 ± 0.905^{d}
n- butanol	90.288 ± 0.240^{e}
aqueous	18.945 ± 0.749^{b}
crude extract	36.731 ± 0.485^{c}

(*) Data in table are given as mean ± standard deviation (SD) of triplicate experiments. Statistical comparison between groups applied using Post hoc Tukey test. Values with different alphabets within each column are significantly different (*P<0.05*)

For bioactive fraction, the ethanolic extract of *E. prostrata* was further isolated by column chromatography using celite as a stationary phase to obtain 12 fractions (Figure 4.12). The yields of each fraction were shown in Table 4.11. Five fractions, which gave TLC pattern related to *n*-butanol fraction from previous data, were selected to evaluate their antioxidant activity (Figure 4.13).

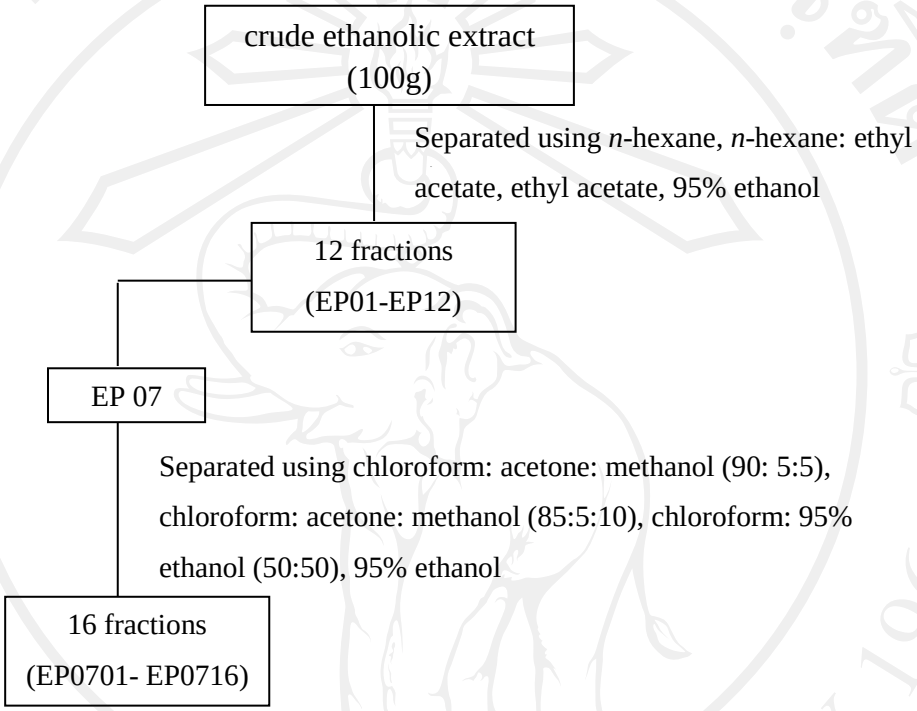

Figure 4.12 Schematic diagram shows the isolation procedure of *E. prostrata* by column chromatography

Table 4.11 Percentage yield of each fraction from *E. prostrata* (100 g) after separating by column chromatography using celite as a stationary phase

Fractions	**Weight (g)**	**Yield (%)**
EP01	23.94	23.94
EP02	0.49	0.49
EP03	0.63	0.63
EP04	2.70	2.70
EP05	2.09	2.09
EP06	0.50	0.50
EP07	0.85	0.85
EP08	6.40	6.40
EP09	1.00	1.00
EP10	0.64	0.64
EP11	42.42	42.42
EP12	7.06	7.06

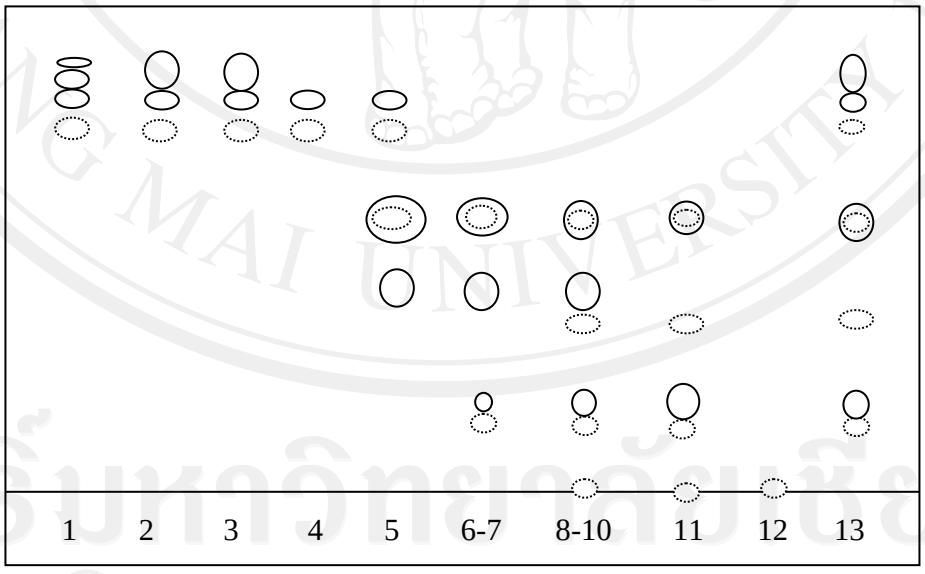

Figure 4.13 TLC Chromatograms of isolated fractions of 12 sub-fraction of *E. prostrata* (EP01-EP12) (1-12) compared to *n*-butanol fraction (13) at wavelength 254 nm (----) and 366 nm (—) using chloroform: methanol (85: 15) as a mobile phase.

The result of antioxidant activity was given in Table 4.12-4.13. As a result, EP07 fraction gave the highest antioxidant activity in both ABTS and DPPH methods and also gave the highest total phenolic content. EP07 gave the highest ABTS radical scavenging activity with TEAC values of 132.845 mg TE/g extract followed by EP08, EP11, EP05 and EP 12 with TEAC values of 106.499, 86.584, 65.759 and 7.497 mg TE/ g extract. Furthermore, EP07 also gave the highest DPPH radical scavenging activity with GAE value of 81.612 mg GAE/ g extract followed by EP08, EP11, EP05 and EP12 by 59.699, 57.041, 52.785 and 35.369 mg GAE/g extract, respectively. For total phenolic content, EP07 fraction had the highest phenolic content of 164.069 mg GAE/ g extract followed by EP08, EP11, EP05 and EP05 by 146.483, 87.650, 84.373 and 56.635 mg GAE/ g extract, respectively. Therefore, this study was indicated that EP07 fraction had the highest ABTS and DPPH radical scavenging activity and also gave the highest total phenolic content so this fraction was suitable in further isolated to obtain active compounds.

Table 4.12 Antioxidant activity of *E. prostrata* fractions by ABTS, DPPH method and total phenolic content

Fractions	**ABTS TEAC (mg TE/g of extract)*±SD**	**DPPH GAE (mg GAE/g of extract)*±SD**
EP05	65.759 ± 0.969^{b}	52.785 ± 0.336^{c}
EP07	132.845 ± 0.239^{f}	81.612 ± 0.328^{f}
EP08	106.499 ± 0.925^{e}	59.699 ± 0.664^{e}
EP11	86.584 ± 1.430^{c}	57.041 ± 2.544^{d}
EP12	7.497 ± 0.054^{a}	35.369 ± 0.187^{b}
Crude extract	90.447 ± 0.122^{d}	23.462 ± 0.806^{a}

(*) Data in table are given as mean ± standard deviation (SD) of triplicate experiments. Statistical comparison between groups applied using Post hoc Duncan test. Values with different alphabets within each column are significantly different ($P<0.05$)

Table 4.13 Total phenolic content of *E. prostrata* fractions

Fractions	**Total phenolic content GAE (mg GAE/g of extract)*±SD**
EP05	84.373 ± 7.925^{c}
EP07	164.069 ± 6.512^{e}
EP08	146.483 ± 8.636^{d}
EP11	87.650 ± 1.912^{c}
EP12	56.635 ± 1.114^{b}
Crude extract	36.731 ± 0.485^{a}

(*) Data in table are given as mean ± standard deviation (SD) of triplicate experiments. Statistical comparison between groups applied using Post hoc Duncan test. Values with different alphabets within each column are significantly different ($P<0.05$).

Therefore, EP07 fraction was further isolated using column chromatography to obtain 16 fractions. The percentage yield of 16 fractions was shown in Table 4.14. The fraction EP07-13 showed the highest percentage yield with 0.0184 % followed by EP07-12, EP07-07, EP07-09 and EP07-10 by 0.0150, 0.0124, 0.0116 and 0.0110 % while the fraction EP07-16 had the lowest percentage yield with 0.0018 % (Table 4.17). After that all fractions were further determined for antioxidant activity using DPPH assay and total phenolic content evaluation.

Table 4.14 Percentage yield of 16 subfraction by column chromatography using silica gel as stationary phase

Fractions	**Weight (g)**	**Yield (%)**
EP07-01	0.0149	0.0055
EP07-02	0.0132	0.0048
EP07-03	0.0126	0.0046
EP07-04	0.0164	0.0060
EP07-05	0.0148	0.0054
EP07-06	0.0194	0.0071
EP07-07	0.034	0.0124
EP07-08	0.0234	0.0086
EP07-09	0.0316	0.0116
EP07-10	0.0304	0.0111
EP07-11	0.0233	0.0085
EP07-12	0.0409	0.0150
EP07-13	0.0503	0.0184
EP07-14	0.021	0.0077
EP07-15	0.0257	0.0094
EP07-16	0.0049	0.0018

For the results of antioxidant activity, DPPH and total phenolic content were determined in this study present in Table 4.15. For DPPH assay, it was found that fraction EP07-07 showed the highest antioxidant activity with 142.918 mg GAE/g extract followed by EP07-06, EP07-15, EP07-08, and EP07-10 fractions with GAE values 119.887, 107.542, 71.965 and 70.475 mg GAE / g extracts, respectively. On the other hand, the fraction EP07-13 had the lowest DPPH radical scavenging ability with GAE value 15.241 mg GAE/ g extract. For total phenolic content determination, fraction EP07-07 had significantly the highest total phenolic contents with 493.205 mg GAE/g extract compare with other fractions. Consequently, the fractions EP07-06, EP07-15, EP07-10, EP07-08 with GAE values 377.698, 310.152, 276.978, 258.193 mg GAE/g extracts, respectively. However, fractions EP07-02, EP07-03, EP07-04,

EP07-05 and EP07-16 had low percentage yield, thus they could not determine for antioxidant and total phenolic content.

In the overall trend, this result indicated that after fractionation this plant gave antioxidant activity and total phenolic content higher than crude extract. These results revealed that the ethanolic extract of *E. prostrata* had higher ability to scavenge DPPH radical more than aqueous extract. After fractionation, the ability of plant extract to scavenge DPPH radical has been significant increasing. Likewise, Gurusamy and Saranya (2010) found that the ethanolic extracts of this plant showed high reducing activity in DPPH. In addition, ethanolic extract of *E. prostrata* had high antioxidant activities, which analyzed by DPPH, 2,2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assays and scavenging of nitric oxide radical with IC_{50} 21.35, 41.8, 17.55 μg/ml, respectively (Baldi *et al.,* 2011).

For phytochemical analysis, flavonoids, and hydrolysable tannins were major constituents in ethanolic extract while, only flavonoids were found in fraction EP07 (Table 4.16). Similar results were earlier reported by previous worker found that triterpenoids, flavonoids and phenolc acids, which are major constituents in methanolic extract of *E. prostrata* (Lee *et al.,* 2010). This result was similar to the report that revealed tannins, flavonoids, coumestans, saponin and alkaloids were present in methanolic extract of this plant (Dalal *et al.,* 2010). The presence of flavonoids and phenolics were likely to be responsible for the free radical scavenging observed as there were major compounds.

Table 4.15 Antioxidant activity of *E. prostrata* fractions by DPPH radical scavenging assay and total phenolic content

Fraction	DPPH GAE (mg GAE/g of extract)*±SD	Total phenolic content GAE (mg TE /g of extract)*±SD
EP07-01	31.633 ± 0.392^{c}	135.891 ± 2.544^{c}
EP07-02	-	-
EP07-03	-	-
EP07-04	-	-
EP07-05	-	-
EP07-06	119.887 ± 0.607^{i}	377.698 ± 3.391^{j}
EP07-07	142.918 ± 0.762^{j}	493.205 ± 10.174^{k}
EP07-08	71.965 ± 0.322^{g}	258.193 ± 2.544^{g}
EP07-09	54.381 ± 0.170^{d}	205.835 ± 5.087^{f}
EP07-10	70.475 ± 0.237^{f}	276.978 ± 0.848^{h}
EP07-11	30.902 ± 0.627^{c}	151.479 ± 2.544^{d}
EP07-12	23.414 ± 0.273^{b}	116.307 ± 1.696^{b}
EP07-13	15.241 ± 0.411^{a}	89.528 ± 4.239^{a}
EP07-14	54.140 ± 0.428^{d}	180.256 ± 11.022^{e}
EP07-15	107.542 ± 0.417^{h}	310.152 ± 4.239^{i}
EP07-16	-	-
EP07	63.018 ± 0.340^{e}	205.046 ± 2.544^{f}

(*) Data in table are given as mean ± standard deviation (SD) of triplicate experiments. Statistical comparison between groups applied using Post hoc Duncan test. Values with different alphabets within each column are significantly different ($P<0.05$)

(-) Not determined

Table 4.16 Primary chemical screening test of crude ethanolic extract of *E. prostrata*

Test	**Crude ethanolic extract**	**Fraction EP07-07**
1. Alkaloids		
Dragendroff's reagent	-	-
Wagner' reagent	-	-
Hager's reagent	-	-
Mayer's regent	-	-
2. Flavonoids	+	+
3. Coumarins	-	-
4. Saponins	-	-
5. Cardiac glycosides		
Liebermann- Burchard's test (steroidal nucleus)	-	-
Keller - Kikiani's test (deoxy sugar)	-	-
6. Antraquinone glycosides	-	-
7. Tannins		
1% gelatin test	+	-
1% $FeCl_3$ test	+	-
Formaldehyde – HCl test	-	-
Vanillin – HCl test	-	-
$CaOH_2$ solution test	+	-
Lead acetate test	+	-
8. Phenolics	+	+

(+) = Positive result (-) = Negative result

2) Isolation of bioactive fractions of *Hiptage* sp.

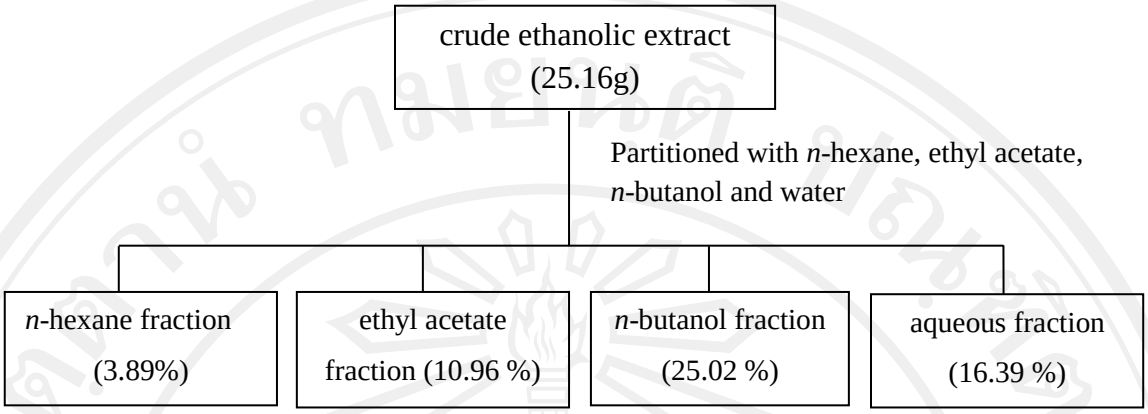

Figure 4.14 Schematic diagram shows the isolation procedure of *Hiptage* sp. by partition technique

The crude ethanolic extract of *Hiptage* sp. was separated by partition technique to obtain four fractions including *n*-hexane, ethyl acetate, *n*-butanol and aqueous fractions. From our result, it was found that *n*-butanol fraction had the highest percentage yield of 25.02%, followed by aqueous, ethyl acetate and *n*-hexane fractions of 16.39, 10.96 and 3.89 %, respectively (Figure 4.14). The result of antioxidant activity and total phenolic content was shown in Table 4.17-4.18. For ABTS assay, ethyl acetate fraction showed the highest antioxidant activity with TEAC value 496.835 mg TE/g extract followed by n-butanol, water and hexane with TEAC values 370.110, 184.300 and 32.884 mg TE/g extract, respectively. For DPPH assay, ethyl acetate fraction also gave the highest antioxidant activity with GAE value 191.508 mg GAE/g extract followed by *n*-butanol, aqueous and *n*-hexane fraction with GAE value 144.137, 80.342 and 6.700 mg GAE/g extract, respectively. Moreover, ethyl acetate fraction also gave the highest total phenolic content with GAE value 352.038 mg GAE/g extract followed by *n*-butanol, aqueous and *n*-hexane fraction with GAE values 294.884, 77.738 and 14.109 mg GAE/g extract, respectively.

Table 4.17 Antioxidant activity and total phenolic content of crude and fractions of *Hiptage* sp.

Fractions	**ABTS assay TEAC (mg TE/g of extract)*±SD**	**DPPH assay GAE (mg GAE/g of extract)*±SD**
n-hexane	32.884 ± 0.299[a]	6.700 ± 0.027[a]
ethyl acetate	496.835 ± 9.569[d]	191.508 ± 0.756[d]
n- butanol	370.110 ± 4.041[c]	144.137 ± 0.911[c]
aqueous	184.300 ± 5.266[b]	80.342 ± 1.002[b]
Crude extract	199.694 ± 7.742[b]	77.913 ± 1.477[b]

(*) Data in table are given as mean ± standard deviation (SD) of triplicate experiments. Statistical comparison between groups applied using Post hoc Duncan test. Values with different alphabets within each column are significantly different (*P<0.05*)

Table 4.18 Total phenolic content of crude and fractions of *Hiptage* sp.

Fractions	**Total phenolic content GAE (mg/g of extract)*±SD**
n-hexane	14.109 ± 0.604[a]
ethyl acetate	352.038± 20.716[e]
n- butanol	294.884 ± 1.816[d]
aqueous	77.738 ± 2.338[b]
crude extract	132.454 ± 1.971[c]

(*) Data in table are given as mean ± standard deviation (SD) of triplicate experiments. Statistical comparison between groups applied using Post hoc Duncan test. Values with different alphabets within each column are significantly different (*P<0.05*)

For bioactive fraction, the ethanolic extract of *Hiptage* sp. was further isolated by column chromatography using celite as a stationary phase to obtain 8 fractions (Figure 4.15). For percentage yield recovery, the SP04 fraction had the highest percentage yield of 15.03 % followed by SP05, SP06, SP01, SP07, SP02, and SP08 of 14.33, 4.26, 3.71, 2.91 and 1.55%, respectively while SP03 fraction gave the lowest percentage with percentage yield of 0.08% (Table 4.19).

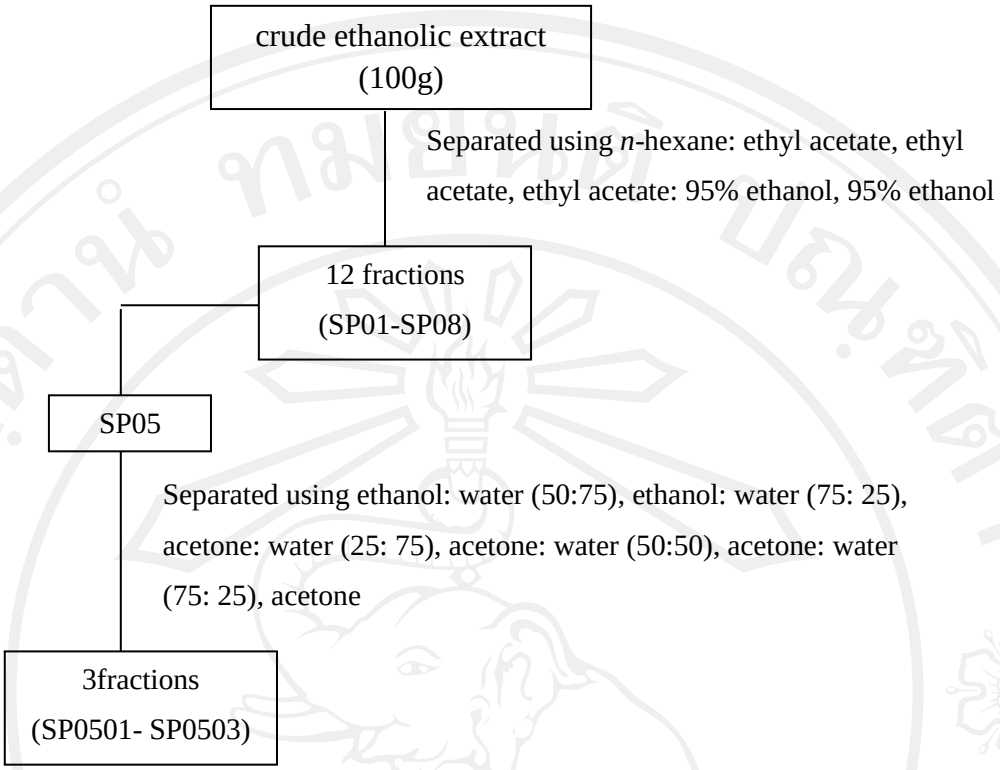

Figure 4.15 Schematic diagram shows the isolation procedure of *Hiptage* sp. by column chromatography

Table 4.19 Percentage yield of each fraction from *Hiptage* sp. (100 g)

Fractions	**Weight (g)**	**Yield (%)**
SP01	5.5702	3.71
SP02	2.3324	1.55
SP03	0.1126	0.08
SP04	22.550	15.03
SP05	21.500	14.33
SP06	6.3894	4.26
SP07	4.4174	2.94
SP08	1.425	0.95

Eight fractions were evaluated for their antioxidant activity by ABTS and DPPH assay and determined total phenolic content by Folin-Ciocalteau method and the results were given in Table 4.20-4.21. For ABTS assay, SP05 fraction had the highest radical scavenging ability of 636.726 mg TE/g extract followed by SP04, SP08, SP07, and SP06, SP02 and SP01 with TEAC values of 415.921, 325.976, 241.801, 202.773, 106.403, 96.939 mg TE/ g extract, respectively. On the contrary, SP03 fraction gave the lowest ABTS radical scavenging activity with TEAC value of 62.197 mg TE/g extract. For DPPH assay, SP05 fraction also had the highest antioxidant activity with GAE value of 190.815 mg GAE/ g extract followed by SP04, SP07, SP08, SP06, SP01 and SP02 with GAE values of 179.146, 168.180, 116.074, 105.747, 70.395 and 65.139 mg GAE/g extract, respectively. The SP03 fraction gave the lowest DPPH radical scavenging activity with GAE value of 35.481 mg GAE/ g extract. For total phenolic content assay, SP05 also had the highest total phenolic content by 323.941 mg GAE/ g extract followed by SP04, SP08, SP06, SP02, SP01, and SP07 with GAE values of 266.387, 241.527, 128.937, 125.260, 106.035 and 104.677 mg GAE/ g extract, respectively. On the other hand, SP03 also gave the lowest total phenolic content with GAE value of 83.733 mg GAE/g extract. Therefore, it was concluded that SP05 fraction had the highest antioxidant activity in all antioxidant testing model including ABTS, DPPH and total phenolic content assay.

Table 4.20 Antioxidant activity of *Hiptage sp.* fractions by ABTS decolorization assay

Fractions	**ABTS assay TEAC (mg TE/g of extract)*±SD**	**DPPH assay GAE (mg GAE/g of extract)*±SD**
SP01	96.939 ±0.741^{b}	70.395 ± 0.452^{c}
SP02	106.403 ± 0.362^{c}	65.139 ±0.622^{b}
SP03	62.197 ± 0.479^{a}	35.481 ±0.276^{a}
SP04	415.921 ± 2.817^{h}	179.146 ±0.531^{h}
SP05	636.726 ± 2.269^{i}	190.815 ±1.239^{i}
SP06	202.773 ± 0.894^{e}	105.747 ±0.518^{e}
SP07	241.801 ± 0.904^{f}	168.180 ± 0.172^{g}
SP08	325.976 ± 1.162^{g}	116.074 ± 3.783^{f}
Crude extract	196.208 ± 1.586^{d}	73.474 ± 0.106^{d}

(*) Data in table are given as mean ± standard deviation (SD) of triplicate experiments. Statistical comparison between groups applied using Post hoc Duncan test. Values with different alphabets within each column are significantly different (*P<0.05*)

Table 4.21 Total phenolic content of *Hiptage* sp. fractions

Fractions	**Total phenolic content GAE (mg GAE /g of extract)*±SD**
SP01	106.035 ± 4.257^{b}
SP02	125.260 ±8.530^{c}
SP03	83.733 ±2.779^{a}
SP04	266.387 ± 22.972^{e}
SP05	636.726 ± 2.269^{i}
SP06	128.937 ± 2.639^{c}
SP07	104.677 ± 0.317^{b}
SP08	241.527 ± 4.325^{d}
Crude extract	136.91 ± 0.360^{c}

(*) Data in table are given as mean ± standard deviation (SD) of triplicate experiments. Statistical comparison between groups applied using Post hoc Duncan test. Values with different alphabets within each column are significantly different (*P<0.05*)

Therefore, SP05 fraction which had the highest antioxidant activity was further isolated by reverse phase column chromatography using sephadex-LH20 as a stationary phase. After fractionation, three fractions was obtained and further determined for antioxidant activity. The percentage yield of these fractions was shown in Table 4.22. The highest yield of extraction was obtained from SP05-3 with 32.90 % followed by SP05-2 and SP05-1 with 22.20 and 14.50 %, respectively.

Table 4.22 Percentage yield of each fraction from SP05 fraction (0.2 g)

Fractions	**Weight (g)**	**Yield (%)**
SP05-1	0.0290	14.50
SP05-2	0.0444	22.20
SP05-3	0.0658	32.90

From the antioxidant activity testing, SP05-2 gave the highest ABTS radical scavenging activity with TEAC value of 323.176 mg TE/g extract followed by SP05-3 and SP05-1 with values of 263.977 and 24.987 mg TE/g extract. For DPPH assay, SP05-2 also gave the highest activity with GAE value of 184.638 mg GAE/ g extract followed by SP05-3 and SP05-1 with values of 97.446 and 3.000 mg GAE/g extract. Moreover, SP05-2 also had the highest total phenolic content with GAE value of 299.720 mg GAE/ g extract followed by SP05-3 and SP05-1 with GAE values of 261.751 and 18.8945 mg GAE/g extract (Table 4.23-4.24).

In the past, *Hiptage* sp. was used as a traditional medicine for traditional medicine in Asia pacific. Leaves and roots are used to relieve back and flank pains, and rheumatism treatment. Moreover, traditional knowledge believes that this plant can provide energy especially in case of exhaustion (Wiart, 2006). However, the pharmacological of this plant are to date unexplored. Few publications reported the *Hiptage* sp. extract have any biological effects on productive activity of male rats. However, antioxidant activity of this plant was not investigated. For phytochemical screening, only SP05 fraction was evaluated and the results have been showed in Table 4.25. It was observed from result that this fraction contained tannin and phenolics.

Table 4.23 Antioxidant activity of SP05 fractions by ABTS decolorization assay

Fractions	**ABTS assay TEAC (mg TE /g of extract)*±SD**	**DPPH assay GAE (mg GAE /g of extract)*±SD**
SP05-1	24.987 ±1.270[a]	3.000± 0.043[a]
SP05-2	323.176 ± 27.190[c]	184.638 ± 8.127[c]
SP05-3	263.977 ± 7.398[b]	97.446 ±5.167[b]
SP05	636.726 ± 2.269[d]	190.815 ±1.239[c]

(*) Data in table are given as mean ± standard deviation (SD) of triplicate experiments. Statistical comparison between groups applied using Post hoc Duncan test. Values with different alphabets within each column are significantly different (*P<0.05*)

Table 4.24 Total phenolic content of SP05 fractions

Fractions	**Total phenolic content GAE (mg GAE /g of extract)*±SD**
SP05-1	18.8945 ± 0.668[a]
SP05-2	299.720 ± 2.861[c]
SP05-3	261.751 ± 15.047[b]
SP05	636.726 ± 2.269[d]

(*) Data in table are given as mean ± standard deviation (SD) of triplicate experiments. Statistical comparison between groups applied using Post hoc Duncan test. Values with different alphabets within each column are significantly different (*P<0.05*)

Table 4.25 Primary chemical screening test of SP05 fraction

Test	Result
1. Alkaloids	
Dragendroff's reagent	-
Wagner' reagent	-
Hager's reagent	-
Mayer's regent	-
2. Flavonoids	-
3. Coumarins	-
4. Saponins	-
5. Cardiac glycosides	
Liebermann- Burchard test's (steroidal nucleus)	-
Keller-Kikiani's test (deoxy sugar)	-
6. Antraquinone glycosides	-
7. Tannins	
1% gelatin test	+
1% $FeCl_3$ test	+
Formaldehyde – HCl test	+
Vanillin – HCl test	+
$CaOH_2$ solution test	+
Lead acetate test	+
8. Phenolics	+

(+) = Positive result (-) = Negative result