CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 In *vitro* study: The effects of antioxidant activity of 30 Thai indigenous plants against diabetic stress in the *in vitro* study.

4.1.1 Indigenous plants with the yield of ethanol extracts.

In this study, 30 Thai indigenous plants which have been used as an alternative treatment in DM were obtained using various varieties of plant parts. They were extracted with 95% ethanol for antioxidant activity analysis. The plant was classified by family name. The high yields of extracts were found in *Momordica charantia* Linn. fruit (12.96%), *Artocarpus heterophyllus* Lamk. leaf (11.96%) and *Allium sativum* Linn. bulb (9.25%) from the families of Cucurbitaceae, Moraceae and Alliaceae, respectively. However, the extract of *Costus speciosus* Koen. J.E. Smith. rhizome in the Costaceae families was found to have the lowest yield (0.24%) as shown in Table 4.1.

Table 4.1 Percentage yield of ethanolic crude extracts in Thai indigenous plants

Family name	Scientific name	Thai name	Fresh weight (g)	Extract weight (g)	%Yield
1. Alliaceae	*Allium sativum* Linn.	กระเทียม	200	18.5	9.25
2. Apiaceae	*Eryngium foetidum* Linn.	ผักชีฝรั่ง	167	9.3	5.56
3. Asclepiadaceae	*Gymnema inodorum* Decne.	ผักเชียงดา	500	28.9	5.78
4. Combretaceae	*Terminalia chebula* Retz	สมอไทย	1000	88.3	8.83
5. Convolvulaceae.	*Ipomcea aquatica* Forsk.	ผักบุ้ง	188	4.3	2.28
6. Costaceae	*Costus speciosus* Koen. J.E. Smith	เอื้องหมายนา	500	1.3	0.24
7. Cucurbitaceae	*Luffa acutangula* (Linn.) Roxb.	บวบเหลี่ยม	500	11.3	2.26
8. Cucurbitaceae	*Coccinia grandis* (L.) Voigt. Syn	ตำลึง	500	8.2	1.64
9. Cucurbitaceae	*Momordica charantia* Linn.	มะระขี้นก	500	64.8	12.96
10. Euphorbiaceae	*Phyllanthus emblica* Linn.	มะขามป้อม	500	12.9	2.58

Table 4.1 (continued)

Family name	Scientific name	Thai name	Fresh weight (g)	Extract weight (g)	%Yield
11. Fabaceae	*Acacia penneata* Linn.	ชะอม	95	4.7	7.35
12. Fabaceae	*Glycine max* Merr.	ถั่วเหลือง	208	15.3	2.58
13. Labiatae	*Ocimum basilicum* Linn.	แมงลัก	200	8.5	4.10
14. Leguminosae	*Pisum sativum* Linn.	ถั่วลันเตา	114	8.4	7.36
15. Malvaceae	*Abelmoschus esculentus* Linn.	กระเจี๊ยบ	300	14.5	4.83
16. Moraceae	*Artocarpus heterophyllus* Lamk.	ขนุน	500	59.8	11.96
17. Musaceae	*Musa sapientum* Linn.	ปลีกล้วย	530	6.7	1.34
18. Papilionaceae	*Sesbania grandiflora* (L.) Desv.	แคแดง	300	17.5	5.83
19. Pipereaceae	*Piper samentosum* Roxb.	ใบชะพลู	500	22.7	4.54
20. Piperaceae	*Piper retrofractum* Vahl.	ดีปลี	213	10.3	4.83

Table 4.1 (continued)

Family name	Scientific name	Thai name	Fresh weight (g)	Extract weight (g)	%Yield
21. Rubiaceae	*Morinda citrifolia* Linn	ยอ	290	17.3	5.97
22. Rutaceae	*Aegle marmelos* correa (L.)	ใบมะตูม	500	28.7	5.74
23. Saururaceae	*Houttuynia cordata* Thunb.	พลูคาว	500	5.8	1.16
24. Solanaceae	*Capsicum frutescens* Linn.	พริกขี้หนู	200	10.9	5.45
25. Solanaceae	*Lycopersicon esculentum* Mill.	มะเขือเทศ	488	16.3	3.34
26. Solanaceae	*Solanum torvum* Sw.	มะเขือพวง	500	23.8	4.76
27. Umbelliferae	*Apium graveolens* Linn.	ผักคื่นไช่	200	8.7	4.35
28. Umbelliferae	*Coriandrum sativum* Linn.	ผักชี	200	8.2	4.10
29. Zingiberaceae	*Kaempferia parviflora* Wall.	กระชายดำ	1000	53.9	5.39
30. Zingiberaceae	*Zingiber officinale* Linn. Adrak	ขิง	500	10.9	2.18

4.1.2 Total antioxidant activity by ABTS

Assessment of the antioxidant activity has been a variety method. In the *in vitro* part of the present study, these are using an ABTS free radical decolorization assay [148], TBARS assay for lipid peroxidation [149] and glycation of protein analysis [150]. Antioxidants can act at different levels in the oxidative sequence. Free radicals are a major cause at the propagation stage of the oxidative process. In diabetic cases, hyperglycemia causes macro- and microvascular oxidative stress damage, leading to many major diabetic complications. Reduction of free radical levels should improve metabolic function of beta cells, vascular endothelial cells, fat and muscle cells, and platelets. Decreased glycosylation and oxidation of proteins should also reduce the complications such as atherosclerosis, retinopathy, nephropathy and neuropathy [140-141]. There is a high potential that scavenging free radicals could inhibit distribution of oxidation [160]. Many plant studies reported the potential of antioxidants and its compounds in radical-scavenging effects [10-19]. Thus, in this *in vitro* study of selected-Thai indigenous plants, the plants with the highest potential antioxidant effect were used as the raw material in the production of biologically fermented Thai plant beverage product. Their bioactive compounds were tested for radical-scavenging antioxidants.

The oxidation process is one of the most important routes for producing free radicals. There has been an interest in the potential of plants, antioxidant action in reducing free radical induced tissue injury. Particularly,

free radicals due to disease or physical stress cause depletion of immune system antioxidants and a change in gene expression inducing abnormal proteins. Strong antioxidants as free radical scavengers may be necessary. In this study, 95% ethanol crude extracts of 30 Thai indigenous plants were analyzed for antioxidant activity and expressed as TEAC levels (mg trolox equivalent/g dry weight of plant extract). The levels of all samples (as shown in Table 4.2) ranged from 0.24 to 226.52 mg trolox /g dry weight of plant extract. The highest antioxidant activity of five plants were *Phyllanthus emblica* Linn. (PE), *Terminalia chebula* Retz. TC, *Morinda citrifolia* Linn. (MC), *Kaempferia parviflora* Wall. (KP) and *Houttuynia cordata* Thunb. (HC) (226.52, 95.02, 32.60, 21.21 and 18.93 mg trolox/g dry weight of plant extract), respectively. However, *Luffa acutangula* (Linn.) Roxb. the fruit from Cucurbitaceae had the lowest level of antioxidant activity (0.24 mg trolox/g dry weight of plant extract). The highest levels, which indicated a strong antioxidant index, were from several families with an astringent taste. In addition, the results exhibited that they had a high antioxidant activity level, similarly as observed in the previous studies [29, 161]. Many reports indicate consumption of fruits and vegetables has been associated with a lowered incidence of degenerative diseases including cancer, diabetes, heart disease, inflammation, immune system diminish and brain dysfunction [10, 13-17, 26, 52, 96, 98, 114-115]. Especially, when protective effects in diabetes are considered, in large part, it is related to the various antioxidants contained in them. There is great evidence to

indicate that free radicals cause oxidative damage to lipids, proteins, and nucleic acids. Antioxidants, which can inhibit or decrease the oxidation of an oxidisable substrate in a chain reaction, could be used for reducing oxidative stress in diabetes [3, 5, 6-7, 10-12, 60-61, 67-71, 90-93, 98]. Hence, Thai indigenous plants in this study can be considered good sources of natural antioxidant, since their extracts were found to have high antioxidant activity levels. The five highest antioxidant activity levels were detected in PE, TC, MC, KP and HC, respectively. These plants will be investigated for total polyphenolic compounds and the active components present, especially phenols, flavonoids and tannins.

Table 4.2 Total antioxidant activity of ethanolic crude extracts of Thai indigenous plants by ABTS free radicals decolorization assay

Plants name	**TEAC (mg Trolox/g crude extract)**[a]
Phyllanthus elica Linn.	226.52±0.02
Terminalia chebula Retz.	95.02±0.00
Morinda citrifolia Linn.	32.60±0.00
Kaempferia parviflora Wall.	21.21±0.00
Houttuynia cordata Thunb.	18.93±0.00
Artocarpus heterophyllus Lamk	18.73±0.00
Ocimum basilicum Linn.	10.01±0.01
Coccinia grandis (L.) Voigt. Syn.	9.02±0.04
Zingiber officinale Linn. Adrak.	7.72±0.01
Musa sapientum Linn.	6.52±0.00
Eryngium foetidum Linn.	5.94±0.02
Ipomcea aquatica Forsk.	4.67±0.00
Aegle marmelos Correa	4.65±0.02
Gymnema inodorum Decne.	3.76±0.01
Capsicum frutescens Linn.	3.71 ±0.02
Solanum torvum SW.	3.68 ±0.00
Acacia penneata Linn	2.46±0.00

Table 4.2 (continued)

Plants name	TEAC (mg Trolox/g crude extract)[a]
Coriandrum sativum Linn.	2.23±0.04
Piper samentosum Roxb	2.20±0.01
Costus speciosus Koen. J.E. Smith.	2.02±0.01
Sesbania grandiflora (L.) Desv	1.93±0.01
Apium graveolens Linn.	1.93±0.01
Piper retrofractum Vahl.	1.82±0.01
Momordica charantia Linn.	1.20±0.01
Pisum sativum Linn.	1.19±0.01
Glycine max Merr.	1.17±0.00
Abelmoschus esculentus Linn.	0.77±0.01
Allium sativum Linn.	0.72±0.00
Lycopersicon esculentum Mill.	0.43±0.00
Luffa acutangula (Linn.) Roxb.	0.24±0.32

Note a: values expressed means ± SD (n = 5)

4.1.3 Lipid peroxidation study of diabetic oxidative stress

The main mechanism of antioxidant action in food is radical scavenging, inhibiting the function of various membrane proteins such as lipid peroxidation or glycation *in vitro* [13]. In this model, oxidative stress was generated by blood plasma of diabetes patients and reaction of lipid peroxidation by TBARS. Plant extracts scavenged free radicals by their antioxidant activity. Table 4.3 shows the results of co-incubation with plant extracts at a concentration of 1 µg/ml. Among the 30 plants, *Phyllanthus emblica* Linn. had the highest lipid peroxidation inhibition following by *Lycopersicon esculentum* Mill., *Solanum torvum* Sw., *Houttuynia cordata* Thunb. and *Ocimum basilicum* Linn. (0.07, 0.22, 0.30, 0.45 and 0.90 µM MDA), respectively, defined as free radical scavenging activity in lipid peroxidation. This is significant, confirming the antioxidant mechanism, which explains the etiology and pathophysiology of the biological effects, especially in regards to cell damage and cellular degeneration, as seen in complications of diabetes [5, 162]. As many studies have reported, plants have significant antioxidant potential and free radical scavenging activities on lipid peroxidation [13-15], particularly in the diabetes model [11, 76, 80-82, 91-92, 98].

Table 4.3 Total antioxidant activity of ethanolic crude extracts of Thai indigenous plants by TBARS assay for lipid peroxidation

Plants name	**MDA (μM)[b]**
Phyllanthus emblica Linn.	0.07±0.01
Lycopersicon esculentum Mill.	0.22±0.01
Solanum torvum Sw.	0.30±0.00
Houttuynia cordata Thunb.	0.45±0.01
Ocimum basilicum Linn.	0.90±0.04
Aegle marmelos Correa.	1.12±0.03
Pisum sativum Linn.	1.19±0.03
Terminalia chebula Retz.	1.25±0.02
Artocarpus heterophyllus Lamk.	1.27±0.01
Costus speciosus Koen. J.E. Smith.	1.27 ± 0.08
Gymnema inodorum Decne.	1.27±0.11
Luffa acutangula (Linn.) Roxb.	1.34±0.03
Musa sapientum Linn.	1.34±0.01
Momordica charantia Linn.	1.57±0.06
Eryngium foetidum Linn.	2.01±0.07
Abelmoschus esculentus Linn.	2.24±0.02
Glycine max Merr.	2.54±0.03

Table 4.3 (continued)

Plants name	**MDA (µM)[b]**
Sesbania grandiflora (L.) Desv.	2.69 ±0.03
Zingiber officinale Linn. Adrak.	2.69 ±0.03
Allium sativum Linn.	2.69±0.00
Piper samentosum Roxb.	2.76±0.07
Coccinia grandis (L.) Voigt. Syn.	2.84±0.04
Piper retrofractum Vahl.	3.13±0.01
Morinda citrifolia Linn.	3.43±0.01
Kaempferia parviflora Wall.	4.08±0.05
Acacia penneata Linn.	4.78±0.07
Ipomcea aquatica Forsk.	5.07±0.06
Capsicum frutescens Linn.	5.30±0.02
Coriandrum sativum Linn.	6.87±0.05
Apium graveolens Linn.	6.94±0.07

Note b: values expressed means ± SD (n = 5)

4.1.4 Free radical scavenging activity on diabetic oxidative stress using glycation assay

Bovine serum albumin (BSA) is modified by glucose creating advanced glycation end products (AGEs) formation, which contribute to the complications in diabetes and other degenerative disorder diseases [163]. The free radicals have been shown to react in AGEs formation, which could be inhibited by using antioxidants, that is free radical scavengers. It is proposed that therapeutic strategies of anti-glycation are the use of alpha-oxoaldehyde scavengers, advanced glycation endproduct receptor antagonists and inducers of enzymatic antiglycation defense [164]. In this experiment, we found the protective effect of all plant extracts on glycation as shown in Table 4.4. The IC50 value was determined based on scavenging activity per unit mass of inhibition of glycation in μg/ml. *Phyllanthus emblica* Linn. had the highest level of inhibition of protein glycation follow by *Morinda citrifolia* Linn., *Terminalia chebula* Retz., *Piper samentosum* Roxb. and *Artocarpus heterophyllus* Lamk. (0.01, 0.02, 0.06, 0.10 and 1.65 μg/ml), respectively. The result may imply that bioactive compounds in plants persisting in the extracts would compete with glucose to bind onto protein molecules, leading to inhibiting protein glycation. Chaiyasut & Chansakaow studied the inhibitory effects of *Phyllanthus emblica* and *Kaempferia parviflora* extracts. Results reported *Phyllanthus emblica* achieved 88.09 % inhibition against protein glycation formation at 10.0 μg/ml whereas, *Kaempferia parviflora* achieved

28.10 % inhibition at 50 µg/ml. [77]. Moreover, *Phyllanthus emblica* improved glucose metabolism in streptozotocin-induced diabetic rats and relieved the oxidative stress by strong inhibition of the production of advanced glycosylated end products, in a dose-dependently significant pattern [76]. The results showed that *Phyllanthus emblica* extracts had a potential use as an anti-aging agent against oxidative protein damage. In India, a beneficial effect of *Phyllanthus emblica* against glycoproteins in STZ-induced diabetes in rats was exhibited and a rationale for the use of *Terminalia chebula* in Ayurvedic medicinal treatment was provided [78]. In addition, Rudnicki et al. reported effective protection against protein damage induced by iron and glucose. These findings demonstrate that the plant extracts have potent *in vitro* and *ex vivo* antioxidant properties as shown and might be considered as possible new sources of natural antioxidants [13] as found in this the study.

Table 4.4 Glycation inhibitory activity assay of ethanolic crude extracts of Thai indigenous plants

Plants name	**Glycation inhibition ($IC_{50;}$ µg/ml)**
Phyllanthus emblica Linn.	0.01
Musa sapientum Linn.	3.63
Terminalia chebula Retz.	0.63
Artocarpus heterophyllus Lamk.	1.65
Morinda citrifolia Linn.	0.02
Gymnema inodorum Decne.	2.82
Sesbania grandiflora (L.) Desv.	1.75
Solanum torvum Sw.	3.48
Kaempferia parviflora Wall.	2.80
Momordica charantia Linn.	8.89
Lycopersicon esculentum Mill.	3.30
Houttuynia cordata Thunb.	4.82
Capsicum frutescens Linn.	2.61
Piper retrofractum Vahl.	4.57
Allium sativum Linn.	4.87
Zingiber officinale Linn. Adrak.	2.22

Table 4.4 (continued)

Plants name	**Glycation inhibition ($IC_{50;}$ µg/ml)**
Costus speciosus Koen. J.E. Smith.	3.98
Ocimum basilicum Linn.	2.96
Aegle marmelos Correa.	3.21
Coccinia grandis (L.) Voigt. Syn.	2.23
Eryngium foetidum Linn.	3.29
Ipomcea aquatica Forsk.	2.88
Acacia penneata Linn.	7.72
Coriandrum sativum Linn.	4.91
Luffa acutangula (Linn.) Roxb.	2.43
Piper samentosum Roxb.	0.10
Apium graveolens Linn.	4.43
Pisum sativum Linn.	4.91
Abelmoschus esculentus Linn.	4.24
Glycine max Merr.	3.84

4.1.5 Analysis of polyphenolic compounds by HPLC

The standard polyphenolic compounds studied in this research are rutin (3.29 min), gallic acid (5.56 min), pyrolgallol (6.35 min), catechin (22.72 min) and caffeic acid (30.24 min). HPLC chromatogram shows the major components of the plant extracts in Figure 4.1a, Figure 4.1b and Figure 4.1c. The major polyphenols in *Phyllanthus emblica* Linn. (PE), *Terminalia chebula* Retz. (TC) and *Houttuynia cordata* Thunb. (HC) are rutin, gallic acid, pyrolgallol and catechin. *Morinda citrifolia* Linn. (MC) consists of rutin, gallic acid, pyrolgallol and caffeic acid. *Kaempferia parviflora* Wall. (KP) possibly consists of rutin.

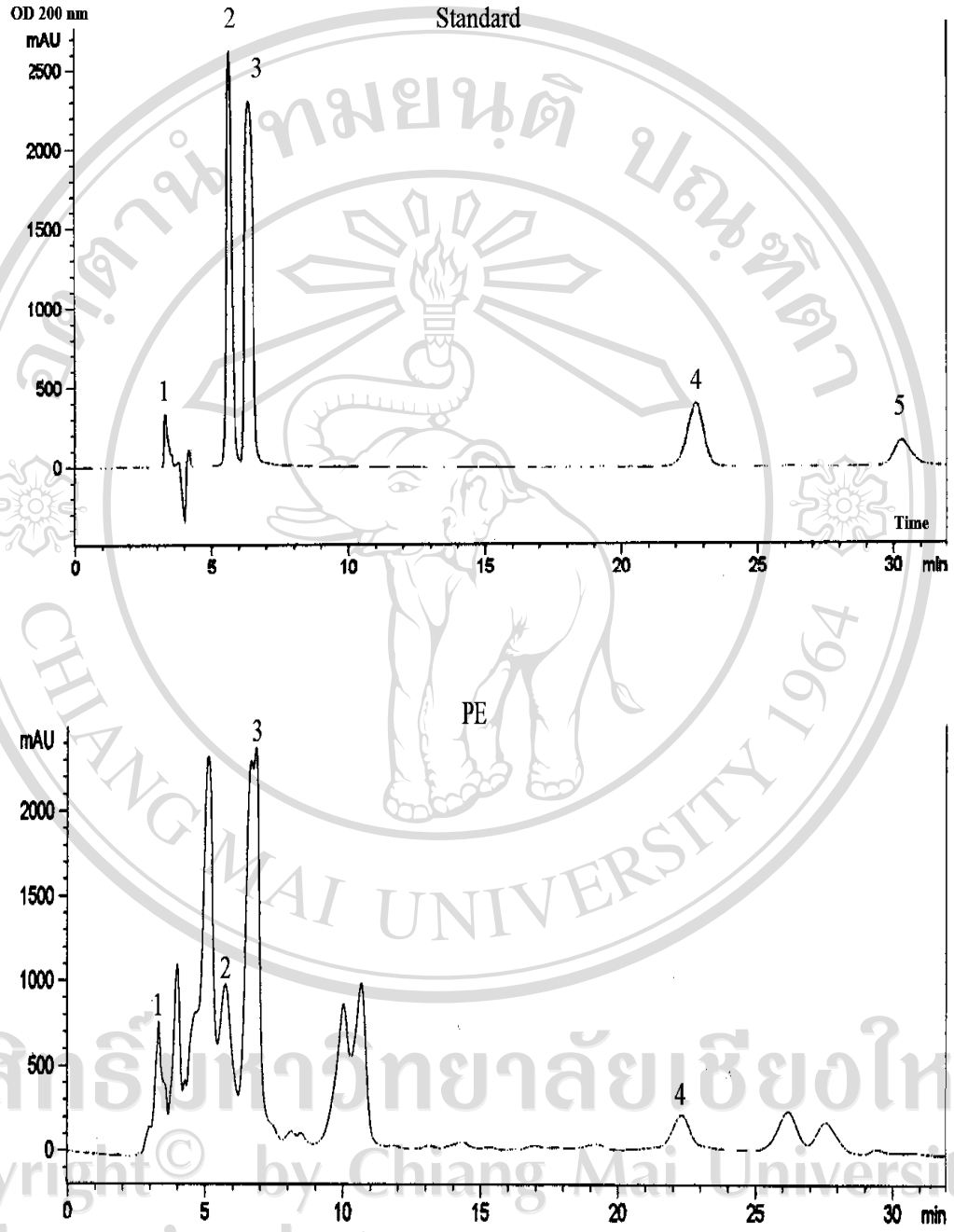


Figure 4.1a) HPLC analysis of polyphenols from standard and PE plant extract was detected at a wavelength of 200 nm, reference of standard compounds using rutin (1), gallic acid (2), pyrolgallol (3), catechin (4) and caffeic acid (5).

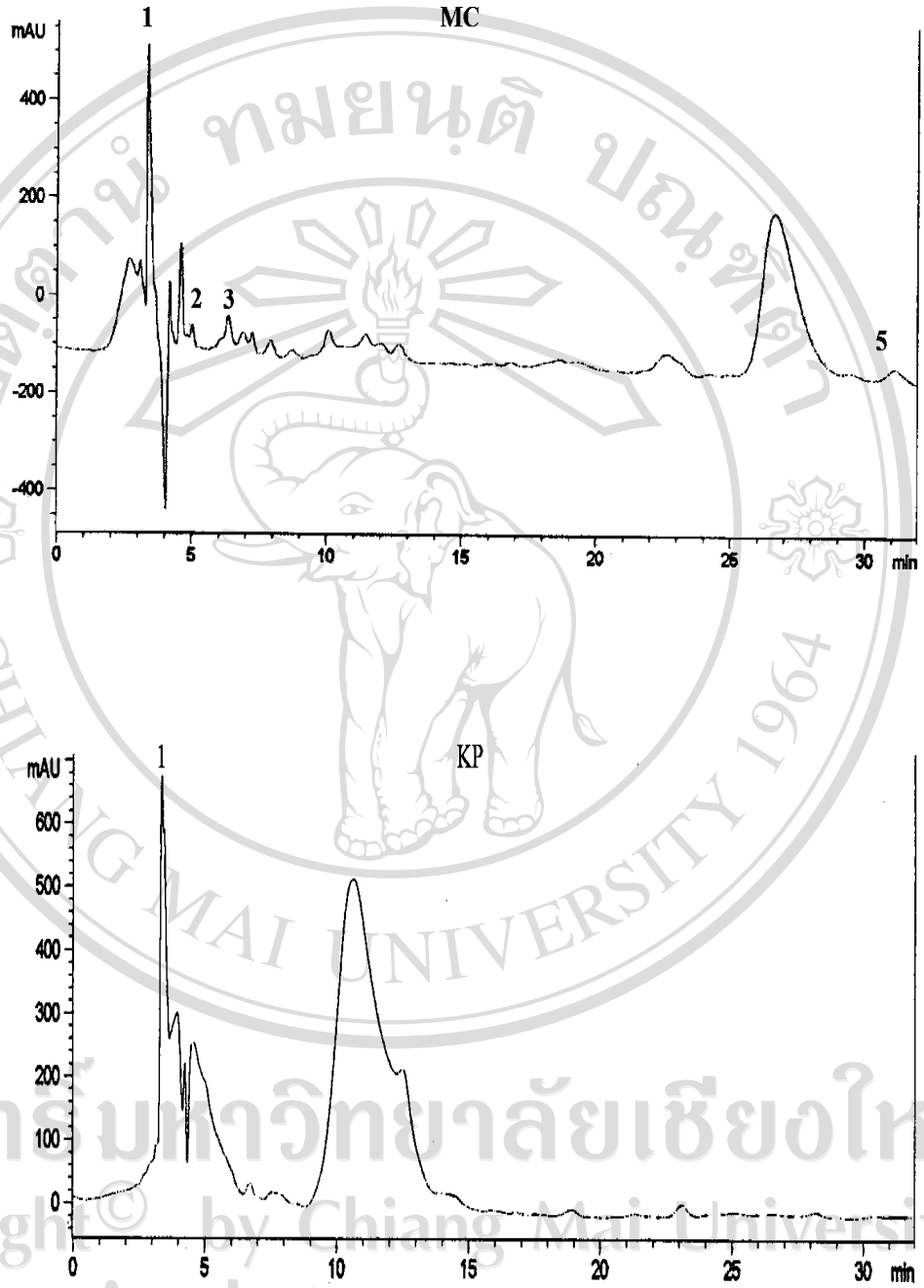


Figure 4.1b) HPLC Chromatograms of MC and KP plant extracts were detected at a wavelength of 200 nm, reference of standard compounds (Figure 4.1a) using rutin (1), gallic acid (2), pyrolgallol (3), catechin (4) and caffeic acid (5).

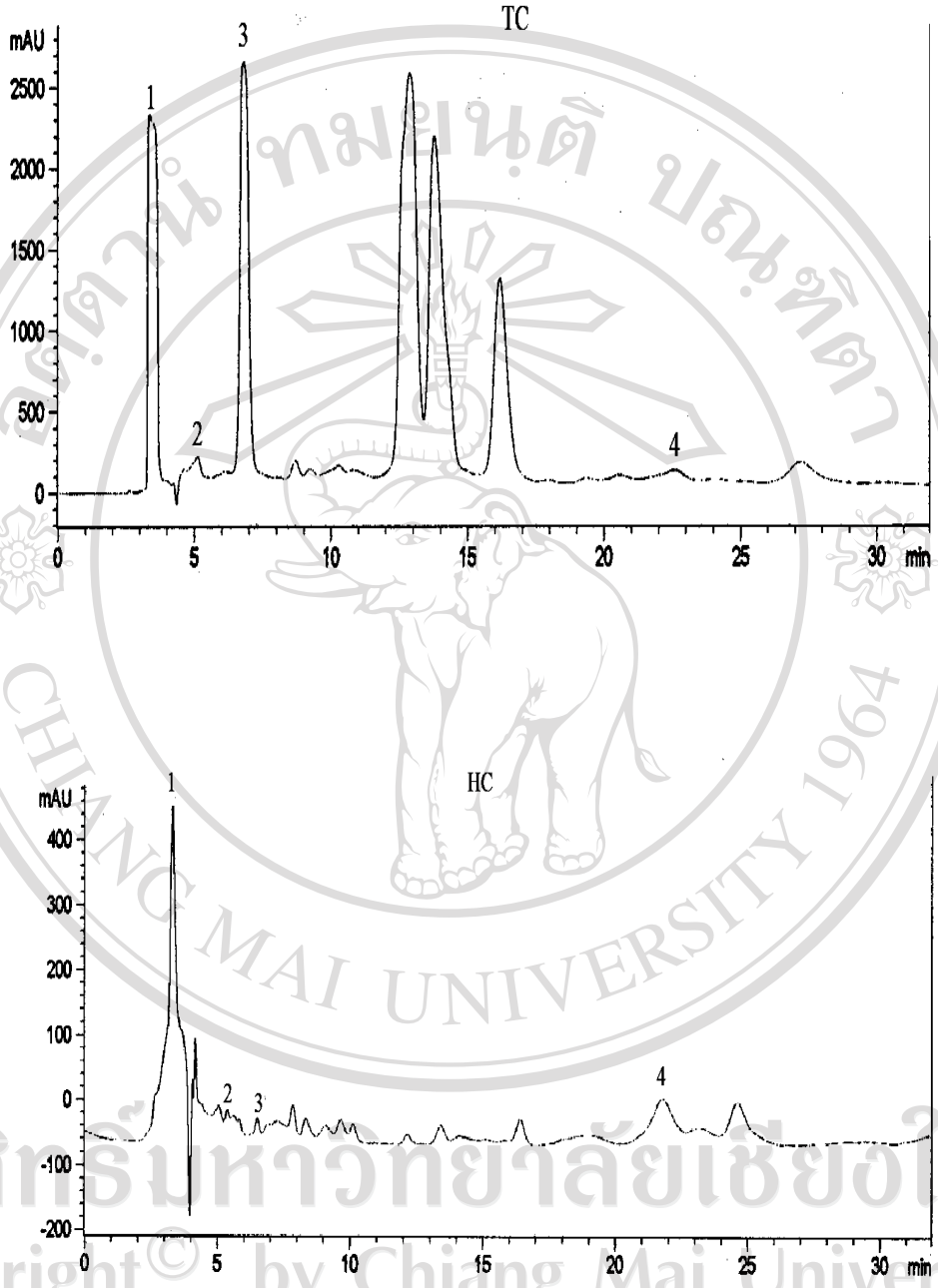


Figure 4.1c) HPLC Chromatograms of TC and HC plant extracts were detected at a wavelength of 200 nm, reference of standard compounds (Figure 4.1a) using rutin (1), gallic acid (2), pyrolgallol (3), catechin (4) and caffeic acid (5).

4.1.6 Analysis of polyphenol compounds by TLC

Table 4.5 showed TLC values of the rate of flow (R_f) of five plants (PE, TC, MC, KP and HC) and standards (rutin, quercetin, pyrolgallol, gallic acid, caffeic acid and catechin) on a) condition n-hexane: ethyl acetate: acetic acid (20:19:1) and b) condition dichloromethane: methanol: acetic acid (28:18:1). Their TLC chromatograms were exhibited in Figure 4.2.

Table 4.5 a) TLC values of the rate of flow of five plants and standards on condition n-hexane: ethyl acetate: acetic acid (20:19:1)

Plant	Colour	R_f value
PE	brown	0.46
	brown	0.72
TC	brown	0.40
	brown	0.62
	brown	0.85
	green	0.99
MC	brown	0.04
	brown	0.62
HC	brown	0.04
	brown	0.12
	brown	0.62
	Green	0.77
	green	0.94
KP	yellow	0.77
rutin	brown	0.04
quercetin	brown	0.73
pyrolgallol	brown	0.85
Gallic acid	brown	0.46
Caffeic acid	brown	0.68
catechin	orange	0.27

Table 4.5 b) TLC values of the rate of flow of five plants and standards on condition dichloromethane: methanol: acetic acid (28:18:1)

Plant	**Colour**	**R_f value**
PE	brown	0.32
	brown	0.56
TC	brown	0.46
MC	brown	0.68
KP	green	0.78
HC	brown	0.54
	brown	0.61
	green	0.82
rutin	brown	0.73
quercetin	brown	0.86
pyrolgallol	brown	0.79
Gallic acid	brown	0.73
Caffeic acid	brown	0.79
catechin	orange	0.81

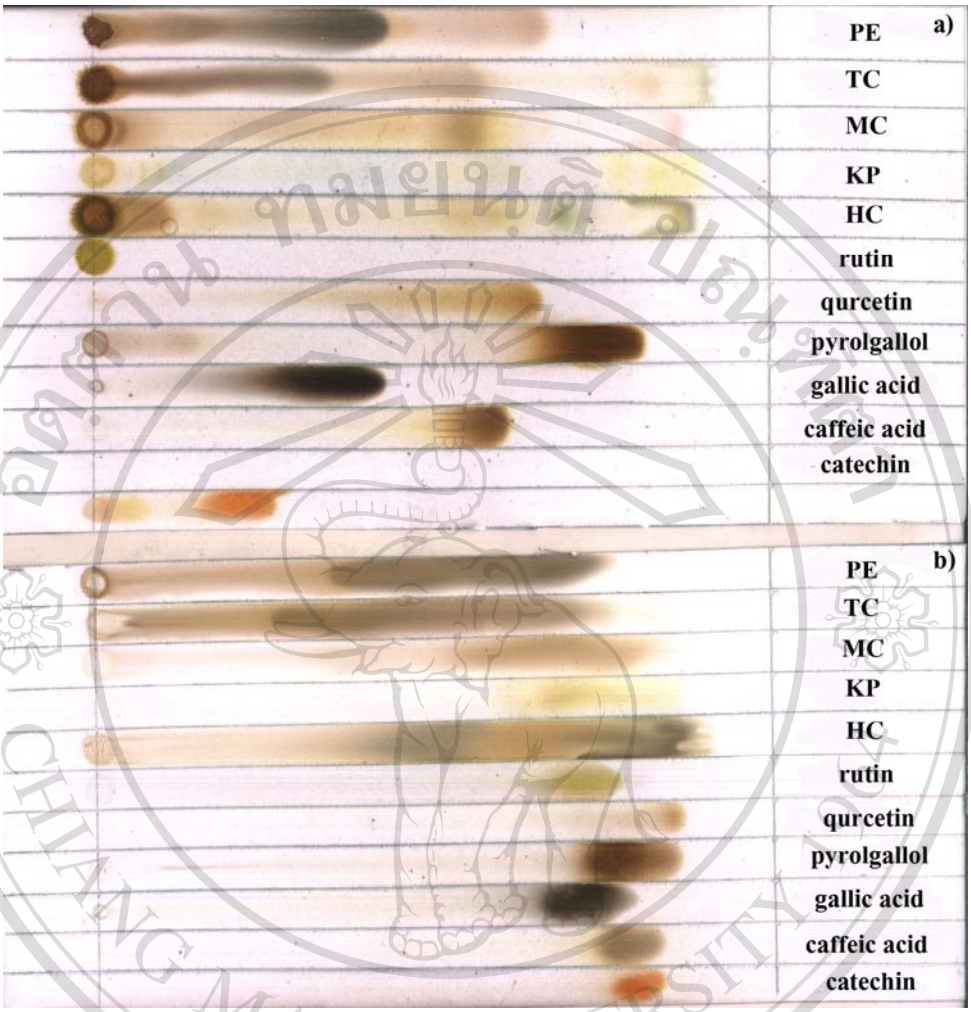


Figure 4.2 TLC chromatograms of PE, TC, MC, KP and HC plant extracts and also the polyphenol compounds standard; rutin, quercetin, pyrolgallol, gallic acid, caffeic acid and catechin. a) condition = n-hexane: ethyl acetate: acetic acid (20:19:1) and b) condition = dichloromethane: methanol: acetic acid (28:18:1)

4.1.7 Determination of total polyphenolic compounds properties; total phenols, flavonoids and tannins content of five plants with strong antioxidant activity

The antioxidant activity of plants is mainly contributed by the active compounds present in them. The five plants with a strong antioxidant activity index and appropriate ability against oxidative stress were *Phyllanthus emblica* Linn., *Terminalia chebula* Retz., *Morinda citrifolia* Linn., *Kaempferia parviflora* Wall. and *Houttuynia cordata* Thunb. Thus, these plant extracts with a high potency for antioxidant ability were selected to study total polyphenolic compound properties. They were studied for total phenols, flavonoids and tannins contents. These are very important plant constituents. Their antioxidant activity includes scavenging or chelating free radicals, preventing lipid peroxidation and glycation [10, 13, 35, 42, 44, 46-47, 49, 60, 72, 76, 80, 98, 165-167]. In this study, we found that different plant extracts contain different levels of total polyphenolic compounds (Table 4.6). *Phyllanthus emblica* Linn. showed the highest total polyphenol and tannin content (2,536 mg GAE/g dry weight of plant extract and 461 mg TAE/g dry weight of plant extract) but showed the lowest total flavonoid content (49.91 mg QE/g of dry weight of plant extract) compared to other plants. *Houttuynia cordata* Thunb. had the highest flavonoid content (191.17 mg QE/g dry weight of plant extract). In addition, *Kaempferia parviflora* Wall. was high in total flavonoid content

(104.36 mg QE/g dry weight of plant extract). Moreover, *Terminalia chebula* Retz. showed high levels of total polyphenol and tannin content (2,276 mg GAE/g dry weight of plant extract and 330.86 mg TAE /g dry weight of plant extract). However, *Morinda citrifolia* Linn. had the lowest total polyphenol and tannin content (49.60 mg GAE/g of dry weight of plant extract and 44.24 mg TAE /g dry weight of plant extract).

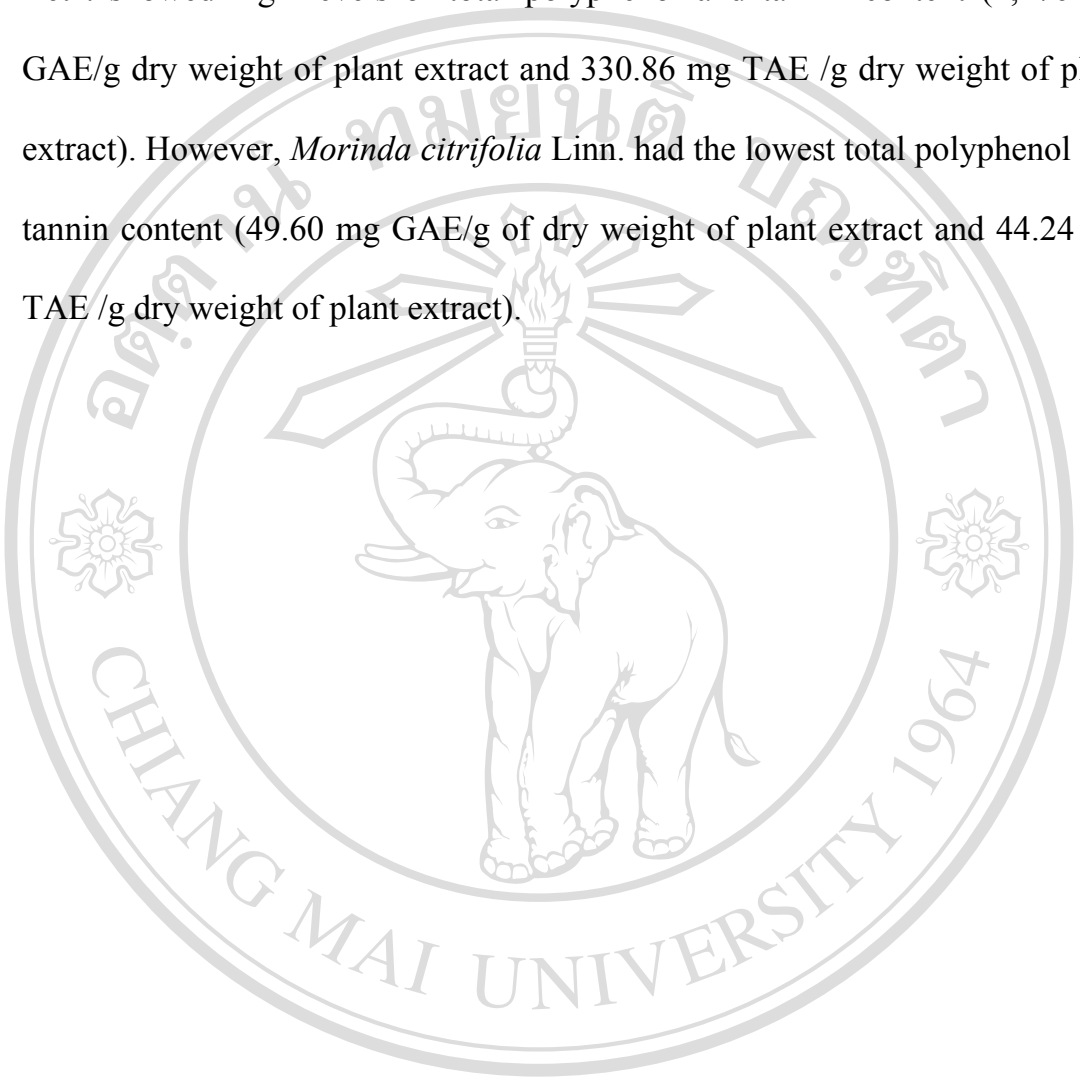


Table 4.6 Total antioxidant activity and contents of main polyphenolic compounds in ethanol extract of five plants

Plants	Antioxidant activity (mg ascorbic acid /g)	Phenolic acid (mg gallic acid/g)	Flavonoid (mg quercetin/g)	Tannin (mg tannic acid/g)
Phyllanthus emblica Linn.	1,266 ± 4.60	2,535.45 ± 68.52	49.91 ± 1.23	461.26 ± 84.90
Terminalia chebula Retz.	939 ± 1.38	2,275.45 ± 436.35	99.21 ± 5.99	330.86 ± 55.30
Morinda citrifolia Linn.	658 ± 1.84	49.60 ± 7.78	68.06 ± 0.43	44.24 ± 4.61
Houttuynia cordata Thunb.	397 ± 10.59	295.20 ± 22.49	191.17 ± 17.71	96.40 ± 14.56
Kaempferia parviflora Wall.	285 ± 3.68	100.15 ± 8.34	104.36 ± 0.43	48.00 ± 11.93

Note Data were expressed as means ± SD (n = 5)

4.2 The effects of biologically fermented Thai indigenous plant beverage against diabetic oxidative stress in streptozotocin-induced rats.

4.2.1 GC/MS and HPLC analytic of mixed BFPB extract components and Contents of main polyphenolic compounds

GC/MS profile in Figure 4.3 shows that gallic acid (MW = 170.12, retention time 5.56 min), pyrolgallol (MW = 126.11, retention time 8.5 min) and one unidentified compound (retention time 4.5 min) are major constituents of the BFPB. In addition, HPLC chromatogram of the BFPB extract demonstrates the major components with reference to standard polyphenolic compounds; rutin (retention time 3.29 min), gallic acid (retention time 5.56 min), pyrogallol (retention time 6.35 min), catechin (retention time 22.72 min) and caffeic acid (retention time 30.24 min) in Figure 4.4(a). We found that the polyphenolic compounds in the BFPB were rutin, gallic acid, pyrolgallol and catechin as shows in Figure 4.4(b). The observation of other UV absorbance possibly identified flavonoids group. The figure might implies a finger print of final product of BFPB.

1 ml of BFPB was found to have different levels of subclasses from polyphenolic compounds (Table 4.7). Contents were phenols 27.35 mg gallic acid equivalent, total flavonoid 2.24 mg quercetin equialent and tannins 0.05

mg tannic acid equivalent**,** and also had an antioxidant activity index of 31.31 mg ascorbic acid equivalent/ml of BFPB**.**

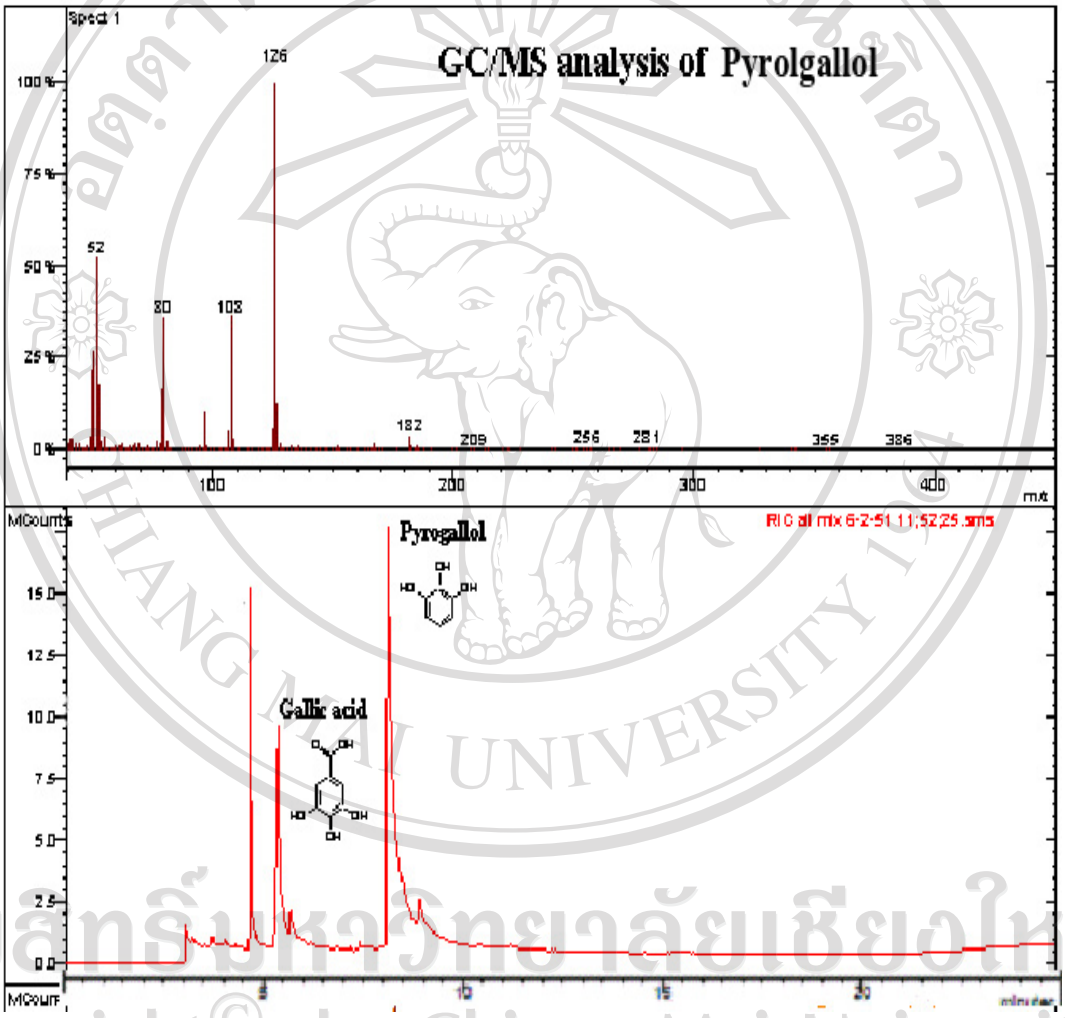
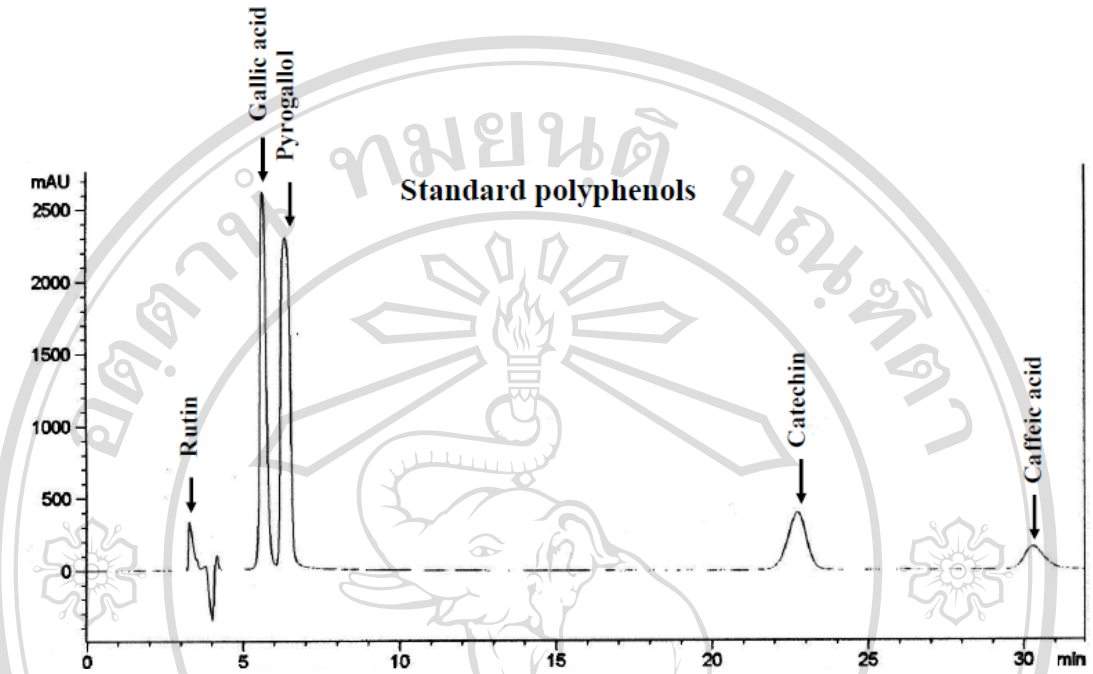


Figure 4.3 GC/MS identification of active ingredients of mixed BFPB extract

(a)



(b)

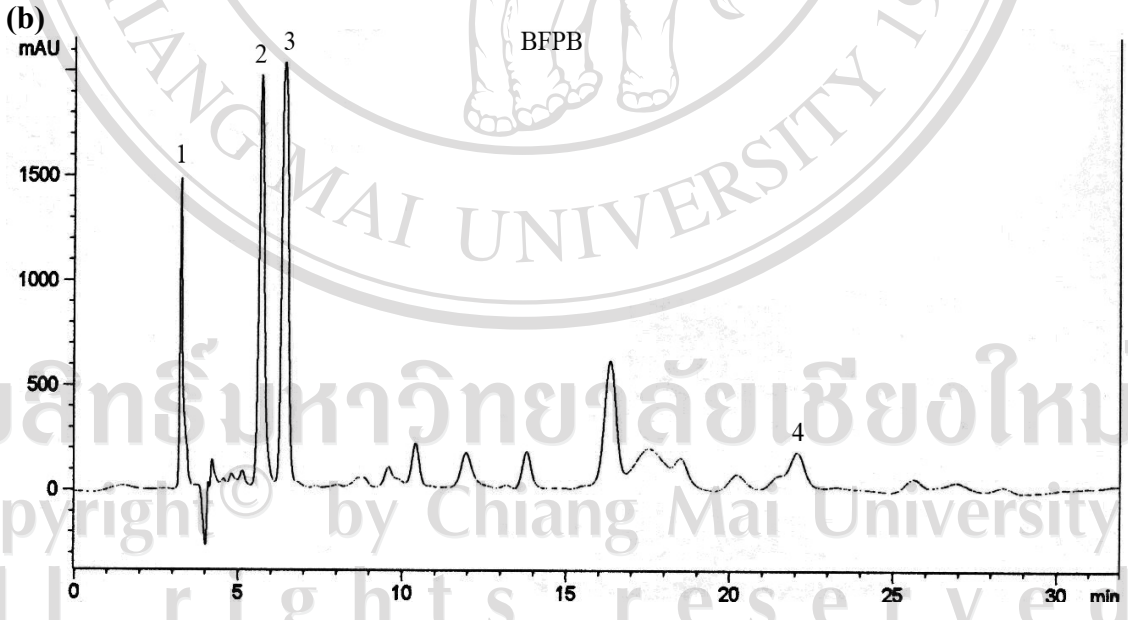


Figure 4.4 HPLC chromatograms of the polyphenol standard (a); rutin (1), gallic acid (2), pyrolgallol (3), catechin (4) and caffeic acid (5) and mixed BFPB (b).

Table 4.7 Contents of main polyphenolic compounds in mixed BFPB

Polyphenolic compounds	**Amounts** (mg/ml)
Phenols (mg gallic acid equivalent/ml of BFPB)	27.35±1.134
Flavonoids (mg quercetin equivalent/ml of BFPB)	2.24±0.017
Tannins (mg tannic acid equivalent/ml of BFPB)	0.05±0.002
Antioxidant activity (mg ascorbic acid equivalent/ml of BFPB)	31.31±0.036

Values expressed means ± SD (*n*=5)

4.2.2 Effect of BFPB on body weight of rats

Table 4.8, Figure 4.5 and Figure 4.6 showed the effects of BFPB on the changes in body weight of rats after the 6 week experimental period. The body weight of rats with STZ-induced diabetes (diabetic control group) was initially 170.4±3.0 g and progressively increased to 313.5±12.5 g (increase 37.7%) at the 6th week. The weight gain in the normal control group during the experimental period was 47.6% (166.7±4.8 g to 436.7±11.7 g). The increase in body weight of the diabetic control group was significantly lower than normal rats (p<0.05) at the beginning of treatment (week 0). The weight gain of the diabetic control group was significantly less than the normal group from week 0 to the 6th week with p<0.001. The BFPB treated groups had slightly higher weight gain than diabetic rats untreated (control group) at the 2nd week and the 6th week, but was not statistically significant. In addition, we found that the body weight gains in diabetic group-treated with 2 ml BFPB/kg BW/day from 2nd week to 6th week was slightly higher (42.7%) than diabetic group treated with 6 ml BFPB/kg BW/day (41.6%). In Figure 4.7, we found that from before treatment until the 6th week, the experimental period, the changes in body weight of all groups increased, which was significantly different (p<0.001) when comparing the normal and diabetic group.

Percentage of weight gain and body weights during the 6 weeks of the experimental period was significantly lower in the diabetic control group

when compared to the normal group. The observation made during the experiment in the diabetic groups found that increased food (hyperphagia) and water intake, also accompanied polyuria and glucosuria (data not shown). It is suggested that an elevated food intake in diabetic rats were an adaptative behavior in response to the loss of calories and cellular starvation. In response to cellular hunger of glucose in diabetes, lipolysis and proteolysis were enchanced [168]. The increase in blood glucose levels in diabetes leads to overproduction of free radicals, defined as an imbalance between oxidants and antioxidants. Glucose auto-oxidises in the presence of transition metal ions generating oxygen-free radicals, make the membrane vulnerable to oxidative damage [2, 4]. Fermented plant product**,** a product rich in antioxidant activity and a potentially useful probiotic, used to improve health as well as an alternative therapy, has anti-inflammatory properties, enhances anti-oxidative activity, has an effect on the activity of antioxidant enzymes in liver, and also has been a useful antigenotoxic antioxidant by scavenging free radicals, inhibiting lipid peroxidation and protecting against oxidative DNA damage [24-25, 34, 101-104, 108, 169-170]. The abnormalities of metabolism responsible for the destruction of *β*-cells and disorder of insulin secretion in the diabetic state could cause the abnormal changes in weight gain by oxidative damage. Type 2 diabetes is not caused only by a defect in β-cell function and insulin resistance, but also by a α-cell dysfunction with relative glucagon excess. Glucagon inhibits glucose-utilization pathways and the storage of

metabllic fuels and also activates hepatic gluconeogenesis, glycogenolysis and lipolysis [171]. Hence, this probably accounted for the lower body weight, weight gain and hyperglycemia in diabetic rats. During the study period, diabetic rats had hyperphagia related to a marked hyperglycemia. In addition, the consequent glucosuria occurred along with polydypsia and polyuria in diabetic rats. Hyperglycemia, the primary clinical indication of diabetes, was accompanied by glucose in the urine when the plasma glucose was higher than 180 mg/dl in human. As glucose is osmotically active, renal excretion of a large amount of glucose leads to loss of water (osmotic diuresis) and the passing of large volumes of urine (polyuria) as well. The resulting fluid loss finally leads to dehydration, therefore, the diabetic rats have to drink a lot of water (polydypsia) [168].

BFPB in this study was composed of five Thai indigenous plants, rich in antioxidant activities and their polyphenolic compounds such as rutin, gallic acid, pyrolgallol and catechin. In the study, a dose of 2 ml and 6 ml BFPB/kg BW/day was administered to diabetic rats tending to improve the loss of body weight in diabetic rats. Regarding the findings, BFPB treatment could improve body weight loss, the clinical signs of diabetes that occurred in STZ-induced diabetic rats.

Table 4.8 Effect of BFPB on body weight in STZ-induced diabetic rats

Group	Weight (g)				
	1 wk before STZ-induction	Week 0	Week 2	Week 4	Week 6
Normal (*n*=9)	166.7±4.8	295.9±19.9	378.3±13.8	419.4±13.4	436.7±11.7
DM (*n*=13)	170.4±3.0	227.7± 9.1	270.8±10.3	306.5 ±11.2	313.5 ±12.5
DM+2 ml BFPB /kg BW /day (*n*=12)	169.6±2.4	227.2±9.5	272.5 ± 12.4	302.5±17.9	324.2 ±18.8
DM+6 ml BFPB /kg BW /day (*n*=11)	174.1±3.4	225.9±7.6	273.6 ±10.6	305.0±13.6	318.6 ±17.8

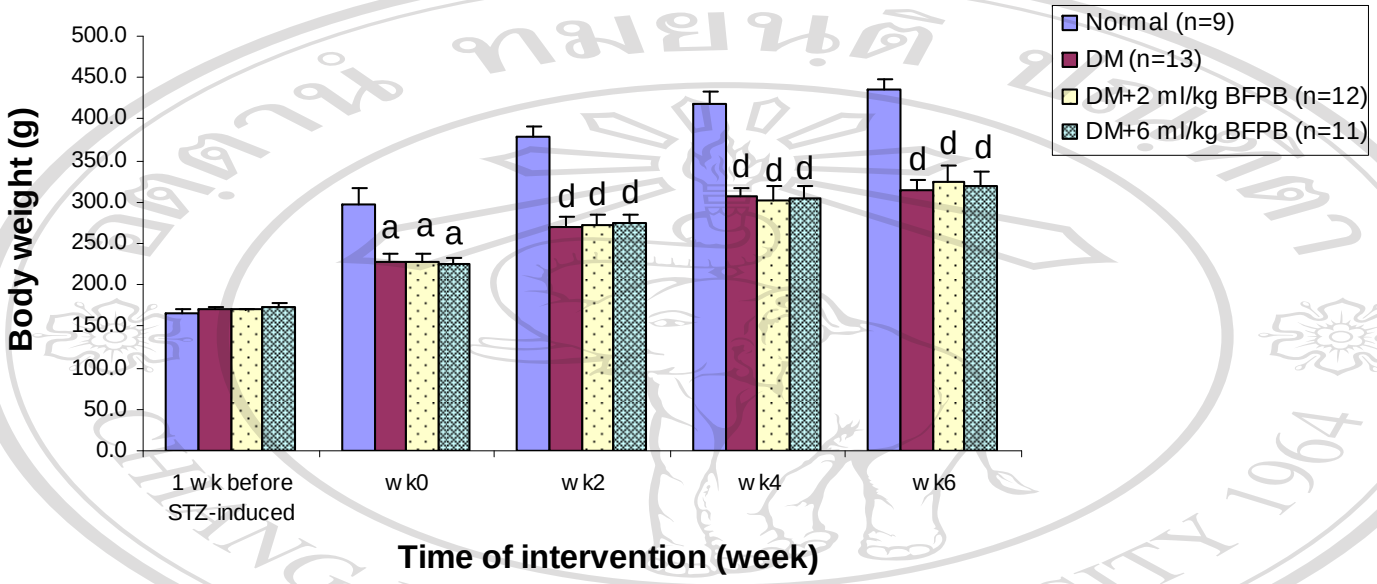


Figure 4.5 Effect of BFPB on body weights in STZ-induced diabetic rats (DM). Values are expressed as means±S.E.M. compared with normal rats group ($^{a}P< 0.05$ and $^{d}P< 0.001$).

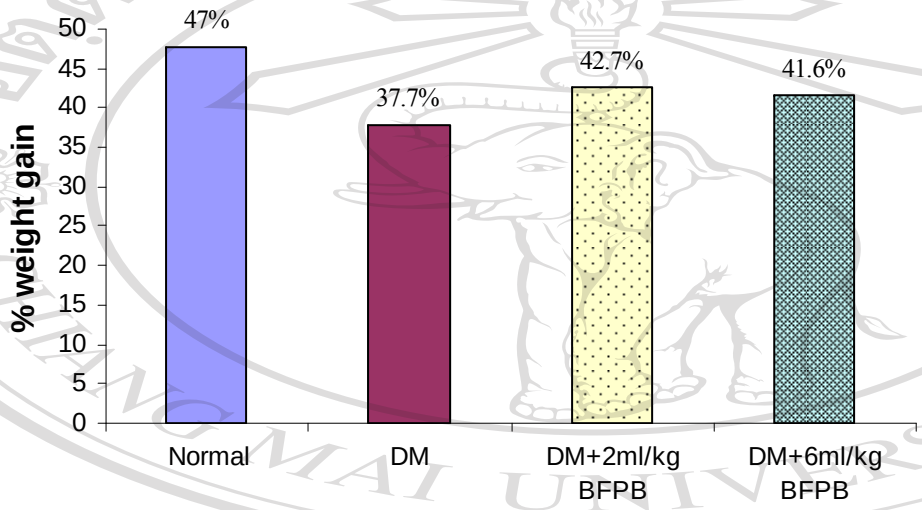


Figure 4.6 Change of body weight in each STZ-induced diabetic rat and normal rat group, from prior to treatment until the end of 6 weeks. Data are expressed as the percentage of weight gain.

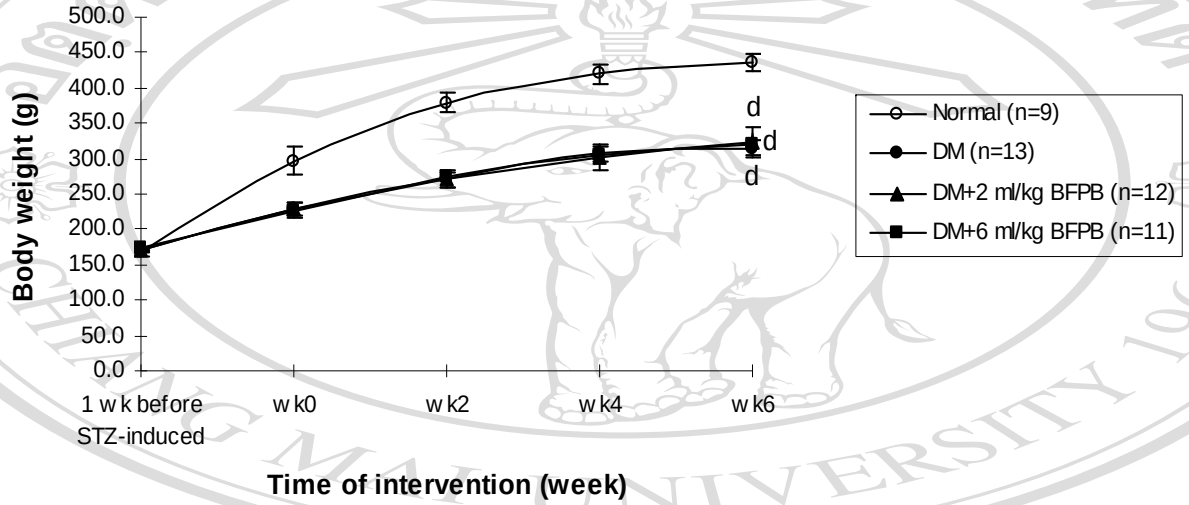


Figure 4.7 Dose response of BFPB on body weight in each STZ-induced diabetic rat and normal rat group, from prior to treatment until the end of 6 weeks. Data are expressed as means±S.E.M. compared with normal rat group ($^{d}P< 0.001$).

4.2.3 Effect of BFPB on plasma glucose levels in STZ-induced diabetic rats

As Table 4.9, Figure 4.8 and Figure 4.9, the plasma level of glucose was significantly increased ($P< 0.05$) in rats with STZ-induced diabetes (29% from 276.6 mg/dl to 356.7 mg/dl) during entire the experimental period when compared with the normal rats group (6.4% from 143.1 mg/dl to 152.2 mg/dl). However, the administration of a dose of 6 ml/kg /day BFPB reduced the progressive increase of plasma glucose levels during week 1-6 (increased only 13.1%) when compared to the diabetic control group (29%) and low dose group (21.1%), but not significantly. In Figure 4.10, we found that before treatment until the 4th week of the experimental period, the plasma glucose increased in each diabetic group and then slightly decreased in the 6th week. In addition, the levels of plasma glucose significantly increased in all diabetic groups ($p<0.05$) when compared with the normal group.

The rats with STZ-induced diabetes showed loss of body weight gain and increased plasma blood glucose. The relationship between diabetes and premature vascular disease is well established. Recent prospective studies indicate that long-term glycemic control is an important predictor not only of microvascular disease, but also of macrovascular complications. It has been suggested that, in diabetes, oxidative stress plays a key role in the pathogenesis

of vascular complications, both microvascular and macrovascular, and an early marker of such damage is the development of an endothelial dysfunction [2].

Hence, the administration of a dose of 6 ml/kg /day of BFPB tended to reduce the physiological changes associated with diabetes via reducing the plasma glucose level in diabetic rats. This implies that high doses of BFPB normalized energy utilization and metabolism and possibly suppressed glucose production through the gluconeogenesis pathway. It may be a potential novel therapy for inhibition of micro- and macrovascular complications in diabetes.

Table 4.9 Effect of BFPB on plasma glucose levels in STZ-induced diabetic rats

Group	Plasma glucose levels (mg/dl)			
	Week 0	**Week 2**	**Week 4**	**Week 6**
Normal (*n*=9)	143.1±7.3	154.4±6.3	160.3±10.2	152.2±4.8
DM (*n*=13)	276.6±26.0	343.1±42.7	374.3±50.6	356.7 ±51.8
DM+2 ml BFPB /kg BW /day (*n*=12)	272.5±28.1	326.4±42.1	344.0 ±49.2	330.1±55.0
DM+6 ml BFPB /kg BW /day (*n*=11)	279.6±29.1	312.5±41.5	339.9 ±55.3	316.3±49.0

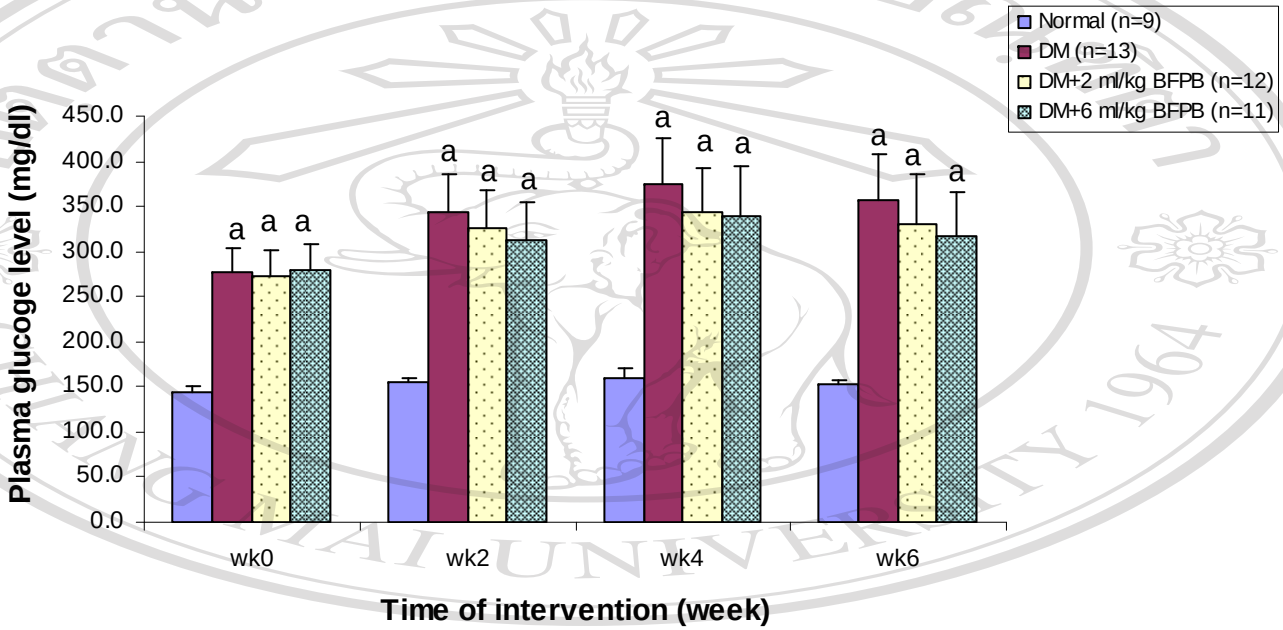


Figure 4.8 Effect of BFPB on plasma glucose levels in STZ-induced diabetic rats. Values are expressed as means±S.E.M. compared with normal rats group ($^{a}P< 0.05$).

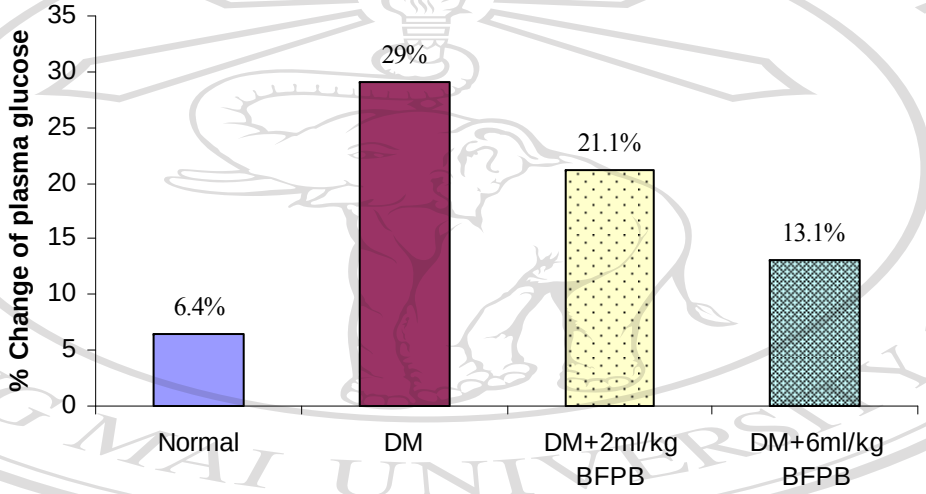


Figure 4.9 Change of plasma glucose in each STZ-induced diabetic rat and normal rat group, from prior to treatment until the end of 6 weeks. Data are expressed as the increasing percentage of plasma glucose.

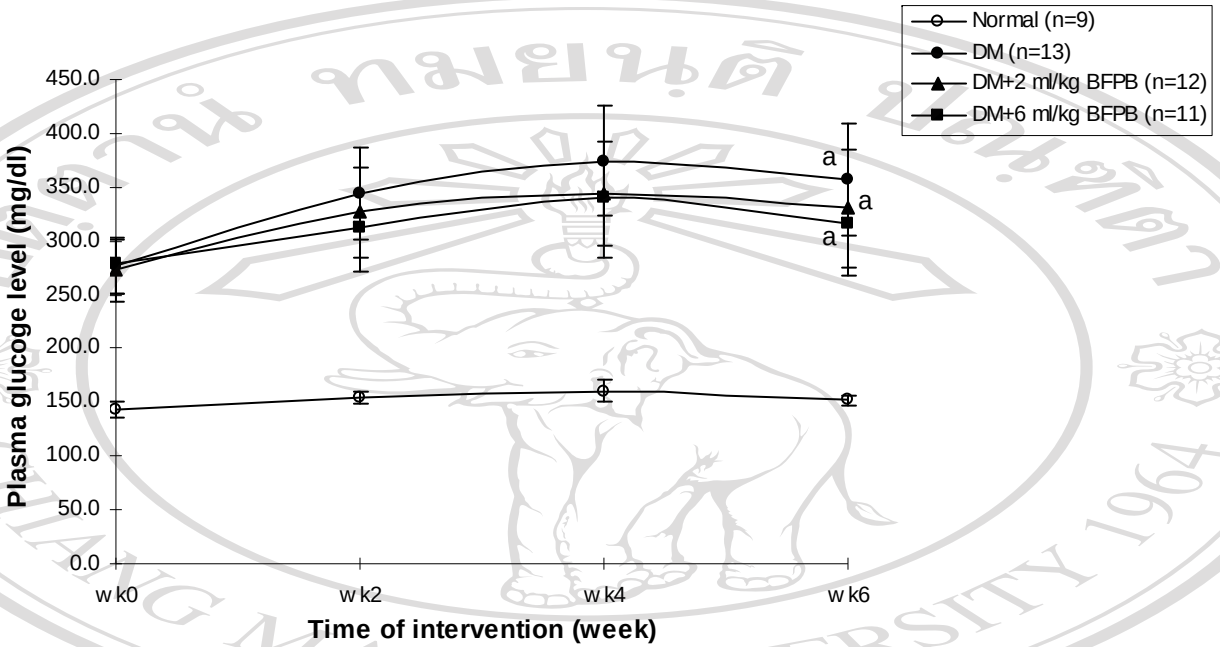


Figure 4.10 Dose response of BFPB on plasma glucose concentrations in each STZ-induced diabetic rat and normal rat group, from prior to treatment until the end of 6 weeks. Data are expressed as means±S.E.M. compared with normal rat group ($^{a}P< 0.05$).

4.2.4 Effect of BFPB on plasma TBARS levels in STZ-induced diabetic rats

The increased MDA level may have an important role in pancreatic damage associated with diabetes. Under diabetic conditions, the level of lipid peroxidation in the pancreas is much higher than in non-diabetic rats. During the experimental period, diabetic rats had higher lipid peroxidation in plasma than normal rats (Table 4.10 and Figure 4.11). The administration of BFPB significantly reduced MDA level at the 6th week in a dose dependently manner when compared to the diabetic control (P<0.05). Throughout the experiment the lipid peroxidation marker, MDA had a tendency to decrease 8.8%, 14.4%, 22.4% and 41.4% (normal group, DM group, DM treated with 2 and 6 ml/kg /day BFPB groups) respectively. However, we found a trend of reducing MDA levels in the diabetic treated group in a dose-dependent manner during weeks 2-6. In addition, by the 6th week the diabetic group treated with 6 ml/kg /day BFPB had a significant decrease in MDA levels (P< 0.05) when compared with the other group of diabetic rats. Moreover, the 6 ml/kg/day dose of BFPB significantly reduced MDA level (P< 0.05) when compared with the diabetic group treated with 2 ml/kg BFPB, from initiation to the 6th week of the experiment (Figure 4.12).

Oxidative stress is associated with lipid peroxidation, which was analyzed by measuring TBARS levels. Lipid peroxidation is a key marker of

oxidative stress. It is the result of a chain reaction induced by ROS and eventually leads to extensive membrane damage and dysfunction [108]. Significant increase in lipid peroxidation products have been reported in diabetes [172-173]. Content of lipid peroxidation products may indicate oxidative stress associated with the diabetic condition, leading to diabetic complications. Tissue and blood MDA levels in STZ-induced diabetic rats increased because of lipid peroxidation [4, 27, 174-175]. Previous studies, both *in vitro* and *in vivo*, have shown anti-oxidation activity to inhibit and reduce the degeneration of cells, through its antioxidation activity. Studies in Japan, Korea and Taiwan reported that natural fermented product reduced lipid peroxidation [34, 168-169, 176]. In this study, the administration of BFPB at doses of 2 and 6 ml/kg significantly decreased MDA levels of diabetic rats during the experimental period in a dose-dependent manner at the 6^{th} week. The study exhibited a relief of oxidative stress, a diabetic pathological condition, through the inhibition of lipid peroxidation. The results indicate that BFPB and their polyphenols (such as phenols and flavonoids) show promise as therapeutic agents for various disorders involving free radical reactions [80]. Antioxidant actions of BFPB could act by chain-breaking antioxidants, by combining with the intermediate radical produced from hyperglycemia in diabetes. It should be stressed that many antioxidants have multiple mechanisms of action [52, 118].

Table 4.10 Effect of BFPB on plasma TBARS levels in STZ-induced diabetic rats

Group	Plasma TBARS levels (µM)			
	Week 0	**Week 2**	**Week 4**	**Week 6**
Normal (*n*=9)	1.47±0.32	1.76±0.20	1.88±0.22	1.34±0.09
DM (*n*=13)	3.26±0.84	2.81±0.17	2.72±0.23	2.79±0.29
DM+2 ml BFPB /kg BW /day (*n*=12)	2.54±.0.40	2.76±0.22	2.58±0.25	1.97±0.08
DM+6 ml BFPB /kg BW /day (*n*=11)	2.66±0.54	2.50±0.19	2.22±0.25	1.56±0.07

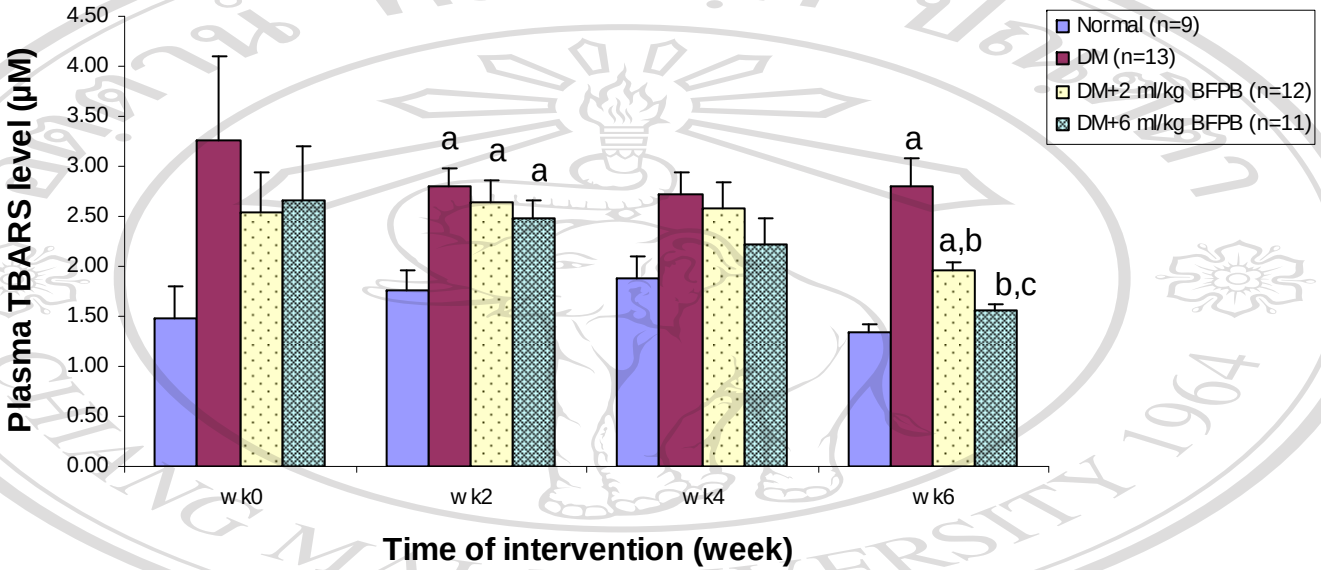


Figure 4.11 Effect of BFPB on plasma TBARS levels in STZ-induced diabetic rats. Values are expressed as means±S.E.M. compared with normal group ($^{a}P< 0.05$), DM group ($^{b}P< 0.05$) and compared with DM group treated with 2ml/kg BFPB/day ($^{c}P< 0.05$).

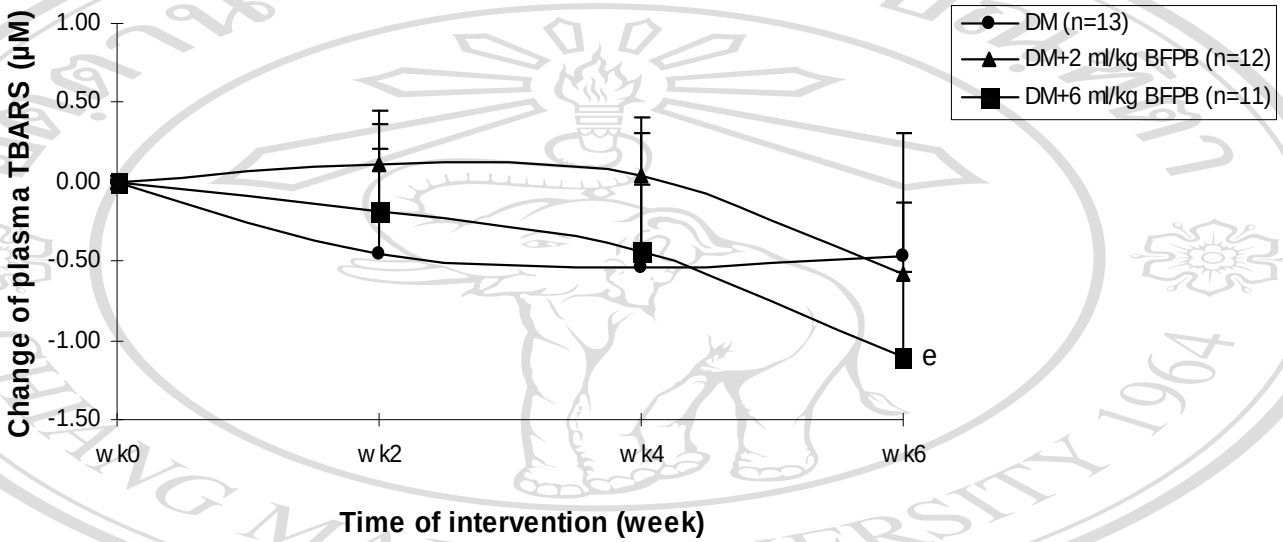


Figure 4.12 Dose response of BFPB on plasma TBARS (MDA equivalents) concentrations in each STZ-induced DM group, from prior to treatment until the end of 6 weeks. Data are expressed as mean±S.E.M compared with DM group treated with 2ml/kg BFPB ($^{e}P< 0.001$).

4.2.5 Effect of BFPB on levels of red blood cells oxidative stress in STZ-induced diabetic rats

Table 4.11 and Figure 4.13 show the inhibitory effects of BFPB on RBCs oxidative stress levels (erythrocyte ROS level). The diabetic rats had higher RBCs oxidative stress than normal rats during the entire experiment. The administration of BFPB resulted in a decrease (85.8% and 79.7%) in the levels of erythrocyte ROS levels at doses of 2 and 6 ml/kg BW daily to diabetic rats from initiation to the 6th week compared with diabetic rats control group (70.8%), not significantly. However, the dose response of BFPB on erythrocyte ROS levels in each diabetic group, before treatment until week 6 was significantly lowered ($P< 0.05$) when compared within the same group (Figure 4.14).

In vivo conditions, higher baseline levels of free radicals might not be specific for cancer, thalassemia or cardiovascular disease but can be found in other clinical situations such as diabetes. The availability of a flow cytometer has been used as standard equipment in the laboratory for indicating RBCs oxidative stress status [157, 177]. Over the experimental period, diabetic rats had higher RBCs oxidative stress levels than normal rats, reflecting the primary pathological diabetic complication. Although, the administration of BFPB overall during 6 weeks did not significant reduce RBCs free radicals when compared with the diabetic control group. However, at the 4th and 6th

weeks of the experiment RBCs oxidative stress tended to reducing in the diabetic treatment group. It indicates that RBCs oxidative stress tended to scavenge by antioxidant activity, obtained from such active ingredients as polyphenolic compounds.

Based on these results, we may expect BFPB to prevent the development of diabetes and its complication. On the other hand, it was observed that in case of diabetes without BFPB treatment, free radicals of RBCs was similarly decreased continuously until week 4 and slightly increased there after (Figure 4.14). It is proposed that early stage pathology of RBCs oxidative stress, the auto-defense mechanism in the rat may be compensated and slightly depleted by week 4. In previous *in vivo* studies, long-term effects of plant product supplementation on antioxidative status in the human organism and on the red blood cell parameters have been investigated. There is considerable and consistent evidence for antioxidant activity [178-182]. However, the data from the *in vivo* studies of BFPB is still limited. Beneficial health effects of polyphenolics depend on free radical scavenging activities, and also due to the influence on gene expression, and the cell signaling cascades [183-184].

Table 4.11 Effect of BFPB on levels of red blood cells oxidative stress in STZ-induced diabetic rats

Group	Levels of red blood cells oxidative stress (FI unit)			
	Week 0	**Week 2**	**Week 4**	**Week 6**
Normal (*n*=9)	15.90±2.71	16.71±3.18	15.20±2.57	14.01±3.68
DM (*n*=13)	55.75±7.89	38.41±5.44	16.37±3.67	16.30±3.50
DM+2 ml BFPB /kg BW /day (*n*=12)	54.60±5.29	39.42±9.13	8.06±1.09	7.76±1.17
DM+6 ml BFPB /kg BW /day (*n*=11)	51.34±10.72	39.82±13.26	11.73±2.43	10.45±2.51

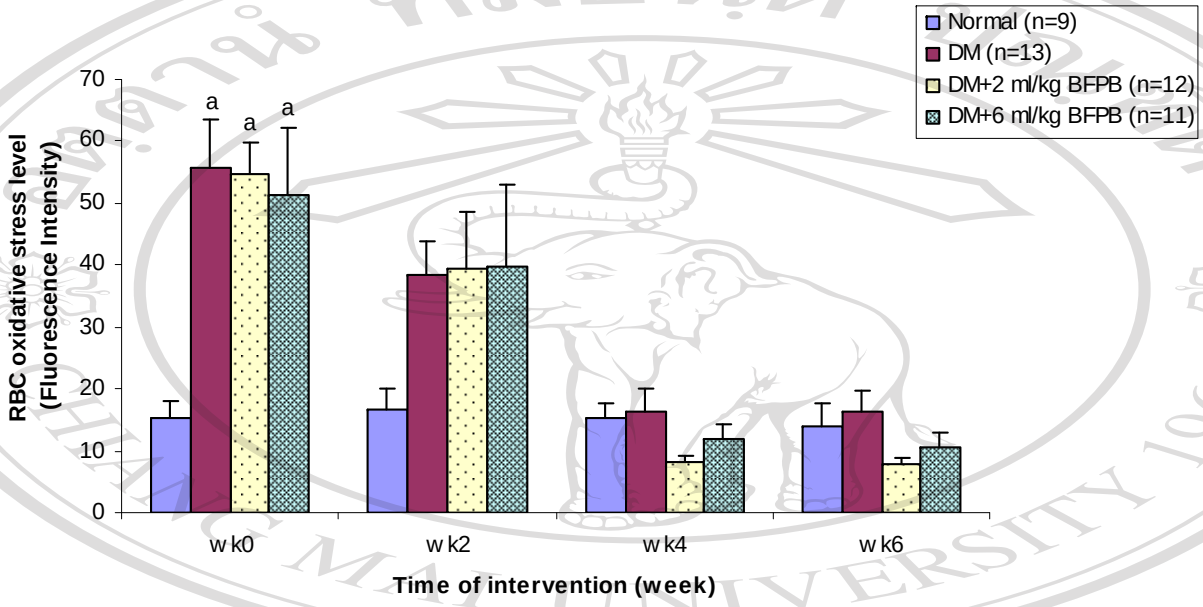


Figure 4.13 Effect of BFPB on levels of red blood cells oxidative stress in STZ-induced diabetic rats.

Values are expressed as means ± S.E.M. compared with normal rats group ($^{a}P< 0.05$).

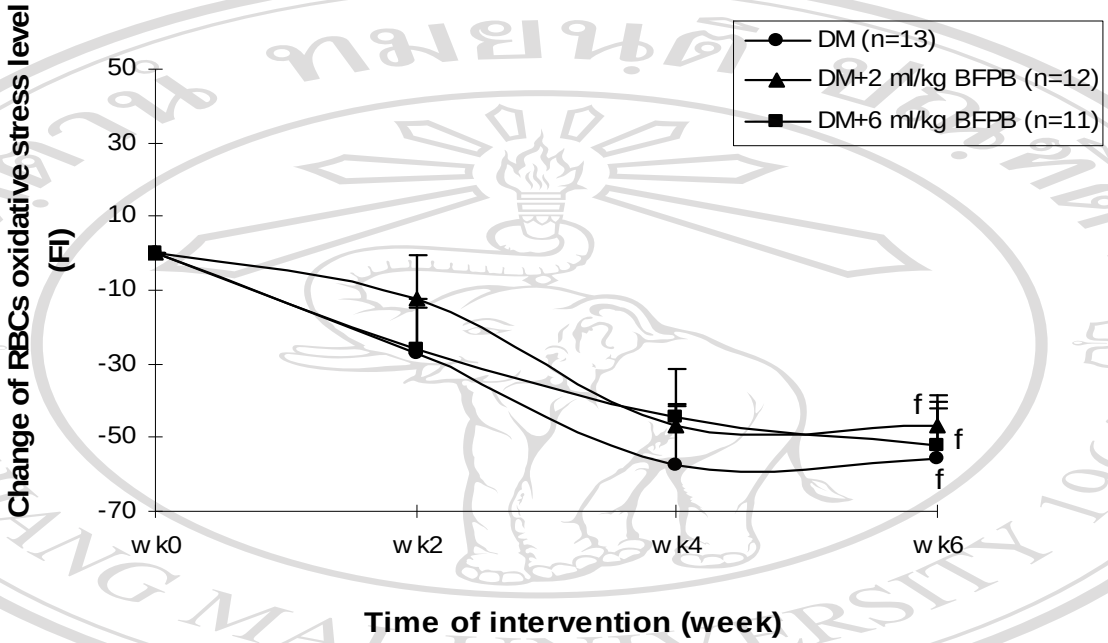


Figure 4.14 Dose response of BFPB on erythrocyte ROS levels in each STZ-induced diabetic group, from prior to treatment until the end of 6 weeks. Data are expressed as mean ± S.E.M. compared with the same group ($^{f}P < 0.05$).

4.2.6 Effect of BFPB on plasma superoxide anion in STZ-induced diabetic rats

From Table 4.12 and Figure 4.15, the diabetic rats had higher plasma superoxide anion levels (OD_{560nm} 0.016±0.003) than normal rats (OD_{560nm} 0.011±0.003), except at 4 weeks. The administration of BFPB at doses of 2 and 6 ml/kg BW daily to diabetic rats resulted in a decrease in the levels of plasma superoxide anion compared with those of diabetic rats control at the 4^{th} and 6^{th} weeks (except 2 ml/kg BW dose on 4^{th} week, increasing more than the other groups), no significant differences were found between groups throughout this investigation. In addition, at end of the study, levels of superoxide anion radical in diabetic rats treated with 2 ml/kg BW/day BFPB decreased 7.7% (from OD_{560nm} 0.013±0.002 to OD_{560nm} 0.012±0.003) less than the untreated diabetic group, which lowered by 18.2%. On the other hand, the dose 6 ml BFPB/kg BW/day increased the levels of superoxide anion radical 7.1% (OD_{560nm} 0.014±0.003 to OD_{560nm} 0.015±0.006). While the dose response of BFPB on plasma superoxide anion levels in each diabetic group, before treatment until the 6^{th} week, as shown in Figure 4.16, seemed to fluctuate during the study, probably because the half life of O_2^- is too short to measure.

Under diabetic conditions, hyperglycemia leads to overproduction of O_2^- and NO. O_2^- is an attractive candidate as a mediator of endothelial dysfunction in diabetes. Increased production of O_2^- occurs as a result of auto-

oxidation of glucose and/or nonenzymatic protein glycation. NO is responsible for harmful effects on β-cell function and it interacts with O_2^- to form the hydroxyl radicals, which target intracellular antioxidative enzymes, leading to highly reactive oxidative damage associated with diabetes [2, 9, 185-186]. Previous studies have demonstrated that some traditional medicines have superoxide and peroxynitrite scavenging properties [98, 187]. Our results showed that rats with STZ-induced diabetes had high plasma levels of O_2^-, implying that STZ leads to oxidative stress, which will ultimately affect the β-cell function, resulting in the production of the superoxide, and damages DNA. DNA damage is an obligatory stimulus for the activation of nuclear enzymes, such as poly (ADP-ribose), polymerase (PARP), which activation, in turn, depletes the intracellular concentration of its substrate NAD^+, slowing the rate of glycolysis, electron transport, and ATP formation, and produces an ADP-ribosylation of the GAPDH. This process results in acute endothelial dysfunction in diabetic blood vessels, which contributes to the development of diabetic complications. NF-κB activation also induces a proinflammatory condition and adhesion molecule overexpression. All these alterations produce the final picture of diabetic complications [2]. BFPB reduced mitochondrial overproduction of superoxide. Reducing DNA damage property may be a good property for causal intracellular antioxidants. This study showed that effects of BFPB on O_2^- of rats plasma are uncertain. Since the half life of O_2^- is too short to measure. However, these findings suggest that low dose of BFPB might act

as a free radical scavenger of O_2^- and provide protection against the oxidative stress induced by hyperglycemia. On the other hand, high dose of BFPB would be pro-oxidant to promote plasma superoxide generation in diabetes. The utilization of primary antioxidant defenses aims to protect against oxidant damage, improve damage removal and increase repair enzymes to remove and/or repair molecules that do get damaged. Cells have certain defense mechanisms to protect from ROS-induced damage by endogeneous ROS scavenging enzymes, and the consumption of food containing high antioxidant phytochemicals such as polyphenols and carotenoids help break down H_2O_2 to oxygen and water, and converts $O_2^{\bullet-}$ into H_2O_2 [52, 118, 188]. It is important for further study to emphasize that flavonoids and phenolic acids under certain conditions *in vivo* are able to exert pro-oxidant chemistry, avoiding formation of, rather than scavenging of free radicals that could lead to undesired toxic effects on animal or human organism [189-190].

Table 4.12 Effect of BFPB on plasma superoxide anion level in STZ-induced diabetic rats.

Group	Plasma superoxide anion levels (Absorbance)			
	Week 0	**Week 2**	**Week 4**	**Week 6**
Normal (*n*=9)	0.011±0.003	0.008±0.002	0.013±0.004	0.009±0.003
DM (*n*=13)	0.016±0.003	0.009±0.002	0.011±0.003	0.015±0.005
DM+2 ml BFPB /kg BW /day (*n*=12)	0.013±0.002	0.013±0.002	0.016±0.003	0.012±0.003
DM+6 ml BFPB /kg BW /day (*n*=11)	0.014±0.003	0.017±0.004	0.010±0.002	0.015±0.006

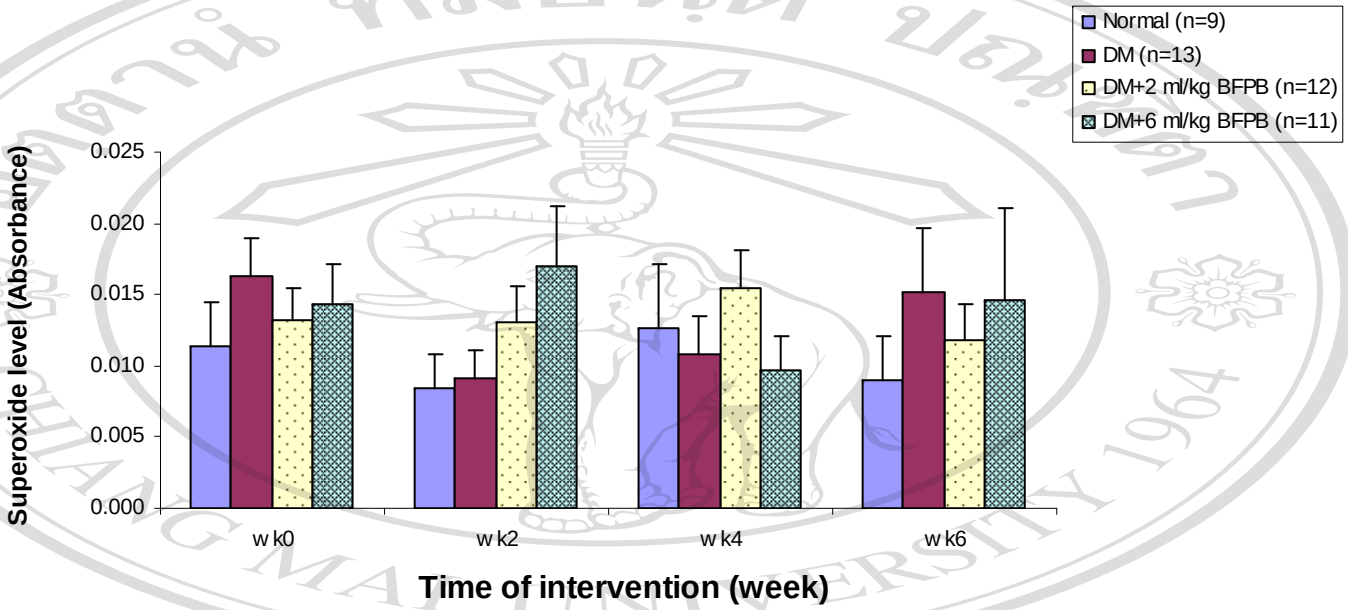


Figure 4.15 Effect of BFPB on plasma superoxide anion in STZ-induced diabetic rats. Values are expressed as means $\pm$ S.E.M.

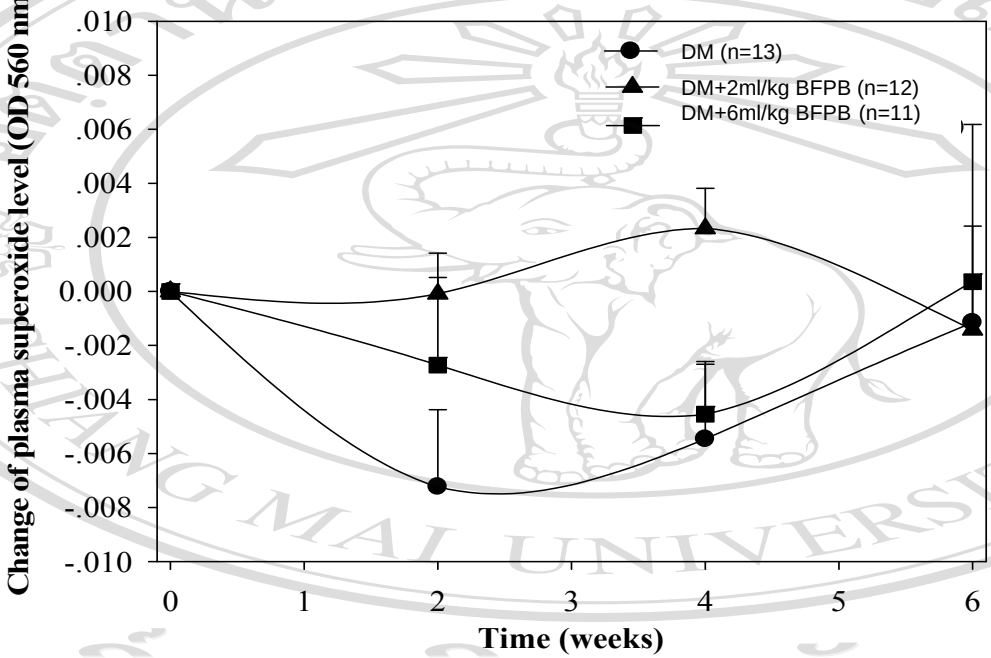


Figure 4.16 Dose response of BFPB on plasma superoxide anion levels in each STZ-induced diabetic rat, from prior to treatment until the end of 6 weeks. Data are expresed as mean+S.E.M.

4.2.7 Effect of BFPB on plasma nitric oxide levels in STZ-induced diabetic rats

As shown in Table 4.13, Figure 4.17 and Figure 4.18, the levels of nitric oxide in plasma in normal rats have a tendency to increase (0.031±0.004 to 0.050±0.008 μM). On the other hand, plasma nitric oxide levels of all the diabetic rats decreased, except diabetic rats treated with high dose of BFPB, which tended to increase by the 6^{th} week (0.048±0.007 to 0.050±0.007 μM). No significant differences were found between any of the groups throughout this investigation. Plasma nitric oxide concentrations fluctuated during the study in all groups, except for diabetic rats treated with low-dose BFPB, which progressively lowered throughout the treatment. Most importantly, nitric oxide is labile in the plasma compartment and can be auto-oxidized to be nitrite and then nitrate, leading to underestimated values. It is essential that the concentration of plasma nitric oxide be measured immediately.

In diabetes, hyperglycemia leads to overproduction of O_2^- and NO. O_2^- overproduction reduces eNOS activity, but through NF-κB and protein kinase C (PKC) activates NAD(P)H and increases iNOS expression, the final effect is an increased NO generation. This condition favors the formation of the strong oxidant peroxynitrite, which in turn produces iNOS and eNOS, an uncoupled state. NO is responsible for harmful effects on *β*-cell function and it interacts with O_2^- to produce the peroxynitrite radicals, which target

intracellular antioxidative enzymes, leading to highly reactive oxidative damage associated with diabetes [2, 9, 185]. It is well recognized that endothelial nitric oxide (NO) plays a significant role in maintaining normal vascular function and preventing cardiovascular disease [191]. The present study suggests that BFPB may improve endothelial function by the activation of NO production. NO is a signaling molecule synthesized by three isoforms of NO synthases (NOS), i.e., bNOS, iNOS, and eNOS. NO production from each NOS isoform is tightly controlled at the transcription, translation, and posttranslational levels [192]. NO produced by eNOS is described as "low output" pathway whereas iNOS generates NO in a "high output" manner, shown in Table 4.12. Results demonstrate that BFPB selectively enhanced eNOS expression. Thus, BFPB may potentially be used as a supplement for vascular endothelial health promotion. The major phytoconstituents of BFPB are polyphenolic compounds such as phenols, flavonoids and tannins. Their effects showed that low-dose BFPB decreased NO induced by diabetes. Since the half life of NO are too short to measure, so the effect of BFPB on them are uncertain. Interestingly, a mixture of fermented grain foods (called antioxidant biofactors) had strong antioxidant activity and stimulated endothelial nitric oxide synthase activity [104]. Similarly, these finding suggest that anti-oxidative BFPB at low dose is the optimal dose that might provide hypoglycemic effects and protection against the oxidative stress induced by hyperglycemia. It has been suggested that overproduction of the free radical

NO under the influence of STZ may play a crucial role in the dysfunction of the β-cells during the development of diabetes.

Table 4.13 Effect of BFPB on plasma nitric oxide levels in STZ-induced diabetic rats

Group	Plasma nitric oxide levels (μM nitrite equivalent)			
	Week 0	Week 2	Week 4	Week 6
Normal (*n*=9)	0.031±0.004	0.028±0.006	0.041±0.008	0.050±0.008
DM (*n*=13)	0.057±0.006	0.038±0.005	0.044±0.006	0.038±0.009
DM+2 ml BFPB /kg BW /day (*n*=12)	0.061±0.008	0.054±0.007	0.053±0.007	0.045±0.007
DM+6 ml BFPB /kg BW /day (*n*=11)	0.048±0.007	0.049±0.008	0.029±0.006	0.050±0.007

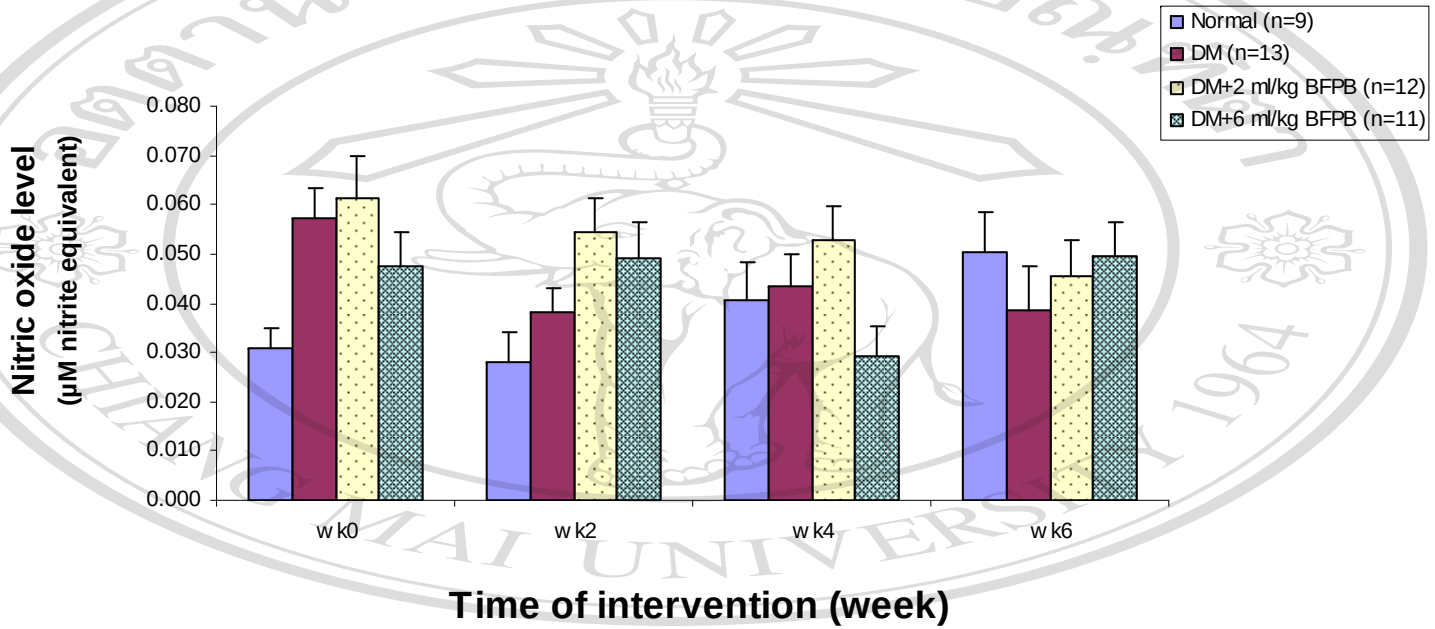


Figure 4.17 Effect of BFPB on plasma nitric oxide in STZ-induced diabetic rats. Values are expressed as means ± S.E.M.

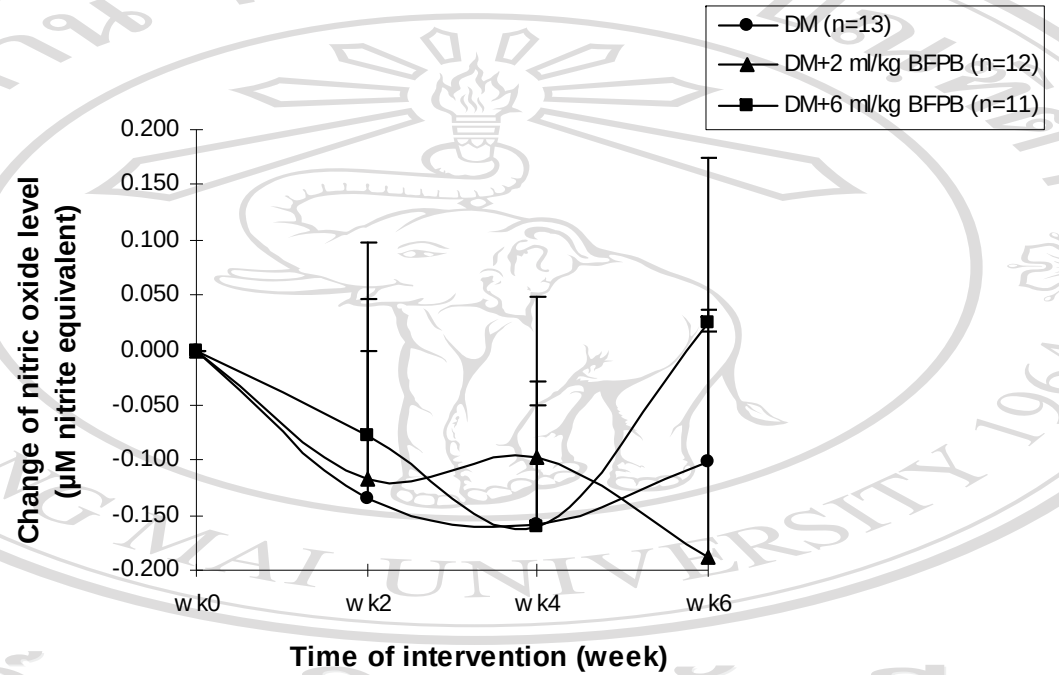


Figure 4.18 Dose response of BFPB on plasma nitric oxide levels in each STZ-induced diabetic rat, from prior to treatment until the end of 6 weeks. Data are expressed as mean±S.E.M.